CHAPTER VII

ENANTIOSEPARATION OF (*S*)-AMLODIPINE FROM PHARMACEUTICAL INDUSTRY WASTEWATER BY SPECIFIC STRIPPING PHASE RECOVERY VIA HFSLM: POLARITY OF DILUENT AND MEMBRANE STABILITY INVESTIGATION

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7.1 ABSTRACT

Pharmaceutical wastewater may contain high-value pharmaceutically active compounds such as amlodipine. A hollow-fiber supported liquid membrane (HFSLM) process was developed and applied in the pretreatment of pharmaceutical wastewater for (S)-amlodipine recovery. The HFSLM system contained O,O'-dibenzoyl-(2S,3S)-tartaric acid ((+)-DBTA) in the liquid membrane phase and β-cyclodextrin in the stripping phase. The effects of various chemical parameters, including the concentration of the chiral selector in the stripping phase, as well as the type of organic diluent and the carrier concentration in the membrane, were also investigated. Several diluents – hexane, 1-decanol, chlorobenzene, benzene, dichloromethane, ethylene dichloride, and chloroform – with different polarity indexes, from 0.1–4.1, were used. The results found that the polarity of the diluents was the main factor influencing the extraction performance and stability of the liquid membrane. Decreasing the polarity of the diluent could prolong membrane stability, but the percentages of extraction and stripping decreased as well. The longest lifetime (150 min) was obtained by using 1-decanol, with a polarity index of 1.8, as a diluent.

7.2 INTRODUCTION

The treatment of pharmaceutical wastewater presents a real challenge for wastewater engineers. A solution to the pharmaceutical water pollution problem has become a matter of great urgency [1]. Recently, most drugs have been manufactured through chemical synthesis processes. Chemical synthesis-based pharmaceutical wastewater contains a variety of organics, including solvents, additives, reactants, and high-value pharmaceutically active compounds [2]. Although pharmaceutically active compounds are present in low concentrations in the environment, such drugs can have adverse effects on aquatic organisms [3]. These effects are chronic rather than acutely toxic, and depend on exposure, susceptibility to the compound in question, and the degradability of the compound [4, 5]. Moreover, high-value drug substances can also be recycled for use in the pharmaceutical industry [6].

Currently, much attention has been directed toward waste reduction and recycling of materials and high-value products that are used repeatedly in pharmaceutical production processes. A primary objective in wastewater treatment is the recovery of pharmaceutically active compounds [7]. Many such compounds are used as racemic mixtures. The enantiomers may have different effects on a drug's pharmacological activity, metabolic processes, and toxicity in the human body [8].

Like other chiral drugs, amlodipine – (3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate) – is a racemic drug that belongs to the calcium channel blocker group; these are used for treating hypertension and angina pectoris [9]. As a racemic mixture, amlodipine contains (S)-amlodipine (Figure 1(a)) and (R)-amlodipine (Figure 1(b)); but only (S)-amlodipine, as the active moiety, possesses therapeutic activity [10]. Based on pharmacological research, it remains uncertain if (S)-amlodipine alone has similar efficacy and fewer associated adverse events compared with the racemic mixtures [11]. (S)-amlodipine exhibits vasodilating properties [12]. (R)-amlodipine is inactive, and is thought to be responsible for pedal edema observed with racemic amlodipine [13]. (S)-amlodipine is the more potent calcium channel blocker, showing about 2,000 times the potency in *in vitro* evaluation in the rat aorta compared to (R)-amlodipine [14]. In addition to its longer duration of action, (S)-amlodipine reduces the chances of reflex tachycardia, and its clearance is subject to much less inter-subject variation than (R)-amlodipine [15].

Enantiomeric separation is a very important method for preparative separation of enantiomers [16–18]. Various sources in the literature and patents have reported on the separation of (S)-amlodipine from its racemic mixture. Conventional methods, e.g. crystallization [19], chromatography [20] and capillary electrophoresis [21], have accelerated research on chiral compounds; however, some defects still exist in the separation process for most racemic compounds. The most often employed method of isolating (S)-amlodipine involves selective diastereomeric salt crystallization; but this technique requires many time-consuming and expensive steps, increasing the complexity of the process and leading to a considerable loss of product [22]. Recently, the use of a hollow-fiber supported liquid membrane (HFSLM) has come to be regarded as an effective method for the simultaneous extraction and

recovery of components of interest from a very dilute solution in the feed, by means of a single-unit operation [23–25]. The advantages of HFSLM over traditional separation techniques include lower capital and operating costs, lower energy consumption, low diluent usage, and high selectivity [26–28]. These advantages make HFSLM particularly suitable for application in the enantioseparation of compounds in pharmaceutical industrial wastewater.

The aim of the present study was to investigate the racemic resolution of amlodipine. A selective separation method for (S)-amlodipine used a HFSLM system based on O,O'-dibenzoyl-(2S,3S)-tartaric acid ((+)-DBTA) (Fig. 1(c)) as a chiral extractant. β-cyclodextrin was utilized as a stripping phase selector (Fig. 1(d)). This work not only investigated the separation of (S)-amlodipine by using HFSLM, but also the prolongation of liquid membrane stability. Several diluents – hexane, 1-decanol, chlorobenzene, benzene, dichloromethane, ethylene dichloride, and chloroform – with different polarity indexes, from 0.1–4.1, were chosen. The effect of polarity index was analyzed, focusing on the stability and extractability of the hollow-fiber supported liquid membrane system.


(a)

(b)

(c)

(d)
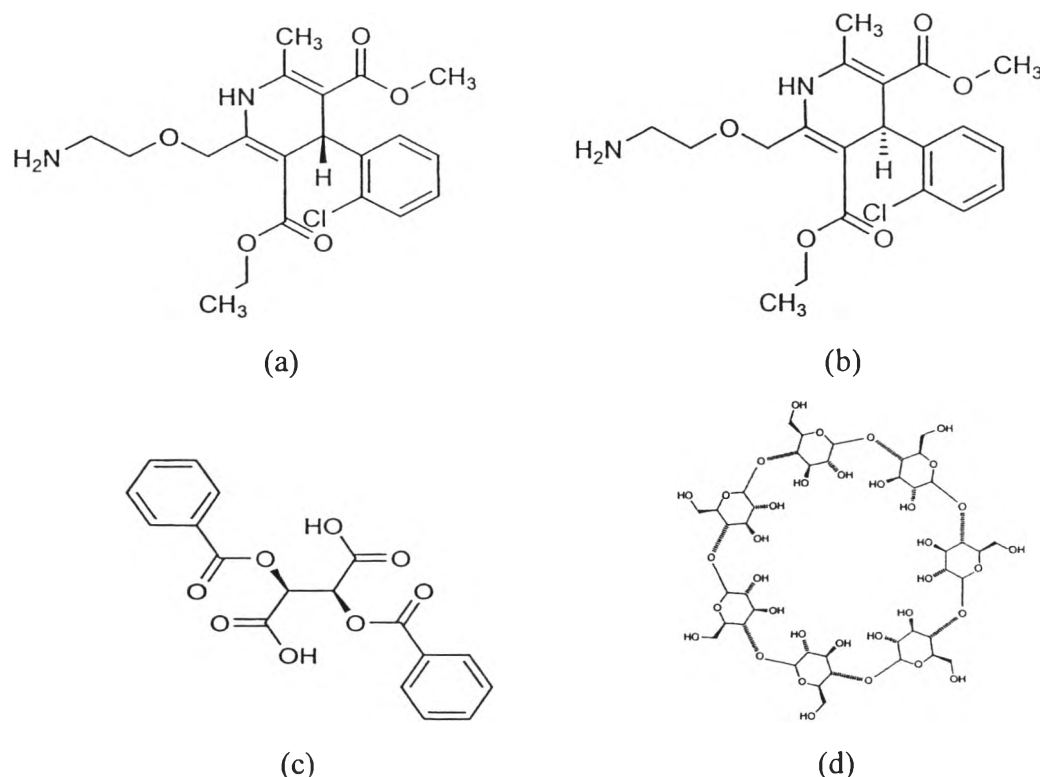
Figure 7.1 Structures of (a) (S)-amlodipine, (b) (R)-amlodipine (c) O,O'-dibenzoyl-(2S, 3S)-tartaric acid, (d) β- cyclodextrin.

