THE EFFECT OF CONCENTRATION OF CARRIER, pH AND TIME OF EXTRACTION ON SEPARATION'S FACTOR OF PENICILLIN G - PHENYL ACETATE BY REACTIVE EXTRACTION

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ABSTRACT

The aim of this research is to study the effect of concentration carrier, pH and time of extraction on separation's factor of penicillin g - phenyl acetate by reactive extraction technique. The 10 mL aqueous solution with variation of pH : 5, 6 contains 0.001 M penicillin G and 0.001 M phenyl acetate has been extracted with 10 mL n-butyl acetate contains dioctylamine as carrier. Variation concentration of carrier were 0.000; 0.002; 0.004; 0.006 and 0.008 M. Variation time of extraction were 1, 5, 10, 15 and 20 min. The penicillin G and phenyl acetate that dissolved in organic phase ha been reextracted with 10 mL aqueous with variation of pH : 7, 8. The optimum condition obtained as follow : concentration dioctylamine was 0.002M ; pH the first phase water was 5 and the second phase water was 8 ; and the time of extraction was 10 min.

***Keywords**: Separation factor, Reactive extraction*

INTRODUCTION

The range of width penicillin G in the use of the clinic and as the raw material in the production of synthetic penicillin like amoxicillin, ampicillin, cefadroxil, and cephalexin, caused the requirement for penicillin G occupied the majority compared with another antibiotic [1].

The separation of penicillin G from other compounds in the fermentation medium is the important step especially in the separation phenyl acetic acid in order to obtain penicillin G with high quality for the medical purpose. The separation and purification of penicillin G from phenyl acetic acid (PAA) is really difficult to be carried out because the two compounds are weak acid and the two compounds can change between one to another by influence of pH, as the reaction as follow Fig 1.

For the commercial purpose, the separation of penicillin G and phenyl acetic acid untill this time still used by solvent extraction technique. Solvent extraction technique is one of the most widely used for the recovery of antibiotics [3]. Nevertheless solvent extraction technique has many limitations and low economic value [4] and penicillin G decompose at low pH [5], therefore the price of penicillin G is relatively expensive. These limitations cause scientist try to search and develop other separation techniques.

Until now about ten techniques of penicillin G separation has been developed . Those are : micro-filtration [6], reactive solvent extraction [4], ion exchange [7], electrodialysis [8], Vapour phase point extraction [9], supported liquid membrane [10], reactive extraction in hollow fiber [11] and emulsion liquid membrane [12]. Base on the scientific publication from several separation techniques the reactive extraction in hollow-fiber and the emulsion liquid membrane potential to be developed for technique at industrial purpose. Nevertheless the separation of penicillin G by hollow fiber technique needed technology that higher than the emulsion liquid membrane [13].In economical point of view, the emulsion liquid membrane more economic than the hollow fiber.

In this research the separation of penicillin G from phenyl acetic will be carried out by reactive extraction technique. To obtain the optimal separation will be studied several parameters that influence the procees, that is : the concentration of carrier, pH and time of extraction.

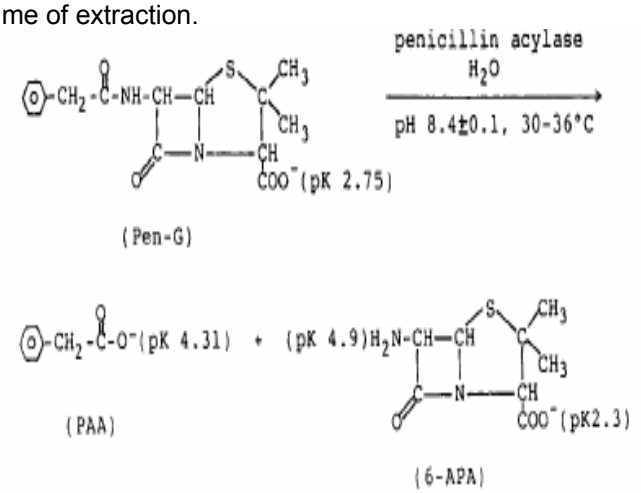

Fig 1. The influence of pH an hydrolysis of penicillin G [2].

