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Facilitated Transport of Penicillin G by Bulk Liquid Membrane with TBP as Carrier

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Abstract The facilitated transport of penicillin G from aqueous solutions to the stripping phase through bulk liquid membrane (BLM) containing TBP in 3% iso-octanol and *n*-butyl acetate was studied. Na_2CO_3 solution was used as the stripping phase. Experiments were performed as a function of stirring rate, TBP concentration and type of diluent in the liquid membrane phase, pH, and initial penicillin G concentration in the feed phase, Na_2CO_3 concentration in the stripping phase, etc. The results showed that the BLM process could carry out the simultaneous separation and concentration of penicillin G from dilute aqueous solutions, and arise "up-hill" effect due to the characteristic of non-equilibrium mass transfer. The diffusion of penicillin G complex in the liquid membrane phase played an important role in BLM process. The mass transfer mechanism of BLM for this system was also discussed.

Keywords Bulk liquid membrane · Penicillin G · Facilitated transport · TBP

Introduction

Penicillin G is one of the most popular antibiotics, and is also an important raw material for semisynthetic penicillin [1]. The commercially viable technique for recovery of penicillin G from fermentation broth is solvent extraction [2, 3]. However, in the commercial solvent extraction process with *n*-butyl acetate as solvent [4], a considerable amount of penicillin G (10%–15%) is lost due to its instability at pH of 1.8–2.5. Reschke and Schügerl [4–6] first suggested the reactive extraction penicillin G with amine-based extractants under a mild pH condition where penicillin G is more stable, re-extraction was carried out at pH 6.8–8.0 by a carbonate or phosphate buffer. Although many types of complex extractants, such as amine-based solvent, aliphatic alcohol, and phosphorus ester, etc. had been applied to recover penicillin G in many studies [7–11], the reactive extraction process had not been adopted in

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industrial process because the consumption of solvent in the extraction process is large and the solvent is usually expensive [7].

Liquid membrane techniques (LMs) have obtained special attention in separation and purification of biochemical products in recent years because LMs have characteristics of carrying out simultaneous extraction and stripping processes in the same stage, non-equilibrium mass transfer, "up-hill" effect, and low consumption of solvent, etc. Many researchers had applied liquid membrane techniques for penicillin G recovery [12–15].

Neutral phosphorus extractant has the nature of demulsification and good extraction capability. Tri-*n*-butyl phosphate (TBP) was reported to be an alternative choice [10]. Moreover, the re-extraction of penicillin G with TBP as carrier is much easier than that of an amine-based extractant.

In this paper, the facilitated transport of penicillin G in a bulk liquid membrane (BLM) process was investigated. TBP was used as a carrier, *n*-butyl acetate as a diluent, iso-octanol as a modifier, and Na_2CO_3 solution as the stripping phase. The effects of stirring rate, carrier, and diluent concentrations in the liquid membrane phase, pH, and initial penicillin G concentration in the feed solution, Na_2CO_3 concentration in the stripping phase, etc. on the mass transfer performance of the BLM process were studied. The mass transfer mechanism was also discussed.

Material and Methods

Reagents and Procedures

The sodium salt of penicillin G with an activity of 1,667 units mg^{-1} was purchased from Huabei Medicine Producing Plant. *n*-Butyl acetate, tri-*n*-butyl phosphate (TBP), anhydrous sodium carbonate KH_2PO_4, H_2SO_4, and NaOH were all analytical grade reagents from Beijing Beihua Refined Chemical Co. Ltd. Iso-octanol was an analytical grade reagent from Beijing Yili Refined Chemical Co. Ltd.

The aqueous phase was prepared by dissolving sodium salt of penicillin G in KH_2PO_4 (0.05 mol dm^{-3}) aqueous solutions. Concentrations of 10% H_2SO_4 and 0.1 mol·L^{-1} NaOH aqueous solution were used to adjust the pH value.