7.3 THEORY

7.3.1 Hollow fiber supported liquid membrane

The liquid membrane system consists of an aqueous feed containing enantiomeric compound and a stripping solution. The liquid membrane is between the feed and stripping phases, and contains an organic carrier which reacts with the enantiomeric compound as shown in Figure 7.2. The hollow fiber module contains many hollow fibers aligned horizontally, with the liquid membrane embedded inside them. The organic phase fills the pores of the fibers by capillary force [29].
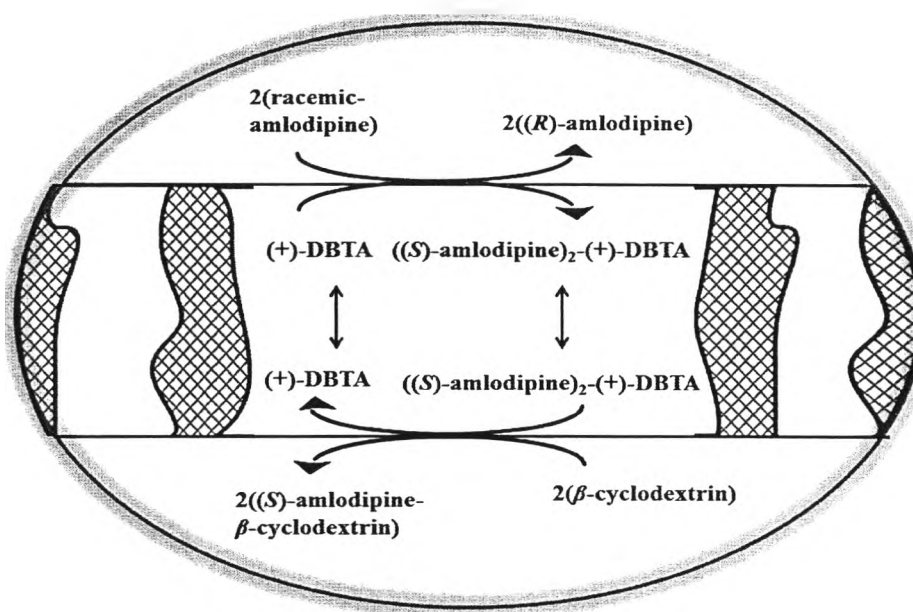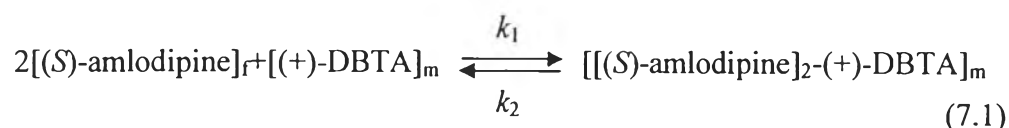

Figure 7.2 Transport scheme for chiral extractant

7.3.2 Transport mechanisms of separation of (S)-amlodipine

The supported chiral liquid membrane consists of an organic solution of a chiral selector as the extractant, which is held in polymeric micropores by capillary action [30]. The enantioselector O,O'-dibenzoyl-(2S,3S)-tartaric acid ((+)-DBTA) resides in the liquid membrane, trapped in the hydrophobic microporous hollow-fiber module. (+)-DBTA forms enantioselective complexes with (S)-amlodipine by

hydrogen bonding [31]. The transport mechanism of (S)-amlodipine through the liquid membrane is shown in Figure 7.2.
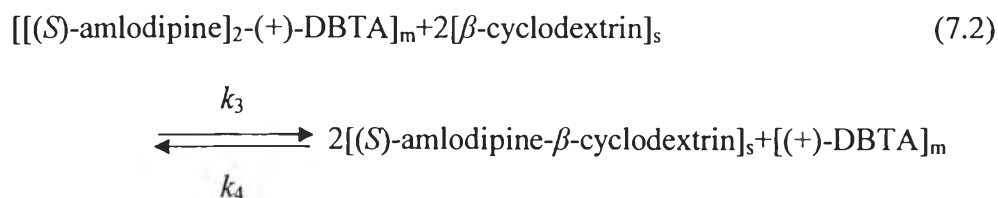
The mechanism and the enantioselective transport kinetics scheme of amlodipine enantiomers through a hollow fiber supported liquid membrane are described in Eqs. (7.1) and (7.2):

$$2[(S)\text{-amlodipine}]_f + [(+)\text{-DBTA}]_m \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [[(S)\text{-amlodipine}]_2\text{-}(+)\text{-DBTA}]_m \tag{7.1}$$

where k_1 and k_2 are the apparent rate constants of feed–membrane interfacial transport and membrane–strip interfacial transport of amlodipine enantiomers, respectively. The index suffixes, f and m, indicate feed phase and membrane phase, respectively.

According to the literature reporting the advantages of tartaric acid derivatives as chiral selectors [32-34], they are acidic compound containing two carboxylic acid groups. (S)-amlodipine can form a complex with (+)-DBTA. The good complex-forming abilities of enantiomeric compounds and (+)-DBTA have been demonstrated in several previous reports [35-37]. The carboxylic acid groups of amlodipine and (+)-DBTA can donate protons for hydrogen bonding, while they can also behave as a proton acceptor due to the eight oxygen atoms they contain [38-40]. The benzoyl groups can take part in hydrophobic interactions, while the other parts of the molecule contain polar hydrophilic groups [41, 42]. The concentration of (S)-amlodipine$_2$-(+)-DBTA complex in membrane phase was obtained from the material balance of (S)-amlodipine.

The stripping phase (β-cyclodextrin) reacts with [[(S)-amlodipine]$_2$-(+)-DBTA]$_m$ to recover (S)-amlodipine into the stripping phase:

$$[[(S)\text{-amlodipine}]_2\text{-}(+)\text{-DBTA}]_m + 2[\beta\text{-cyclodextrin}]_s \tag{7.2}$$

$$\underset{k_4}{\overset{k_3}{\rightleftharpoons}} 2[(S)\text{-amlodipine-}\beta\text{-cyclodextrin}]_s + [(+)\text{-DBTA}]_m$$

where k_3 and k_4 are the apparent rate constants of feed–membrane interfacial transport and membrane–strip interfacial transport of amlodipine enantiomers,

respectively. The index suffixes, m and s, indicate membrane phase and stripping solution phase, respectively.

7.3.3 Extraction equilibrium constant and distribution ratio

The extraction equilibrium constant $(K_{ex(S)})$ of (S)-amlodipine extracted by (+)-DBTA can be written as:

$$K_{ex(s)} = \frac{[[(S)\text{-}amlodipine]_2\text{-}(+)\text{-}DBTA]_m}{[(S)\text{-}amlodipine]_f^2[(+)\text{-}DBTA]_m} \tag{7.3}$$

The enantioselectivity of a process may be expressed as the operational selectivity [43]. For the current system, (S)-amlodipine is preferentially extracted. The distribution ratios of (S)-amlodipine and (R)-amlodipine, D_S and D_R, respectively, were determined as in Eqs. (7.1) and (7.2).

The distribution ratio for (S)-amlodipine (D_S) is given by:

$$D_s = \frac{[[(S)\text{-}amlodipine]_2\text{-}(+)\text{-}DBTA]_m}{[(S)\text{-}amlodipine]_f} \tag{7.4}$$

The distribution ratio for (R)-amlodipine D_R is given by:

$$D_R = \frac{[[(R)\text{-}amlodipine]_2\text{-}(+)\text{-}DBTA]_m}{[(R)\text{-}amlodipine]_f} \tag{7.5}$$

According to Eq. (7.4), the distribution ratio could then be derived as a function of the extraction equilibrium constant as follows:

$$D_S = K_{ex(S)}[(S)\text{-}amlodipine]_f[(+)\text{-}DBTA]_m \tag{7.6}$$

The selectivity is defined as enantioselectivity. The enantioselectivity of the membrane process is given in terms of the separation factor (α) and the enantiomeric excess (% $e.e.$). Enantioselectivity is one of the important parameters for estimating

the separation performance of the process, which can be calculated by the following formulae:

$$\alpha = \frac{D_S}{D_R} \tag{7.7}$$

In this work, the extractability of (S)-amlodipine was determined by the percentage of extraction:

$$\%e.e. = \frac{|D_S - D_R|}{(D_S + D_R)} \times 100 \tag{7.8}$$

$$\%\text{Extraction} = \frac{C_{f,in} - C_{f,out}}{C_{f,in}} \times 100 \tag{7.9}$$

The percentage of recovery was calculated by:

$$\%\text{Stripping} = \frac{C_{s,out}}{C_{f,in}} \times 100 \tag{7.10}$$

where $C_{f,in}$ and $C_{f,out}$ are the inlet and outlet feed concentrations of component i (mmol/L), and $C_{s,in}$ is the outlet stripping concentration of component i (mmol/L).