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EXPERIMENTAL SECTION

Material

The Chemical used in this experiment are : Phenyl acetic (SIGMA), Potassium benzilpenicillin SIGMA, Citric Acid crystal $(C_6H_8)_7.H_2O$) SIGMA, salt citrates crystal $(C_6H_5O_7Na_3.2H_2O)$ SIGMA. salt phosphate crystal $(Na_2HPO_4.2H_2O)$ SIGMA, salt dehydrogen phosphate crystal $(NaH_2PO_4.H_2O)$ SIGMA, n-butyl acetate, kerosene (SIGMA), dioctylamin (Aldrich). All chemicals used in this research had proanalysis (pa) grade.

Apparatus

During the extraction the concentration of penicillin G and phenyl acetate has been monitored by HPLC Waters 247, equiped by UV detector. pH has been determined by pH meter HANNA. Glasware used in this investigation were the standard laboratory.

Procedures

Determination of Separation Factor

Pipet 10 mL the first phase water of the pH = 5 (buffer citrate) containing the mixture of 0.001 M. penicillin G (372.5 ppm) and 0.001 M. phenyl acetic (136.5 ppm) into a 250 mL separatory funnel and add 10 mL of *n*-butyl acetate containing 0.002 M dioctylamin. The mixture shaked for 2.5 min. Allow the layers to separate. The first phase water was separated from organic phase then in separatory funnel added the second phase water that is buffered by phosphate solution at pH = 8. The mixture has been extracted for 2.5 minutes. Afterwards the mixture was allowed through to separate. Penicillin G and phenyl acetate in the second phase water separated, and concentration has been determined by HPLC with (the condition for the analysis was as follows: eluen 0.01M $NH_4H_2PO_4$: Methanol = 7: 3; flow rate 1 mL/min; the Column C8. λ_{max} = 225 nm).

Three repititive run with the same procedure as above for different time of extraction i.e. 10; 15; and 20 min.

Influence of the concentration of dioctylamin and the pH on the separation factor of penicillin G - phenyl acetate by reactive extraction. The pH of the first phase water was 5 and the second phase water was 7 and 8 respectively.

Prepared 5 separatory funnel 250 of mL capacity. Each in separatory funnel add 10 mL of consist of citrate buffer pH 5 0.001 M penicillin G and 0.001 M phenyl acetate. Afterwards add 10 of mL *n*-butylacetate contain dioctylamin 0.000 M in the first ; 0.002 M dioctylamin in the second ; 0.004 M. dioctylamin in the third separatory; 0.006 M dioctylamin in the fourth and 0.008 M. dioctylamin in the fifth separatory funnel . The mixture shaked vigorously for 2.5 min and allow until the layer to separate. Draw off the the first phase water to the organic phase add 10 mL the second phase water that is the solution of phosphate buffer pH = 7. The mixture was extracted again for 2.5 min. Afterwards let it kept, then separated. Penicillin G and phenyl acetate in the second phase water separated, and concentration has been determined by HPLC with (the condition for the analysis was as follows: eluen 0.01M $NH_4H_2PO_4$: Methanol = 7: 3; flow rate 1 mL/min; the Column C8. λ_{max} = 225 nm). Repititive run with the same procedure as above for different solution phosphate buffer pH = 8.

Two repititive run with the same procedure as above for different solution pH the first phase water = 6, pH the second phase water = 7 and 8.

