CHAPTER II

ENANTIOSELECTIVE SEPARATION OF RACEMIC AMLODIPINE BY TWO-PHASE CHIRAL EXTRACTION CONTAINING *O,O'*-DIBENZOYL-(2*S*,3*S*)-TARTARIC ACID AS CHIRAL SELECTOR

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2.1 ABSTRACT

A two-phase chiral extraction system containing *O,O'*-dibenzoyl-(2*S*,3*S*)-tartaric acid ((+)-DBTA) in 1-decanol organic phase and aqueous phase was developed for the chiral resolution of amlodipine. The effects of extractant concentration, equilibrium time, and pH of the aqueous phase on the separation performance were investigated. The results indicated that the system afforded a strong chiral separation ability; the (+)-DBTA showed a higher recognition ability toward (*S*)-amlodipine than the (*R*)-amlodipine. Upon a single extraction, the enantiomeric excess (%) of (*S*)-amlodipine could be enriched to 24.27%. The product recovery ratio was 0.74. The distribution ratios for (*S*)-amlodipine (D_S), (*R*)-amlodipine (D_R) and separation factor (α) were 1.28, 0.78 and 1.64, respectively. Therefore, the pH and concentration of the extractant have the great effects on chiral separation ability. Two-phase chiral extraction has great significance for preparative separation of (*S*)-amlodipine; it can also be used to design and scale up the enantioselective separation process.

2.2 INTRODUCTION

Amlodipine (2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl), 1, 4 dihydroxy -6-methyl-3, 5-pyridine dicarboxylic acid, 3-ethyl-5-methyl ester) is a third-generation dihydropyridine calcium channel antagonist. Amlodipine functions as a selective inhibitor of calcium influx across cell membranes, resulting in a greater effect on vascular smooth muscle than cardiac muscle cells [1-4]. Amlodipine is indicated for the treatment of hypertension, myocardial ischemia and angina [5, 6]. It is used as an enantiomeric mixture of salt forms, but the two enantiomers of amlodipine and their salts have different pharmacological profiles [7, 8]. (*S*)-Amlodipine (Figure 2.1(a)) is the more potent calcium channel blocker, showing about 2,000 times greater potency in *in-vitro* evaluation in the rat aorta than (*R*)-amlodipine (Figure 2.1(b)) [9, 10]. The (*S*)-amlodipine is a potent drug for the treatment of hypertension, while (*R*)-amlodipine has been shown to release nitric oxide in the peripheral blood vessels, which may lead to peripheral edema [11, 12]. Hence, in order to reduce the incidence of peripheral edema and other side effects, it

is beneficial to separate (*S*)-amlodipine from racemic amlodipine, and use (*S*)-amlodipine as a single enantiomeric drug.

The enantioselective separation of enantiomeric compounds poses glaring challenges to the pharmaceutical industry. Recently, numerous attempts have been made to develop efficient separation and concentration processes to elucidate racemic mixtures into enantiomerically pure compounds [13-15]. Various sources in the literature and patents have reported the separation of (*S*)-amlodipine from its racemate [16], by methods such as crystallization, kinetic resolution, chromatography and capillary electrophoresis [16-19]. The most used preparation of (*S*)-amlodipine is by selective diastereomeric salt crystallization method; but this technique requires a considerable number of different steps, and is time-consuming and cost-inefficient [20, 21]. Kinetic resolution is very expensive due to its single separation state, while chromatography and capillary electrophoresis are not suitable for large-quantity production of chiral substances [22-26]. Solvent extraction is a good alternative method for chiral separation because it is usually preformed by dispersion of one immiscible phase in the other [27, 28]. Moreover, it can be used on an industrial scale, and can be performed continuously with good efficiency and high recovery [29, 30].

As a simple, low-cost, and easily scalable technique, solvent extraction has attracted many researchers who have explored its use [31-34]. Solvent extraction is an important analytic technique; and it is mainly used in analysis and separation. As a potential large-scale production technique, chiral solvent extraction has recently attracted the attention of many researchers [35-38]. There are several chiral extractants, such as tartaric acid derivatives. The tartaric acid derivatives are acidic compound contain two carboxylic acid groups; moreover, several literature reports the advantage of tartraric acid derivative chiral selector [39-41]. The enantiomeric complex is formed to the good complex-forming of tartaric acid derivatives and enantiomer. Furthermore, tartaric acid derivatives are distributed predominantly in organic solvents such as alcohol, alkyl halide and hexane. The partitioning of the tartaric acid derivatives cannot be presented as a function of pH because they are distributed predominantly only in the organic solvent [42]. The *O,O'*-dibenzoyl-(2*S*,3*S*)-tartaric acid ((+)-DBTA) (Figure 2.1(c)) is one of the tartaric acid derivatives. The (+)-DBTA is known to be a chiral selector of enantiomers, such as ephedrine, chiral alcohols and *n*-methyl-amphetamine [41, 43]. Moreover, has also been

observed that (+)-DBTA can form complexes with some racemic alcohols [44]. Verification of the good complex-forming abilities of enantiomeric compound and (+)-DBTA arise from a number of facts [39-41]. 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 [40-42]. The benzoyl groups can take part in hydrophobic interactions while the other parts of the molecule contain polar hydrophilic groups [44]. (+)-DBTA evidently has the strongest ability to separate a chiral drug. For these reasons, the aim of this work is to develop a selective separation method for (*S*)-amlodipine by using two-phase chiral separation technology based on (+)-DBTA as an extractant.

