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Chemical Engineering Journal 79 (2000) 61–67

Chemical Engineering Journal

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Continuous extraction of penicillin G by emulsion liquid membranes with optimal surfactant compositions

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Received 20 June 1999; received in revised form 27 March 2000; accepted 1 April 2000

Abstract

In order to find optimal surfactant compositions of a membrane phase for volume ratios of internal aqueous phase to membrane phase (*w*/*o* ratios) suitable for continuous extraction of penicillin G, batch extraction by an emulsion liquid membrane (ELM) was studied by varying the volume percentages of Span 80 in a binary surfactant mixture in the membrane phase. The optimal volume percent of Span 80 for each *w*/*o* ratio was 5 or 10% (v/v) of total volume of the binary surfactant mixture. The continuous extraction was investigated using a column of the Oldshue–Rushton type. Total flow rate, *w*/*o* ratio and flow rate of a dispersed emulsion phase were considered as the operational variables for the continuous extraction. In most of the runs, actual degrees of extraction at the bottom of the column were higher than 95%. However, the highest concentration of penicillin G in the internal phase of the emulsion effluent from the column was about 2.4 times higher than that in the influent continuous phase. The low concentration of penicillin G resulted from dilution of the internal phase due to swelling of the emulsion phase. In addition, strategies for reduction of the swelling were investigated.

Keywords: Penicillin G; Emulsion liquid membrane; Continuous extraction; Optimal surfactant composition; Swelling

1. Introduction

Since Reschke and Schügerl [1,2] first proposed reactive extraction of penicillin G with amines, reactive extraction has been applied to liquid membrane processes [3–11] as well as solvent extraction processes [12,13] for the separation and concentration of penicillin G. In particular, we have extracted penicillin G from model media by emulsion liquid membranes (ELMs), which can accomplish both extraction and stripping in one step. Since a combination of the extraction and stripping processes can remove equilibrium limitations, solute concentrations in the feed phase can be reduced to very low levels. Simultaneously, the solute can be concentrated in a receiving phase due to a high volume ratio of the feed phase to the receiving phase. Even though the two substantial problems of the ELMs, membrane breakage and emulsion swelling, still need a solution, we have continued to investigate the commercial applicability of penicillin G extraction by the ELMs.

Amberlite LA-2 was proved to be the best extractant of penicillin G in our previous ELM study [3]. The driving force of the ELM system is the pH gradient between an external phase (feed phase) and an internal phase (receiving phase). However, pH control of the external and internal phases is required because penicillin G is stable in the pH range 5–8 [1,3]. For the pH control of the internal phase, Na_2CO_3 was selected as the most suitable internal reagent. With a suitable Na_2CO_3 concentration, its extraction rate was maintained high until pH of the internal phase decreased to the pH value where penicillin G was stable, and the pH of the internal phase was within the range after termination of extraction [3]. When the same Na_2CO_3 concentration is used in systems with different *w*/*o* ratios, the reaction capacity of the internal phase for capturing penicillin G increases with the ratio. Thus, the use of the same Na_2CO_3 mass in the internal phase is more meaningful for a comparison between extraction efficiencies of these systems.

Lee et al. [5] showed that the degree of extraction of penicillin G with the use of a surfactant mixture of a polyamine surfactant (ECA 4360J) and Span 80 as an emulsifier could be higher than that with the use of a single polyamine surfactant. This investigation was limited to illuminate the effect of a surfactant composition on extraction only at a specific *w*/*o* ratio. Later, however, an optimal composition of the binary surfactant mixture for each *w*/*o* ratio was obtained keeping the Na_2CO_3 mass constant in a batch system [7]. More

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1385-8947/00/$ – see front matter
PII: S1385-8947(00)00173-X

detailed explanations about the batch system are also treated in this paper.

The objective of this study is to present experimental results on the extraction of penicillin G in a continuous extractor of the Oldshue–Rushton type with an optimal surfactant composition in a batch system. The effects of various operational conditions on the degree of extraction and emulsion swelling are investigated. In addition, the practical applicability of the continuous ELM process to the extraction of penicillin G is discussed.

