Equilibrium Studies on Enantioselective Extraction of Oxybutynin Enantiomers by Hydrophilic β-Cyclodextrin Derivatives

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The enantioselective extraction of hydrophobic oxybutynin (OBN) enantiomers by hydrophilic β-cyclodextrin (β-CD) derivatives was studied. The efficiency of extraction depends strongly on a number of process variables such as types of organic solvents and β-CD derivatives, concentration of selector, pH, and temperature. The experimental data were described by a reactive extraction model with a homogeneous aqueous phase reaction of R,S-OBN with β-CD. Important parameters of this model were determined experimentally. The physical distribution coefficients for molecular and ionic OBN were 4.96 × 10^{-3} and 9.52, respectively. The equilibrium constants of the complexation reactions were 1770 and 1340 L/mol for S- and R-OBN, respectively. By modeling and experiment, an optimal extraction condition with pH of 5 and HP-β-CD concentration of 0.1 mol/L was obtained with enantioselectivity (α) of 1.26, which was close to the theoretical maximum of 1.32 and performance factor (pf_i) of 0.036. The model was verified experimentally with excellent results. © 2011 American Institute of Chemical Engineers *AIChE J,* 57: 3027–3036, 2011
Keywords: reactive extraction, chiral separation, oxybutynin enantiomers, β-cyclodextrin, modeling

Introduction

There is a growing demand for the enantiopure compounds in the fragrance, pharmaceutical, and food industries, because it is found that different enantiomers may exhibit large differences in biological activity and/or toxicity and the unwanted enantiomer may cause unwanted side effects.[1,2] The most common technique for obtaining enantiopure compounds is the separation of enantiomers. Various separation methods including crystallization,[3] chromatography,[4] liquid membrane,[5] and so forth, have been developed, but the methods are not always applicable for most racemic compounds. Compared to the methods mentioned above, chiral solvent extraction is a potentially attractive technique, which is cheaper and easier to scale up to commercial scale and has a large application range. Many researchers have attempted the separation of optically active compound by chiral solvent extraction in recent years.[6–29] Although ample literature is available for enantioselective extraction, only a few studies provide fundamental insights in the reaction engineering mechanism.[26–29]

The chiral extraction process requires an enantioselective extractant dissolved in the extract phase, which reacts with the solutes of enantiomers in the feed. Enantioselectivity (α) is the most important parameter for chiral extraction. For

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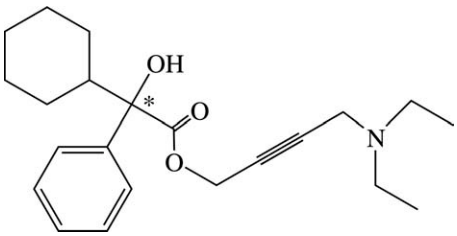

Figure 1. Chemical structure of OBN.

example,[7] for a 99% pure product ($R/S = 100$) about 190 theoretical stages are required for an enantioselectivity of 1.05, a number decreasing to ~30 when α increases to a value of 1.20. There are several normal chiral extractants, such as tartaric acid derivatives.[6–11,27] crown ethers,[12–16,28] cinchona alkaloids,[17–20,29] and so on.[21–26] However, the enantioselectivities of the chiral extractants are somewhat low, and a large number of theoretical stages are required in the chiral solvent extraction process. Until now, few new types of extractants have been tested to separate enantiomers.

Hydrophilic β-CDs possess hydrophobic cavities in the molecular structures and can form host–guest complexes with various guest molecules by including them in the cavities.[30] Several weak intermolecular forces between host and guest, such as dipole–dipole, hydrophobic, Van der Waals, electrostatic, and hydrogen bonding interaction, cooperatively contribute to the formation of diastereomeric complexes between enantiomers and β-CDs, which enable β-CDs to recognize enantiomers.[31,32] Hydrophilic β-CDs show negligible solubilities in organic solvents and can be modified to achieve high solubility in water. Cyclodextrins have been used for chiral separation by electrophoresis[33,34] and liquid chromatography,[35] and used for extraction of toluene, *o*-xylene from heptane and benzyl alcohol from toluene.[36] Enantioselectivities for extraction of some aromatic acid enantiomers have been improved greatly by hydrophilic β-CDs in recent work.[8,9,11]

Oxybutynin (OBN) has been widely prescribed for the treatment of urinary tract disorders (in Figure 1). However, the *S*-enantiomer is proposed to display an improved therapeutical profile compared to its racemic form.[37] Although analytical methods are available for the separation of OBN enantiomers,[38,39] there is no feasible method for the large-scale production of optically pure OBN enantiomers. This article reports the enantioselective extraction of hydrophobic OBN enantiomers by hydrophilic β-cyclodextrin (β-CD) derivatives. The effects of process variables such as types of organic solvents and β-CD derivatives, concentration of selector, pH, and temperature, on extraction efficiency were investigated. A reactive extraction model with a homogeneous aqueous phase reaction of *R,S*-OBN with β-CD has been established to optimize the extraction process.