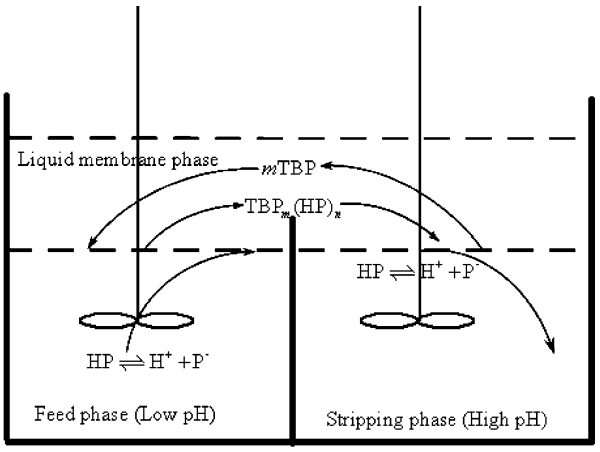

Fig. 1 Transport mechanism of penicillin G through BLM

The experiments were carried out in a self-designed glass transport cell as described in Fig 1. A volume of 150 mL penicillin G aqueous solution was added to one of the chambers, 150 mL 0.1 mol L^{-1} Na_2CO_3 solution as the stripping phase was added to another chamber. A volume of 300 mL liquid membrane solution, 15% TBP and 3% iso-octanol dissolved in *n*-butyl acetate, was slowly added the upper of the cell. The feed phase and stripping phase were stirred at 150 rpm with stirring paddles in opposite directions. All the experiments were performed at room temperature. The samples were taken out from the feed solution and stripping phase at scheduled time intervals, pH, and the penicillin G concentration were determined. Each experiment was duplicated under identical conditions.

Analysis Methods

The pH value was measured by a digital precision ionometer model PXS-450 (Shanghai Dapu Co. Ltd.) with a combined glass electrode (±0.01 pH). The concentration of penicillin G in the aqueous phase was determined by HPLC (Shimadzu, LC-20A) with an ODS C18 (5 μm) column and a UV detection wavelength set at λ=225 nm. The mobile phase was a mixture of 0.02 mol·L^{-1} KH_2PO_4 and methanol in a volume ratio of 38:62, the flow rate was 1.0 ml·min^{-1}. The penicillin G concentration in the organic phase was calculated from mass balance.

Results and Discussion

The transport efficiency of penicillin G through BLM, E, was calculated as follows:

$$E = \frac{C_s V_s}{C_{f,0} V_f} \times 100\% \tag{1}$$

Where C_s is the penicillin G concentration in the stripping phase, $C_{f,0}$ the initial penicillin G concentration in the feed phase, V_s the volume of stripping phase, and V_f the volume of feed phase.

Mass Transfer of Penicillin G in BLM Process

The mechanism of carrier-facilitated transport of penicillin G with TBP through BLM is shown in Fig. 1. A reversible reaction of TBP with penicillin G occurs at the interfaces between aqueous and membrane phases as follows [3, 10]:

$$m\mathrm{TBP}_{\mathrm{org}} + n\mathrm{H}^{+}_{\mathrm{aq}} + n\mathrm{P}^{-}_{\mathrm{aq}} \Leftrightarrow \mathrm{TBP}_m(\mathrm{HP})_{n\ \mathrm{org}} \tag{2}$$

The transport process consists of following steps: diffusion of penicillin G across stagnant films of the feed and strip phases, diffusion of carrier and its complex in the membrane phase, and chemical reaction of penicillin G and carrier at both aqueous-membrane interfaces.

