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UNIFAC Model for Liquid-Liquid Phase Equilibrium of Penicillin G and 6-APA System

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Abstract

This study investigated the effect of pH and type of solvent on liquid-liquid phase equilibrium in the system of pure penicillin G and mixed penicillin G with 6-APA. Penicillin G extraction was carried out in a pH range of 2.0–5.0 at 4 ^{o}C using several types of solvents. The liquid-liquid phase equilibrium mathematical model was prepared by assuming that a single stage of thermodynamic equilibrium occurs in a batch process of liquid-liquid extraction. The coefficient of activity was calculated by the UNIFAC method. From the experiment, it was found that the extraction process of penicillin G was strongly influenced by pH of the solution. The highest yield of extraction was achieved with different solvents in the two types of solution. For pure penicillin G system, the highest yield was obtained in n-butyl acetate solvent (95.51%) while for penicillin G mixtured with 6-APA, it was obtained in methyl iso-butyl ketone solvent (92.6%). The UNIFAC model has been tested against five three-component liquid-liquid phase equilibrium systems at pH 2.0 and 2.5. It was able to estimate the concentration of penicillin G in the organic phase with a relatively average error between experiment and calculation of 8.32%.

Keywords: Extraction, penicillin G, 6-APA, UNIFAC.

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1. INTRODUCTION

The use of semi-synthetic penicillin as a natural penicillin derivative product is now increasingly widespread due to the nature of pathogenic microorganisms that are starting to be resistant to natural penicillin. The advantage of semi-synthetic penicillin is that it is resistant to beta-lactamase, broad-spectrum, and stable in acidic conditions (Singh and Goyal, 2014). This component is produced from the hydrolysis of penicillin G and V using the enzyme penicillin acylase or from a chemical reaction (Dhal, 2018; Haagensen, 2018; Sheldon and van Pelt, 2013; Szewczuk, 2018). Commercially, semi-synthetic penicillin is produced from 6-aminopenisilic acid (6-APA) through an enzymatic equilibrium reaction with penicillin acylase which yields in a mixture of 6-APA components, penicillin G, and phenylacetic acid (PAA) (Carleysmith and Lilly, 2018; Deo and Gaucher, 2018; Haagensen, 2018; Harvey, 2018; Park, 2018; Warburton, 2018). Purification of 6-APA resulting from enzymatic conversion from penicillin G is carried out by extracting penicillin G which is not converted at low pH. 6-APA in the phase of raffinate is concentrated and followed by crystallization in the isoelectric pH region (Deo and Gaucher, 2018; Haagensen, 2018; Karlsen and Villadsen, 2018). Separation of penicillin G with liquid-liquid extraction is conducted by utilizing the solubility difference of penicillin G in the liquid phase and in the organic phase. Previously, the effect of pH and type of solvent on the solubility of pure penicillin G at 25 ^{o}C has been studied (Reschke and Schügerl, 1984a).

The thermodynamic model is assumed for one stage of equilibrium in liquid-liquid extraction of penicillin G in batches and is arranged to obtain operating conditions and types of solvents suitable for the extraction of penicillin G and a mixture of penicillin G with 6-APA. The liquid-liquid phase equilibrium is expressed in terms of the similarity of the activity coefficients in both phases. The activity coefficient is a quantity that cannot be

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measured directly but can only be calculated, for example by using the Gibbs excess function of indexed Margules 2 (1895), Van Laar (1910), Felich-Kister (1948), and Black (1999) or by composition models Locals from Wilson (1964), NRTL (Non Random-Two Liquid) from Renon (1966) and UNIFAC (UNIquac Functional group Activity Coefficient) from Fredenslund (1975) (Sørensen, Magnussen, Rasmussen, and Fredenslund, 1979). The UNIFAC method has advantages in terms of the ability to determine the activity coefficient of a mixture without using experimental data. This method only requires three group parameters that form the components in the mixture, namely relative volume (R_k), relative surface area (Q_k), and interaction parameters (a_{mn}) of each functional group (Sørensen *et al.*, 1979).