7.3.4 Permeability coefficient

The permeation of (S)-amlodipine can be expressed in terms of the permeability coefficient (P), as proposed by Danesi [44] in Eq. (7.18):

$$-V_f \ln\left(\frac{C_f}{C_{f,o}}\right) = AP\frac{\beta}{\beta+1}t \tag{7.18}$$

where P is the permeability coefficient (cm/s), V_f is the volume of the feed (cm^3), $C_{f,0}$ is the initial (S)-amlodipine concentration (mol/L) at time $t = 0$, C_f is the (S)-amlodipine concentration at time t (mol/L), A is the effective area of the hollow-fiber module (cm^2), and t is the time (min).

$$\beta = \frac{Q_f}{PL\varepsilon\pi Nr_i}$$ (7.19)

$AP(\beta /(\beta + 1))$ is the slope of the plot between $-V_f \ln (C_f /C_{f,0})$ versus t in Eq. (7.18), and P can be obtained by Eq. (7.19), where Q_f is the volumetric flow rate of feed solution (cm^3/s), L is the length of the hollow fiber (cm), ε is the porosity of the hollow fiber (%), N is number of hollow fibers in the module and r_i is the internal radius of the hollow-fiber module (cm).

To determine mass-transfer coefficients for (S)-amlodipine enantio-separation by HFSLM, the mass-transfer model and permeability coefficient (P) are employed. The permeability coefficient depends on mass transfer resistance, which is the reciprocal of the mass-transfer coefficients as follow

$$\frac{1}{P} = \frac{1}{k_f} + \frac{r_i}{r_{lm}}\frac{1}{P_m} + \frac{r_i}{r_o}\frac{1}{k_s}$$ (7.20)

where r_{lm} is the log-mean radius of the hollow fiber, r_o is the external radius of the hollow fiber module (cm), k_f is the aqueous mass-transfer coefficient in the tube side, k_s is the stripping mass-transfer coefficient in the shell side, and P_m is the membrane permeability coefficient.

The relationship between P_m and the distribution ratio (D_S) is as follows:

$$P_m = D_s k_m$$ (7.21)

Combining Eq. (7.6) and Eq. (7.21), thus:

$$P_m = K_{ex} k_m[(S)\text{-amlodipine}]_f [(+)\text{-DBTA}]_m$$ (7.22)

where k_m is the mass-transfer coefficient of the membrane, and the value of the liquid-membrane permeability coefficient (P_m) from Eq. (7.22) is substituted into Eq. (7.20).

Assuming that the stripping reaction is instantaneous and the contribution of the stripping phase is neglected, Eq. (7.20) becomes:

$$\frac{1}{P} = \frac{1}{k_f} + \frac{r_i}{r_{lm}}\frac{1}{K_{ex} k_m[(S)\text{-amlodipine}]_f [(+)\text{-DBTA}]_m}$$ (7.23)

where k_f is the mass transfer coefficient of the feed solution.

7.4 EXPERIMENT

7.4.1 Pharmaceutical wastewater containing amlodipine

The wastewater used in this study was taken from a chemical synthesis-based amlodipine pharmaceutical plant of the Government Pharmaceutical Organization, Bangkok, Thailand. The wastewater was generated from the synthesis and chiral separation processes of amlodipine manufacturing, and contained a variety of diluents, additives, reactants, and high-value finished amlodipine products. The generation rate of the wastewater was 20 L per batch. The properties of the wastewater are shown in Table 7.1.

Table 7.1 Characteristics of the raw pharmaceutical wastewater

Parameter	Value
Color	Brownish
(R,S)-amlodipine (mmol/L)	~4.0
Temperature (K)	298.5
pH	6.5
TSS (mg/L)	720
BOD (mg/L)	1,700
COD (mg/L)	3,100
Conductivity (µs/cm)	2,800

7.4.2 Chemicals and reagents

All reagents (pharmaceutical grade) used in this work – (R)-amlodipine, (S)-amlodipine, and (R,S)-amlodipine – were provided by the Government Pharmaceutical Organization (Thailand). O,O'-Dibenzoyl-$(2S,3S)$-tartaric acid ((+)-DBTA) and β-cyclodextrin were obtained from Acros, USA. The diluents N,N-dimethylformamide, cyclohexane, 1-decanol, chlorobenzene, benzene, dichloro methane, ethylene dichloride and chloroform and 1-propanol were all of analytical reagent grade and obtained from Merck, Germany. All reagents used in this experiment were GR grade and also purchased from Merck, Germany. Aqueous

solutions were prepared using Milli-Q® deionized water (Millipore®, USA). Doubly deionized water was used throughout the experiments.

7.4.3 Apparatus

The hollow-fiber supported liquid membrane (HFSLM) system (Liqui-Cel® Extra-Flow 2.5 in × 8 in membrane contactor) was manufactured by Membrana/Celgard (Charlotte NC, USA). The module uses celgard microporous polypropylene fibers that are woven into fabric and wrapped around a central tube feeder that supplies the shell side fluid. The woven fabrics provided more uniform fiber spacing, which in turn leads to higher mass transfer coefficients than those obtained with individual fibers [45]. The properties of the hollow fiber module are specified in Table 7.2.

Table 7.2 Physical characteristics of the hollow fiber module

| Properties	Descriptions
Material	Polypropylene
Inside diameter of hollow fiber	240 μm
Outside diameter of hollow fiber	300 μm
Effective length of hollow fiber	15 cm
Number of hollow fibers	35,000
Average pore size	0.03 μm
Porosity	30%
Effective surface area	1.4×10^4 cm^2
Area per unit volume	29.3 cm^2/cm^3
Module diameter	6.3 cm
Module length	20.3 cm
Contact area	30%
Tortuosity factor	2.6
Operating temperature	273.15-333.15 K

7.4.4 Liquid membrane preparation

The chiral selector, O,O'-Dibenzoyl-(2S,3S)-tartaric acid ((+)-DBTA), was dissolved in organic solvent as the diluent. In this work, the diluent was investigated in several diluents: hexane, 1-decanol, chlorobenzene, benzene, dichloromethane, ethylene dichloride and chloroform. All chemicals were analytical grade and purchased from Merck Ltd, Germany.

7.4.5 Procedures

The single-module operation is shown in Figure 7.3. The selected organic carrier (+)-DBTA was dissolved in diluent (500 mL) and then simultaneously pumped into the tube and shell sides of the hollow-fiber module for 50 min to ensure that the extractant was entirely embedded in the micropores of the hollow fibers. Subsequently, 5 L (each) of feed solution and stripping solution were fed counter-currently into the tube side and the shell side of the single-module operation, respectively.

The concentration of feed solution was deliberately varied in order to determine the optimum value for (S)-amlodipine extraction. The concentration of the chiral selector (+)-DBTA in the liquid membrane, the volumetric flow rates of feed and stripping solutions, the number of separation cycles, and the stability of the HFSLM were each investigated in turn. The operating time for each run was 50 min. The concentrations of (S)-amlodipine in samples from feed and stripping solutions were determined by high-performance liquid chromatography (HPLC) in accordance with U.S. Patent No. 6646131 B2 [46] to estimate the percentages of extraction and stripping. To achieve higher enantioseparation and to study membrane stability, the number of separation cycles was varied. The feed of the second cycle was obtained from the first outlet feed solution and so on, whereas the inlet stripping solution was fresh.
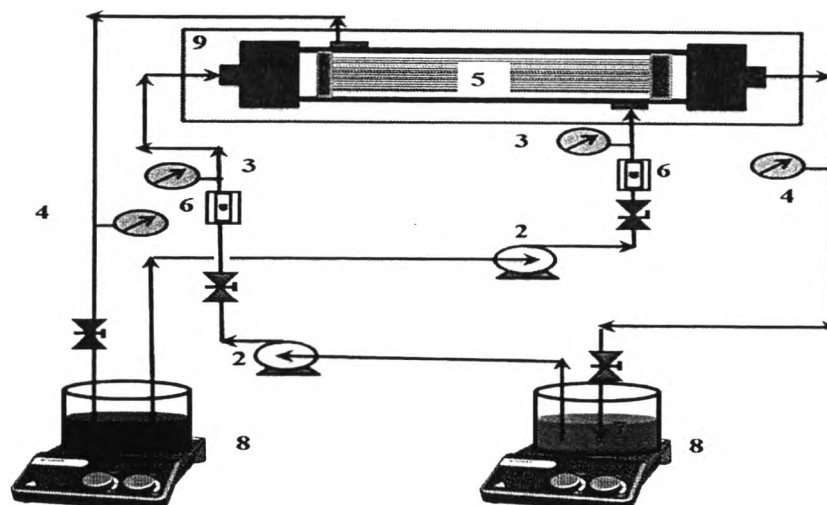
Figure 7.3 Schematic representation of the counter-current flow diagram for batch-mode operation in HFSLM: 1) feed reservoir, 2) gear pumps, 3) inlet pressure gauges, 4) outlet pressure gauges, 5) the hollow-fiber module, 6) flow meters, 7) stripping reservoir, 8) stirrer with temperature controller, and 9) temperature control box

7.4.6 Analytical instruments and chromatographic conditions

The chromatographic system consisted of an Agilent® 1100 Compact LC series (Agilent Technologies, Palo Alto CA, USA). The chromatographic system was equipped with a built-in solvent degasser, quaternary pump, column compartment, photodiode array detector with variable injector and auto sampler. Data analysis was carried out using ChemStation® version B.04.01 software (Agilent).