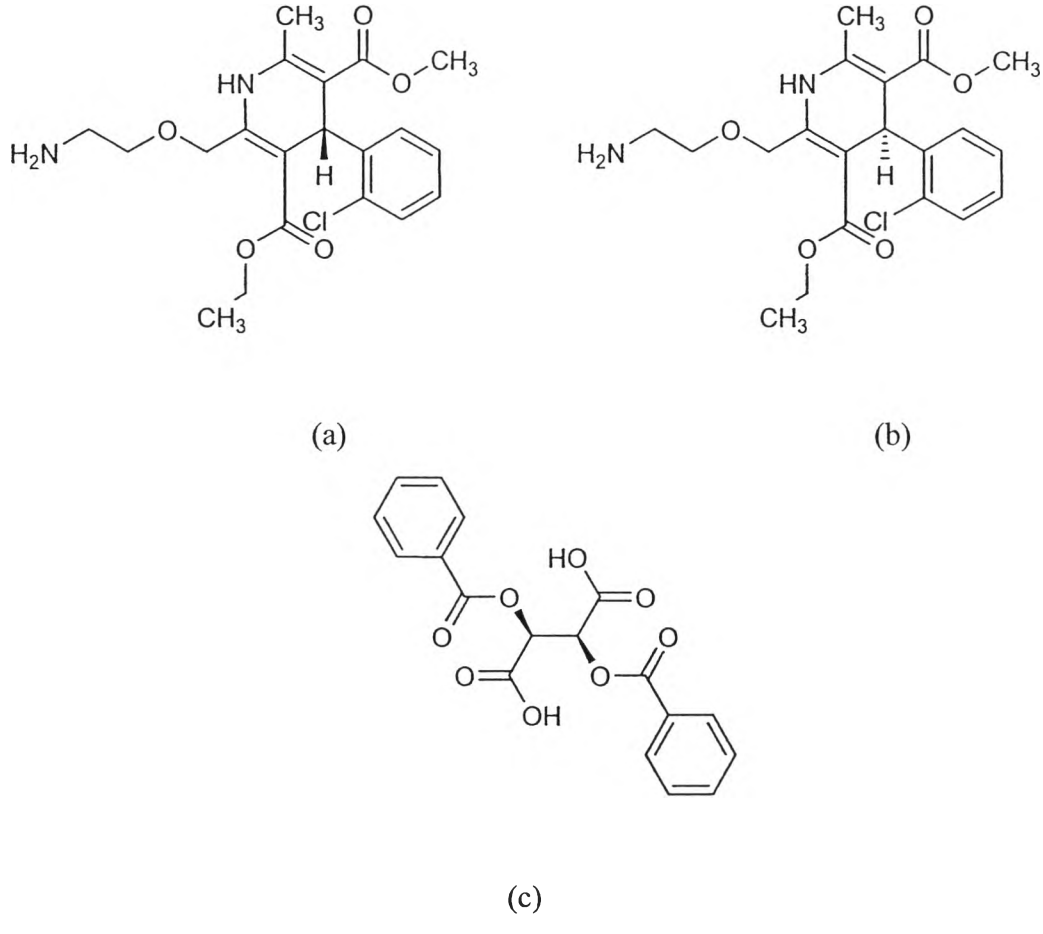


Figure 2.1 Structures of (a) (*S*)-amlodipine, (b) (*R*)-amlodipine
(c) *O,O'*-dibenzoyl-(2*S*, 3*S*)-tartaric acid

The present work was conducted to better understand the equilibrium and distribution behavior of amlodipine enantiomers in a two-phase chiral extraction system containing (+)-DBTA in organic phase. This work used initial parameter as our previous study [16] to confirm and make understand about the operating conditions. The condition parameter in recent work such as the buffer pH in aqueous phase, the type of organic solvents, the contact time, and the concentration ratio of racemic compound to extractant could influence the chiral extraction and enantioseparation efficiency. This condition is very important to apply chiral solvent extraction system in pharmaceutical industry. Solvent two-phase chiral extraction is of great interest as a promising method for the efficient separation and high recovery of chiral drugs with very low concentrations from aqueous solutions. Two parameters – the separation factor (α), a measure of the separation efficiency of the process; and the percentage enantiomeric excess (% *e.e.*), a measure of the purity of the product were evaluated. The product recovery ratio (defined as the ratio of the quantity of the enantiomer recovered over its initial quantity) will be also referred to as an indicator of the productivity of the process.

2.3 EXPERIMENT

2.3.1 Chemicals and reagents

All reagents (pharmaceutical grade) used in this work – (*R*)-amlodipine, (*S*)-amlodipine and racemic amlodipine – were provided by the Government Pharmaceutical Organization (Thailand). *O,O'*-Dibenzoyl-(2*S*,3*S*)-tartaric acid ((+)-DBTA) was obtained from Acros Organics (Morris Plains NJ, USA). *n,n*-Dimethylformamide was obtained from Merck (Darmstadt, Germany). 1-Propanol, cyclohexane, and 1-decanol were also obtained from Merck. All chemicals used in these experiments were analytical reagent grade and all reagents were GR grade (Merck). Aqueous solutions were prepared using Milli-Q® deionized water (Millipore, Billerica MA, USA). Doubly deionized water was used throughout the experiments.

2.3.2 Analytical apparatus

The analysis was performed following U.S. Patent No 6646131 B2 [45]. The chromatographic procedure was carried out using an Ultron ES-OVM, ovomucoid chiral column (5 μm, 4.6 x150 mm) (Agilent®). 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 wavelength and auto sampler. Data analysis was carried out using ChemStation® version B.04.01 software (Agilent). The pH of the aqueous phase was measured with a SevenMulti™ pH meter with modular expansion (Mettler-Toledo, Greifensee, Switzerland).

2.3.3 Procedures

The extraction experiments were performed in 50 mL baffled flasks. Aqueous phase: (*R,S*)-amlodipine was dissolved into 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer solution. Organic phase: chiral selector ((+)-DBTA) was dissolved into the suitable organic phase (such as 1-decanol). The solution extraction experiments were conducted by pipetting 5 mL of aqueous phase and 5 mL of organic phase, and adding them into 50 mL baffled flasks. The flasks were then vigorously shaken in an orbital shaker for the desired contact time to reach the maximum extract of (*S*)-amlodipine into the organic phase. The experiments were run at a temperature of 25 °C until the distributive behavior achieved equilibrium. The mixing time was sufficient to reach the equilibrium state. The sample obtained from aqueous phase was analyzed by HPLC. Each experiment was duplicated under identical conditions. Owing to the change of volume is very small and can be neglect, the concentration of (*R*)-amlodipine and (*S*)-amlodipine in organic phase were determined from a mass balance method.