This study examined the effect of pH and type of solvent on the liquid-liquid phase equilibrium in the penicillin G system and the mixture of penicillin G with 6-APA. The purpose of this study was to investigate the effect of pH and type of solvent on the distribution of penicillin G and 6-APA in the organic phase and the aquatic phase, determine the parameters of the UNIFAC ternary system interaction parameters (penicillin G-organic solvents and water) under certain pH conditions, and estimate concentration penicillin G in both phases under equilibrium conditions through the model.

2. MATERIALS AND METHODS

Extraction of Penicillin G and 6-APA

Extraction was carried out in a 100 mL separating funnel at 4 ^oC. Before the extraction process was carried out, all materials and tools used were first cooled in a 4 ^oC temperature room. Penicillin G and 6-APA solutions were prepared by dissolving the material in a buffer solution at a certain pH. This solution was extracted using organic solvents with equal volume ratio of the aquatic phase and organic phase. Stirring was done manually with a predetermined time. After the two phases separate, the concentration of penicillin G in the aquatic phase was measured by HPLC and the pH of the solution was measured by a pH-meter. The distribution coefficient (D) was determined by using equation (1).

$$\boldsymbol{D = \frac{[HP]_{org}}{[HP]_{raf}} = \frac{C_o - C_a}{C_a}} \quad \textbf{(1)}$$

where C_o is the initial penicillin G concentration in the aquatic phase and C_a is penicillin G concentration in the raffinate phase. The extraction percentage was calculated by equation (2)

$$\%\mathbf{E} = \frac{[HP]_{org}}{[HP]_{awal}} \times \mathbf{100\%} = \frac{C_o - C_a}{C_o} \times \mathbf{100\%} \quad \textbf{(2)}$$

The extraction of penicillin G from the the mixture of Penicillin G/6-APA was carried out the same as the extraction process on pure penicillin G. In pure penicillin G extraction, a buffer solution of pH 2 from a mixture of HCl and KCl; pH 3, 4, and 5 of a mixture of citric acid and disodium hydrogen phosphate were used. Meanwhile, for a mixture of penicillin G with 6-APA, the citrate-phosphate buffer solution cannot be used because the peak of the chromatogram produced by 6-APA coincides with the peak of the glycine and HCl mixture buffer solution (pH 3) and Na-acetic acid-acetic acid for pH 4 and 5.

Determination of Partition Coefficient (Kd) and pKa

Based on the distribution coefficient obtained form experimental results at various pH for each solvent, the value of pKa penicillin G was calculated by equation (3) using a correction factor at the initial pKa value of 2.75. The pKa value was determined by the Simplex method using the objective function of the difference between the least squares between the coefficients of the calculated distribution and the experimental data. The partition coefficient for each solvent was alculated by equation (3) using the pKa value of the results of optimization.

$$\mathbf{D} = \frac{K_d}{1+10^{(pH-pKa)}} \quad \textbf{(3)}$$

Analysis Method

The analysis of penicillin G content was carried out with a Shimadzu HPLC type C-R4A device using a column made of stainless steel with a diameter of 4.6 mm and a length of 25 cm with a stationary phase C-18. HPLC operating conditions are shown in Table 1.

Chromatographic data analysis was done qualitatively based on the suitability of retention time between samples and standard components. Meanwhile, quantitative analysis was carried out based on the peak chromatogram area obtained from the

integrator by calibration outside the standard penicillin G peak.

Table 1. Operation conditions for HPLC

Parameters	Value
Column temperature	33^{o}C
Detector	UV (λ = 220 nm)
Sample volume	5 µm
Mobile phase	Acetonitrile: phosphate buffer (0.025 M) pH 7 = 2:8
Flowrate of the mobile phase	1 mL/min

3. RESULTS AND DISCUSSION

Determination of Stirring Time

Stirring time is the minimum time required to obtain the phase equilibrium, which is indicated by the concentration of penicillin G. The residual in the raffinate phase has not changed with the variation of the stirring time. Based on observations, the stirring time does not significantly affect the extraction percentage (Figure 1).