2. Experimental

2.1. Reagents preparation

Citrate buffer solution of 0.408 mol/dm^3 was prepared by dissolving a mixture of citric acid and trisodium citrate (EP grade, Junsei Chemical) in deionized water so as to reduce emulsion swelling and minimize pH changes of the solution throughout the experiments. Two buffer solutions of pH 5.0 and 4.8 containing 20 mmol/dm^3 of penicillin G were used as an external aqueous phase in a batch reactor and a continuous extraction column, respectively. A surfactant mixture of PARABAR 9551 (nonionic polyamine, Exxon Chemical, Singapore) and Span 80 (sorbitan monooleate, Sigma Chemical) was used as an emulsifier. An organic membrane phase consisted of the surfactant mixture at 8% by volume of the membrane phase and 20 mmol/dm^3 of Amberlite LA-2 (secondary amine, Sigma Chemical) as an extractant dissolved in kerosene (commercial GR grade, Junsei Chemical). An internal aqueous solution was prepared by dissolving sodium carbonate (commercial EP grade, Junsei Chemical) in deionized water. Emulsions of the water-in-oil (W/O) type were prepared by adding the internal aqueous phase to the organic phase and then emulsifying with a homogenizer (T25, IKA Lab) for 10 min at 12 000 rpm.

2.2. Experimental apparatus and procedure

Batch experiments for extraction of penicillin G were carried out in the same stirred glass reactor that we used in the previous works [3–7]. W/O emulsion of 70 cm^3 was dispersed in a batch reactor containing 420 cm^3 of the feed solution having pH 5.0 and the mixed solution was stirred at 330 rpm to give a double emulsion. As soon as samples were taken from the reactor at given intervals, the external phase was separated from the emulsion phase by filtration. The concentration of penicillin G in the external phase was analyzed by a UV–Vis spectrophotometer (UV2-100, ATI Unicam) calibrated at 258 nm. The batch extraction was investigated at varying *w/o* ratios of the emulsion and compositions of the surfactant mixture in the membrane phase.

The extractor of the Oldshue–Rushton type shown schematically in Fig. 1 was used for continuous extraction of penicillin G. A column of the extractor was made of an acrylic tube of 6.0 cm inner diameter and 85.5 cm length. It consisted of 14 compartments each 4.6 cm high formed by stator-ring baffles, each fitted with four vertical baffles and each diameter of stator openings was 3.25 cm. Working volume of the column was 1670.5 cm^3. Stirring was carried out at 330 rpm using four-flat blade turbine impellers located centrally in each stage. A continuous aqueous phase (feed solution) was fed to the top of the extraction column and a dispersed emulsion phase was countercurrently injected through a nozzle attached to the bottom of the column. Samples were taken from the bottom of the column and four sampling ports installed on the side wall of the column at steady state. Dispersed emulsion phase holdup was determined by measuring volume of the W/O emulsion phase in the column after the inlet and outlet valves of the continuous and the dispersed phases were simultaneously closed. In addition, water content in the emulsion phase effluent from the top of the column was measured by a Karl Fisher titrator (AF7, Orion) so as to obtain the extent of emulsion swelling. The extent of membrane breakage was measured using lithium ion as a tracer. Since the portion of lithium ion in the emulsion phase leaked to the continuous phase was less than 1% in the batch and the continuous systems, the effect of the leakage on the degree of extraction in each system was not considered any more.