RESULT AND DISCUSSION

Percent of Extraction, Distribution ratio and the Separation factor at Reactive Extraction Technique

The formula extraction percent that was used :

$$\%E = \frac{[A-X]}{[X]} x100\% \quad (1)$$

Equation that often has been used in determination of the separation factor in the extraction of solvent begin with extraction percent, because extraction percent has correlation with the distribution ratio [14]. The formula was reduced begin : if D was the distribution ratio; A= penicillin at first; X = penicillin that remnants; Vw = the volume of water and Vo = the organic volume then:

$$D = \frac{\frac{A-X}{Vo}}{\frac{X}{Vw}} \quad (2)$$

$$D = \frac{Vw(A-X)}{XVo} \quad (3)$$

$$DXVo = AVw - VwX \quad (4)$$

$$DXVo + VwX = AVw \quad (5)$$

$$X(DVo + Vw) = AVw \quad (6)$$

$$\text{Then : } X = \frac{AVw}{DVo + Vw} \quad (7)$$

If penicillin was known at first and penicillin that was extracted could be determined by the HPLC, then penicillin that remnants (was not extracted) could be counted. Therefore the distribution ratio also could be determined. Another formulation also could be used to determine the distribution ratio like this formula below.

$$\%E = \frac{100D}{D + (Va/Vo)} \quad (8)$$

Where Va = Vo then:

$$D = \frac{\%E}{100 - \%E} \quad (9)$$

The effectiveness of the separation was based on the capacity to separate a certain component against the other component. The equation was known as the separation factor. The separation factor was the comparison between the distribution ratio at first component (in this case penicillin G) towards the distribution ratio at second component (in this case phenyl acetate) in the same solvent. The formula :

$$\beta = \frac{D_{penG}}{D_{Fenas}} \quad (10)$$

The Time Extraction effect on separation factor of penicillin g- phenyl acetate

The formula has been used for calculation of the extraction percent, distribution ratio and the separation factor formula was 7 or 9 and 10 above. From results of this research, for penicillin G standard with the concentration 372.5 ppm gave retention time 5.67 minutes and peak area 438108. Phenyl acetate with the concentration 136.5 ppm gave retention time 3.08 minutes and peak area 432889.

From Table 1 and Fig 2 were seen that the extraction percent of penicillin G and phenyl acetate increased with the length of time extraction. Phenyl acetate gave extraction percent higher than extraction percent of penicillin G. Increasing of extraction percent phenyl acetate was correlated with the distribution ratio like seen in the Fig 3 and Table 2. Phenyl acetate gave the distribution ratio of 139.84 when the time extraction 20 minutes, whereas penicillin G gave the distribution ratio 2.56.

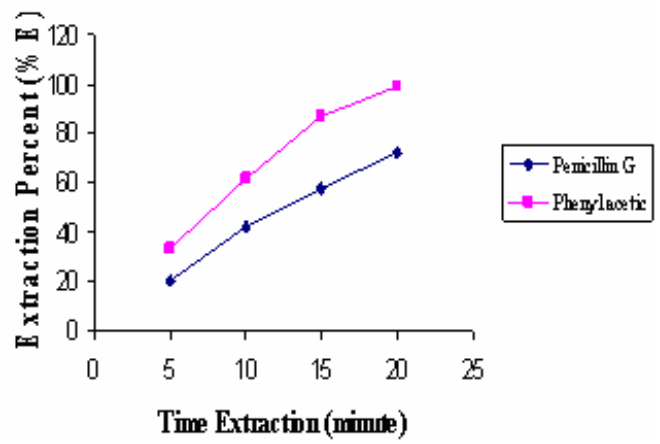

Fig 2. Curve the extraction percent of penicillin G and phenyl acetate

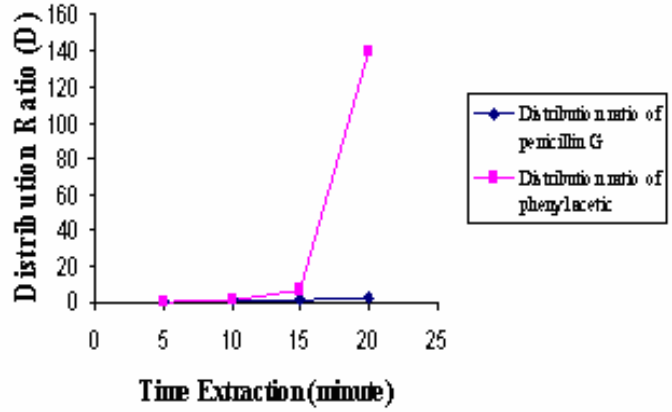

Fig 3. Curve time extraction towards the distribution ratio of penicillin G and phenyl acetate