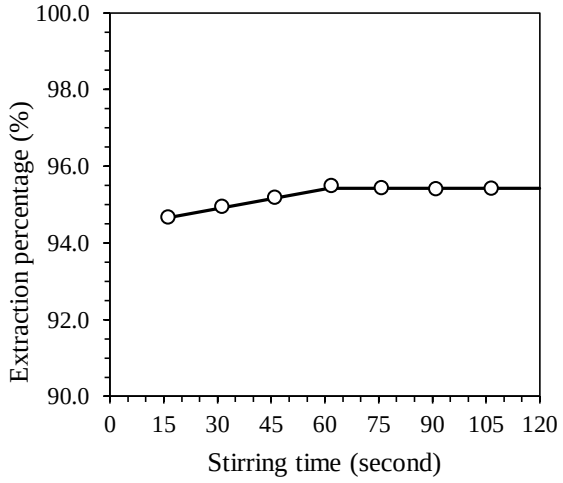

Figure 1. The extraction percentage at a various stirring time

The extraction percentage price is in the range of 94.5-95.5%. When the stirring time is less than 60 seconds, the extraction percentage tends to increase. This might be due to the contact between the organic phase and the aquatic phase is still not perfect and the extraction conditions have not reached equilibrium. The residual concentration of penicillin G in the raffinate phase is relatively constant during the stirring time of 60 seconds. After 60 seconds, the addition of the stirring time did not significantly affect the concentration of penicillin G in the raffinate phase and the conditions had reached equilibrium. Therefore, the stirring time used in this study was 60 seconds (1 minute).

Partition Coefficient and Extraction Percentage of Pure Penicillin G System

The partitioning coefficient of penicillin G in various solvents shown in Table 2 is obtained from the optimum pKa which is the result of optimization of the difference in the smallest square between the distribution coefficient of the experimental data with the distribution coefficient of the calculated results. The experimental results show that the largest partition coefficient of penicillin G was obtained in the solvent n-butyl acetate (21.47) and the lowest partition coefficient in chloroform (5.885).

Table 2. Partition coefficient of penicillin G in pure penicillin G system (pKa = 3.015)

Solvent	Kd	Kd (Reschke & Schugerl, 1984b)
N-butyl acetate	21.47	48
isobutyl acetate	13.62	37
Isoamyl acetate	12.05	22
Methyl isobutyl ketone	8.017	-
Chloroform	5.885	12.5

A noticeable difference between the experimental results and the partition coefficient data were obtained by (Reschke and Schügerl, 1984b). This may be influenced by a decrease in temperature, causing the penicillin G solubility to get smaller. In the literature, experiments were carried out at 8 ^{o}C, whereas in this study, 4 ^{o}C was chosen. This was supported by (Jing, 2010) that reported data on the solubility of penicillin sulfoxide in various solvents with the temperature range of 273.15-298.55K, the solubility decreased in a lower temperature due to the differences in solvent polarity, intermolecular interactions, hydrogen bonds, and others. Furthermore, the number of solvent mole fractions can also determined the solubility of penicillin, where the higher the mole fraction of water, the solubility of penicillin G in the aquatic phase will increase

because of its ionic and polar property. If the solubility of penicillin G increases in the aquatic phase, while in the raffinate phase does not change significantly, the partitions coefficient will be even greater. This is consistent with the thermodynamic data obtained for penicillin V potassium (PVK) in the ethanol-water system and the 1-butyl alcohol-water system at 278.15-313.15 K using the correlation of Van not Hoff and modified Apelblat (Wei, 2015).

The percentage of extraction of penicillin G at various pH from calculations using partition coefficient and pKa obtained from the optimization is shown in Figure 2. The results show that at pH around 2, the extraction percentage is not so affected by slight changes in pH (tends to remain) but at pH above 3, there was a sharp decrease in extraction due to changes in pH. For example, for n-butyl acetate solvents at pH 4 and 5, the percentage of extractions was 66.83% and 18.03%, respectively. Observation of the five types of solvents used showed that the largest percentage of extraction was given by n-butyl acetate, iso-butyl acetate, isoamyl acetate, methyl iso-butyl ketone, and chloroform.

