Penicillin G extraction from simulated media by emulsion liquid membrane

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ABSTRACT

Penicillin G extraction by an emulsion liquid membrane was studied under various operational conditions in a batch system. Span 80 (sorbitan monooleate), TOA (Trioctylamine) and a mixture of n-butyl acetate and paraffin were used as surfactant, carrier and diluent, respectively. The effects of stirrer speed, volume ratio of membrane to external phases, initial penicillin G concentration, pH and buffer concentration in the external phase, sodium carbonate concentration in the internal phase, surfactant, volume ratio of diluents and carriers on the extraction rate were examined. Extraction rate was nearly 95% and a concentration greater than 12.67 times of the initial concentration of penicillin G in the external phase was obtained in the internal phase. The pH of external phase, containing a basic salt was theoretically calculated by the amount of penicillin G which was transported in the internal phase. The calculated results agreed well with the experimental data and extraction of penicillin G was successfully performed by the emulsion liquid membrane process through adjustment of the pH of both internal and external phases to an optimum values.

Keywords: Penicillin G extraction, Emulsion liquid membrane

INTRODUCTION

Penicillin G, a weak acid (pk_a=2.75), is extracted with n-butyl acetate at pH 2-2.5.In this pH range, penicillin G is unstable and decomposes, therefore the aqueous medium in fermentation broth is cooled to 0^{o}C and extracted in centrifugal extractors, to keep the contact time as short as possible (1). Re-extraction is carried out at pH 6.8-8.0 using either carbonate or phosphate buffers. However, in spite of low temperature (0^{o}C) and short time in the centrifugal extractor, penicillin G is prone to decomposition and losses of penicillin G during recovery is usually considerable (10-15%) (1). Reactive extraction by secondary amines, have been developed and used to avoid the loss of penicillin G due to decomposition (2-5).Extraction and re-extraction have also been performed at pH 5 and 7.5 respectively, where penicillin G is relatively stable. Although reactive extraction greatly reduces the loss of Penicillin G during recovery, several economic problems have been determined (6,7).First; an organic solvent and a carrier are used in excess. Moreover, a re-extraction step is required to recover penicillin G from the organic phase. Finally, the degree of re-extraction diminishes as the ratio of the throughput of organic phase to that of aqueous phase increases in order to concentrate penicillin G. Recently; a liquid membrane process has been utilized to avoid the disadvantages of reactive extraction of penicillin G. One of the advantages of liquid membrane processes is high permeation rate due to large interfacial area resulting in short contact time necessary for a high level of extraction .This is well suitable for separation and enrichment of unstable antibiotics. Marches et al. (8) were the first to perform the liquid process, where they used a supported liquid membrane (SLM) and described the facilitated transport of Penicillin acid anion as an ionic pair with tetrabutyl ammonium cations. The ELM process, using dioctylamine as a carrier and a mixture of kerosene and n-butyl acetate as solvent and ECA 4360 j as surfactant, to extract and concentrate of penicillin G from simulated media, have also been reported (9, 10).The pH of the external aqueous phase was adjusted to 6 using citrate buffer, but that of the internal aqueous phase was very unstable under their experimental conditions. They also reported that amines reacted with penicillin G to form 1:1 complex. The emulsion

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liquid membrane using Amberlite LA-2 as a carrier to recover the penicillin G from simulated media, have also been applied (6,7) and it has been reported that solvent and surfactant were a mixture of kerosene and n-butyl acetate, ECA 4360 j respectively .The pH of the external aqueous phase was adjusted to 5 using citrate buffer, and the initial Na_2CO_3 concentration in the internal aqueous phase was experimentally determined in order to keep the pH of the internal aqueous phase within the stable range for penicillin G at the end of extraction. The degree of extraction which was achieved was 90% under specific conditions. In this study, the effect of various experimental conditions on separation of penicillin G by emulsion liquid membrane was investigated. Due to the fact that the pH in the internal phase should be within the relatively stable range for penicillin G at the end of extraction, the initial pH of internal phase has been determined.

