EXTRACTION OF PENICILLIN WITH LIQUID SURFACTANT MEMBRANE

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Introduction

Penicillin, one of the most typical antibioitcs, is produced by fermentation, although a variety of semi-synthetic penicillins are prepared from natural penicillin. Since penicillin is unstable and tends to decompose, the fermented broths are cooled immediately and sent to a recovery process after filtering out the microbes. A solvent extraction method is employed widely to recover the penicillin. To prevent its decomposition, the pH of the cell-free medium is adjusted to 2.0–2.5 and extraction is carried out in a short contact time by use of centrifugal extractors like the Podbielniak. Instead of physical extraction in

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such a low pH range, where the decomposition rate is high, reactive extraction employing various alkylamines like Amberlite LA-2, di-*n*-octylamine and tri-*n*-octylamine as the extractant has been investigated by Schügerl and co-workers in the middle pH range, where penicillin is relatively stable.[6,9] In this extraction method the antibiotics transported to the organic phase must be recovered in the next step by reextraction.

As an innovative separation process which could perform both extraction and reextraction in a single stage, Li *et al.*[5] proposed a liquid membrane process in 1968. This process has been studied for the recovery of metals exclusively. Recently, the recovery of bioproducts from fermented broths by liquid membrane processes has been investigated.[11] We have reported the separation and concentration of various amino acids[1,3] and organic acids[2-4] with liquid surfactant membranes (LSM) where the extraction proceeds quickly due to low membrane thickness and extremely large interfacial area per unit volume. LSM processes are considered to be well suited to the separation and concentration of unstable antibiotics.[10] This paper discusses the recovery of penicillin G with LSM, using various amines as the carrier.

1. Experimental

W/O emulsions were prepared by mixing 20 cm^3 of oil and 15 cm^3 of internal water phases in a homogenizer at 167 s^{-1} for 10 min. The oil phase was composed of di-*n*-octylamine (DOA) as carrier, ECA4360J (Exxon Chem. Co.) as surfactant and *n*-butyl acetate, kerosene, or a mixture of the two as solvent. Aqueous Na_2CO_3 solution was used as the internal water phase for stripping. LSM operation was performed at 298 K by pouring 35 cm^3 of the W/O emulsion into 200 cm^3 of aqueous solution of penicillin G and agitating the contents at 4.2 s^{-1}. The pH of the aqueous penicillin G solutions was adjusted to 6 by citrate buffer. In runs made to examine the contribution of physical extraction (**Fig. 4**), the mixture was agitated at 0.5 s^{-1} to maintain a free flat interface between the W/O emulsion and the penicillin G solution. The concentration of penicillin G in the aqueous solution was measured by UV absorption at 257 nm. The W/O emulsion after extraction was demulsified in a high-A.C. electric field and the concentration of penicillin G in the internal water phase was measured.

2. Results and Discussion

In the conventional physical extraction of penicillin, *n*-butyl acetate is usually used as the organic solvent. Schügerl *et al.*[9] investigated the reactive extraction in *n*-butyl acetate solvent. However, stable W/O emulsion could not be prepared when *n*-butyl acetate

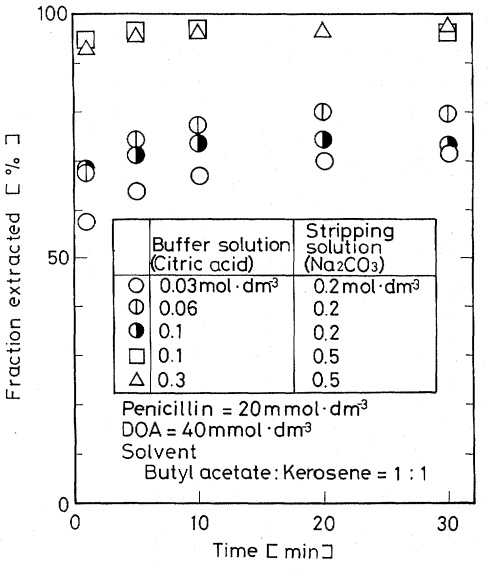

Fig. 1. Effects of concentrations of Na_2CO_3 in internal water phase and citric acid in external water phase on extent of extraction

was used together with Span80 as the surfactant. Therefore, various combinations of organic solvents and surfactants were tested to obtain a stable emulsion. It was found that a stable emulsion could be otained by using a mixture of kerosene and *n*-butyl acetate as solvent and ECA4360J (polyamine) as surfactant. Even in this case the emulsion was unstable when the volumetric ratio of *n*-butyl acetate in a solvent exceeded 50%.

Figure 1 shows the time-course of penicillin G extraction at various concentrations of Na_2CO_3 in the internal water phase and those of citrate buffer solution in the external water phase. A high extent of extraction was obtained by increasing concentrations of both stripping and buffer reagent. When the concentration of buffer solution was low, the pH of the external water phase increased gradually during the run. Therefore, it is clear that the pH difference between the internal and external water phases must be kept high to raise the final extent of extraction. The extraction proceeded quickly after pouring the emulsion. This is very desirable since the decomposition of penicillin G during the operation can be suppressed.