Table 1. Extraction percent of penicillin G and Phenyl acetate

Extraction time (min)	Penicullin G			Phenyl acetic		
	tR	Peak area	Extraction Percent (%E)	tR	Peak area	Extraction Percent (%E)
5	6.17	87665	20.01	3.84	143109	33.06
10	6.30	182029	42.05	3.44	265665	61.37
15	6.18	248047	57.30	3.66	374473	86.50
20	5.82	311387	71.93	3.35	429795	99.29

Table 2. Table of time extraction data towards the distribution ratio of penicillin G and phenyl acetate

Time Extraction (min)	$D_{Pen\ G}$	D_{phen}
5	0.25	0.49
10	0.73	1.59
15	1.34	6.41
20	2.56	139.84

Table 3. Table of time extraction data towards separation factor (β) penicillin G - phenyl acetate

Time Extraction (min)	$\beta = D_{Pen\ G}/ D_{phen}$
5	0.51
10	0.46
15	0.21
20	0.02

Table 4. Table of the effect concentration dioctylamin data towards the separation factor penicillin G- phenyl acetate

Concentration of Dioctylamin (M)	Separation factor (β)			
	pH the first phase water 5, pH the second phase water 7	pH the first phase water 5, pH the second phase water 8	pH the first phase water 6, pH the second phase water 7	pH the first phase water 6, pH the second phase water 8
0.000	0.17	0.34	0.07	0.06
0.002	0.71	0.77	0.31	0.17
0.004	0.46	0.43	0.07	0.12
0.006	0.44	0.38	0.10	0.04
0.008	0.01	0.39	0.03	0.03

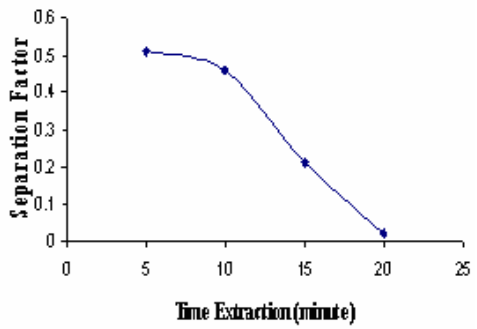

Fig 4. Curve time extraction towards the separation factor penicillin G-phenyl acetate

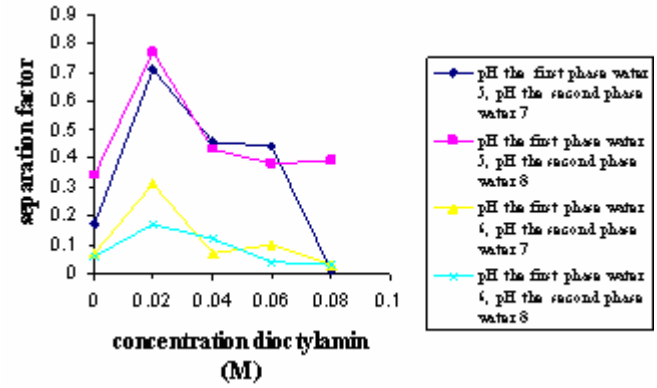

Fig 5. Curve of concentration effect dioctylamin towards the separation factor penicillin G- phenyl acetate

If the distribution ratio of penicillin G (DPen G) compare with the distribution ratio phenyl acetate (Dphen), then will be obtained of separation factor like the Table 3 and Fig 4. The separation factor penicillin G-phenyl acetate tended to descend in the 10th minute. It was the optimum condition where in the second phase water (the recipient phase) the extraction results were more often contained phenyl acetate than penicillin G.