MATERIAL AND METHODS

Material

Penicillin G potassium salt was purchased from Synpac Co. (Portugal). Trioctyl amine, n-butyl acetate, sodium carbonate, paraffin oil and Span 80 (sorbitan monooleate) were all from E. Merck (Darmestat, Germany).

Methods

Estimation of hydrogen ion concentration in the internal phase

Since penicillin G is unstable at elevated pH, optimal pH value was determined in order to increase the extraction rate and simultaneously to reduce losses of penicillin G in the internal phase. The pH of the internal phase containing a basic salt must be initially high and then decreases rapidly with the penicillin G transport and after termination of extraction, the pH of the internal phase must be within the range of 5.5-7.5 in which penicillin G is stable(11). Although Na_2CO_3 has been selected as the most suitable internal phase for capturing penicillin G in ELM (6,12,13), further optimization of the initial concentration of Na_2CO_3 in the internal phase is important. The Na_2CO_3 in the internal phase was assumed to be in chemical equilibrium with transported penicillin G and decomposition of penicillin G was ignored. When the initial concentration of Na_2CO_3 in the solution is C_b, and the concentration of penicillin G in the internal phase at any time is C_a, the charge balance equation is:

$$[H^+]+ [Na^+] = [HCO_3^-] +2[CO_3^{2-}] + [OH^-] + [P^-]$$

The equilibrium expression for the weak acid and diacidic base are:

$$HP \leftrightarrows [H^+] + [P^-],\ K_a= [H^+][P^-] / [HP]$$
$$[CO_3^{2-}]+H_2O \leftrightarrows [HCO_3^-] + [OH^-]$$
$$K_{b1}= [HCO_3^-] [OH^-]/ [CO_3^{2-}]$$
$$[HCO_3^-]+ H_2O \leftrightarrows [H_2CO_3] + [OH^-],$$
$$K_{b2}= [H_2CO_3] [OH^-]/ [HCO_3^-]$$

The mass balance of equation is:

$$Ca= [HP] + [P^-]$$
$$Cb= [CO_3^{2-}] + [HCO3^-] + [H_2CO3] = [Na^+]/2$$

The above equations may be rearranged to give:

$$[H^+]^5+[H^+]^4\{K_{b1}+K_a+2\ Cb\}+ [H^+]^3\{K_{b1}\ K_{b2}+ K_{b1}\ K_a+ K_{b1}C_b+2K_aCb-K_w-K_aC_a\}+[H^+]^2\{ K_{b1}\ K_{b2}\ K_a + K_{b1}\ K_a\ C_b - K_{b1}\ K_w -K_a\ K_w –K_{b1}K_aC_a\}-[H^+]\{ K_{b1}\ K_{b2}\ K_w+ K_{b1}\ K_a\ K_w+ K_{b1}K_{b2}\ K_aC_a\}=K_{b1}\ K_{b2}\ K_w\ K_a$$

The above equation was solved by the FORTRAN and the calculated results are shown in Fig. (1).

pH change of an aqueous solution of penicillin G containing Na_2CO_3.

Since free penicillin G was not available, experiments were performed through two steps, specified as extraction and re-extraction of penicillin G. Initially, the aqueous solution for extraction was prepared by dissolving penicillin G potassium salt in 0.2 mole/dm^3 citrate buffer at pH 5.5. Trioctyl amine was dissolved in 20 mmole n-butyl acetate by stirring at 1000 rpm for at least 25 min. and equal volume of the solvent was added to the equal volume of penicillin G solution. Concentration of penicillin G in the aqueous phase was assessed using a UV/VIS spectrophotometer at 260 nm and the quantity in the organic phase was estimated using mass balance. For re-extraction the organic solution was shaken with the aqueous solution containing 0.05-0.5 mole/dm^3 Na_2CO_3 at various volume ratios for 5 min. After separation of the phases, the concentration of penicillin G in the aqueous solution and the pH of solution were determined.