The analysis was performed following U.S. Patent No 6646131 B2 [46]. The chromatographic procedure was carried out using an Ultron ES-OVM ovomucoid chiral column (5 μm, 4.6 ×150 mm) (Agilent®). The column was thermostated at 298.15 K using a column heater. The mobile phase was a mixture of disodium hydrogen phosphate buffer (20 mmol/L) and acetonitrile (80:20). Flow rate of the mobile phase was 0.3 mL/min; injection volume was 20 μL. The relative retention time of (R)-amlodipine was about 1.0, and for (S)-amlodipine about 1.2, as detected by a UV-spectrophotometer set at 237 nm. The analysis time was set at 20 min per sample to eliminate potential interference from late eluting peaks. The pH of the

aqueous phase was measured with a SevenMulti™ pH meter with modular expansion (Mettler-Toledo, Greifensee, Switzerland).

7.5 RESULTS AND DISCUSSION

7.5.1 Optimization of initial parameters for (S)-amlodipine separation via HFSLM

The separation efficiency gives the overall mass transfer of the analytes diffusing across the HFSLM. This is controlled by several initial parameters, including: pH, concentration of the feed phase, flow rates of feed and stripping solutions, and temperature of enantioseparation of (R,S)-amlodipine via HFSLM. The optimal conditions are shown in Table 7.3 [31,36–38]. Some of these parameters can be determined by examining the physical properties of the compounds. The feed phase was operated at an optimized pH of 5.0 by using NaH_2PO_4/H_3PO_4 buffer to adjust the pH values [31]. The influence of the pH of the feed phase is also an important parameter on the enantiomeric excess (% e.e.) [21, 31]. The concentration of the feed phase was 4 mmol/L. The flow rates of feed and stripping solutions were 100 mL/min [31, 47-49].

Table 7.3 Optimized operation using HFSLM in enantioseparation

Phase	Chemical reagent	Concentration	Flow rate
Feed	(R,S)-amlodipine pKa = 8.6 at 298.15 K	~4 mmol/L	100 mL/min
Membrane	(+)-DBTA	4 mmol/L	-
Strip	β-cyclodextrin	-	100 mL/min

7.5.2 Influences of organic diluents

Organic diluents influence the performance of many liquid membrane systems. The role of the diluent is not only to improve the physical properties of the liquid membrane system, but also to improve the (S)-amlodipine$_2$-(+)-DBTA complex. This needs be taken into account in the choice of diluents [50]. As is known from liquid membrane theory, the main factor affecting liquid membrane stability is the type of diluent [51, 52]. In this study, several diluents with different polarity indexes were chosen: hexane (polarity index 0.1); 1-decanol (1.8); chlorobenzene (2.5); benzene (2.7); dichloromethane (3.1); ethylene dichloride (3.5); and chloroform (4.1) [53]. From Table 7.4, we can see the extraction performance of the different kinds of organic solvents, alcohol > alkyl halide > hexane; their performance might be related to the polarity and interaction of different organic diluents with the solute. The relationship between the polarity of diluents and the percentage of extraction is shown in Figure 7.4, indicating that 1-decanol is a suitable organic diluent for the extraction of (S)-amlodipine.

Table 7.4 Lifetime and performance of HFSLM with diluents different polarities

| Diluents	Polarity (-)	Lifetime(min)	%Extraction	%Stripping	% $e.e.$
Hexane	0.10	150	69.00	62.00	44.76
1-decanol	1.80	150	77.50	74.50	57.58
Chlorobenzene	2.50	120	76.80	72.33	54.00
Benzene	2.70	120	75.50	70.00	50.54
Dichloromethane	3.10	90	72.80	68.60	50.00
Ethylenedichloride	3.50	90	71.00	65.00	48.45
Chloroform	4.10	90	70.00	63.00	46.53
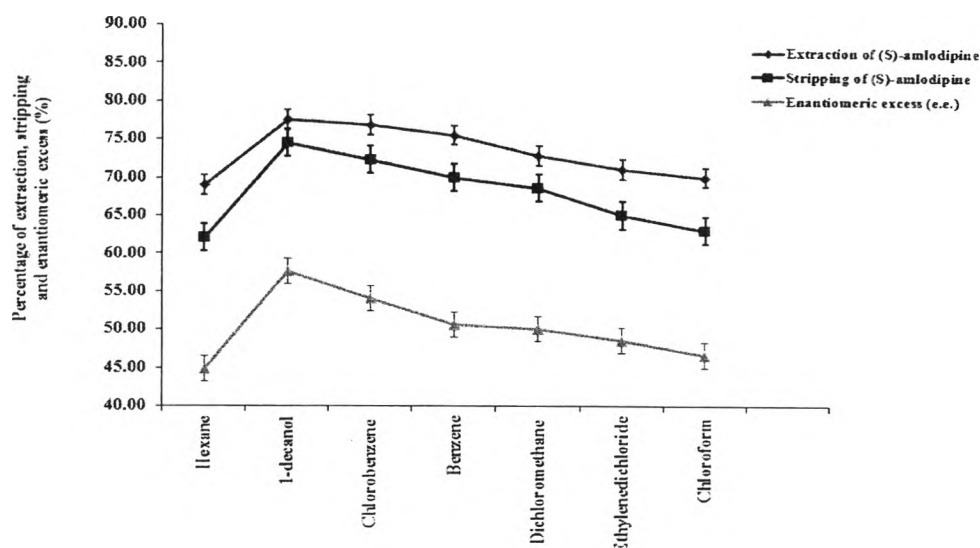
Figure 7.4 The relationship between the polarity of diluents on percentages of extraction and recovery of (S)-amlodipine and the enantiomeric excess (% *e.e.*)