Experimental

Materials

Methyl-β-cyclodextrin (Me-β-CD), hydroxyethyl-β-cyclodextrin (HE-β-CD), 2-hydroxyethyl-β-cyclodextrin (2-HE-β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), and sulfobutylether-β-cyclodextrin (SBE-β-CD) were bought from Qianhui Fine Chemical (Shandong, China). Oxybutynin (OBN, racemate, purity $\geq$99.5%) was purchased from Hengshuo pharmaceutical chemical (Hubei, China). Solvent for chromatography was of HPLC grade. All other chemicals were of analytical-reagent grade and bought from different suppliers.

Determination of physical distribution coefficients P_0 and P_i of molecular and ionic OBN

Experiments to determine physical distribution coefficient P_0 and P_i of molecular and ionic OBN over the aqueous and dichloroethane phases were carried out in a water batch at 5°C. The organic phase was prepared by dissolving 1×10^{-3} mol/L OBN in dichloromethane. The aqueous phases were 0.1 mol/L K_2HPO_4/H_3PO_4 buffer solutions with a series of pH values in the range (2–7). Equal volumes of the two phases were placed together, and shaken sufficiently (5 h) before being kept in a water bath at 5°C to reach equilibrium. The concentration of OBN in the aqueous phase was analyzed by HPLC. The concentration of OBN in organic phase was determined from a mass balance.

Determination of complexation equilibrium constants K_R and K_S

Solubility measurements were carried out according to the method of Higuchi and Connors.[40] Excess amounts of racemic OBN, exceeding its solubility, were added to aqueous solutions containing increasing amounts of HP-β-CD (0, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.075, and 0.1 mol/L). The suspensions were shaken for 24 h in a water bath at 5°C. After equilibration, the suspensions were filtered through 0.45 μm membrane filters, appropriately diluted with the mobile phase and the total concentrations of OBN enantiomers were analyzed by HPLC. The apparent stability constants of the complexes are calculated from the straight lines of the phase solubility diagram.

Extraction experiments

The aqueous phase was prepared by dissolving (Me-, HE, 2-HE, HP-, or SBE-) β-CD in 0.1 mol/L KH_2PO_4/H_3PO_4 buffer solution and the organic phase was prepared by dissolving racemic OBN in organic solvent. Extraction experiment was performed in a 10 mL plastic centrifuge tube. Equal volumes (each 2 mL) of the aqueous and the organic phase were placed together and shaken sufficiently (5 h) before being kept in a water bath at a fixed temperature to reach equilibrium. After phase separation, the concentrations of OBN enantiomers in the aqueous phase were analyzed by HPLC. The total amounts of *R,S*-enantiomer in the organic and aqueous phases after extracting were consistent with their initial amounts included in organic phase. The concentrations of *R*- and *S*-OBN in the organic phase were calculated from a mass balance.

Extraction experiments with 1 mmol/L racemic OBN in different organic solvents and 0.1 mol/L HP-β-CD in aqueous phases were repeated with the solvent to feed ratio of 1/3 to investigate the influence of the solvent to feed ratio on extraction efficiency.

Analytical method

The quantification of OBN enantiomers in the aqueous phase was performed by HPLC using an Agilent LC 1200

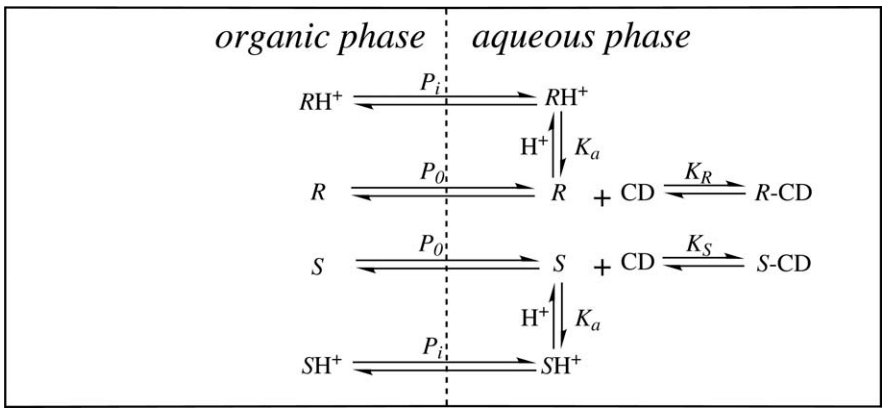

Figure 2. Diagram of the mechanism of reaction extraction.