2.3.3 Analytical instruments and chromatographic conditions

The quantification of amlodipine enantiomers in the aqueous phase was performed following U.S. Patent No 6646131 B2 [45]. 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 25°C by using a column heater. Mobile phase is a mixture of disodium hydrogen phosphate buffer (20 mmol/L) and acetonitrile (80:20%, v/v). A flow rate of mobile phase was 0.3 mL/min. The injection volume was 20 μL. The retention times for (*R*)-amlodipine was about 4.8 min, and for (*S*)-amlodipine was about 5.6 min, as detected by HPLC system with a photodiode array detector set at UV 237 nm. The analysis time was set at 20 min per sample to eliminate potential interference from late eluting peaks. The chromatogram was shown in Figure 2.2.

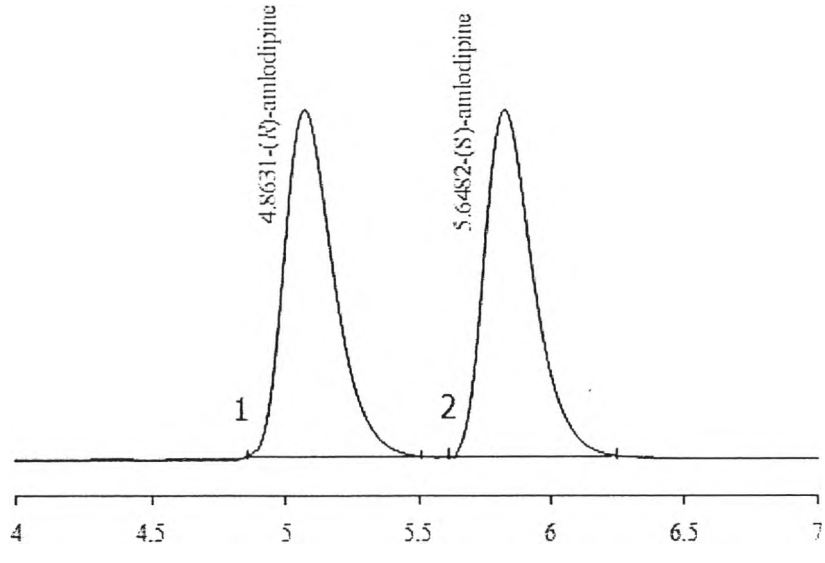


Figure 2.2 Chromatogram of resolution of amlodipine by HPLC in aqueous phase before two-phase chiral extraction system. (1): (*R*)-amlodipine, (2): (*S*)-amlodipine.

2.4 THEORY

Enantioselective extractions were first reported in the late 1960's [44, 46]. Since then, numerous studies on the origin of enantioselectivity at a molecular level have been published [47, 48]. The enantioselectivity of a process may be expressed as the operational selectivity [49]. For the current system, the (*S*)-amlodipine is preferentially extracted and the distribution coefficients of (*S*)-amlodipine and (*R*)-amlodipine, D_S and D_R, extracted from aqueous into organic phase were determined as in Eq. (2.1) and Eq. (2.2):

$$D_S = \frac{[(\mathrm{S})\text{ - amlodipine}]_o}{[(S)\text{ - amlodipine}]_a} \tag{2.1}$$

$$D_R = \frac{[(R)\text{ - amlodipine}]_o}{[(R)\text{ - amlodipine}]_a} \tag{2.2}$$

The enantioselectivity was calculated in terms of the separation factor (α) and the enantiomeric excess (% *e.e.*):

$$\alpha = \frac{D_S}{D_R} \tag{2.3}$$

$$\%e.e. = \frac{|D_S - D_R|}{(D_S + D_R)} \times 100 \tag{2.4}$$

2.4.1 Mechanisms of amlodipine enantiomers via two-phase chiral extraction

(+)-DBTA can form diastereomeric complexes with enantiomeric drugs. The carboxylic acid groups of (+)-DBTA can donate protons for hydrogen bonding, while it can also behave as a proton acceptor due to the eight oxygen atoms it contains. The benzoyl groups can take part in hydrophobic interactions, while the other part of the molecule contains polar hydrophilic groups [42, 50]. According to the

abovementioned, it seemed promising to justify the next step of carrier-mediated transport in a two-phase chiral extraction to determine the feasibility of amlodipine enantiomer enrichment by a (+)-DBTA facilitated two-phase chiral extraction. (*R*)-Amlodipine and (*S*)-amlodipine form two diastereomeric complexes with (+)-DBTA through coulombic interactions, hydrogen bonding and van der Waals interactions [51]. The mechanism and the enantioselective transport kinetics of amlodipine enantiomers through two-phase chiral extraction are schematically described in Eq. (2.5). At the interface of the aqueous and organic phases, (*S*)-amlodipine forms complexes with (+)-DBTA, and [(*S*)-amlodipine]$_2$-(+)-DBTA complex dissolves in the organic phase:

$$2[(S)\text{-amlodipine}]_a + [(+)\text{-DBTA}]_o \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [[(S)\text{-amlodipine}]_2\text{-}(+)\text{-DBTA}]_o \quad (2.5)$$

where k_1 and k_2 are the apparent rate constants of the aqueous phase interfacial and organic phase interfacial transport of amlodipine enantiomers, respectively (16). The transport mechanism of (*S*)-amlodipine through two-phase chiral extraction is shown in Figure 2.3.