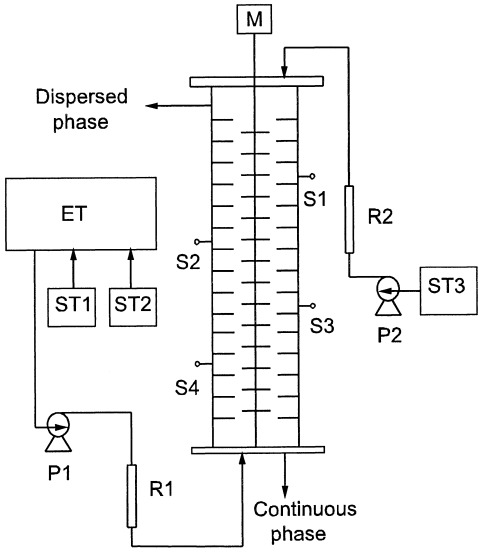

Fig. 1. Schematic diagram of experimental apparatus. ET: emulsion tank; M: DC motor; P1, P2: pumps for dispersed emulsion and continuous phases; S1–S4: sampling ports; ST1–3: storage tanks for internal, membrane and external phases; R1, R2: flowmeters for dispersed emulsion and continuous phases.

3. Results and discussion

3.1. Permeation mechanism through ELM

Actually, two carriers including Amberlite LA-2 exist in the present ELM system because PARABAR 9551 functions

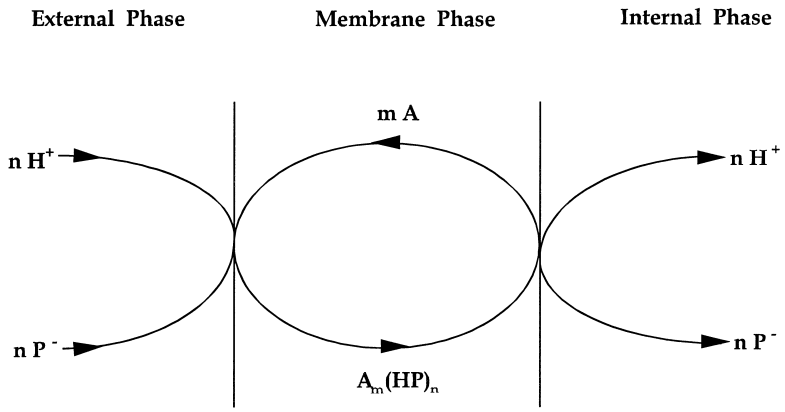

Fig. 2. Schematic diagram for co-transport mechanism of hydrogen ion and penicillin acid anion through membrane phase.

as a carrier of penicillin G as well as an emulsifier [3,4,6]. The mechanism of facilitated transport of penicillin G using the amines is shown in Fig. 2. A general reaction of an amine with hydrogen ion and penicillin acid anion occurs at the external and internal interfaces between the aqueous and membrane phases as follows:

$$m\mathrm{A(org.)} + n\mathrm{H^+(aq.)} + n\mathrm{P^-(aq.)} \Leftrightarrow \mathrm{A}_m\mathrm{(HP)}_n\mathrm{(org.)} \quad (1)$$

n moles of hydrogen ion (H^+) and penicillin acid anion (P^-) react with m moles of amine (A, Amberlite LA-2 or PARABAR 9551) at the external interface to form 1 mol of complex [$A_m(HP)_n$]. The complex then diffuses across the membrane phase until it reaches an internal droplet. At the internal interface, hydrogen ion and penicillin acid anion are released to the internal phase by the stripping reaction because of an extremely high pH of the internal phase. The uncharged amine diffuses back across the membrane to repeat the separation cycle. Since the volume ratio of the internal phase to the external phase is very small, separation and concentration of penicillin G occur simultaneously during its transport from the external phase to the internal phase.

3.2. Batch extraction of penicillin G

Batch extraction experiments were performed by varying the volume percent of Span 80 of the binary surfactant mixture in order to obtain optimal surfactant compositions of the membrane phase for the *w*/*o* ratios which would be used for continuous extraction of penicillin G by the ELM. For the sake of convenience, the terminology "apparent degree of extraction" on the ordinates of Figs. 3 and 4 was used instead of the terminology "actual degree of extraction", because its use does not make a serious difference to an analysis of the present batch system in which emulsion swelling does not seem to be high. The apparent degree of extraction means a degree of extraction of penicillin G in the external phase observed without taking into consideration any breakage or swelling.