series apparatus (Agilent Technologies Co. Ltd.). An UV detector operated at 223 nm was applied. The column was Diamonsil C18, 5 μm particle size of the Packing Material, 200 mm $\times$ 4.6 mm I.D. (Dikma Technologies). The mobile phase was acetonitrile: 0.03 mol/L potassium dihydrogen phosphate buffer solution (pH = 4.0, adjusted with phosphoric acid) (20:80, v/v), containing 0.06 mol/L HP-β-CD. The flow rate was set at 0.8 mL/min and the column temperature was set at 25°C.

Mechanism and Modeling

Mechanism of reaction extraction

Knowledge of the reaction mechanism is very useful for optimization of a reactive extraction process. In reactive extraction systems, the reactions may take place in either the organic phase, the aqueous phase or at the interface. In the reactive extraction of *R*- and *S*-OBN with HP-β-CD as chiral selector, the extractant, HP-β-CD, is highly hydrophilic, which excludes the possibility that the reaction takes place in the organic phase. Depending on the solubility of the solutes of *R*- and *S*-OBN in the aqueous phase, the complexation reactions will either be limited to the interface or may take place in the aqueous phase. It is found that the solutes of *R*- and *S*-OBN distribute over the organic and aqueous phases. Therefore, we have applied the homogeneous aqueous phase reaction mechanism here. Further in this article, we will validate this mechanism for the system under study.

The homogeneous aqueous phase reaction mechanism is depicted in Figure 2 and a three-step mechanism is assumed. The first step is a physical process of mass transfer of OBN enantiomers. In this step, OBN enantiomers molecules transfer from the organic phase to the aqueous phase. In the second step, two diastereomeric complexes between HP-β-CD and *R*- and *S*-OBN enantiomers form, due to such molecular interactions as dipole–dipole, hydrophobic, Van de Waals, electrostatic, and hydrogen bonding interaction, and two acid-base dissociation equilibria exist in the aqueous phase. In the third step, the ionic solutes of RH^+ and SH^+ in the aqueous phase partition into the organic phase.

The chiral extraction is carried out by the formation of two inclusion complexes between OBN enantiomers and HP-β-CD. The difference in free energy between the two complexes [$-\Delta(\Delta G)$] is the driving forces for separation of enantiomers.

Distribution ratios (k) and enantioselectivity (α) are two important parameters for chiral solvent extraction. The distribution ratios of *R*- and *S*-OBN can be calculated from the following formulas, respectively

$$k_S = C_{S,w}/C_{S,o} \tag{1}$$

$$k_R = C_{R,w}/C_{R,o} \tag{2}$$

among which $C_{S,o}$ and $C_{S,w}$ represent concentrations of *S*-OBN in organic phase and aqueous phase, respectively; $C_{R,o}$ and $C_{R,w}$ represent the concentrations of *R*-OBN in organic phase and aqueous phase, respectively.

Enantioselectivity (operational selectivity) is defined by the ratio of the distribution ratios. Its upper limit is the intrinsic selectivity a_{int}, which is the ratio of the complexation constants:

$$a_{op} = k_S/k_R, \text{ assuming } k_S > k_R \tag{3}$$

$$a_{int} = K_S/K_R \tag{4}$$

where K_R and K_S are the complexation constants of *R*- and *S*-OBN with HP-β-CD, respectively.

The operational difference in free energy is

$$-\Delta(\Delta G)_{op} = RT \ln \alpha \tag{5}$$

The intrinsic difference in free energy is

$$-\Delta(\Delta G)_{int} = RT \ln \alpha_{int} \tag{6}$$

Modeling of the extraction equilibrium

The reaction extraction system depicted in Figure 2 may be modeled by a series of coupled equilibrium relations and mass balance equations, as follows. The physical partition coefficient of molecular *R*- and *S*-OBN, P_0, can be written as follows:

$$P_0 = \frac{[R]_w}{[R]_o} = \frac{[S]_w}{[S]_o} \tag{7}$$

where $[R]_o$ and $[S]_o$ are the concentrations of the free *R*- and *S*-OBN in the organic phase at equilibrium, respectively, and $[R]_w$ and $[S]_w$ are the concentrations of the free *R*- and *S*-OBN in the aqueous phase at equilibrium, respectively.