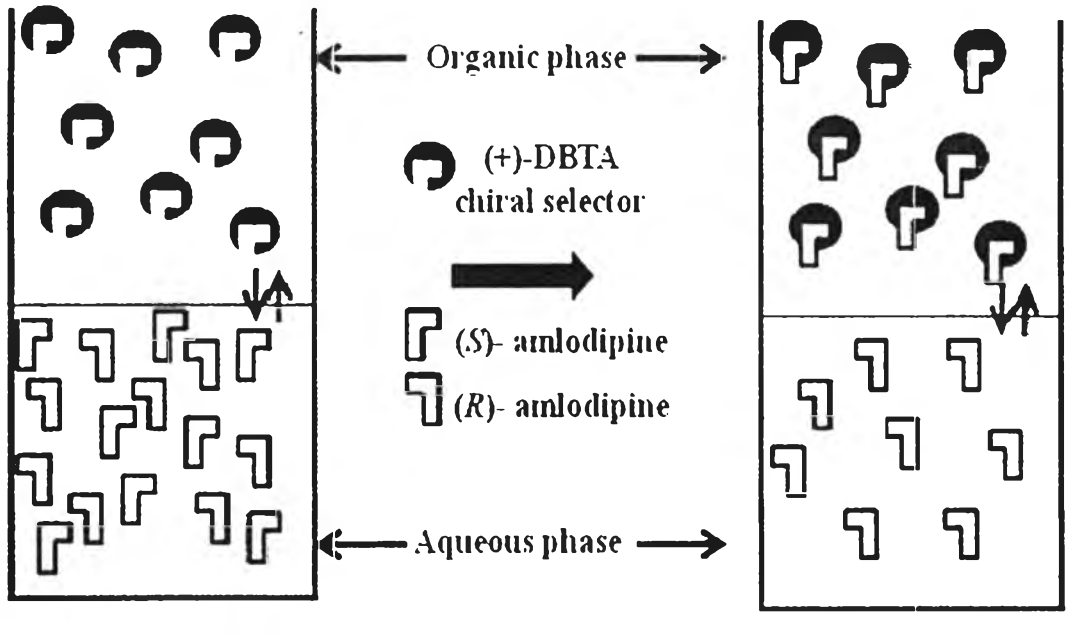


Figure 2.3 Distribution behavior scheme for enantioselective separation of racemic amlodipine by (+)-DBTA

2.4.2 Extraction equilibrium constant

The extraction equilibrium constant (K_{ex}) of (*S*)-amlodipine extracted by [(+)-DBTA in Eq. (2.5) was derived from the experimental data and calculated from the following equation:

$$K_{ex} = \frac{[[(S)\text{-amlodipine}]_2\text{-}(+)\text{-DBTA}]_b}{[(S)\text{-amlodipine}]_a^2[(+)\text{-DBTA}]_b} \quad (2.6)$$

where the value of K_{ex} for (*S*)-amlodipine extracted with (+)-DBTA was found to be 1.3251 $L^2/mmol^2$. The result of experiment was showed in section 2.5.6. This result showed promising agreement with the previous study [16].

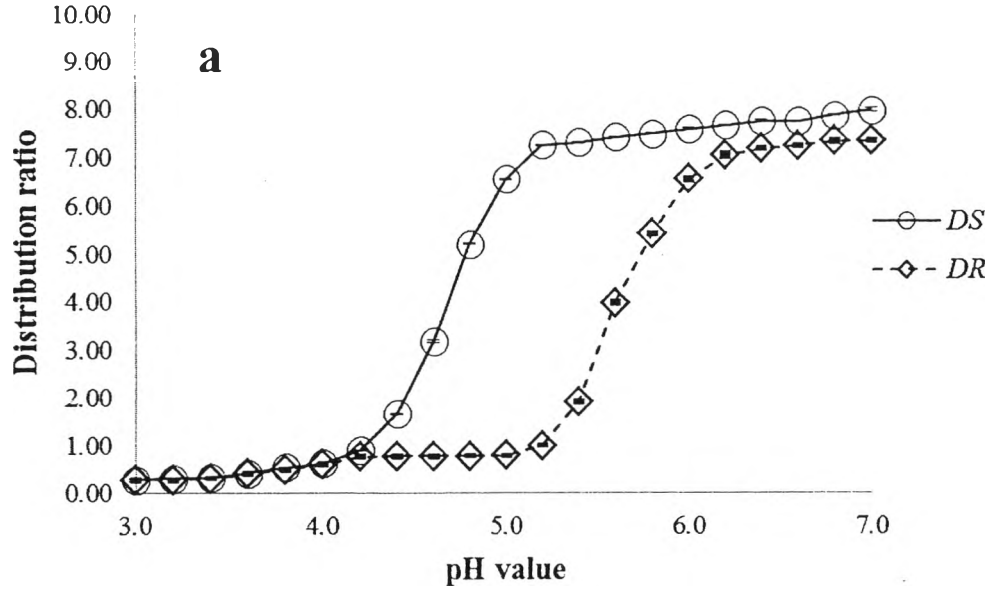
2.5 RESULTS AND DISCUSSION

2.5.1 The effect of the buffer pH in aqueous phase on the distribution coefficient and the enantioselectivity (α)

The pH is an important factor for consideration in the separation of enantiomers, as it impacts the states of amlodipine enantiomers. The distribution ratios (D_S and D_R) and the enantioselectivity (α) of amlodipine enantiomers were studied in 4 mmol/L (+)-DBTA in 1-decanol, and 4 mmol/L racemic amlodipine in NaH_2PO_4/H_3PO_4 buffer at different pH values. The pH dependence plot (Figure 2.4 (b)) was derived from plot (Figure 2.4 (a)), which represented distribution ratios of diastereomeric salts (D_S or D_R). The pKa of amlodipine was found that the pKa is 8.6 based according to M. Zandkarimi [25, 26]. From the structure of amlodipine in Figure 2.1 (a) is a weak base in aqueous solution, amlodipine exists in two states of neutral and positively charged molecules. From Henderson-Hasselbach Equation [52]:

$$pH = pKa + \log \text{Base [A-; B]} / \text{Acid [HA; BH +]} \quad (2.7)$$

The pH below 5.0 (pH3.0-4.5) enantiomers of amlodipine exists in states of cation (ionized form). Percent ionization of amlodipine is nearly 100%. But at pH 5.0 percent ionization of amlodipine is 99.97%. That mean unionized form is about 0.03%. The pH over 5.0 (pH6.0-8.0) enantiomers of amlodipine exists in states of neutral molecule (unionized form). That means amlodipine can distributed into the organic phase was increased, but from Figure 2.4 (a). The concentrations of amlodipine in organic phase were increased in the pH range from pH 4.0 to nearly pH 5.0. From this reason (*S*)-amlodipine and (*R*)-amlodipine can move into the organic phase (+)-DBTA has the ability to select specific binding with (*S*)-amlodipine more than (*R*)-amlodipine resulted in the D_S is greater than D_R and the enantioselectivity increased when the pH to near 5.0. But at pH greater than 5.0 amlodipine exists in states of neutral molecule (unionized form). (*S*)-amlodipine and (*R*)-amlodipine were increased able to enter the organic phase. The results were shown in Figure 2.4 (a) that D_R is closing to D_S. The enantioselectivity decreased as illustrated in Figure 2.4 (b). These experiments were performed five times using five independent samples. In order to show the precision and reproducibility, the plots have included error bars. This result agreed with our earlier reported [16]. So, pH 5.0 was an appropriate choice in view of the greater enantioselectivity of the enantioselective extraction.