Extraction of penicillin G by ELM

The experimental apparatus used was a batch-type stirred glass cell of 6.5 cm inner diameter and 10 cm depth. The vessel was fitted with a four baffles and stirring was carried out using a turbine impeller with six flat blades, each 3.75 cm diameter, connected with a speed controller.

A water-in-oil emulsion was prepared by slow addition of the internal aqueous solution to the organic solution while mixing by a homogenizer. A 35 m^3 value of W/O emulsion was dispersed in the vessel containing 200m^3 of feed solution of penicillin G potassium salt dissolved in citrate buffer solution and stirred at a constant speed. Samples were taken from the vessel at intervals. The external phase was separated from emulsion

phase by filtration using a filter paper and the concentration of penicillin G in the external phase was determined. Generally one parameter was changed and the other experimental parameters presented in table 1 were kept constant. The volume ratio of internal phase to membrane phase was kept constant at 3:4, emulsification speed was 6000 rpm and emulsification time was selected as 15 min.

RESULTS AND DISCUSSION

pH change of an aqueous solution of penicillin G containing Na2CO3

In figure 1, the pH values of solution with various Na_2CO_3 concentrations as a function of concentration of added penicillin G are shown. The results agreed with the experimental data without considering the amount of penicillin G transported into the internal phase. The use of a higher Na_2CO_3 concentration to increase the extraction rate in the ELM process might result in losses of penicillin G due to a high pH. For example after termination of extraction, if the concentration of penicillin G in the internal phase is about 200 mmole/dm^3, when 500mmole/dm^3 of Na_2CO_3 solution is used as the internal phase, the pH (higher than 8) was within the range where penicillin G will be more unstable. In other case at low Na_2CO_3 concentration, it is impossible to obtain penicillin G, higher than 210 mmole/dm^3 for 100 mmole/dm^3 Na_2CO_3 solution. The optimal Na_2CO_3 concentration was found to be 200 mmole/dm^3 Na_2CO_3. Thus, an appropriate concentration of Na_2CO_3 in the internal phase must be selected by estimation of the final concentration of penicillin G in the internal phase after the termination of extraction.

Extraction of penicillin G by ELM

Effect of stirrer speed

The effect of stirrer speed on the extraction is shown in figure 2. Emulsion drop size decreased as stirring speed was increased. Thus, initial extraction rate increased with stirrer speed because of the increase of the mass transfer coefficient in the external aqueous phase and the large interfacial area of emulsion drops. In addition, when the initial extraction rate is higher, the losses of penicillin G in the internal phase due to decomposition may be reduced since the pH decreases more rapidly.

However, at 400 rpm at the beginning of extraction, since the pH gradient between the external and internal phases is high and the concentration of penicillin G in the internal phase is low, the permeation rate, due to diffusion into the emulsion drops, is much higher than the leakage rate, due to membrane breakage. Thus, the degree of extraction was increased during this early stage. The permeation rate decreased rapidly with time while the leakage rate increased. Finally, the leakage rate equated to permeation rate after 25 min. However an optimal stirrer speeds was found 300 rpm.

Effect of volume ratio of membrane phase to external aqueous phase

In figure 3, higher extraction rate were observed at high volume ratio of membrane phase to external phase, because the higher volume membrane phase had larger capacity for extraction of penicillin G (14). Thus an optimal volume ratio of membrane phase to external phase was selected at 35:200.

Effect of initial penicillin G concentration in the external aqueous phase

Figure 4 shows the effect of the initial concentration of penicillin G in the external phase on the extraction. The higher the initial concentration of penicillin G, the higher was the initial extraction rate. In the case of 35 mmole/dm^3 of penicillin G, the degree of extraction did not increase after 30 min because most of the internal droplets were saturated with penicillin G. Therefore, the best initial penicillin G in the external aqueous phase was found at 20 mmole/dm^3.