7.5.3 Influence of chiral selector concentration on the membrane phase

The chiral selector, *O,O'*-Dibenzoyl-(2S,3S)-tartaric acid ((+)-DBTA), concentration in the liquid membrane plays a vital role in the overall extraction behavior. The concentration of (+)-DBTA in 1-decanol was studied in the ranges of 2-10 mmol/L, while the other operating conditions were constant. The results were shown in Figure 7.5. When (+)-DBTA concentration was increased from 2-10 mmol/L, the percentage of the extraction and stripping of (S)-amlodipine rose abruptly. Nevertheless, when the (+)-DBTA concentration exceeded 6 mmol/L, both percentages of extraction and stripping decreased; this is because an excessive increase in extractant concentration leads to an increase in the absolute viscosity of the membrane, which generates a lower diffusion speed of the species and affects the mass transfer process [50]. Based on these results, a concentration of 4 mmol/L was selected as the optimum carrier concentration. The highest percentage of (S)-amlodipine extraction was 78.50%, and the percentage of enantiomeric excess was 57.58%. Percentages of extraction and enantiomeric excess of (S)-amlodipine of about 77.50% and 54.67%, respectively, were obtained in a previous work [31]; the present result was better because of the effects of diluent and specific stripping solution, as explained below.
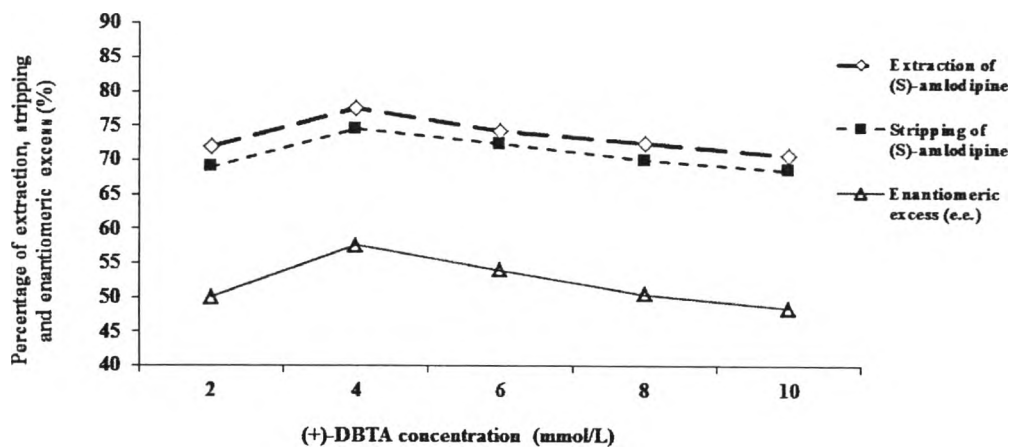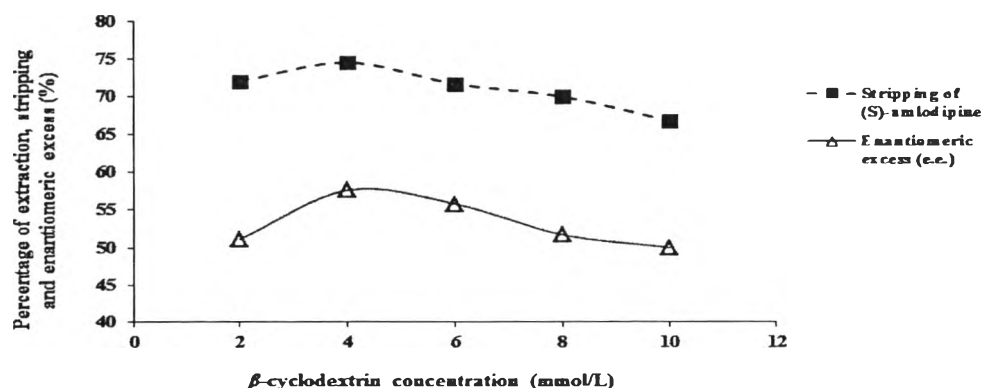
Figure 7.5 Influence of chiral selector ((+)-DBTA) concentration in the membrane phase on percentages of extraction and recovery of (S)-amlodipine and the enantiomeric excess (% e.e.)

7.5.4 Influence of stripping phase concentration

Another parameter that affects the transport of (S)-amlodipine is the stripping agent and its concentration. Since the extraction and stripping reactions in HFSLM are performed simultaneously, it is important to explore the effect of reagent concentration in the stripping solution in order to enhance effective (S)-amlodipine transport and ensure an efficient stripping reaction at the interface of the membrane and the stripping solution [31]. The concentrations of β-cyclodextrin were studied in a range of 2–10 mmol/L. The results indicates that β-cyclodextrin recognizes (S)-amlodipine, which means that β-cyclodextrin forms complexes with (S)-amlodipine before being transferred to the stripping phase. From previous work it is evident that the pH of the feed phase has a very strong influence on the enantiomeric excess (%e.e.) [21, 31, 55, 56]. The maximum % e.e. at pH 5.0 is explained by the formation of unionized enantiomers of amlodipine. However, the pH of the stripping phase can be influenced by the concentration of β-cyclodextrin in the stripping solution. A

previous work [31] investigated the use of benzenesulfonic acid as a stripping solution. Since benzenesulfonic acid is an achiral molecule, it does not specifically form a complex with (S)-amlodipine. Hence, the percentage of stripping of (S)-amlodipine was about 72.50%, while the enantiomeric excess was only 54.67% [31].

The aim of the present study was to improve a HFSLM system for use in the pharmaceutical industry. Based on the chiral selector theory, β-cyclodextrin was selected as the stripping solution. β-cyclodextrin exhibited higher stripping results by creating a complex with the (S)-enantiomer by various electrostatic forces and hydrogen bonding. Complex formation depends largely on the size, shape and polarity of the (S)-enantiomer [21, 55, 56]. This means that the configuration of (S)-amlodipine should be compatible with the cavity of β-cyclodextrin, and that the stability of the generated complex is subject to the shape, polarity and side groups of (S)-amlodipine. The highest stripping of (S)-amlodipine, about 74.50%, was achieved at a β-cyclodextrin concentration of 4 mmol/L. The maximum enantiomeric excess of (S)-amlodipine was 57.58%. The results were shown in Figure 7.6.

Figure 7.6 Influence of stripping phase concentration on percentages of extraction and recovery of (S)-amlodipine and the enantiomeric excess (% $e.e.$)

7.5.5 Extraction equilibrium constant

Considering the ideal behavior in the membrane phase. the extraction equilibrium constant (Kex) of (S)-amlodipine with (+)-DBTA can be described by Eq. 7.3 and was calculated from the slope shown in Figure 7.7. It was found to be 1.3160 $(L/mmol)^2$.
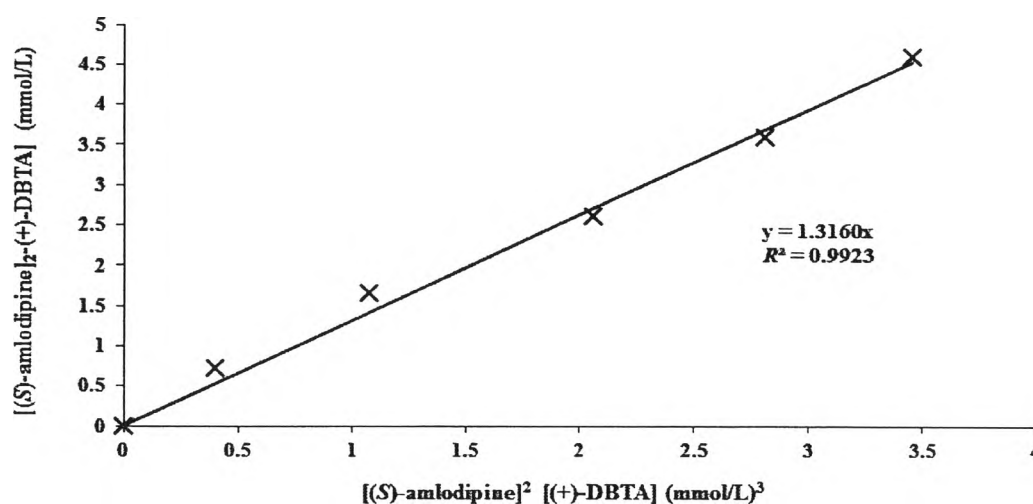
Figure 7.7 (S)-amlodipine extraction with (+)-DBTA as a function of equilibrium $[(S)\text{-amlodipine}]^2[(+)\text{-DBTA}]$.

7.5.6 Permeability and mass transfer coefficients

The permeability coefficient (P) of (S)-amlodipine as function of concentration of (+)-DBTA from 2 to 8 mmol/L, were calculated by the slope obtained in Figure 7.8. The permeability coefficients (P) were obtained from Eqs. 7.18, 7.19 and by a plot between $-Vf \ln(C_f/C_{f,0})$ versus t, as expressed by ($AP\beta/(\beta+1)$). The permeability coefficients of (S)-amlodipine increases with increasing carrier concentration. Eq. 7.22 was attained by substituting the membrane permeability coefficient (P_m) and assuming that the stripping reaction of (S)-amlodipine was instantaneous and with no contribution of the stripping phase. Eq. 7.23 was used to calculate the aqueous mass transfer coefficient (k_f) and the membrane mass transfer