As shown in Fig. 3, the highest apparent degrees of extraction at most of the sampling intervals for each *w*/*o* ratio were obtained with the use of a surfactant mixture with a specific composition as an emulsifier rather than with the sole use of PARABAR 9551. The specific surfactant composition is called as an optimal surfactant composition. The binary surfactant mixture had the optimal surfactant composition for each *w*/*o* ratio due to the following reason: when surfactants such as PARABAR 9551 and Span 80 are used to stabilize emulsion, they form surfactant layers at the interface between aqueous and organic phases. The surfactant layers become barriers to mass transfer in an ELM system [14]. PARABAR 9551 is a kind of polyamine surfactant having physicochemical properties similar to ECA 4360J which was used in the previous works [3,8]. Since the polyamine surfactants form more condensed adsorption films at the interface than Span 80, their interfacial mass transfer resistance is larger than that of Span 80. On the other hand, the more condensed film by the polyamine surfactant increases emulsion stability [15]. From these concepts, we deduced the fact that the combined use of the two surfactants as an emulsifier would have a positive effect on the extraction of penicillin G, which was also proved by the present and the previous experimental results [7]. As a volume percent of Span 80 of the binary surfactant mixture increased over its optimal value, the apparent degree of extraction for each *w*/*o* ratio in Fig. 3 decreased. From the result, it could be assumed that the amounts of Span 80 occupied by adsorption at the interface between aqueous and organic phases would be almost constant at the range over the optimal volume percent of Span 80. Since the surfactant mixture was fixed as 8% by volume of the membrane phase, the concentration of PARABAR 9551 as a carrier of penicillin G decreased with an increase in Span 80 concentration. Finally, it was considered that the decrease in PARABAR 9551 concentration brought about the decrease in the apparent degree of extraction.

In our previous work [3], the degree of extraction of penicillin G increased with the increase in the Na_2CO_3 concentration which was in between 0.05 and 0.5 mol/dm^3. However, penicillin G in the internal phase was highly

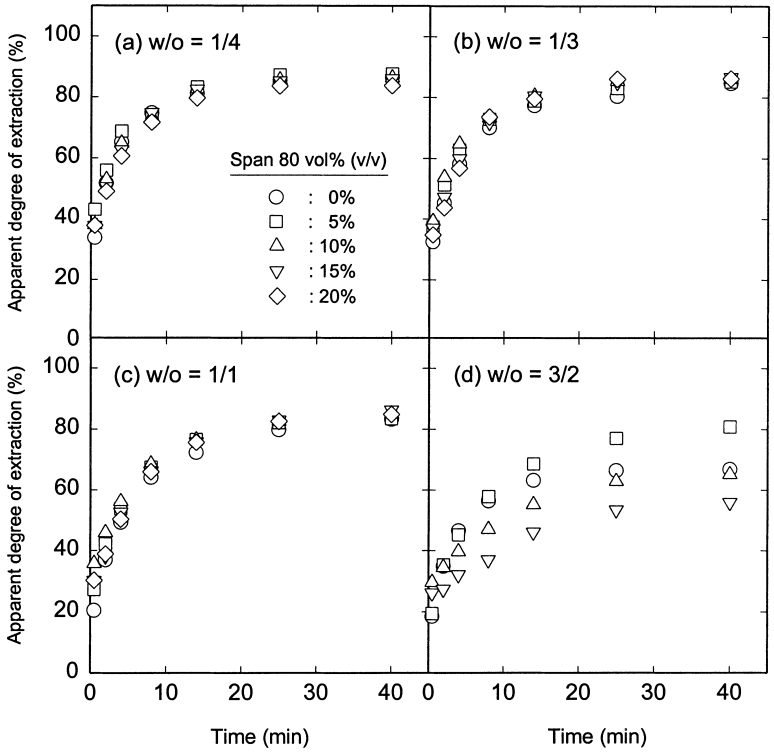

Fig. 3. Effect of volume percent of Span 80 of a binary surfactant mixture on apparent degrees of extraction for different *w/o* ratios in a batch reactor (citrate buffer solution=0.408 M; pH 5.0; mass of Na_2CO_3=3.5×10^{-3} mol).