The physical partition coefficient of ionic *R*- and *S*-OBN, P_i, can be calculated by

$$P_i = \frac{[SH^+]_w}{[SH^+]_o} = \frac{[RH^+]_w}{[RH^+]_o} \quad (8)$$

where $[RH^+]_O$ and $[SH^+]_O$ are the concentrations of the ionic *R*- and *S*-OBN in the organic phase at equilibrium, respectively, $[RH^+]_W$ and $[SH^+]_W$ are the concentrations of the ionic *R*- and *S*-OBN in the aqueous phase at equilibrium, respectively.

The dissociation constant of ionic *R*- and *S*-OBN is

$$K_a = \frac{[S]_w[H^+]}{[SH^+]_w} = \frac{[R]_w[H^+]}{[RH^+]_w} \quad (9)$$

The complexation equilibrium constants of HP-β-CD with OBN enantiomers in aqueous phase can be written as follows:

$$K_S = \frac{[S-CD]_w}{[S]_w[CD]_w} \quad (10)$$

$$K_R = \frac{[R-CD]_w}{[R]_w[CD]_w} \quad (11)$$

Due to $V_w = V_o$, the following equations represent mass balances for *R*- and *S*-OBN:

$$C_R = [R]_o + [RH^+]_o + [R]_w + [RH^+]_w + [R-CD]_w \quad (12)$$

$$C_S = [S]_o + [SH^+]_o + [S]_w + [SH^+]_w + [S-D]_w \quad (13)$$

where C_R and C_S are the initial concentrations of *R*- and *S*-OBN in organic phase, respectively, and $[R - CD]_w$ and $[S - CD]_w$ are the concentrations of the complexes of *R-CD* and *S-CD* in aqueous phase, respectively.

Combining Eqs. 7–11, Eqs. 12 and 13 are deduced to

$$C_R = \frac{[R]_w}{P_0} + \frac{[R]_w[H^+]}{P_iK_a} + [R]_w + \frac{[R]_w[H^+]}{K_a} + K_R[R]_w[CD]_w \quad (14)$$

$$C_S = \frac{[S]_w}{P_0} + \frac{[S]_w[H^+]}{P_iK_a} + [S]_w + \frac{[S]_w[H^+]}{K_a} + K_S[S]_w[CD]_w \quad (15)$$

where C_{CD} is the initial concentration of HP-β-CD in aqueous phase and $[CD]_w$ is the concentration of free HP-β-CD in aqueous phase.

There is the following equation for mass balance for HP-β-CD:

$$C_{CD} = [CD]_w w + [R-CD]_w + [S-CD]_w \quad (16)$$

Combining Eqs. 10 and 11, Eq. 16 is deduced to

$$C_{CD} = [CD]_w + K_R[R]_w[CD]_w + K_S[S]_w[CD]_w \quad (17)$$

Let $A = 1 + \frac{1}{P_0} + \frac{[H^+]}{K_a} + \frac{[H^+]}{P_iK_a}$, C_{CD} can be defined as

$$C_{CD} = [CD]_w + \frac{C_RK_R[CD]_w}{K_R[CD]_w + A} + \frac{C_SK_S[CD]_w}{K_S[CD]_w + A} \quad (18)$$

With a further treatment of Eq. 18, the following equation can be deduced

$$K_RK_S[CD]_w^3 + (AK_R + AK_S + K_SK_RC_R + K_SK_RC_S - K_RK_SC_{CD})[CD]_w^2 + (A^2 + AK_RC_R + AK_SC_S - AK_SC_{CD} - AK_RC_{CD})[CD]_w - A^2C_{CD} = 0 \quad (19)$$

$[CD]_w$ can be calculated from the Eq. 19, and distribution ratios can be written as follows:

$$k_R = \frac{P_0P_iK_a(1 + \frac{[H^+]}{K_a} + K_R[CD]_w)}{P_iK_a + P_0[H^+]} \quad (20)$$

$$k_S = \frac{P_0P_iK_a(1 + \frac{[H^+]}{K_a} + K_S[CD]_w)}{P_iK_a + P_0[H^+]} \quad (21)$$

Enantioselectivity is given by

$$\alpha_{op} = \frac{1 + \frac{[H^+]}{K_a} + K_S[CD]_w}{1 + \frac{[H^+]}{K_a} + K_R[CD]_w} \quad (22)$$