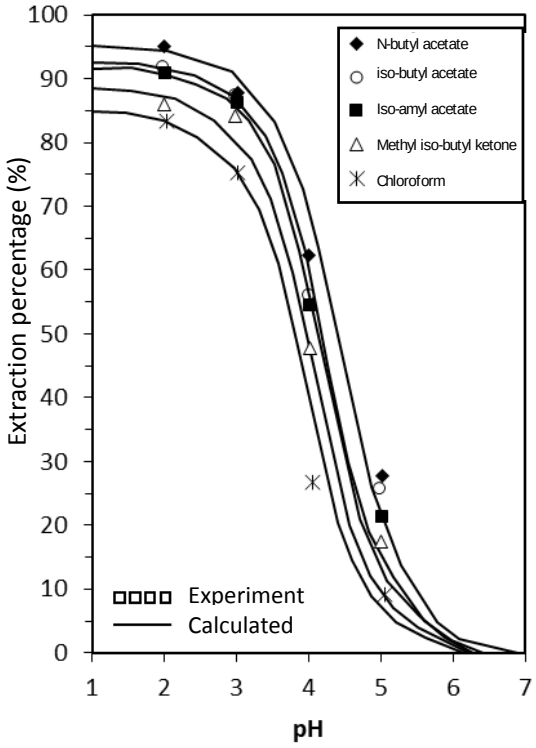

Figure 2. Extraction of penicillin G at various pH

Partition Coefficient and Extraction Percentage of Penicillin G Mixed Systems with 6-APA

The result shows that 6-APA is difficult to extract from the mixture by organic solvents. This phenomenon may be attributed to the fact that 6-APA is an amphoteric component (acidic and basic) with pKa_1 = 2.3 (COOH) and pKa_2 = 4.9 (NH_3+). At low pH (below pKa_1), 6-APA is more dominant in acidic form and more soluble in water. If the pH of the solution is greater than pKa_2, 6-APA is dominated by anionic form. Meanwhile, if the pH of the solution is between pKa_1 and pKa_2, acids and bases are in the mixture in the solution. This condition is known as zwitterion.

The largest percentage of 6-APA extraction is given by chloroform solvents, while the smallest percentage is given by iso-butyl acetate solvents, as shown in Figure 3. This may be due to the quite different chemical properties of each solvent. Because 6-APA is a nonpolar component, it is more soluble in nonpolar solvents. In addition, the dependence of the extraction percentage of 6-APA on the pH for each solvent is quite diverse but generally shows that at pH in the iso-electrocuted area (pI 6-APA = 4.3), the percentage of 6-APA extraction is in optimum condition. In the pI region, 6-APA is dominant in the form of neutral components so that it is easily extracted into organic solvents. Meanwhile, at pH outside the area, 6-APA is dominant in the form of charged ions (Su *et al.*, 2016).

In the mixture of penicillin G with 6-APA, the percentage of penicillin G extraction also changes when compared to the pure penicillin G system. Based on the distribution coefficient data of the experimental and by using the pKa penicillin G price obtained from the extraction of pure penicillin G (pKa = 3.015), the partition coefficient values are shown in Table 3.

Tabel 3. Penicillin G partition coefficient on penicillin G/6-APA systems (pKa = 3.015)

Solvent	Kd	f(x) residue
N-butyl acetate	10.91	1.294
isobutyl acetate	11.17	2.491
Isoamyl acetate	10.53	7.046
Methyl isobutyl ketone	12.98	7.546
Chloroform	7.20	0.727