decomposed at the highest Na_2CO_3 concentration because Na_2CO_3 mass in the internal phase was excessive for neutralization of all penicillin G transported to the internal phase. Likewise, losses of more penicillin G occurred at a higher *w/o* ratio, keeping Na_2CO_3 concentration in the internal phase constant independent of *w/o* ratios [4]. Thus, the Na_2CO_3 concentration at a high *w/o* ratio should be lowered so that penicillin G in the internal phase might be stable after termination of extraction. Besides, the reaction capacity of the internal phase for capturing penicillin G increased with the *w/o* ratio. Actually, comparisons between the experimental results (such as degree of extraction and emulsion swelling) obtained in the systems with different reaction capacities make no sense at all. Finally, the masses of Na_2CO_3 in the internal phase for all runs in Fig. 3 were fixed as 3.5×10^{-5} mol so as to investigate the effect of the *w/o* ratio on the extraction under the same trapping mass of Na_2CO_3 for penicillin G.

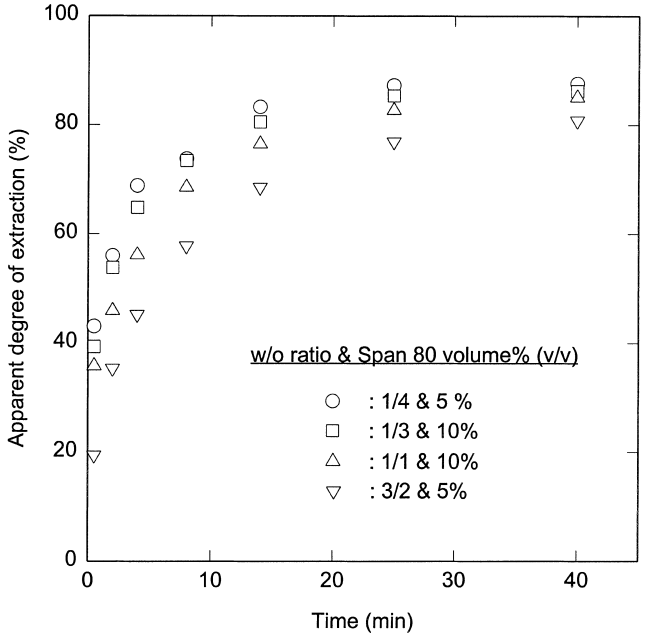

Fig. 4. Comparison between apparent degrees of extraction at different *w/o* ratios with their optimal surfactant compositions in a batch reactor (citrate buffer solution=0.408 M; pH 5.0; mass of Na_2CO_3=3.5×10^{-3} mol).

The optimal volume percent of Span 80 for each *w/o* ratio was 5 or 10% (v/v) of total volume of the binary surfactant mixture as shown in Fig. 4. The apparent degree of extraction was higher at the lower *w/o* ratio. We did not consider continuous extraction with the *w/o* ratio of $\frac{3}{2}$ because the apparent degree of extraction at this *w/o* ratio was much lower than those at the other *w/o* ratios.

3.3. Continuous extraction of penicillin G

In order to increase the degree of extraction, we could consider the use of the internal phase with the highest possible Na_2CO_3 mass with which penicillin G in the emulsion phase effluent from the continuous extraction column

Table 1
Dispersed phase holdup, % swelling and concentration of penicillin G in the internal phase of the effluent emulsion, and degree of extraction at the bottom of the column for each run

Run No.	*w/o* ratio & vol.% of Span 80 of binary surfactants	Na_2CO_3 conc. in the internal phase (mol/dm^3)	Flow rates of continuous & dispersed phases (cm^3/min)	Dispersed phase holdup (ϕ)	% swelling (E_s) of the effluent emulsion	Conc. of penicillin G in the internal phase of the effluent emulsion (mmol/dm^3)	Actual degree of extraction at the bottom of the column (%)
1	1/1 & 10	0.1750	60 & 10	0.233	120.0	48.11	94.6
2	1/3 & 10	0.3500	60 & 10	0.351	228.0	30.52	95.2
3	1/4 & 5	0.4375	60 & 10	0.365	246.4	25.92	95.0
4	1/1 & 10	0.1167	60 & 15	0.342	198.5	31.48	96.6
5	1/1 & 10	0.1167	90 & 22.5	0.280	142.9	35.42	88.4