In this section, the dimensionless concentration R was used:

$$R_f = \frac{C_f}{C_{f,0}},\ R_m = \frac{C_m}{C_{f,0}},\ R_s = \frac{C_s}{C_{f,0}} \tag{3}$$

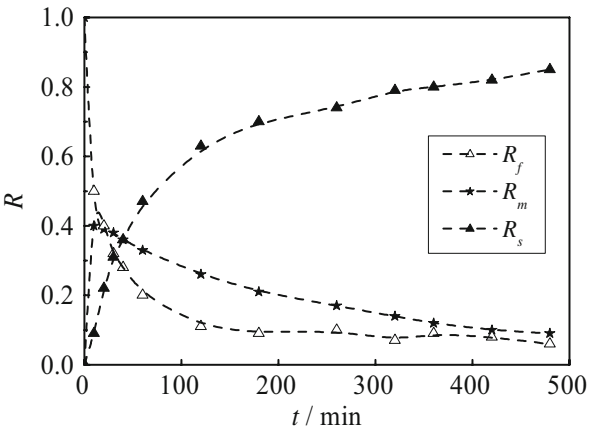

Fig. 2 Variation of penicillin G concentration with time (stirring rate=150 rpm, $C_{f,0}$=30 mmol L^{-1}, pH_f=3.0, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

Where C_f, C_m, and C_s represent the penicillin G concentration in the feed, liquid membrane, and stripping phases, respectively. R_f, R_m, and R_s are the dimensionless penicillin G concentration in the feed, liquid membrane, and stripping phases, respectively.

Figure 2 shows the variation of dimensionless penicillin G concentration with time in the feed, liquid membrane, and stripping phases, respectively. The results show that the bulk liquid membrane can carry out the simultaneous separation and concentration of penicillin G from dilute aqueous solutions. At the beginning of the experiment, penicillin G is transported from the feed phase to the liquid membrane phase rapidly, the penicillin G concentration in the feed phase decreases sharply, and the penicillin G concentrations in the liquid membrane phase and stripping phase increase greatly. With the experiment running, the variations of penicillin G concentration in the feed phase and stripping phase become slow; while the penicillin G concentration in the liquid membrane phase reaches a maximum value first, and then reduces; finally, it reaches a relative stable value in a later experiment. It is also indicated that the diffusion of penicillin G complex in the liquid membrane phase plays an important role in the BLM process, especially in the beginning of the experiment; there is a large accumulation of penicillin G in the membrane phase. At 40min, an “up-hill” effect occurs, penicillin G is transported from low concentration in the feed phase to high concentration in the stripping phase, which also indicates the characteristic of non-equilibrium mass transfer in bulk liquid membrane process.

Effects of Stirring Rate

The stirring rates have significant influences on the transport of penicillin G through BLM from feed phase to the stripping phase. In order to explore the effects of stirring rates, the transport experiments were carried out at four different stirring rates, 70, 100, 150, and 180 rpm. Both chambers were in the same stirring rate in the opposite direction. As shown in Fig. 3, the transport efficiency increases with increasing stirring rate; this is because the higher stirring rate leads to much severer mixing between the aqueous solution and organic phase in both chambers, which could accelerate the chemical reaction rate of penicillin G and carrier, and enhance the mass transfer area between aqueous solution and liquid membrane solution. These could reduce the mass transfer resistances of penicillin G from feed aqueous solution to the liquid membrane phase in the extraction process, and from liquid membrane phase to the stripping phase in the stripping process. However, at a higher

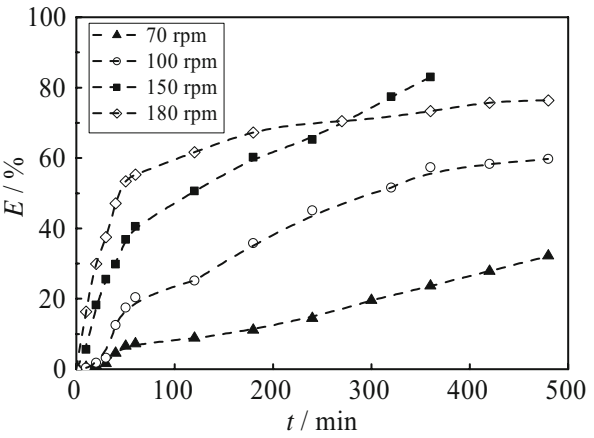

Fig. 3 Effect of stirring rate on the transport of penicillin G ($C_{f,0}$=30 mmol L^{-1}, pH_f=3, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

stirring rate of 180 rpm, with the experiment running, although a stable and non-disturbed mass transfer interface exists, a slight emulsification occurs at the interface between organic and aqueous solutions which do not benefit the transport of penicillin G. On the other hand, a higher stirring rate would increase the risk of leakage between two aqueous phases, which would result in the failure of the transport process through bulk liquid membrane. In later experiments, the stirring rate of 150 rpm is adopted.