The effect of the concentration dioctylamin on separation factor of penicillin G-phenyl acetate in condition the first phase water pH 6, the pH second phase water 8

From Table 4 and Fig 5 could be seen that the maximal concentration carrier dioctylamin that could increase the separation factor of the penicillin G-phenyl acetate in the condition for the first phase water pH 5, the second phase water pH = 8 was 0.002 M.

From Table 4 and Fig 5 could be seen that in the concentration dioctylamin 0,000M the transport process of penicillin G and phenyl acetate could happen. The separation factor penicillin G - phenyl acetate that received was varied, this because influenced by the first phase pH and second water. The concentration of penicillin G and phenyl acetate was made in the first phase water each in treatment at this extraction always constant. Because of the transport process in this situation happened caused the gradient of concentration penicillin G, phenyl acetate and gradient the pH between the first phase and second phase water. Increasingly big ΔpH, causes increase the separation factor. In condition of pH first phase water 5 and the pH second phase water 8 (ΔpH = 3) the separation factor was received 0.34 higher than the separation factor in condition the pH first phase water 6 and the pH second phase water 8 (ΔpH = 2) that is 0.06. In condition the pH first phase water 5 and the pH second phase water 7 (ΔpH = 2) the separation factor was received 0.17 higher than the separation factor in condition for the pH first phase water 6 and the pH second phase water 7 (ΔpH = 1) that is 0.07.

The extraction of penicillin G without the presence carrier (the reactive compound) in organic phase produced free acid penicillin G. Whereas free acid penicillin G in n-butyl acetate was unstable. The constanta instability was 9.52 x 10^{-3} k (h^{-1}) [15]. Because of that the small size of the separation factor above was caused decomposition of penicillin G in organic phase. Increasingly the separation factor has been seen cause increase the concentration of dioctylamin 0.002 M. In this situation penicillin G that was extracted in organic phase was formed the complex compound (AHP) between penicillin G and dioctylamin. This complex compound was stable, so it was not decomposed in organic phase.

$$A + P^- + H^+ \leftrightarrow AHP$$

In variations condition of pH in first phase and second phase water has been seen that the concentration of dioctylamin 0.002 M was the optimum condition. In this

concentration was obtained the separaton factor 0,71 (in the condition of the first phase water 5, the second phase water 7); 0.77 (in the condition of the first phase water 5, the second phase water 8); 0.31 (in the condition of the first phase water 6, the second phase water 7); 0.17 (in the condition of the first phase water 6, the second phase water 8). The separation factor descend in the concentration dioctylamin 0,004 M.; 0,06 M. and 0.008 M. Therefore the concentration dioctylamin 0,002 M was the concentration that will be used in the following extraction. By involved the ion H^+ in the formation reaction of penicillin G complex with dioctylamin, so the condition of the first phase water 5, the second phase water 8 ((ΔpH = 3) was the condition that will be used in the next extraction

CONCLUSION

Increasingly big ΔpH, causes increase the separation factor. In condition of pH first phase water 5 and the pH second phase water 8 (ΔpH = 3) the separation factor was received 0.34 higher than the separation factor in condition the pH first phase water 6 and the pH second phase water 8 (ΔpH = 2) that is 0.06. In condition the pH first phase water 5 and the pH second phase water 7 (ΔpH = 2) the separation factor was received 0.17 higher than the separation factor in condition for the pH first phase water 6 and the pH second phase water 7 (ΔpH = 1) that is 0.07.

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NOTATION

PAA	phenyl acetic acid
λ	wave length
[A-X]	the concentration of penicillin extracted
[A]	the initial concentration of penicillin
[X]	the final concentration of penicillin
D	the distribution ratio
Vw	the volume of water phase
Vo	the volume of organic phase
DpenG	the distribution ratio of penicillin G
Dphen	the distribution ratio of phenylacetic
β	separation factor
AHP	complex of penicillin and carrier
A	carrier
P^-	ion of penicillin
H^+	ion of hydrogen

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