remains stable. The suitable Na_2CO_3 concentration at a particular *w/o* ratio was theoretically estimated by obtaining a pH value of the internal aqueous solution as a function of mass of penicillin G transported to the internal phase in the same way as that reported in earlier work [3,7]. For the calculation, the ratio of the volumetric flow rate of the dispersed emulsion phase to that of the continuous phase in the continuous system was assumed to be the same as the ratio of the volume of the emulsion phase to that of the external phase in the batch system. The mass flow rates of Na_2CO_3 in the internal phase as used for the continuous operation were 8.75×10^{-4} mol/min for runs 1–4 and 1.31×10^{-3} mol/min for run 5.

In order to understand the behavior of the continuous system, dispersed phase holdup, swelling percentage of the emulsion phase and concentration of penicillin G in the internal phase of the emulsion effluent from the top of the column were measured at the steady state and the values are given in Table 1. The dispersed emulsion phase holdup is defined as

$$\phi = \frac{V_{em}}{V_{em} + V_{ex}} \tag{2}$$

where V_{em} and V_{ex} are volumes of the continuous and dispersed phases in the column, respectively. The swelling percentage (E_s) of the emulsion phase is defined as the ratio of the volume increment of the effluent emulsion to the volume of the influent emulsion phase and is expressed by

$$E_s = \frac{(w/o)_e - (w/o)_i}{(w/o)_i + 1} 100(\%) \tag{3}$$

Fig. 5 shows the effect of the *w/o* ratio with the optimal surfactant composition on the apparent degree of extraction during the continuous operation. There were little differences among the degrees of extraction for all *w/o* ratios. If swelling would not occur in the three runs, the highest concentration of penicillin G in the internal phase would be obtained at the *w/o* ratio of $\frac{1}{4}$. However, the higher concentration extent was obtained at the higher *w/o* ratio as described in Table 1 because the swelling was much lower at the higher *w/o* ratio. Despite the high actual degrees of extraction around 95% for all *w/o* ratios, the low concentration extents of penicillin G described in Table 1 seemed to make a practical application of the continuous extraction difficult. In addition, the dispersed phase holdup in the present system was much higher than that obtained in the previous work [8] under the condition of the same continuous and dispersed flow rates. This can be explained by the following arguments: first, Span 80, used only in the present continuous extraction as an auxiliary surfactant, rather than PARABAR 9551 facilitated water transport into the internal phase [16]; secondly, the working volume of the column in the present system was larger than that in the previous system while the factors such as stirring speed, diameter of the stator openings and height of the compartments affecting the dispersed phase holdup were almost the same in the two systems. A longer residence time of the emulsion phase within the column due to the larger working volume resulted in greater swelling and thus a higher holdup of the emulsion phase.

Fig. 6 shows the effect of the flow rate of the dispersed phase on the apparent degree of extraction at a constant flow rate of the continuous phase. Although the flow rate ratios of