Effect of Carrier Concentration

In a carrier-facilitated transport process, the carrier plays an important role in the transport of penicillin G through BLM. The influence of TBP concentration in the liquid membrane phase on penicillin G transport was studied at ranges of 7% to 22% (volume). The results are shown in Fig. 4. When the TBP concentration in the organic phase increases from 7% to 15%, the increment of penicillin G transport through BLM is fast and effective because a higher carrier concentration leads to a higher facilitated transport capacity and a higher distribution coefficient between the aqueous phase and organic phase can enhance the mass transfer driving force of the BLM process. When the TBP concentration increases from

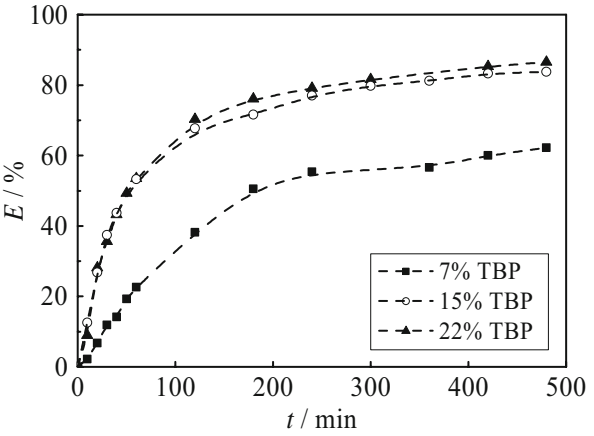

Fig. 4 Effect of TBP concentration on the transport of penicillin G (stirring rate=150 rpm, $C_{f,0}$=30 mmol L^{-1}, pH_f=3, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

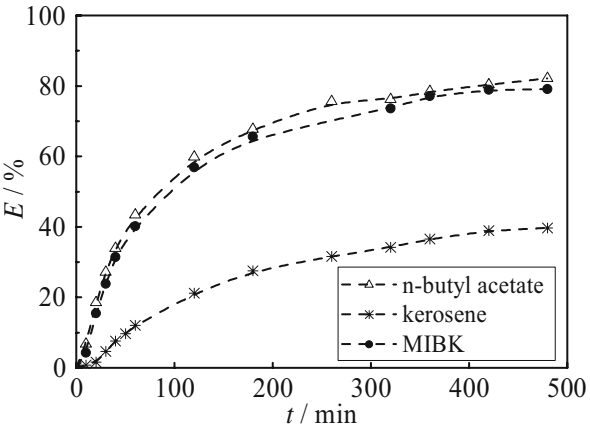

Fig. 5 Effect of diluents on the transport of penicillin G (stirring rate=150 rpm, $C_{f,0}$=30 mmol L^{-1}, pH_f=3, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

15% to 22%, the increment of transport of penicillin G becomes slow. Because the higher TBP concentration in the organic phase would increase the viscosity of the organic phase, which would reduce the diffusional mobility rate of the TBP–penicillin complex in the liquid membrane phase; here, the carrier concentration may be in excess.

Effect of Diluents in Liquid Membrane Phase

Three types of diluents, *n*-butyl acetate, kerosene, and MIBK were used to study the influence on the transport efficiency in BLM process. The results are shown in Fig. 5. The properties of diluents in the liquid membrane phase, such as polarity, viscosity, and other physical properties, have significant influence on the distribution equilibrium of penicillin G between aqueous solution and organic phase with TBP as carrier and iso-octanol as modifier. As a non-polar solvent, kerosene without carrier almost could not extract penicillin G from aqueous solutions. As expected, when kerosene is used as a diluent, the transport efficiency is low (<40%). However, as a result of a polar solvent, there is a high distribution coefficient between *n*-butyl acetate or MIBK and aqueous solution. Also, when *n*-butyl acetate or MIBK is used as a diluent, the transport efficiency is high. Because the penicillin G-TBP complex, $TBP_m(HP)_n$, is more soluble in a polar solvent than in a non-polar solvent [5, 8].