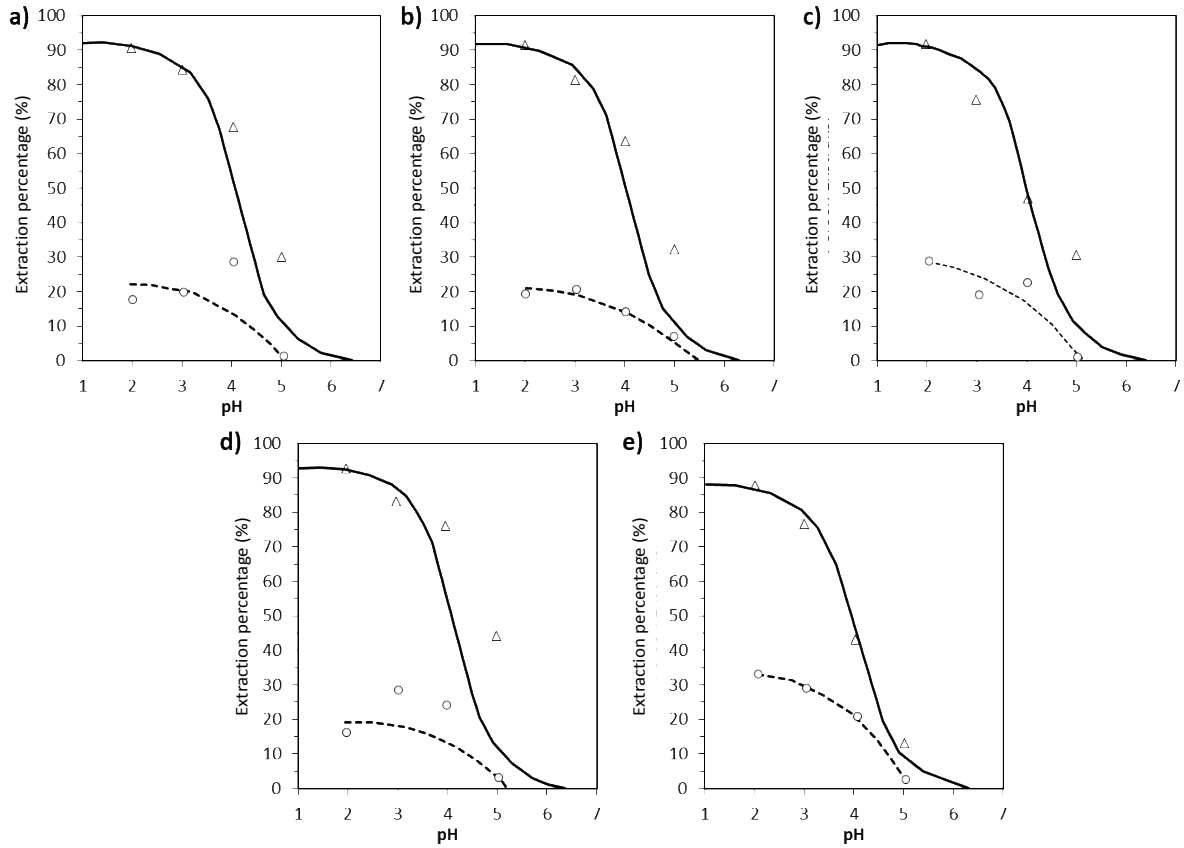

Figure 3. Extraction of penicillin G and 6-APA at 4 ^{o}C with (a) n-butyl acetate, (b) i-butyl acetate, (c) n-amyl acetate, (d) m-i-b-ketone, and (e) chloroform.

The partitioning coefficient of penicillin G in penicillin G/6-APA systems has decreased when compared to the partition coefficient of the pure penicillin G system, except for the methyl iso-butyl ketone and chloroform. This might be due to the influence of 6-APA which can increase the solubility of penicillin G in solvents that have relatively smaller polarity.

Methyl iso-butyl ketone is a suitable solvent used to separate penicillin G/6-APA. As shown in Table 3, methyl iso-butyl ketone provides the greatest partition coefficient. At pH 2, methyl iso-butyl ketone only produces an extraction percentage of less than 20% while the other solvents give an extraction percentage above 30%. In contrast, methyl iso-butyl ketone gives the largest percentage of extraction (92.21%) for penicillin G.

Determination of Penicillin G Concentration in Equilibrium Conditions with the UNIFAC Model

The relative volume (R_k) and the relative surface area (Q_k) of each functional group were calculated by equations (4) and (5). By using V_{wk} and A_{wk} data provided by Daubert (1985), values of R_k and Q_k were obtained and shown in Table 4.

Table 4. Volume and surface area of each component

Solvent	R_k (cm^3/mol)	Q_k (cm^2/mol)
N-butyl acetate	4.82	4.196
isobutyl acetate	4.82	4.192
Isoamyl acetate	5.4944	4.736
Methyl isobutyl ketone	4.60	4.03
Chloroform	2.82	2.40
Water	0.92	1.40
Penicillin G	11.4739	8.476

Refs. (Daubert & Danner, 1985)

For the penicillin G component, the R_k and Q_k data are the sum-up of R_k and Q_k data from the constituent groups.

$$R_k = V_{wk}/15.17 \quad (4)$$

$$Q_k = \frac{A_{wk}}{2.5\times10^9} \quad (5)$$

where V_{wk} is the volume of functional groups and A_{wk} is the surface area of the functional groups.