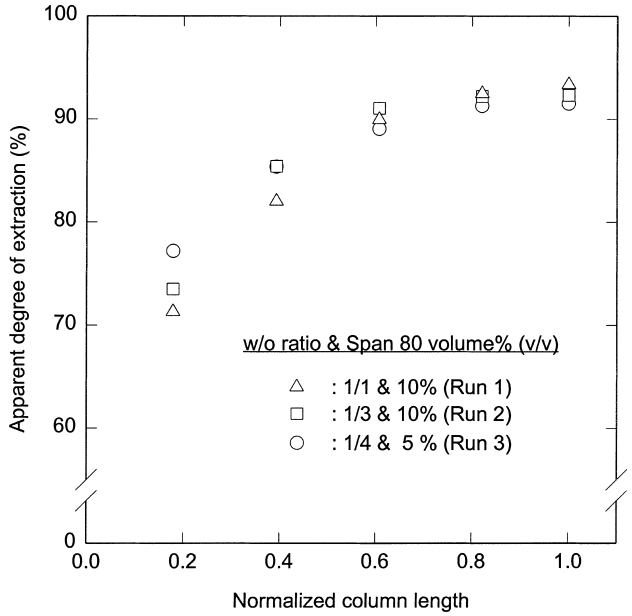

Fig. 5. Effect of *w/o* ratio with its optimal surfactant composition on apparent degree of extraction in a continuous column (q_{ex}/q_{em}=6; q_{em}=10 cm^3/min; mass flow rate of Na_2CO_3=8.75×10^{-4} mol/min).

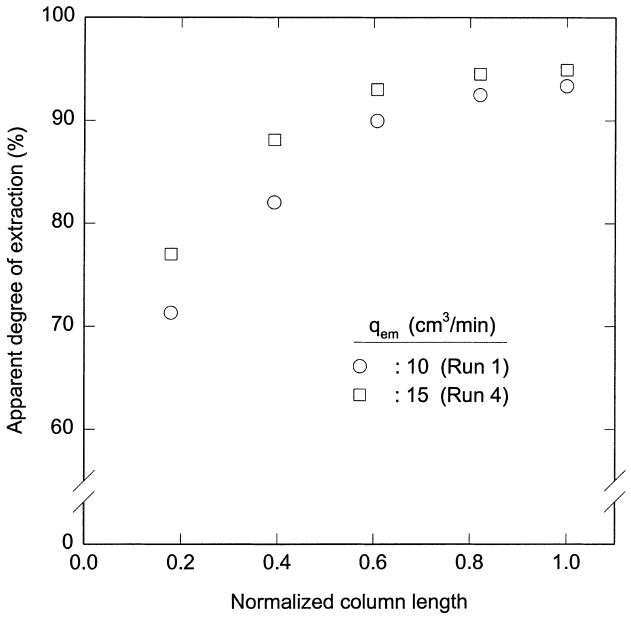

Fig. 6. Effect of flow rate ratio of continuous to dispersed phase on apparent degree of extraction in a continuous column (q_{ex}=60 cm^3/min; *w/o* ratio=1/1; 10% Span 80 (v/v); mass flow rate of Na_2CO_3=8.75×10^{-4} mol/min).

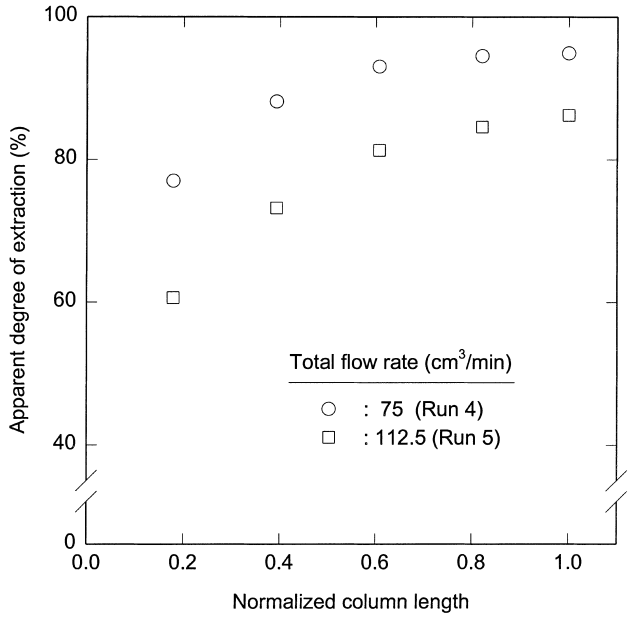

Fig. 7. Effect of total flow rate on apparent degree of extraction in a continuous column (q_{ex}/q_{em}=4; *w/o* ratio=1/1; 10% Span 80 (v/v); mass flow rates of Na_2CO_3=8.75×10^{-4} mol/min for run 4 and 1.31×10^{-3} mol/min for run 5).