Effect of citrate buffer solution concentration and pH

In figure 5 and 6 ,Higher extraction rates were observed at low pH values, because the driving force of the extraction of penicillin G is the pH gradient between the external and internal phase. However, the decomposition of penicillin G is high at low pH (11), therefore the optimum pH was selected as 5.5. Also, at higher concentration of buffer solution, buffer capacity is higher and therefore resistance to pH changes due to transfer of penicillin G from external phase to internal phase could be observed. In this experiment, buffer concentration was selected at 200 mmole/dm^3.

Effect of Na_2CO_3 concentration in the internal phase

The effect of Na_2CO_3 concentration on the extraction is shown in figure 7. The hydrogen ion concentration difference between the external and internal phase has a major influence upon the driving force in the ELM process. Thus, as the Na_2CO_3 concentration in the internal phase increases, the initial rate also increases because the capacity of the internal phase as a sink for penicillin G increases, but as shown in figure 1 when the penicillin G concentration is 200 mmole/dm^3 in the internal phase, the pH value is high for 300 mmole/dm^3 Na_2CO_3, and penicillin G in the internal phase is highly unstable. Therefore the optimal concentration of Na_2CO_3 appears to be 200 mmole/dm^3

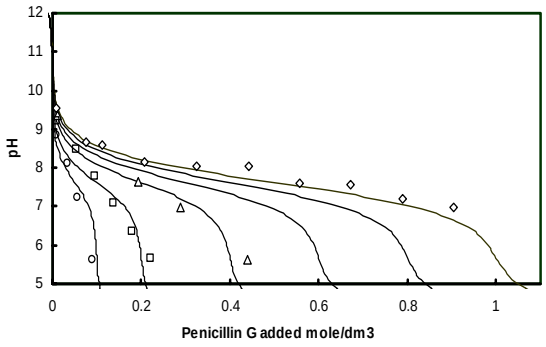

Figure 1. Comparison of the calculated results with the experimental data for the pH change of the internal phase containing various Na_2CO_3 concentrations with penicillin G. ◊: 0.5 mole/dm^3; Δ: 0.2 mole/dm^3; □ : 0.1 mole/dm^3; O : 0.05 mole/dm^3

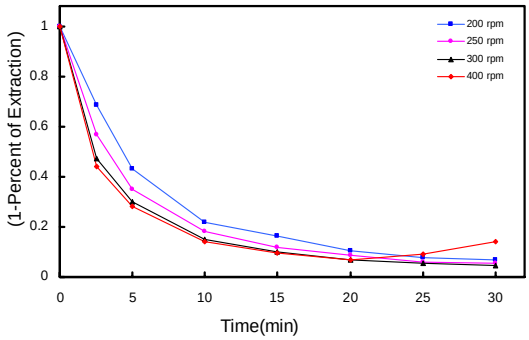

Figure 2. Effect of stirrer speed on the percent of extraction.

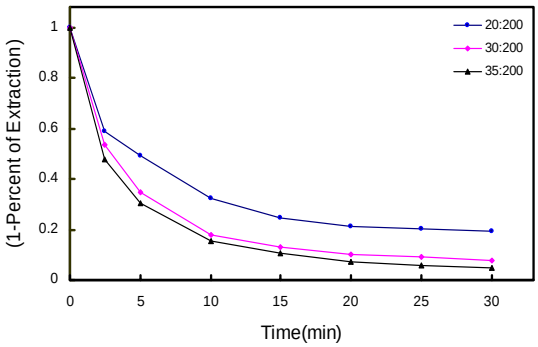

Figure 3. Effect of volume ratio of membrane phase to external aqueous phase on extraction. percent