The enantiomeric excess in the aqueous phases can be expressed in terms of distribution ratios by the following equation:

$$ee_w = \frac{\frac{C_S}{1+1/k_S} - \frac{C_R}{1+1/k_R}}{\frac{C_S}{1+1/k_S} + \frac{C_R}{1+1/k_R}} \quad (23)$$

The fractions of the solute *i* extracted (ϕ_i) into the aqueous phase is given by

$$\phi_i = \frac{C_{i,w}}{C_i} \quad (24)$$

where $C_{i,,w}$ represents the total concentration of the solute *i* in aqueous phase at equilibrium, and C_i represents the initial total concentrations of the solute *i*.

The extraction performance factor (pf_i) is defined as

$$pf_i = \phi_i ee_w \quad (25)$$

Results and Discussion

Physical distribution coefficient P_0 *and* P_i

Physical distribution coefficients for molecular OBN (P_0) and ionic OBN (P_i) were determined through a series of physical extraction experiments. The apparent partition coefficients, P_{app}, were determined at different pH values. As described in Figure 2, both the molecular and ionic OBN distribute between the organic and aqueous phases. Then, P_{app} is given by

$$P_{app} = \frac{[OBN]_w + [OBNH^+]_w}{[OBN]_o + [OBNH^+]_o} \quad (26)$$

where $[OBN]_w$ and $[OBNH^+]_w$ represent the total concentrations of the molecular and ionic *R*- and *S*-OBN enantiomers in the aqueous phase, respectively, and $[OBN]_o$ and $[OBNH^+]_o$ represent the total concentrations of the molecular and ionic *R*- and *S*-OBN enantiomers in the organic phase, respectively.

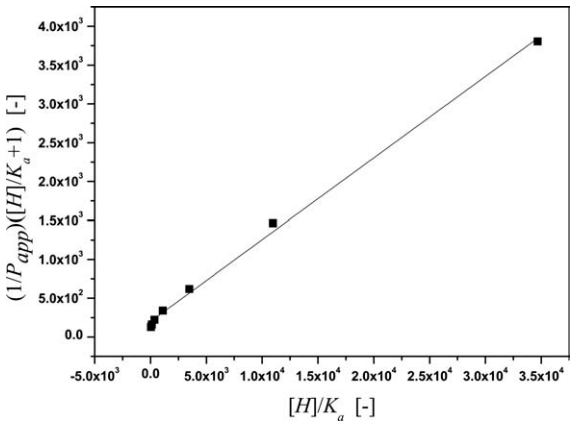

Figure 3. Plot of $(1/P_{app})([H^+]/K_a + 1)$ vs. $[H^+]/K_a$ for OBN in dichloromethane-water two phase system at 5°C; R^2 = 0.997.

Therefore, P_{app} can be derived as

$$P_{app}\left(\frac{1}{P_0}+\frac{1}{P_i}\left(\frac{[H^+]}{K_a}\right)\right)=1+\frac{[H^+]}{K_a} \quad (27)$$

Equation 27 can be transformed into

$$\frac{1}{P_{app}}\left(\frac{[H^+]}{K_a}+1\right)=\frac{1}{P_0}+\frac{1}{P_i}\left(\frac{[H^+]}{K_a}\right) \quad (28)$$

The pK_a of 6.54 was determined by potentiometric titration. The plot of $(1/P_{app})([H^+]/K_a + 1)$ vs. $[H^+]/K_a$ yielded a straight line (in Figure 3), the respective slope and intercept of the line were used to evaluate P_0 as 4.96×10^{-3} and P_i as 9.52, respectively.

Complexation constants K_R *and* K_S

Figure 4 shows the phase distribution diagrams for *S*- and *R*-OBN in the aqueous phase with increasing the concentration of HP-*β*-CD at 5°C. The diagrams indicate that the solubilities of *S*- and *R*-OBN increase linearly with the increase of the concentration of HP-*β*-CD. Consequently, the diagrams can be classified as A_L type, and the stoichiometry of binding between *R*-(or *S*-)OBN and HP-*β*-CD is 1:1. According to the method described in the literature,[40] K_R and K_S calculated from the slope and the intercept of the straight lines of the phase solubility diagrams are 1340 and 1770, respectively. The intrinsic selectivity α_{int} is estimated by Eq. 4 as 1.32.