Based on the concentration data under the equilibrium conditions of the experimental results and by using the objective function in equation (6), the results of the calculation of the interaction parameters at each pH were obtained as shown in Table 5 and Table 6.

$$F(\tau) = \sum_i^j \sum_i^i [x_i^I - \hat{x}_i^I]^2 \quad (6)$$

where x_i^I is concentration of component i in phase I (experiment result), $\hat{x}_i^I$ is concentration of component i in phase I obtained from the calculation, and j is the number of data.

Parameter interactions with the remaining relatively small objective functions for the five liquid-liquid phase equilibrium of the ternary system, except for the water-chloroform-penicillin G system can be observed in Table 5 and Table 6. These tables show that the changes in pH conditions in the aquatic phase also results in the changes in the interaction parameter estimated on the same ternary system.

The relationship of calculated concentration (mole fraction) of penicillin G with the concentration of penicillin G in the feed is given in Figure 4 and Figure 5. These results show that the calculated values are in good agreement with experimental data, especially at low concentrations. Increasing the concentration of penicillin G in the feed causes the greater deviation between estimation and experimental results. This phenomenon may occur because the solubility of penicillin G in the organic phase has exceeded the saturation limit at high penicillin G concentrations (above 10 mmol / L). Thus, an increase in the concentration of penicillin G in the feed does not affect the concentration of penicillin G in the organic phase so that the value of the equilibrium constant decreases.

Table 5. Interaction parameters of the three-component liquid-liquid equilibrium system at pH 2 and 4 ^oC

Component			Interaction parameter (K)						F(x)
1	2	3	a_{12}	a_{13}	a_{21}	a_{23}	a_{31}	a_{32}	residual
Water	N-butyl acetate	Penicillin G	725.06	-751.89	622.64	-498.01	597.77	-3.51	0.001136
	isobutyl acetate		719.81	-555.91	690.50	-366.62	601.37	23.02	0.00398
	Isoamyl acetate		625.14	-614.26	504.28	-441.95	499.31	7.86	0.00387
	Methyl isobutyl ketone		762.10	-939.43	699.25	-780.98	729.44	146.6	0.00047
	Chloroform		696.66	-842.67	776.33	-560.53	499.64	4.03	5.966

Table 6. Interaction parameters of the three-component liquid-liquid equilibrium system at pH 2 and 4 ^oC

Component			Interaction parameter (K)						F(x)
1	2	3	a_{12}	a_{13}	a_{21}	a_{23}	a_{31}	a_{32}	residual
Water	n-b asetat	Penicillin G	720.40	-539.70	695.37	-374.43	592.70	3.12	0.00734
	i-b-asetat		724.16	-499.02	632.53	-368.07	470.80	41.87	0.00259
	i-a-asetat		621.53	-609.14	524.07	-403.00	480.03	-4.85	0.00295
	n-i-b-keton		762.20	-922.86	704.21	-652.08	693.91	-13.7	0.000593
	kloroform		696.66	-842.67	776.33	-560.53	499.64	4.03	5.966