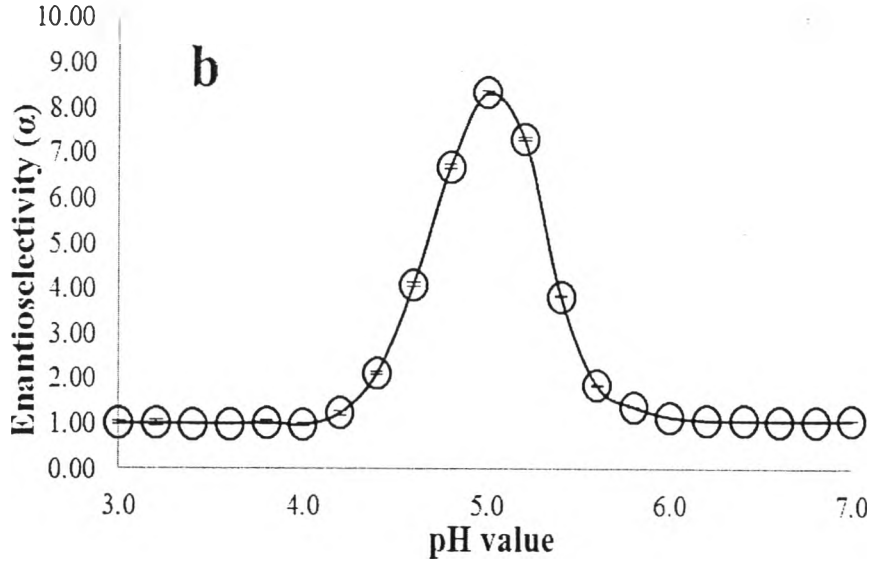


Figure 2.4 (a) The effect of pH on distribution ratios, D_S and D_R

Conditions: At contact time 90 min aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

(b) The effect of pH on enantioselectivity (α)

Conditions: At contact time 90 min aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

2.5.2 The effects of organic solvents

The influences of organic solvents on distribution behavior were investigated. The two-phase chiral extraction system contained 4 mmol/L (+)-DBTA in organic phase and contained 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer in aqueous phase. From Table 2.1, it can be seen the extraction performance of the three different kinds of organic solvents – alcohol > alkyl halide > hexane which could be related to the polarity and interaction of different organic solvents with the diastereomeric complex. For the alcohol group listed in Table 1, the enantioselectivity (α) increased with an increase in the length of the alcohol alkyl chain. It can be seen from Table 2.1 that 1-decanol is a suitable organic solvent for the extraction of racemic amlodipine.

Table 2.1 The effects of organic solvents

Organic solvent	No chiral selector			(+)-DBTA chiral selector		
	D_S	D_R	α	D_S	D_R	α
1-pentanol	0.22	0.22	1.00	2.95	2.90	1.02
1-hexanol	0.27	0.27	1.00	0.94	0.91	1.03
1-heptanol	0.35	0.34	1.03	0.46	0.44	1.05
1-octanol	0.43	0.41	1.05	0.29	0.26	1.12
1-decanol	0.47	0.44	1.07	0.59	0.41	1.44
1,2-dichloroethane	0.10	0.11	0.91	0.10	0.11	0.91
dichloromethane	0.15	0.16	0.94	0.21	0.21	1.00
hexane	0.01	0.02	0.50	0.01	0.02	0.50

2.5.3 The effect of contact time of chiral extraction

In order to investigate the equilibrium time of extraction, 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer pH 5.0 were shaken in an orbital shaker with 4 mmol/L (+)-DBTA in 1-decanol as an organic solvent for the desired contact time. The total amount of each enantiomer existing in the organic and aqueous phases after extracting was consistent with their initial amount included in the aqueous phase, which proved that the (*R*)-amlodipine and (*S*)-amlodipine were not decomposed. The phase concentration and mass-balance data show in the histogram (Figure 2.5-2.6). These Figures show the phase concentration of (*S*)-amlodipine and (*R*)-amlodipine in function of time. The result was found that chiral extraction reaches equilibrium after 90 min and gives a percentage of the enantiomeric excess at 24.27, as shown in Figure 2.7.