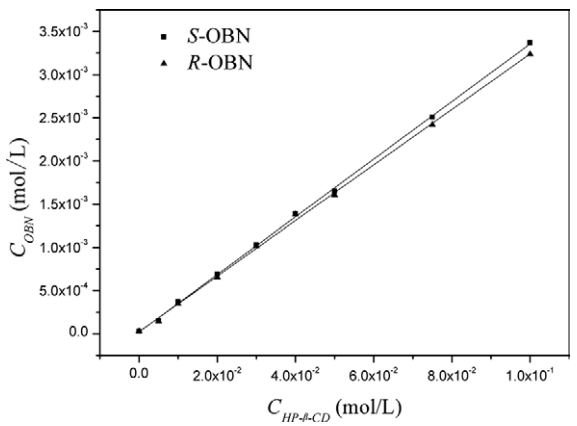

Figure 4. Phase distribution diagrams of *R*- and *S*-OBN as a function of HP-*β*-CD concentration at 5°C; R^2 = 0.999 for *S*-OBN and R^2 = 0.999 for *R*-OBN.

Table 1. Influence of Organic Solvent Type

Organic solvent	k_S	k_R	α
Cyclohexane	9.71	8.48	1.14
n-Heptane	13.92	12.69	1.10
n-Heptanol	4.44	3.78	1.17
n-Octanol	5.86	5.37	1.17
1,2-Dichloroethane	2.32	1.86	1.24
Dichloromethane	1.03	0.82	1.26

Aqueous phase: $C_{HP-\beta-CD}$ = 0.1 mol/L, pH = 5.0; Organic phase: C_{OBN} = 1.0 mmol/L.

Screening of extraction process variables

Screening of Organic Solvents. Distribution behavior of *S*- and *R*-OBN was investigated in various two-phase systems containing 0.1mol/L HP-*β*-CD in aqueous phases and 1.0 mmol/L OBN in different organic solvents (Table 1). As shown in Table 1, the type of the organic solvent has a clear influence on distribution ratio and enantioselectivity. When cyclohexane, *n*-heptane, *n*-heptanol, and *n*-octanol are used as solvents, big distribution ratios are obtained but with relatively low enantioselectivities. With dichloromethane as solvent, the highest enantioselectivity is achieved. The experiments have been repeated with a solvent to feed ratio of 1/3. It is found that the distribution ratios and enantioselectivities obtained with a solvent to feed ratio of 1/3 are all smaller than those with a solvent to feed ratio of 1/1. For example, when cyclohexane was used as organic solvent, the distributions, k_R, k_S and the enantioselectivity are 8.48, 9.71, and 1.14, respectively with a solvent to feed ratio of 1/1, whereas with a solvent to feed ratio of 1/3, k_R, k_S and the enantioselectivity decrease to 6.87, 7.56, and 1.10, respectively. Therefore, dichloromethane is a suitable solvent for extraction of OBN enantiomers.

Screening of β-CD Derivatives. Distribution ratio and enantioselectivity for OBN enantiomers were investigated in several different chiral extraction systems containing different *β*-CD derivatives (Me-*β*-CD, HE-*β*-CD, 2-HE-*β*-CD, HP-*β*-CD, and SBE-*β*-CD) in aqueous phases and OBN enantiomers in dichloromethane organic phase (in Table 2).

It is observed from Table 2 that Me-*β*-CD hardly shows enantioselectivity for OBN enantiomers with low distribution

Table 2. Influence of Hydrophilic Extractant Type

Extractant	k_S	k_R	α
Me-*β*-CD	0.11	0.11	1.00
HE-*β*-CD	0.26	0.22	1.18
2-HE-*β*-CD	0.63	0.59	1.07
HP-*β*-CD	1.03	0.82	1.26
SBE-*β*-CD	2.03	1.68	1.21

Aqueous phase: $C_{Me-\beta-CD}$ = 0.1 mol/L, $C_{HE-\beta-CD}$ = 0.1 mol/L, $C_{2-HE-\beta-CD}$, = 0.1 mol/L, $C_{HP-\beta-CD}$ = 0.1 mol/L, $C_{SBE-\beta-CD}$ = 0.1 mol/L; Organic phase: C_{OBN} = 1.0 mmol/L; temperature: 5°C.