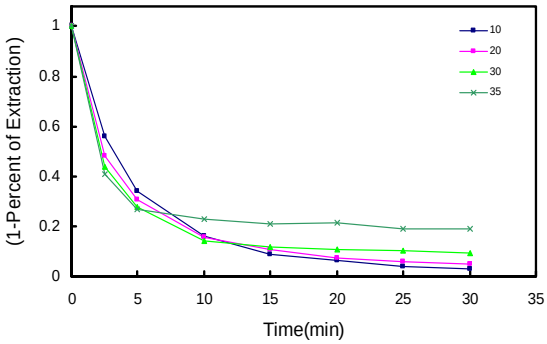

Figure 4. Effect of initial penicillin G concentration in the external aqueous phase on extraction. percent

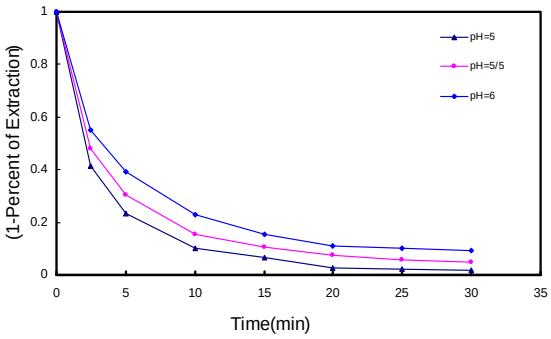

Figure 5. Effect of citrate buffer solution pH on the percent of extraction.

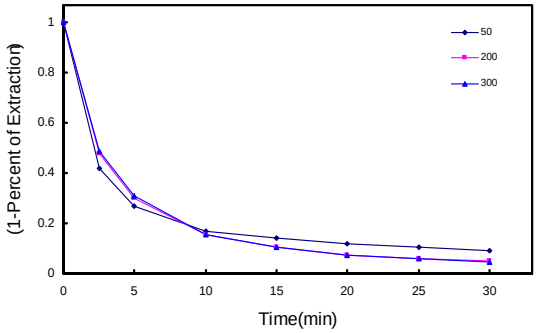

Figure 6. Effect of citrate buffer solution concentration on degree of extraction

Effect of surfactant concentration

The effect of surfactant concentration, on the extraction of penicillin G is shown in figure 8. When the concentration of surfactant increases the emulsion becomes stable but at high surfactant concentration, because of swelling and breakage of emulsion, the degree of extraction after 25 min was decreased. Thus, the optimal surfactant concentration was selected at 8 (%w/w).

Effect of solvent mixture on the extraction of penicillin G

The effect of solvent mixture on the extraction is shown in figure 9.As the volume ratio of paraffin oil to n-butyl acetate increased, the viscosity of the membrane phase increased and thus extraction of penicillin G decreased. Also, since the partition coefficient of physical extraction for n-butyl acetate is higher than paraffin, the initial

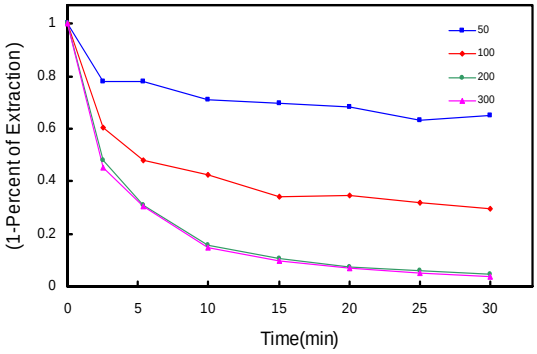

Figure 7. Effect of Na_2CO_3 concentration in the internal phase on the percent of extraction.

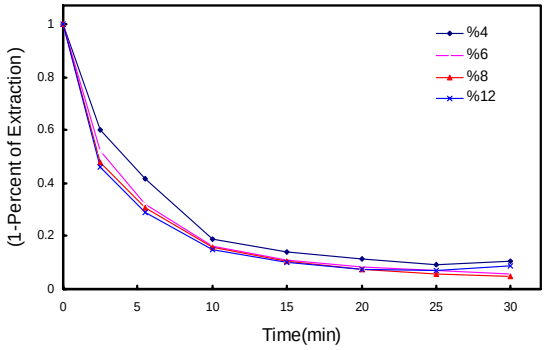

Figure 8. Effect of surfactant concentration on the percent of extraction.