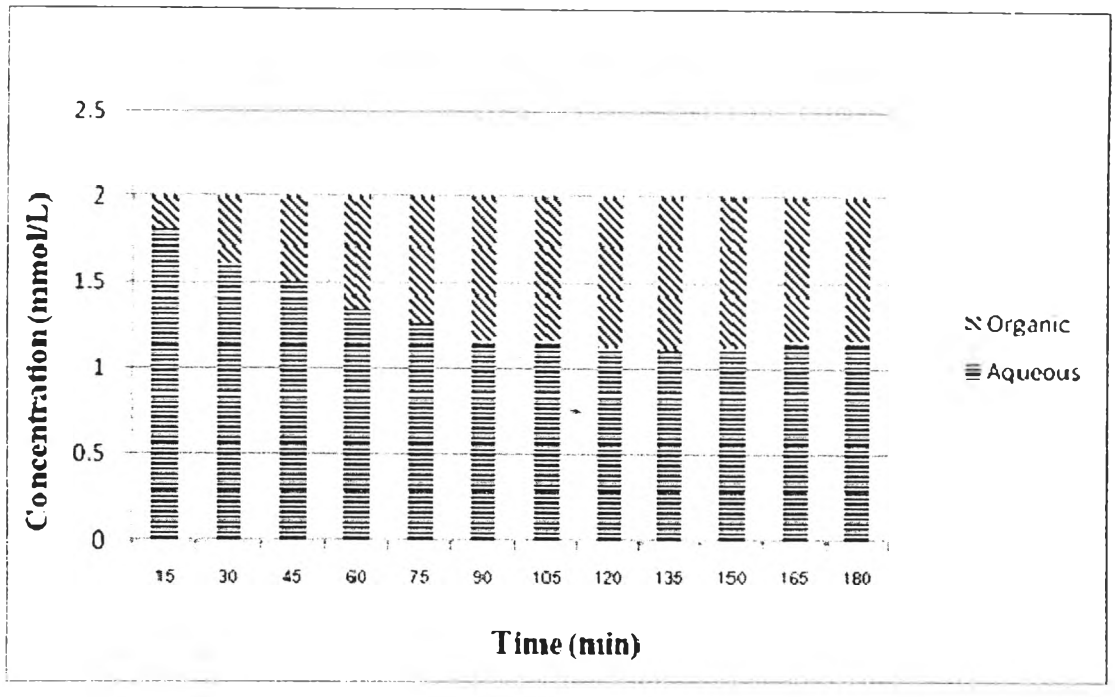


Figure 2.5 Phase concentration of (*S*)-Amlodipine vs time

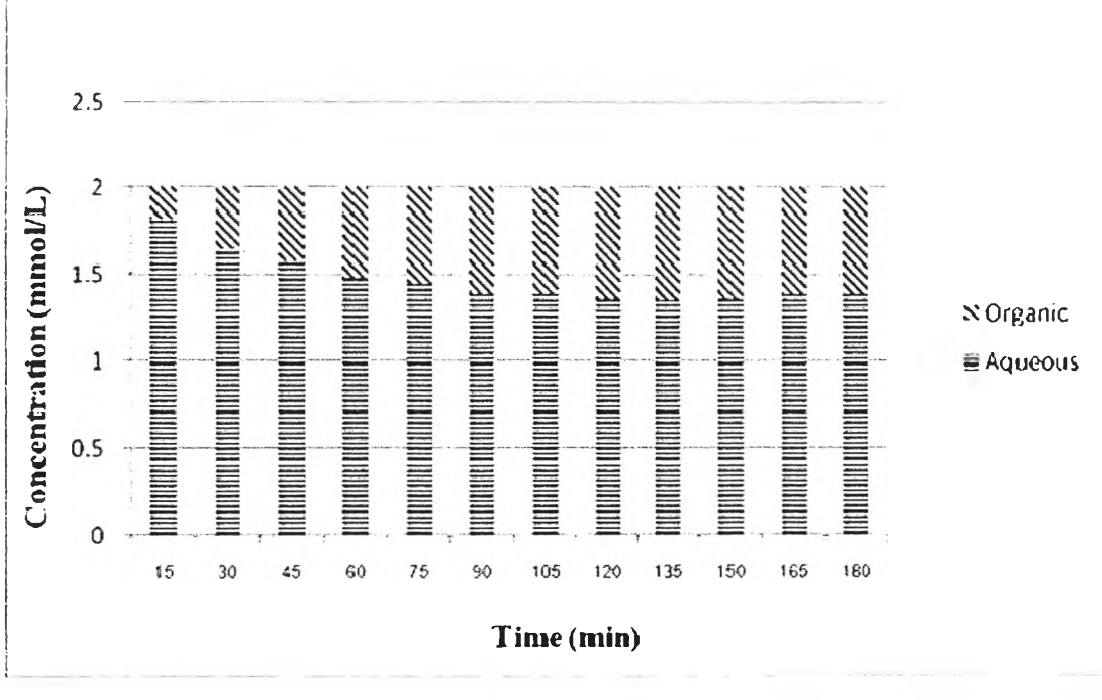


Figure 2.6 Phase concentration of (*R*)-Amlodipine vs time

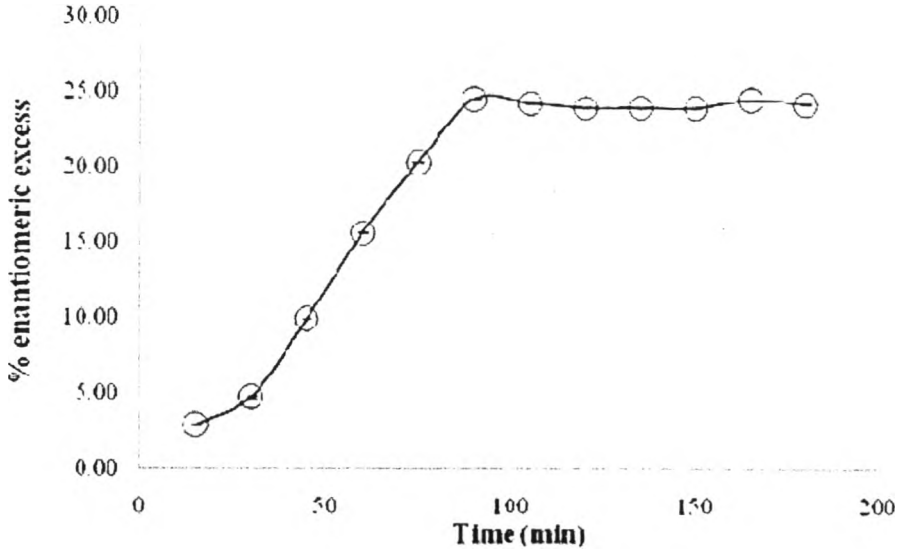


Figure 2.7 The effect of contact time of chiral extraction for amlodipine enantiomers Conditions: Aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer, pH5.0. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

2.5.4 The effect of the concentration ratio of racemic amlodipine to (+)-DBTA on the distribution ratios (D_S and D_R)

When comparing the distribution ratios (D_S and D_R) as a function of the concentration ratio of (+)-DBTA to racemic amlodipine, the enantiomer designated (*S*)-amlodipine had a slightly higher flux in all cases investigated. This means that (+)-DBTA preferentially recognizes (*S*)-amlodipine relative to (*R*)-amlodipine. The distribution coefficient decreases in accordance with transport time in the experiment. This is probably due to the loss of chiral carriers from the organic phase, leading to reduced enantioselectivities and lower distribution ratios and enantioselectivities due to facilitated transport. The distribution ratios, D_S and D_R as shown in Figure 2.8 are 1.28 and 0.78 respectively. These were anticipated, as less carrier was available with this variation of the concentration ratio, which decreased the transport rate of both enantiomers. Further variation of the concentration ratio did not significantly affect the enantioselectivity. However, the time for achieving extraction equilibrium increases with the variation in concentration ratio. To obtain a higher enantioselective extraction rate for all extraction processes, a concentration ratio of 1:1 was selected for the optimal experimental condition.