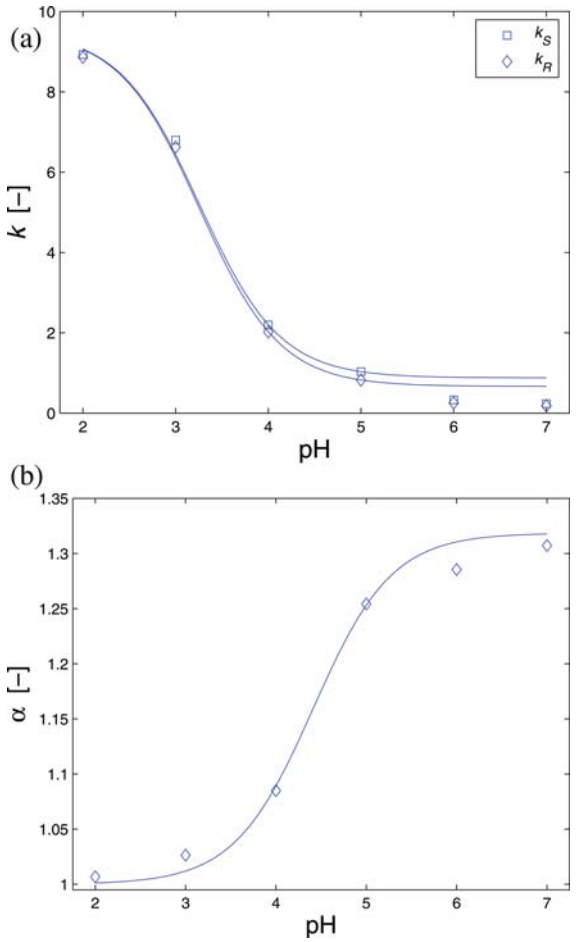

Figure 5. Influence of pH on *k* and α. Solid lines: model predictions.

Symbols: experimental data. C_{OBN} = 1.0 mmol/L, $C_{HP\text{-}\beta\text{-}CD}$ = 0.1 mol/L, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ratios. With HE-β-CD and 2-HE-β-CD as chiral selectors, relatively low distribution ratios and enantioselectivity are obtained. With SBE-β-CD as chiral selector, the highest distribution ratios are obtained, but the enantioselectivity is lower than that with HP-β-CD. Among the β-CD derivatives tested, HP-β-CD has the highest enantioselectivity with relatively high distribution ratios. Therefore, HP-β-CD was chosen as the suitable chiral selector in aqueous phase for extraction of OBN enantiomers.

Influence of pH. When combined with measured physical distribution coefficients, protonation constants and equilibrium formation constants for OBN enantiomers, the two-phase multiple chemical equilibria model provides a means of predicting enantiomer distribution ratio and enantioselectivity in dichloromethane/water two-phase systems as a function of pH. To verify the accuracy of the model predictions, OBN enantiomers were partitioned in a dichloromethane/water two-phase system over a range of pH values. A comparison of the experimental values with the model predictions of distribution ratio and enantioselectivity is shown in Figure 5. It is indicated from Figure 5 that the model predictions of distribution ratios are in moderate agreement with the experiment, whereas the predictions of enantioselectivities are in very good agreement with the experiment, as shown by a mean relative error of 0.87%.

As shown in Figure 5a, k_R and k_S decrease rapidly with the increase of pH value (pH value from 2 to 5), then slightly decrease (pH value from 5 to 7). The possible reason for this may be that most of OBN enantiomers molecules are protonated and dissolved in aqueous phase at low pH. With the increase of pH value, large amount of protonated OBN enantiomers are changed into molecular OBN enantiomers and enter the organic phase, which leads to the decrease of k_R and k_S. When pH value is more than 5, OBN enantiomers mainly exist in the form of neutral molecule. Therefore, with the increase of pH value from 5 to 7, only a very small amount of molecular OBN increase, and k_R and k_S decrease slightly.

It can also be observed from Figure 5b that the operational enantioselectivity increases with the increase of pH value before pH is 5 and then keeps nearly unchanged when pH value continues to increase. HP-β-CD mainly has enantioselective complexation ability for molecular OBN but not for protonated OBN. At pH value less than 5, the amount of