coefficient (k_m). By plotting $1/P$ as a function of $1/[(S)\text{-amlodipine}]_f\,[(+)\text{-DBTA}]_m$ for different carrier concentrations of (+)-DBTA, a straight line with slope $r_i/(r_{lm} \cdot K_{ex} \cdot k_m)$ and ordinate $1/k_f$ for calculation is obtained (Figure 7.9). Thus, the values of k_f and k_m were found to be 2.74×10^{-2} and 2.52×10^{-2} cm/s, respectively.
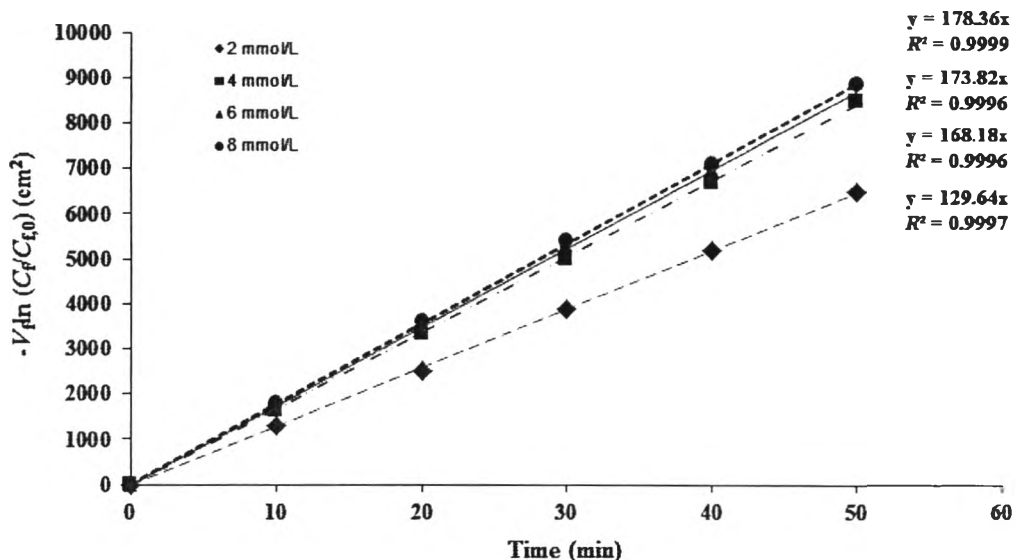

Figure 7.8 Plot of $-V_f \ln(C_f/C_{f.0})$ of (S)-amlodipine in feed solution against time with different (+)-DBTA concentrations
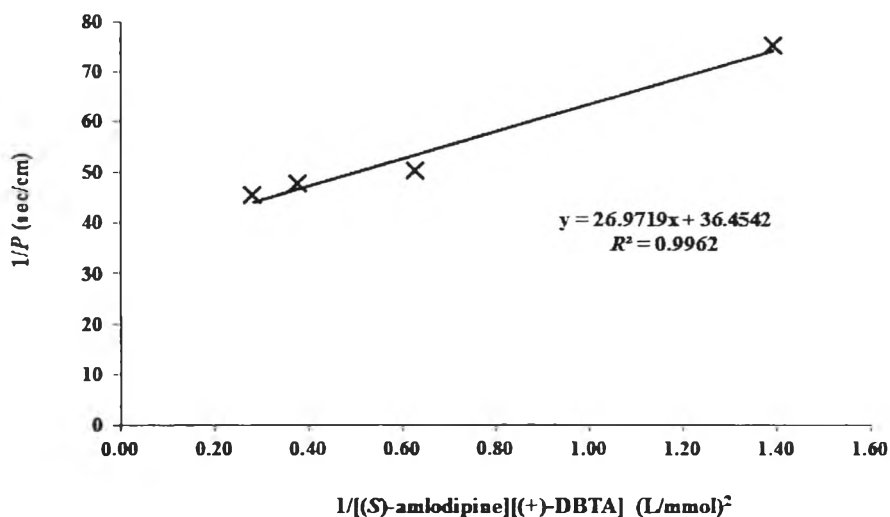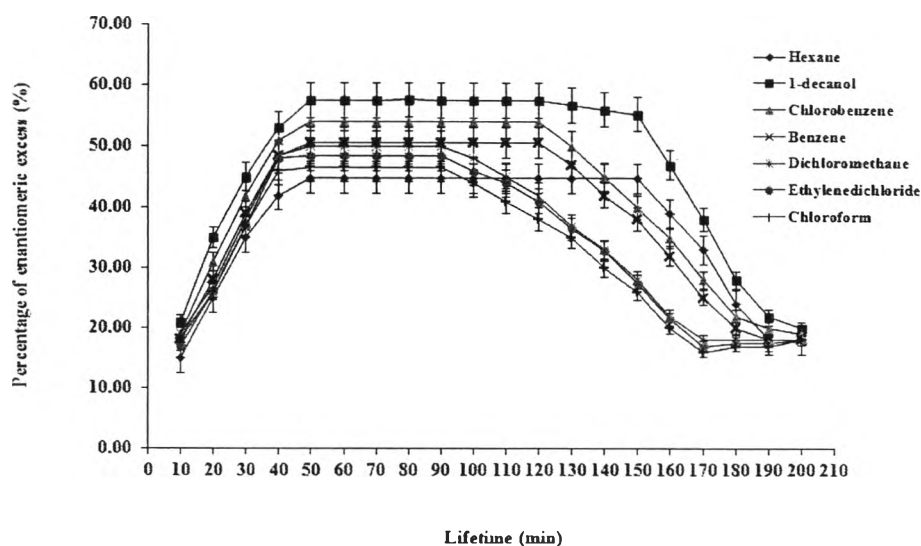

Figure 7.9 Plot of $1/P$ as a function of $1/[(S)\text{-amlodipine}]_f\,[(+)\text{-DBTA}]_m$

7.5.7 Investigation of liquid membrane stability

The effect of diluent polarity on the lifetime of the liquid membrane, as well as the percentages of extraction and stripping of (S)-amlodipine, were investigated. At the optimum conditions, (S)-amlodipine was extracted preferentially (in comparison with (R)-amlodipine) with O,O'-dibenzoyl-($2S,3S$)-tartaric acid ((+)-DBTA). Because (R)-amlodipine was rejected in the feed solution, it can be used as a tracer to determine membrane stability. When (R)-amlodipine is detected in the stripping side, it means that the liquid membrane has been damaged and has leaked out from the hollow fiber pores, allowing (R)-amlodipine to pass through the pores from the feed side to the stripping side.

The effect of diluents on membrane stability was investigated. As shown in Figure 7.10, the diluents were ranked in the following order: 1-decanol > chlorobenzene > benzene > dichloromethane > ethylene dichloride > chloroform > hexane. Figure 7.10 shows the relationship between diluent polarity and the percentage of extraction after 60 min for different diluents under optimal conditions. The percentages of extraction and stripping of (S)-amlodipine were increased when the polarity of the diluent increased. The polarity index of water is 9.0 [44]. It is known that the extraction reaction occurs at the interface feed/liquid membrane layer. When the polarity of the diluent is increased, the diluent is more soluble in the feed phase, as shown in Figure 7.11. Thus, the number of extraction and stripping reactions increased, leading to higher percentages of extraction and stripping. Unfortunately, the large increase in polarity made the interface layers (δ) grow too thick, so the liquid membrane peeled and leaked out from the pores of the hollow fibers by aqueous phase in the feed and the stripping solutions. As a result, higher polarity of the diluent brought about higher extraction performance but lower stability. In particular, when the polarity of the diluent exceeded 3.5, the stability was too low to be trapped in the hollow fiber pores. In light of this, the percentages of extraction and stripping from using chloroform are meaningless. Therefore, in this study, benzene (which has a polarity of 2.7) was found to offer the best compromise between extraction performance and membrane stability.

The correlations between membrane lifetime and percentages of extraction and stripping with different polarity diluents are summarized in Table 7.4. The use of 1-decanol resulted in the longest lifetime, of 150 min. As the results indicate, polarity is a single-parameter correlation. When the polarity of the diluent is decreased, the lifetime or stability of the liquid membrane is significantly increased.

Figure 7.10 (a) Plot of $1/P$ as a function of $1/[(S)\text{-amlodipine}]_f\,[(+)\text{-DBTA}]_m$ at temperature 278-293 K;

(b) Plot of $1/P$ as a function of $1/[(S)\text{-amlodipine}]_f\,[(+)\text{-DBTA}]_m$ at temperature 298-313 K.
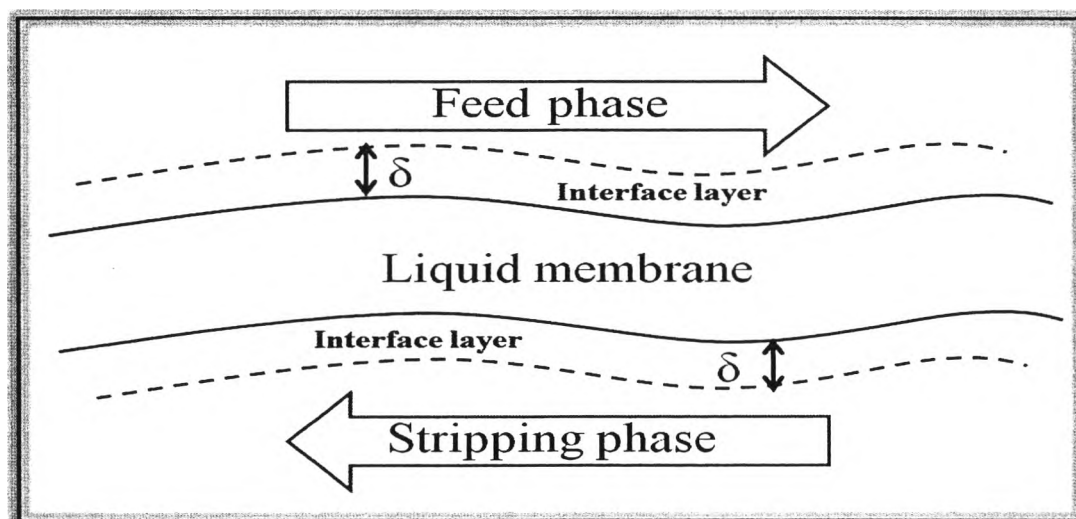
Figure 7.11 Arrhenius plot of (S)-amlodipine transport