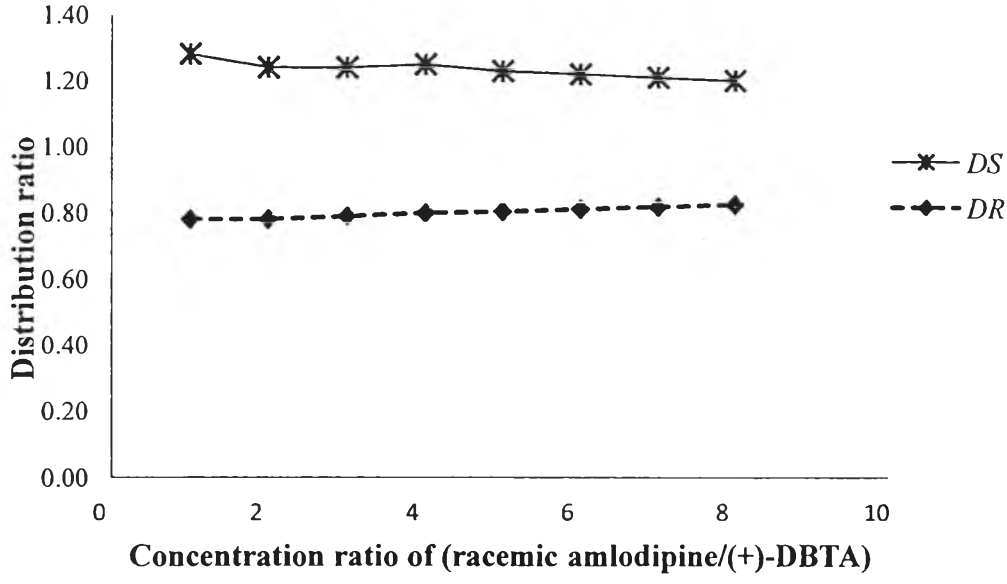


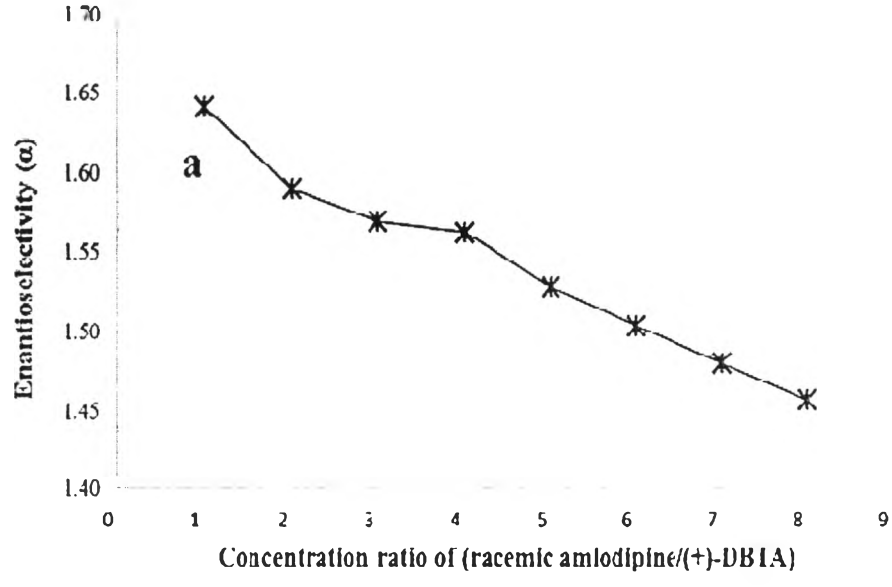
Figure 2.8 The effect of the concentration ratio of racemic amlodipine to (+)-DBTA on distribution ratios (D_S and D_R).

Conditions: At contact time 90 min aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

2.5.5 The effect of the concentration ratio of racemic amlodipine to (+)-DBTA on the enantioselectivity (α) and the enantiomeric excess (%)

Figure 2.9 shows the influences of the concentration ratio of (+)-DBTA to racemic amlodipine on enantioselectivity (α) and enantiomeric excess (%), respectively. The results of the enantioselectivity (α) enantiomeric excess (%) and the product recovery ratio are 1.64 24.27% and 0.74 respectively and decreased in conjunction with transport time in the experiment. This is probably due to the loss of chiral carriers from the organic phase leading to reduced enantioselectivities and lower distribution coefficients and enantioselectivities due to facilitated transport. The enantiomer designated (*S*)-amlodipine had a slightly higher flux in all cases investigated. This means that (+)-DBTA preferentially recognized (*S*)-amlodipine relative to (*R*)-amlodipine. The enantioselectivity (α) and enantiomeric excess (%) also decreased with variation of the concentration ratio from 1:1 to 8:1. This influence was the same as the influence of the concentration ratio of racemic amlodipine to (+)-DBTA on the distribution ratios (D_S and D_R). Further variations of concentration also

did not significantly affect the enantioselectivity. This lesser amount of carrier will decrease the transport rate of both enantiomers. The amount of chiral carrier will perform the required upgrade to the racemic mixture, regardless of initial concentration. The time for achievement of extraction equilibrium increases with the variation in concentration ratio. A concentration ratio of 1:1 was found to provide the best experimental conditions.