Effect of pH in the Feed Solution

Due to the instability of penicillin G in the aqueous solution at low pH and low extraction efficiency at high pH [4, 10], experiments about the effect of pH in the feed solution on the transport efficiency of penicillin G through BLM are conducted at pH ranges of 3.0 to 5.0. As shown in Fig. 6, the pH in the feed solution has an obvious influence on the transport of penicillin G through BLM. At a lower pH value of 3.06 in the feed solution, the higher hydrogen ion concentration leads to the higher distribution coefficient and a higher complex reaction rate, which results in higher transport efficiency of penicillin G as described in Eq 1; the transport efficiency is up to 85.9%. However, when the pH value in the feed solution is >4.0, the transport efficiency sharply reduces due to lower mass transfer driving

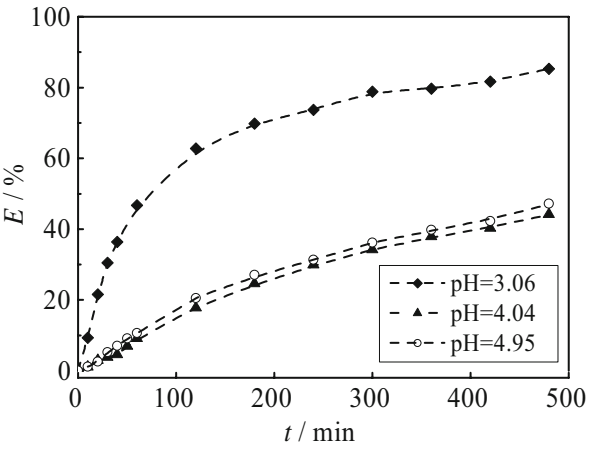

Fig. 6 Effect of pH in the feed solution on the transport of penicillin G (stirring rate=150 rpm, $C_{f,0}$=30 mmol L^{-1}, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

force caused by lower distribution coefficient. This is similar to the behavior of distribution equilibria of penicillin G between the same organic phase and aqueous solution [10].

Effect of Initial Penicillin G Concentration in the Feed Solution

The influence of initial penicillin G concentration in the feed solution on the transport through BLM was studied at ranges of 10 to 30 mmol·L^{-1}. Results were shown in Fig. 7. The transport efficiency of penicillin G decreases with increasing initial penicillin G concentration at ranges from 10 to 20 mmol·L^{-1} in the feed phase, because the lower distribution coefficient, caused by higher initial penicillin G concentration, leads to a higher mass transfer driving force under this case. On the other hand, although the transport fluxes of penicillin G from feed solution to the stripping phase increases with increasing initial penicillin G concentration, the increment of initial penicillin G amount is bigger than that of transport flux. At a higher initial penicillin G concentration, the stripping process in the BLM process, i.e., the transport step of penicillin G from liquid membrane phase to the stripping phase, is changed into the rate-controlling step, then the initial penicillin G concentration has a slight influence on the transport efficiency as shown in Fig. 7.

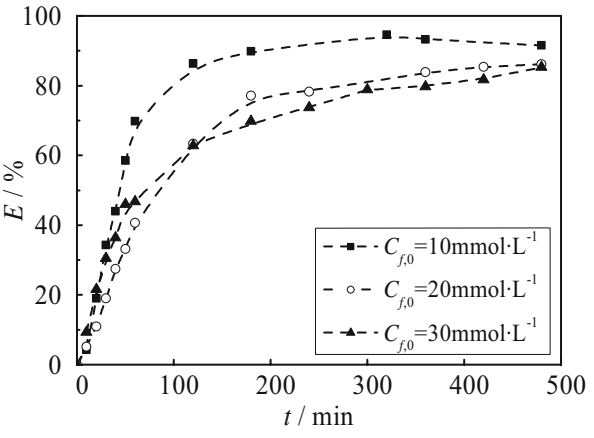

Fig. 7 Effect of initial penicillin G concentration on its transport (stirring rate=150 rpm, pH_f=3, $C_{s,0}$=0.1 mol L^{-1}, pH_s=7.2–7.3)