7.6 CONCLUSIONS

(*S*)-amlodipine was highly selectively separated from pharmaceutical industry wastewater by specific stripping phase recovery via HFSLM. The optimal parameters for operation of the HFSLM system were: an initial feed solution concentration of 4 mmol/L; 4 mmol/L (+)-DBTA; 4 mmol/L β-cyclodextrin; and 100 ml/min of feed and stripping solutions. The percentages of extraction and stripping of (*S*)-amlodipine were 77.50% and 74.50%, respectively. The maximum enantiomeric excess of (*S*)-amlodipine was 57.58%. As the polarity of the diluent decreased, the stability increased. On the contrary, when the polarity index increased, the percentages of extraction and stripping were higher. The diluent 1-decanol provided the longest lifetime, of 150 min. Unfortunately, when the polarity index exceeded 3.5, both the percentages of extraction and stripping abruptly decreased; this resulted from the liquid membrane leaking out because the solubility was too high to be trapped in the hollow fiber pores. The mass transfer coefficients of the aqueous phase (k_f) and organic phase (k_m) were 2.74×10^{-2} and 2.52×10^{-2} cm/s, respectively. The membrane mass transfer coefficient (k_m) was less than the aqueous feed mass transfer coefficient (k_f); mass transfer across the membrane phase is the rate-controlling step. The results demonstrate the energy-saving benefits offered by HFLSM separation and the technique's potential to improve other industrially relevant chiral separations.

7.7 REFERENCES

[1] Chen, Z., Ren, N., Wang, A., Zhang, Z., Shi, Y. A novel application of TPAD–MBR system to the pilot treatment of chemical synthesis-based pharmaceutical wastewater. Water Res. 42 (2008): 3385–3392.

[2] Oktem, Y.A., Ince, O., Sallis, P., Donnelly, T., Ince, B.K. Anaerobic treatment of a chemical synthesis-based pharmaceutical wastewater in a hybrid up flow anaerobic sludge blanket reactor. Bioresour. Technol. 99 (2007): 1089–1096.

[3] Nakada, N., Kiri, K., Shinohara, H., Harada, A., Kuroda, K., Takizawa, S., Takada, H. Evaluation of pharmaceuticals and personal care products as

water soluble molecular markers of sewage. <u>Environ Sci Technol.</u> 42 (2008): 6347-6353.

[4] Alistair, B.A. The environmental side effects of medication. <u>Eur. Mol. Biol. Org.</u> 5 (2004): 1110–1116.

[5] Batt, A.L., Snow, D.D., Aga, D.S. Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. <u>Chemosphere</u> 64 (2006): 1963–1971.

[6] Rodriguez-Donis, I., Gerbaud, V., Arias-Barreto, A., Joulia, X., Heterogeneous batch distillation processes for waste solvent recovery in pharmaceutical industry. <u>Comput. Aided Chem. Eng.</u> 27 (2009): 1119-1124.

[7] Suman Raj, D.S., Anjaneyulu, Y. Evaluation of biokinetic parameters for pharmaceutical wastewaters using aerobic oxidation integrated with chemical treatment. <u>Proc. Biochem.</u> 40 (2005): 165–175.

[8] Breuer, M., Ditrich, K., Habicher, T., Hauer, B., Kesseler, M., Sturmer, R., Zelinski, T. Industrial methods for the production of optically active intermediates. <u>Angew. Chem. Int.</u> 43 (2004): 788–824.

[9] Thompson, A.M., Hu, T., Eshelbrenner, C.L., Reynolds, K., He, J., Bazzano, L.A. Antihypertensive treatment and secondary prevention of cardiovascular disease events among persons without hypertension: A meta-analysis. <u>JAMA</u> 305(9) (2011): 913-922.

[10] Liu, G.S. Wang, K. Zhang M.H. Comparative effect of amlodipine and levamlodipine on nocturnal hypertension in hypertensive patients. <u>J. Med. Postgrad.</u> 14 (2001): 496–499.

[11] Park, J.Y., Kim, K.A., Park, P.W., Lee, O.J., Ryu. J.H., Lee, G.H., Ha, M.C., Kim, J.S., Kang, S.W., Lee, K.R. Pharmacokinetic and pharmacodynamic characteristics of a new S-amlodipine formulation in healthy Korean male subjects: a randomized, open-label, two-period, comparative, cross over study. <u>Clin. Ther.</u> 28 (2006): 1837-1847.

[12] Cheng, Y.Z., Wu, X.G., Chen, X.Y. Clinical trial of therapeutic effects and adverse drug reaction of S-amlodipine. <u>Chin J Med Guide.</u> 4 (2002): 198–200.

[13] Wang, R.X., Jiang, W.P., Li, X.R., Lai, L.H. Effects of (*S*)-amlodipine and (*R*)-amlodipine on L-type calcium channel current of rat ventricular myocytes and cytosolic calcium of aortic smooth muscle cells. Pharmazie. 63 (2008): 470-474.

[14] Yinghua, S., Liang, F., Meng, Z., Wei, L., Ping, M., Li, L., Zhonggui, H. A drug-in-adhesive transdermal patch for *S*-amlodipine free base: In vitro and in vivo characterization. Int. J. Pharm. 382 (2009): 165-171.

[15] Liu, F., Qiu, M., Zhai, S.D. Tolerability and effectiveness of (*S*)-amlodipine compared with racemic amlodipine in hypertension: A systematic review and meta-analysis. Curr. Ther. Res. 71 (2010): 1-29.

[16] Schuur, B., Verkuijl, B.J., Minnaard, A.J., de Vries, J.G., Heeres, H.J., Feringa, B.L. Chiral separation by enantioselective liquid-liquid extraction. Org. Biomol. Chem. 9 (2011): 36-51.

[17] Klein, C., Schneider, R.J., Meyer, M.T., Aga, D.S. Enantiomeric separation of metolachlor and its metabolites using LC–MS and CZE. Chemosphere 62 (2006): 1591–1599.

[18] Tang, K.W., Song, L.T., Liu, Y.B., Jiang, X.Y., Pan, C.Y. Separation of flurbiprofen enantiomers by biphasic recognition chiral extraction. Chem. Eng. J. 158 (2010): 411-417.

[19] Arrowsmith, J.E., Campbell, S.F., Cross, P.E., Stubbs, J.K., Burges, R.A., Gardiner, D.G., Blackburn, K.J. Long-acting dihydropyridine calcium antagonists 1,2-alkoxymethyl derivatives incorporating basic substituents. J. Med. Chem. 29 (1986): 1696-1702.

[20] Luksa, J., Josic, D.J., Podobnik, B., Furlan, B., Kremser, M. Semi-preparative chromatographic purification of the enantiomers *S*-(-)-amlodipine and *R*-(+)-amlodipine. J. Chromatogr. B. 693 (1997): 367-375.

[21] Zandkarimi, M., Shafaati, A., Foroutan, S.M., Lucy, C.A. Rapid enantioseparation of amlodipine by highly sulfated cyclodextrins using short-end injection capillary electrophoresis. DARU 17(4) (2009): 269- 276.

[22] Gotrane, D.M., Deshmukh, R.D., Ranade, P.V., Sonawane, S.P., Bhawal, B.M., Gharpure, M.M., Gurjar, M.K., A novel method for resolution of amlodipine. Org. Process Res.Dev. 13 (2010): 640–643.

[23] Sonawane, J.V., Pabby, A.K., Sastre, A.M. Pseudo-emulsion based hollow fibre strip dispersion (PEHFSD) technique for permeation of Cr(VI) using Cyanex-923 as carrier. J. Hazard. Mater. 174 (2010): 541–547.