Figure 2 shows the effects of carrier (DOA) concentration in the oil phase. The extraction rate was very high, and nearly 100% of the penicillin G was extracted in a few minutes when DOA was added. However, even in the absence of DOA the extraction proceeded rapidly and penicillin G was extracted almost completely. These findings suggested that the surfactant ECA4360J acted as a carrier of the penicillin G anion since it has amino groups, and a small quantity of the penicillin G molecule was extracted in the

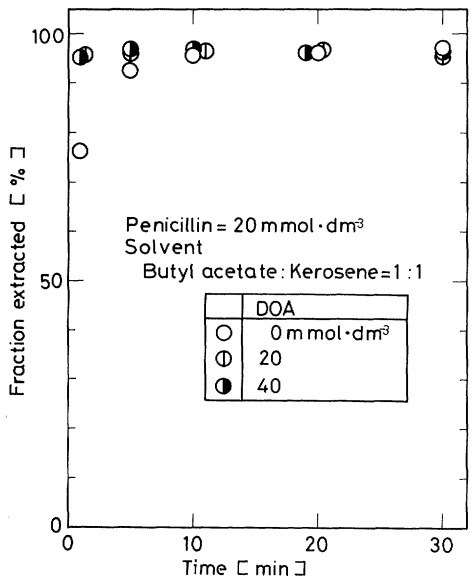

Fig. 2. Effects of carrier (DOA) concentration on extent of extraction

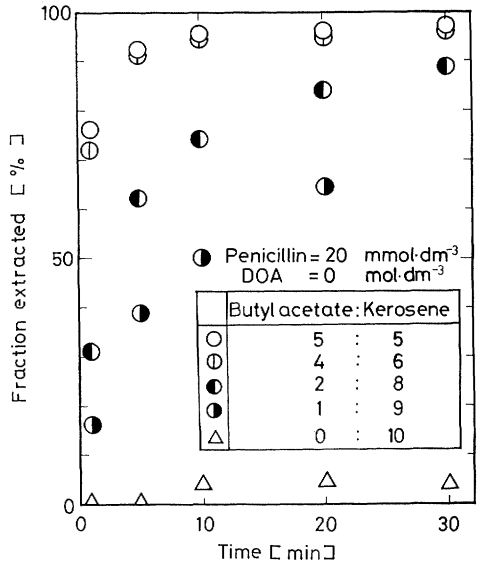

Fig. 3. Effects of oil-phase composition on extent of extraction

oil phase even in the middle pH range. Mori *et al.*[7] also reported that ECA4360J acted as a cerrier and a surfactant simultaneously in the recovery of chromium with LSM.

Figure 3 shows the variation of extraction behavior with changing volume ratio of *n*-butyl acetate and kerosene in a solvent which did not contain DOA as a carrier. Although there was no difference in the final extent of extraction, the extraction rate decreased gradually with increasing volume fraction of kerosene. When the volume fraction of kerosene exceeded 80% it required more than 30 min to accomplish complete extraction, and the advantage of LSM process disappeared. The experimental results shown in Figs. 2 and 3 demonstrate that high solubility of penicillin G into the oil phase is necessary to promote transport through the liquid membrane. The distribution ratio of penicillin G to a mixture of equal volumes of kerosene and *n*-butyl acetate was found to be 2.75×10^{-4} at pH 6. This extremely low distribution ratio, however, cannot explain the quick extraction behavior evident in Fig. 3. Therefore, the contribution of reactive extraction with ECA4360J is considered to be fairly high compared to that of physical extraction in these runs.

To confirm the role of ECA4360J as a carrier, the extraction was performed by using emulsions prepared with various concentrations of ECA4360J. When the concentration of the surfactant was changed, the drop size of W/O emulsion also changed and it was difficult to obtain almost the same interfacial area between the emulsion drops and the external water phase. Therefore, the extraction was carried out under gentle agitation of $0.5\,s^{-1}$ and constant interfacial area by free flat contact between the W/O emulsion and the

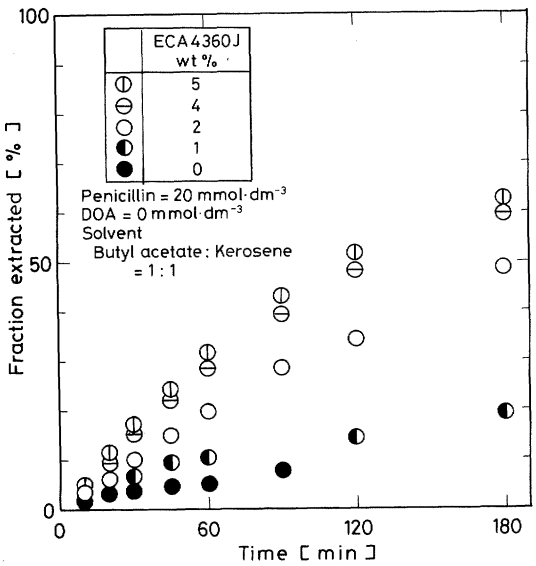

Fig. 4. Effects of ECA4360J concentration on extent of extraction in an extractor with free flat contact

external water phase. The results are shown in Fig. 4. It is clear that the carrier-mediated transport by ECA4360J contributes considerably to the extraction of penicillin G. A reference run without ECA4360J was performed by preparing an emulsion with Span80 which could not react with penicillin G. In a usual LSM operation where 5 wt% surfactant was added to the solvent, the contribution of the reactive extraction with ECA4360J was estimated from Fig. 4 to be about 7 times that of physical extraction.

Though some quantity of water dissolves in *n*-butyl acetate, swelling of W/O emulsion—which is the most serious problem in LSM process[8]—hardly occurred during the extraction. The recovery of penicillin G concentrated into the internal water phase was carried

out by demulsifying the emulsion in a high-A.C. electric field. The mass balance of penicillin G was confirmed by measuring the concentration in the internal water phase.

In conclusion, the experimental results obtained in this study suggested that the penicillin G could be rapidly separated and concentrated by LSM where the oil phase of the emulsions was composed of ECA4360J and a mixture of *n*-butyl acetate and kerosene. Nearly complete extraction was possible by adjusting the pH of both internal and external water phases in an optimum region. No swelling and breakage of W/O emulsion occurred during the operation.

Acknowledgement

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