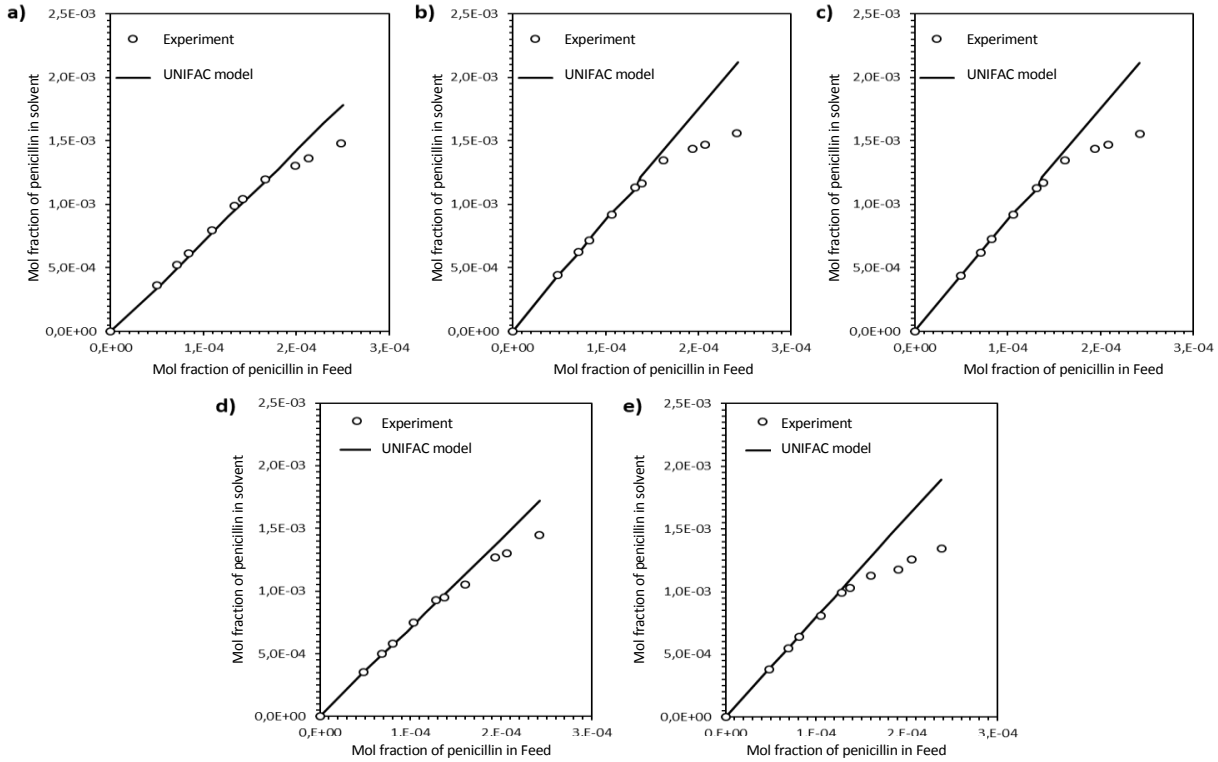

Figure 4. The equilibrium curve of penicillin G in (a) n-butyl acetate, (b) i-butyl acetate, (c) n-amyl acetate, (d) mib-ketone, and (e) chloroform, at pH 2.0 and 4 ^{o}C.