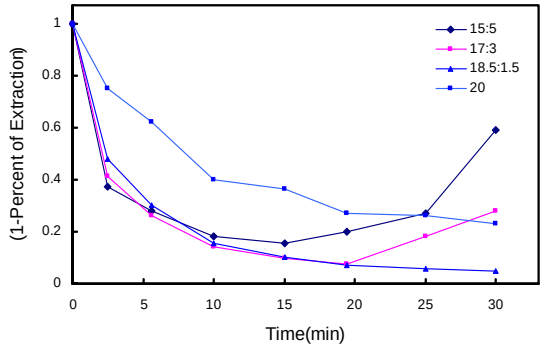

Figure 9. Effect of solvent mixture on the percent of extraction .

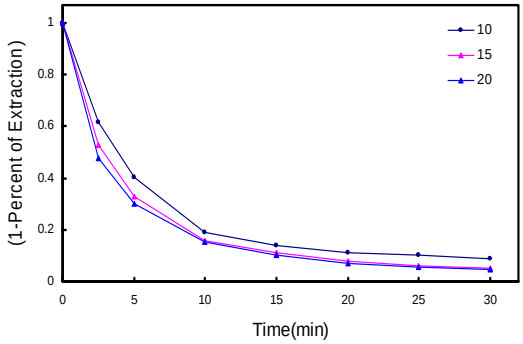

Figure 10. Effect of carrier concentration on the percent of extraction.

Table 1. Experimental Condition

Parameters	Conditions	Typical condition
Stirrer speed	200, 250, 300, 400 rpm	300 rpm
Volume ratio of membrane phase to external phase	20:200, 30:200,35:200	35:200
Penicillin G concentration in the external phase	10, 20, 30, 35 mmole/dm^3	20 mmole/dm^3
Citrate buffer solution concentration	50, 200, 300 mmole/dm^3	200 mmole/dm^3
The pH of external phase	5.5, 6	5.5
Na_2CO_3 concentration in the internal phase	50, 100, 200, 300 mmole/dm^3	200 mmole/dm^3
Surfactant concentration	4, 6, 8, 12 (%w/w)	8 (%w/w)
Volume ratio of paraffin to n-butyl acetate	15:5, 17:3, 18.5:1.5, 20	18.5:1.5
Carrier concentration	10, 15, 20 mmole/dm^3	20 mmole/dm^3

extraction rate of higher amount of n-butyl acetate in this mixture was the highest. However, As shown in figure 9 the ELM using n-butyl acetate (ratio 15:5)after 10 min. was highly unstable. The best result was obtained using the volume ratio of paraffin to n-butyl acetate 18.8 to 1.5.

Effect of carrier concentration

The effect of carrier concentration on the extraction of penicillin G, is shown in figures10. Initially TOA reacted with penicillin acid anion, at the interface between the external and membrane phase to form the complex. The complex then diffuses through the membrane phase to the interface with internal and membrane phase. Thus, the degree of penicillin G transferred increased with increasing of TOA concentration, because of a higher driving force at interface between external and membrane phase to internal and membrane phase. Also, the higher

concentration of TOA swelling may be occurred in membrane. Therefore, the optimal concentration of TOA was selected as 20 mmole/dm^3.

CONCLUSION

The results of this study show that ELM process is very suitable for extraction of penicillin G from feed solutions in a wide range of concentrations. Penicillin G could be rapidly separated and enriched by the ELM when the membrane phase is composed of TOA and a mixture of n-butyl acetate and kerosene. Nearly complete extraction was accomplished by adjustment of the pH of both internal and external phases to an optimum value .Also the optimal concentration of Na_2CO_3 in the internal phase was determined. Therefore the pH should be within the range where penicillin G is stable after termination of extraction.

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