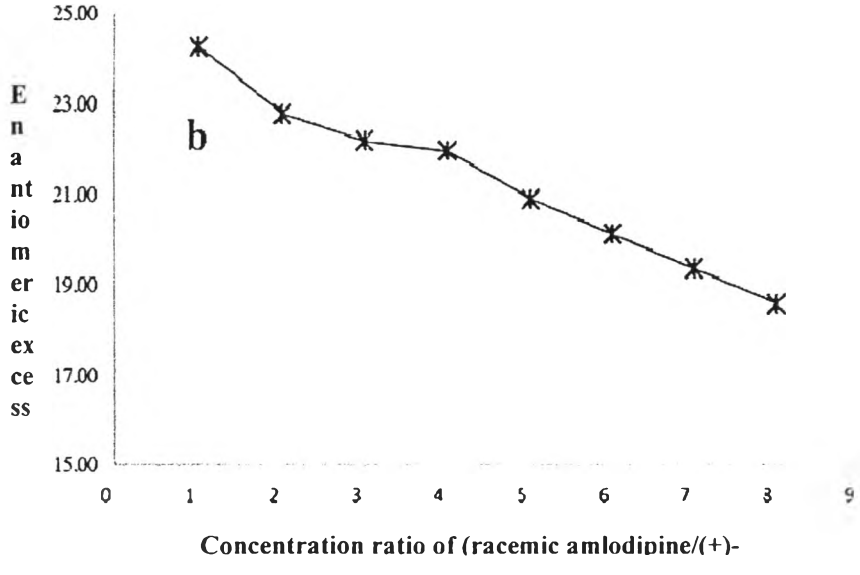


Figure 2.9 (a) The effect of the concentration ratio of racemic amlodipine to (+)-DBTA on enantioselectivity (α)

Conditions: At contact time 90 min aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

(b) The effect of the concentration ratio of racemic amlodipine to (+)-DBTA on enantiomeric excess (%)

Conditions: At contact time 90 min aqueous phase: 4 mmol/L racemic amlodipine in 10 mmol/L NaH_2PO_4 and H_3PO_4 buffer. Organic phase: 4 mmol/L (+)-DBTA in 1-decanol.

2.5.6 Determination of the extraction equilibrium constant

In this study, (*S*)-amlodipine was extracted using (+)-DBTA. When the (*S*)-amlodipine in the aqueous phase reacted with (+)-DBTA in the organic phase, the (*S*)-amlodipine-(+)-DBTA complexes were formed at the interphase and dissolved to the organic phase. The extraction equilibrium constant (K_{ex}) of (*S*)-amlodipine extracted by (+)-DBTA in Eq. (2.5) was derived from the experimental data and calculated from the following Eq. (2.6). The extraction equilibrium constant (K_{ex}) was calculated by the slope of the graph in Figure 2.10. The extraction equilibrium constant (K_{ex}) was calculated found to be 1.3251 $(L/mmol)^2$. This result agreed with our earlier reported [16].

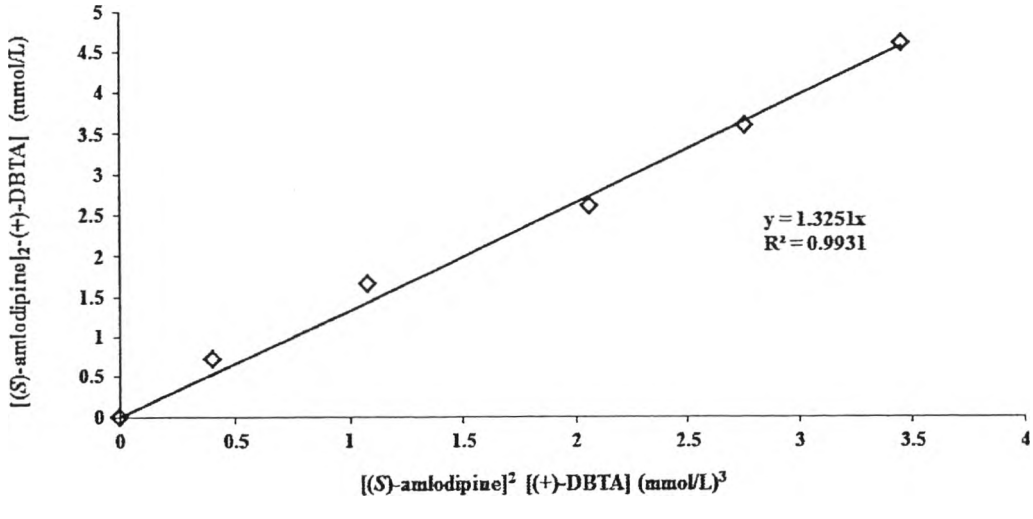


Figure 2.10 (*S*)-amlodipine extraction with (+)-DBTA as a function of equilibrium $[(S)\text{-amlodipine}]^2[(+)\text{-DBTA}]$.

2.6 CONCLUSIONS

The present work investigated the equilibrium enantioselective extraction of racemic amlodipine using two-phase chiral separation technology, using (+)-DBTA in 1-decanol as an extractant. It was shown that (+)-DBTA can form enantioselective complexes with (*S*)-amlodipine. The extraction equilibrium constant (K_{ex}) for (*S*)-amlodipine extracted with (+)-DBTA was 1.3251 $(L/mmol)^2$. This work discussed the variation of the separation factor with contact time, which indicates that any enantioselectivity of a complex of (+)-DBTA for a specific racemic amlodipine in a two-phase chiral separation system is mainly based on the kinetics, and thermodynamically driven. From this work, the two-phase chiral separation is feasible for enantioseparation of (*S*)-amlodipine. The enantiomeric excess (%) of (*S*)-amlodipine could be enriched to 24.27%. The product recovery ratio was 0.74. The distribution ratios for (*S*)-amlodipine (D_S), (*R*)-amlodipine (D_R) and separation factor (α) were 1.28, 0.78 and 1.64, respectively. Therefore, the pH and concentration of the extractant have the great effects on chiral separation ability. This work also discussed the influence of the concentration ratio of racemic amlodipine to (+)-DBTA on distribution ratios and the influence of buffer pH in the aqueous phase on distribution coefficients and enantioselectivity (α). This led to the choice of a concentration ratio of 1:1 and pH 5.0 in aqueous solutions in order to achieve the optimal experimental conditions. These conditions are most important to apply in chiral solvent extraction and separation system.

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