[24] Dzygiel, P., Wieczorek, P.P. Supported liquid membranes and their modifications: definition, classifications, theory, stability, application and perspectives, in: V.S. Kislik (Ed.), Liquid Membranes, Elsevier, pp. 72–140.Amsterdam, 2010.

[25] Lothongkum, A.W., Panchareon, U., Prapasawat, T. Treatment of heavy metals from industrial wastewaters using hollow fiber supported liquid membrane, in Demadis, K. (Ed.), Water Treatment Processes, pp. 299-332. New York: Nova Science Publishers, 2012.

[26] Lin, S.H., Juang, R.S. Mass-transfer in hollow fiber modules for extraction and back-extraction of copper (II) with LIX64N carriers. J. Membr. Sci. 188 (2001): 251–262.

[27] Panja, S., Mohapatra, P.K., Tripathi, S.C., Manchanda, V.K. Facilitated transport of uranium(VI) across supported liquid membranes containing T2EHDGA as the carrier extractant. J. Hazard. Mater. 188 (2011): 281–287.

[28] Lothongkum, A.W., Suren, S., Chaturabul, S., Thamphiphit, N., Pancharoen, U. Simultaneous removal of arsenic and mercury from natural-gas-co-produced water from the Gulf of Thailand using synergistic extractant via HFSLM. J. Membr. Sci. 369 (2011): 350-358.

[29] Schultz, G. Separation techniques with supported liquid membranes. Desalination 68 (1988): 191-202.

[30] Prasad, R., Sirkar, K.K. Dispersion-free solvent extraction with microporous hollow fiber modules. AIChE J. 34 (1988): 177–183.

[31] Sunsandee, N., Leepipatpiboon, N., Ramakul, P., Pancharoen, U. The selective separation of (S)-amlodipine via a hollow fiber supported liquid membrane: modeling and experimental verification. Chem. Eng. J. 180 (2012): 299-308.

[32] Kmecz, I., Simándi, B., Székely, E., Fogassy, E. Resolution of N-methylamphetamine enantiomers with tartaric acid derivatives by supercritical fluid extraction. Tetrahedron: Asymm. 15 (2004): 1841–1845.

[33] Tan, B., Luo, G.S., Wang, J.D. Enantioseparation of amino acids by co-extractants with di(2-ethylhexyl)phosphoric acid and tartaric acid derivatives. Tetrahedron-Asymm. 17 (2006): 883–891.

[34] Tang, K.W., Zhang, P.L., Pan, C.Y., Li, H.J. Equilibrium studies on enantioselective extraction of oxybutynin enantiomers by hydrophilic β-cyclodextrin derivatives. AIChE J. 57 (2011): 3027–3036.

[35] Schuur, B., Winkelman, J.G.M., Heeres, H.J. Equilibrium studies on enantioselective liquid-liquid amino acid extraction using a cinchona alkaloid Extractant. Ind. Eng. Chem. Res. 47 (2008): 10027–10033.

[36] Tang, K.W., Yi, J.M., Liu, Y.B., Jiang, X.Y., Pan, Y. Enantioselective separation of R,S-phenylsuccinic acid by biphasic recognition chiral extraction. Chem. Eng. Sci. 64 (2009): 4081–4088.

[37] Kassai, C., Juvancz, Z., Bálint, J., Fogassy, E., Kozma, D. Optical resolution of racemic alcohols via diastereoisomeric supramolecular compound formation with O,O'-dibenzoyl-$(2R,3R)$-tartaric acid. Tetrahedron 56 (2000): 8355-8359.

[38] Liu, Y.S., Dai, Y.Y., Wang, J.D. Distribution behavior of L-tryptophane by extraction with di(2-ethylhexyl) phosphoric acid. Sep. Sci. Technol. 35 (2000): 1439–1454.

[39] Juang, R.S., Wang, Y.Y., Amino acid separation with D2EHPA by solvent extraction and liquid surfactant membranes. J. Membr. Sci. 207 (2002): 241-252.

[40] Tan, B., Luo, G.S., Qi, X., Wang, J.D. Extractive separation of amino acid enantiomers with co-extractants of tartaric acid derivative and aliquat-336. Sep. Purif. Technol. 53 (2007): 330–336.

[41] Steensma, M., Kuipers, N.J.M., de Haan, A.B., Kwant, G. Identification of enantioselective extractants for chiral separation of amines and amino alcohols. Chirality 18 (2006): 314–328.

[42] Schuur, B., Winkelman, J.G.M., de Vries, J.G., Heeres, H.J., Experimental and modeling studies on the enantio-separation of 3,5-dinitrobenzoyl-$(R),(S)$-leucine by continuous liquid–liquid extraction in a cascade of centrifugal contactor separators. Chem. Eng. Sci. 65 (2010): 4682–4690.

[43] Kazusaki, M., Kawabata H., Matsukura, H. Influence of temperature on enantioseparation employing an amylase-derivative stationary phase. J. Liquid Chromatogr. Relat. Technol. 23 (2000): 2937–2946.

[44] Danesi, P.R. A simplified model for the coupled transport of metal ions through hollow-fiber supported liquid membranes. J. Membr. Sci. 20 (1984): 231-248.

[45] Pancharoen, U., Wongsawa, T., Lothongkum, A.W. A reaction flux model for extraction of Cu(II) with LIX84I in HFSLM. Sep. Sci. Technol. 46 (2011): 2183-2190.

[46] Chung, Y.S., Ha, M.C. Resolution of the enantiomers of amlodipine. US Patent Application 2003/6646131 B2 (November 11, 2003).

[47] Sunsandee, N., Ramakul, P., Thamphiphit, N., Pancharoen, U., Leepipatpiboon, N. The synergistic effect of selective separation of (S)-amlodipine from pharmaceutical wastewaters via hollow fiber supported liquid membrane. Chem. Eng. J. 209 (2012): 201-214.

[48] Sunsandee, N., Leepipatpiboon, N., Ramakul, P., Wongsawa, T., Pancharoen, U. The effects of thermodynamics on mass transfer and enantioseparation of (R,S)-amlodipine across a hollow fiber supported liquid membrane. Sep. Purif. Technol. 102 (2013): 50-61.

[49] Sunsandee, N., Pancharoen, U., Rashatasakhon P., Ramakul, P., Leepipatpiboon, N. Enantioselective separation of racemic amlodipine by two-phase chiral extraction containing O,O'-dibenzoyl-(2S,3S)-tartaric acid as chiral selector. Sep. Sci. Technol. (In-press).

[50] Valenzuela, F., Fonseca, C., Basualto, C., Correa, O., Tapia, C. Removal of copper ions from a waste mine water by a liquid emulsion membrane method. Miner. Eng. 18 (2005): 33-40.

[51] Kyuchoukov, G., Yankov, D., Albet, J., Molinier, J. On the mechanism of lactic acid extraction with quaternary ammonium chloride (Aliquat 336). Ind. Eng. Chem. Res. 44 (2005): 5733-5739.

[52] Mohapatra, P.K., Lakshmi, D.S., Manchanda, V.K. Diluent effect on Sr(II) extraction using di-*tert*-butyl cyclohexano 18 crown 6 as the extractant and its correlation with transport data obtained from supported liquid membrane studies. Desalination 198 (2006): 166–172.

[53] Yaws, C.L. Chemical Properties Handbook. New York: McGraw-Hill, 1999.

[54] Chitra, K.R., Gaikwad, A.G., Surender, G.D., Damodaran, A.D. Studies on ion transport of some rare earth elements through solvating extractants immobilised on supported liquid membrane. J. Membr. Sci. 125 (1997): 257-268.

[55] O'Brien, T., Crocker, L., Thompson, R., Thompson, K., Toma, P.H., Conlon, D.A., Feibush, B., Moeder, C., Bicker G. Grinberg, N. Mechanistic aspects of chiral discrimination on modified cellulose. Anal. Chem. 69 (1997): 1999-2007.

[56] Zandkarimi, M., Shafaati, A., Foroutan, S.M. Chiral separation of basic and zwitterionic drugs by highly sulfated cyclodextrins using short-end injection capillary electrophoresis. Iran. J. Pharm. Res. 7 (2008): 275-281.

[57] Pattaweekongka, W., Vijitchalermpong, N., Pancharoen, U. Selective Recovery of Palladium from Used Aqua Regia by Hollow Fiber Supported with Liquid Membrane. Korean J. Chem. Eng. 20 (2003): 1092-1096.