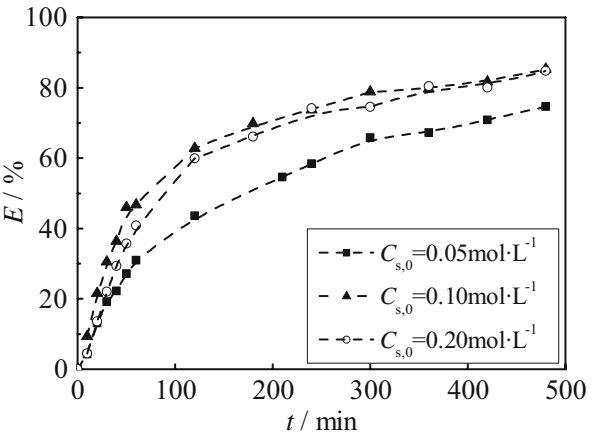

Fig. 8 Effect of Na_2CO_3 concentration on the transport of penicillin G (stirring rate=150 rpm, $C_{f,0}$=30 mmol L^{-1}, pH_f=3, pH_s=7.2–7.3)

Effect of Na_2CO_3 Concentration in the Stripping Phase

The influence of Na_2CO_3 concentration in the stripping phase on penicillin G transport was investigated at three values of 0.05, 0.10, and 0.20 mol·L^{-1}. The results are shown in Fig. 8. Because the proton concentration gradient between the feed and stripping phases is also the mass transfer driving force for this transport process at a lower Na_2CO_3 concentration as shown in Fig. 2, a higher Na_2CO_3 concentration in the stripping phase leads to a higher mass transfer driving force, then the transport efficiency increases with increasing Na_2CO_3 concentration as expected. The higher the Na_2CO_3 concentration leads to a lower proton concentration in the stripping phase, which is beneficial for the decomplexation reaction in the stripping process. Whereas at higher Na_2CO_3 concentration, the stripping phase is in excess, the influence of Na_2CO_3 concentration on the transport efficiency becomes slight.

Conclusions

In this paper, the carrier-facilitated transport of penicillin G through a bulk liquid membrane was investigated. TBP was used as the carrier, iso-octanol as the modifier, and *n*-butyl acetate as the diluent. Na_2CO_3 solution was used as the stripping phase.

The results showed that the bulk liquid membrane can carry out the simultaneous separation and concentration of penicillin G from aqueous solutions. Due to the characteristic of a non-equilibrium mass transfer, the “up-hill” effect was observed. The diffusion of penicillin G complex in the liquid membrane phase played an important role in the BLM process. The transport efficiency increased with increasing stirring rate. At a higher stirring rate, a slight emulsification was observed, which was not beneficial for the transport of penicillin G through BLM. Later, a moderate stirring rate of 150 rpm was adopted.

The transport efficiency increases with increasing TBP concentration in the liquid membrane phase at penicillin G concentration <15% (volume). The diluents, *n*-butyl acetate or MIBK, were better than kerosene for this transport process due to the polarity of the solvent. Due to the influence of the mass transfer driving force caused by the distribution coefficient, the transport efficiency decreases with increasing pH. At a lower pH of 3.06, the transport efficiency can reach 85.9%. Also, the transport efficiency of penicillin G based

on the feed solution decreases with increasing initial penicillin G concentration in the feed phase and increases with increasing Na_2CO_3 concentration in the stripping phase, mainly due to the effects of mass transfer driving force caused by distribution coefficient of penicillin G and pH gradient between the feed phase and stripping phase, respectively.

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