the dispersed phase to the continuous phase were different between two runs, mass flow rates of Na_2CO_3 in the internal aqueous phase were the same. It is known that dispersed phase holdup increased with the increase in flow rate of the dispersed phase in the continuous extraction column with very low swelling [7,17]. Hence, we could assume that the larger interfacial area between the continuous and dispersed phases due to the higher dispersed phase holdup could bring about the higher apparent degree of extraction, even though the initial Na_2CO_3 concentration in the internal phase was somewhat lower at the higher flow rate of the dispersed phase. In addition, a larger amount of surfactant existed in the column at the higher flow rate of the dispersed emulsion phase, which seemed to result in the higher swelling and the dispersed phase holdup as described in Table 1.

Fig. 7 shows the effect of the total flow rate at a constant ratio of the flow rate of the dispersed phase to that of the continuous phase. Since contact time between the continuous and dispersed phases is reduced with an increase in the total flow rate, the apparent (or actual) degree of extraction along the column length was lower at the higher total flow rate. While a higher dispersed phase holdup was obtained at a higher total flow rate in the previous system with very low swelling [8], the dispersed phase holdup in the present system were higher at the lower total flow rate as described in Table 1. This implies that a much lower degree of extraction due to a shorter residence time of the dispersed phase could bring about lower swelling. Finally, the emulsion swelling accompanying the high degree of extraction made the concentration of penicillin G difficult.

One of the most serious problems associated with ELM processes is emulsion stability due to membrane breakage. Current remedies in use for this problem are to increase the concentration of surfactant in the membrane phase and the viscosity of the membrane phase. The remedies tend to reduce the rate of solute transfer by inhibiting any internal motion within emulsion drops, by increasing interfacial mass transfer resistance, or by decreasing molecular diffusivity of a solute in the membrane phase. Moreover, the use of more surfactant can result in higher emulsion swelling. Skelland and Meng [18] converted the membrane phase to appropriate non-Newtonian form, by adding a few percent of polymers, which increased membrane stability without loss in permeability and with marked reduction in swelling. Mok et al. [19] also suggested the possibility of reducing the emulsion swelling by the surfactant mixture of Span 80 and Span 85. Span 85 was more hydrophobic than Span 80. In the future, the above results of these works [18,19] will be used to apply the continuous ELM process to the industrial process for the extraction of penicillin G.

4. Conclusions

Continuous extraction of penicillin G was carried out in an extractor of the Oldshue–Rushton type. The experimental conditions for the continuous operation were obtained from the present batch extraction and our previous works. Especially, an optimal surfactant composition for each *w/o* ratio was obtained in a batch system and it was used to exam-

ine the possibility of a practical use of the continuous ELM process.

In the continuous operation, most of the actual degrees of extraction were higher than 95%, but the concentration of penicillin G obtained in the internal phase was lower than 2.5 times the initial penicillin G concentration in the external phase. This resulted from swelling of emulsion. It seemed that Span 80 had an important effect on the swelling, which brought about high dispersed phase holdup. Finally, the commercial applicability of penicillin G extraction by the ELMs depended on how much the swelling could be reduced. Further study of the reduction of the swelling is required prior to the commercial application.

5. Nomenclature

A	amine, Amberlite LA-2 or PARABAR 9551
$A_m(HP)_n$	amine–penicillin G complex
aq.	aqueous phase
E_s	swelling percentage
org.	organic phase
P^-	penicillin acid anion
q_{ex}	flow rate of continuous phase (cm^3/min)
q_{em}	flow rate of dispersed phase (cm^3/min)
V_{em}	total volume of dispersed phase at steady state (m^3)
V_{ex}	total volume of continuous phase at steady state (m^3)
$(w/o)_e$	volume ratio of internal to membrane phase for effluent emulsion
$(w/o)_i$	volume ratio of internal to membrane phase for influent emulsion
ϕ	dispersed phase holdup

Acknowledgements

The author wishes to acknowledge the financial support of the Korea Research Foundation for the program year of 1997. He is also grateful to Exxon Chemical, Singapore for the supply of PARABAR 9551.

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