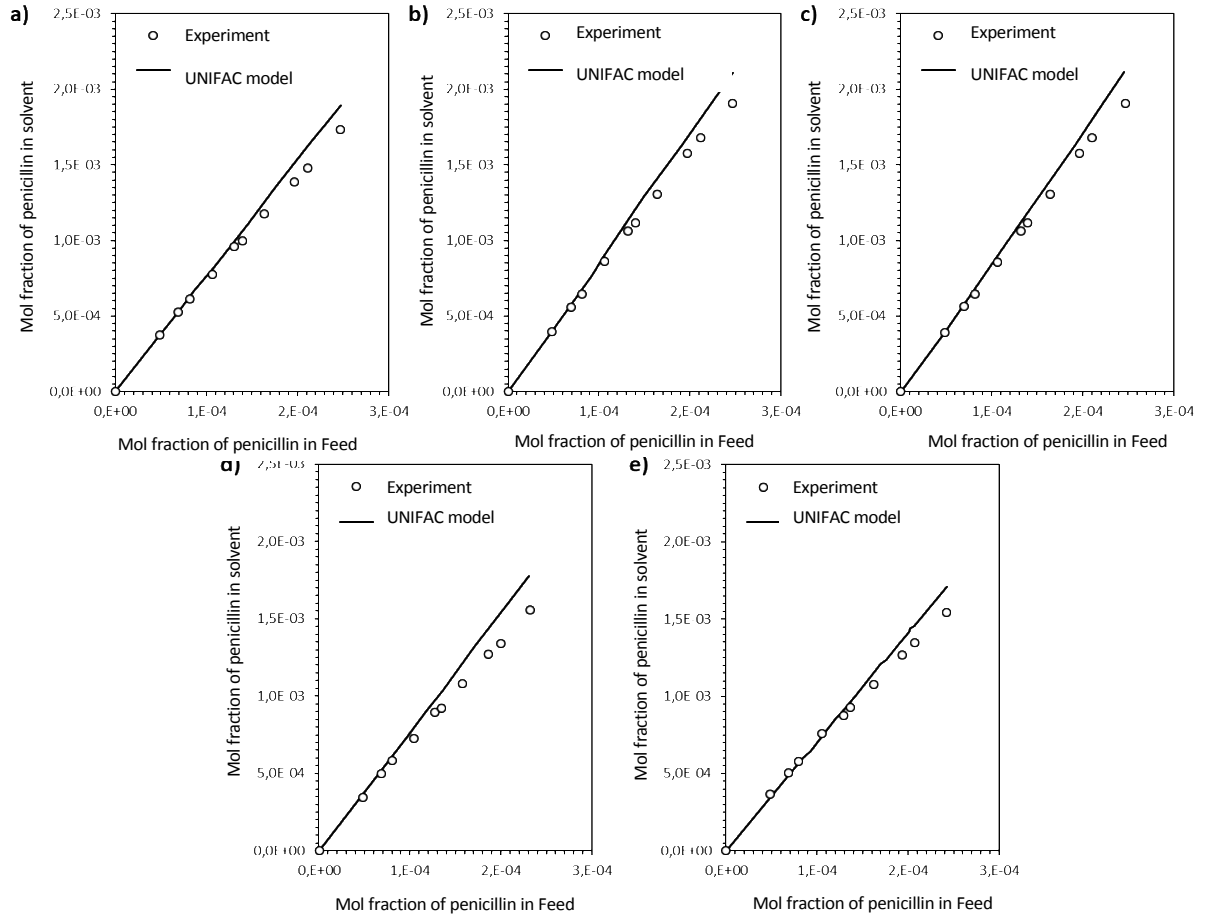

Figure 5. Penicillin G equilibrium curves in (a) n-butyl acetate, (b) i-butyl acetate, (c) n-amyl acetate, (d) mib ketone, and (e) chloroform, at pH 2.5 and 4 ^{o}C.

Table 7. The relative error in determining the concentration of penicillin G in the organic phase calculated by UNIFAC model and experimental data

Solvent	R_k (cm^3/mol)	Q_k (cm^2/mol)
Water – n-b-asetate – penicillin G	2.0	6.156
Water – n-b-asetate – penicillin G	2.5	5.554
Water – i-b-asetate – penicillin G	2.0	12.198
Water – i-b-asetate – penicillin G	2.5	6.826
Water – i-a-asetate – penicillin G	2.0	9.536
Water – i-a-asetate – penicillin G	2.5	6.862
Water – m-i-b-asetate – penicillin G	2.0	6.058
Water – m-i-b-asetate – penicillin G	2.5	10.345
Water – Chloroform – penicillin G	2.0	13.783
Water – Chloroform – penicillin G	2.5	5.894

Based on errors analysis, deviations between the calculation with experimental data are listed in Table 7. The results of calculations through the model give an average of 8.32%. The largest deviation was obtained in the water system (1)-chloroform (2)-penicillin G (3) at pH 2 with a relative error of 13.782%. The smallest deviation was obtained in the water system (1)- n-butyl acetate (2)- penicillin G (3) at pH 2.5 with a relative error of 5.554%.

4. CONCLUSION

The percentage of extraction and distribution coefficient of penicillin G and 6-APA in various solvents is influenced by the pH of the aquatic phase. The highest extraction percentage for pure penicillin G system is obtained in the n-butyl acetate solvent (95.51%) and for the mixture of penicillin G and 6-APA obtained in the methyl iso-butyl ketone solvent (92.6%). The best solvent for the separation of penicillin G from a penicillin G/6-APA mixture is methyl isobutyl ketone. Mathematical model of equilibrium of penicillin G in water and organic solvents is made to determine the activity coefficient using the UNIFAC method. This model is able to estimate penicillin G concentrations in the organic phase with an average relative error of 8.32%.

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