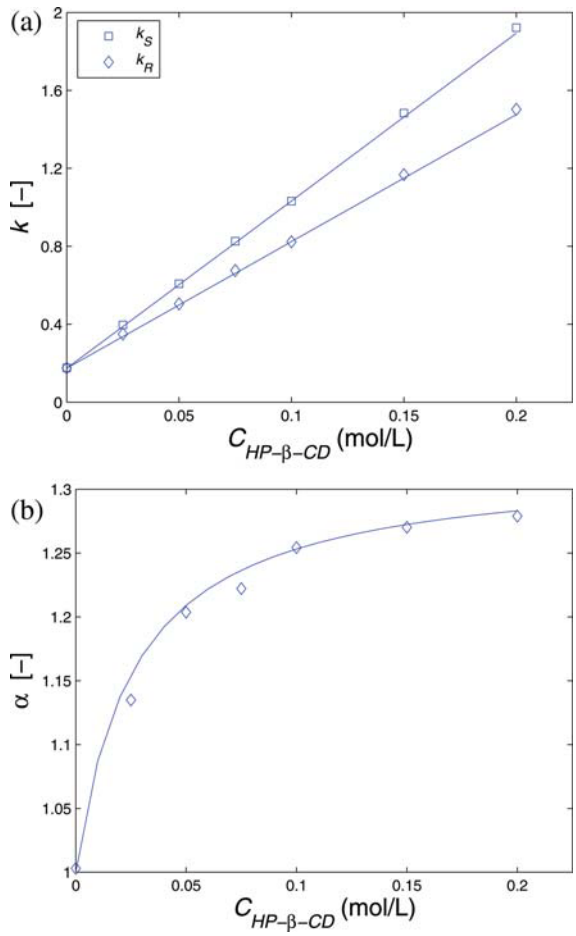

Figure 6. Influence of HP-β-CD concentration on *k* and α.

Solid lines: model predictions. Symbols: experimental data. C_{OBN} = 1.0 mmol/L, pH = 5.0, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

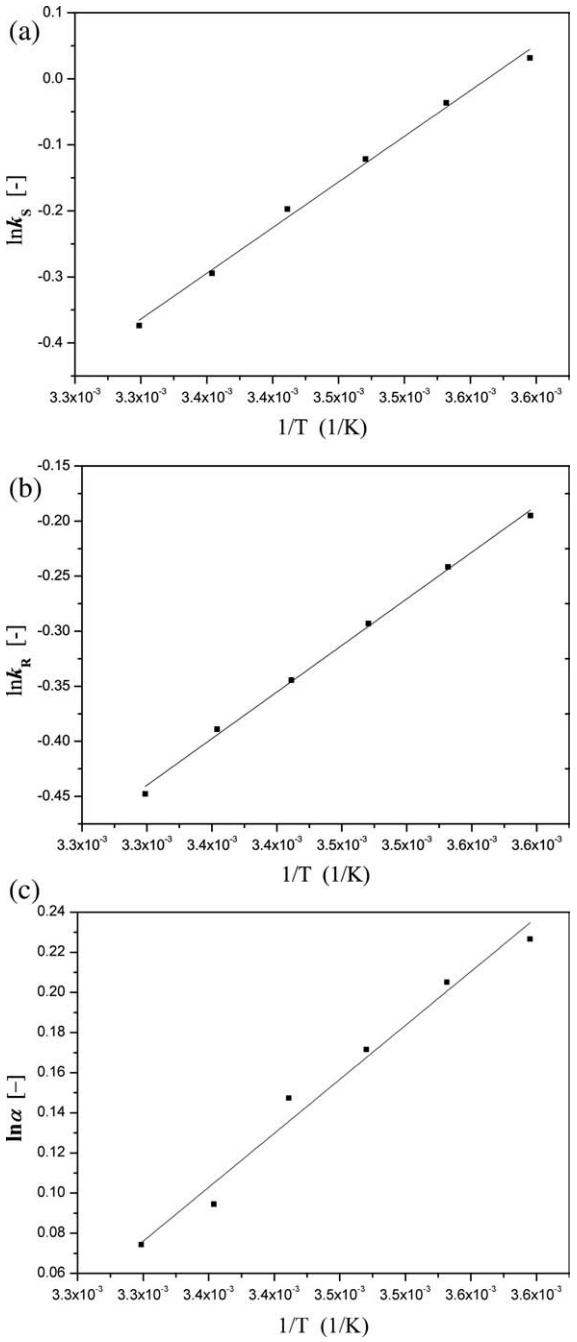

Figure 7. Plots of ln$k$ and lnα vs. 1/T; R^2 = 0.994 for lnk_S, R^2 = 0.997 for lnk_R and R^2 = 0.997 for lnα.

C_{OBN} = 1.0 mmol/L, $C_{HP\text{-}\beta\text{-}CD}$ = 0.1 mol/L, pH = 5.0.

molecular OBN increases with the increase of pH value and more molecular OBN enantiomers interact with HP-β-CD in the aqueous phase to produce diastereomeric complexes. As a result, enantioselectivity increases with the increase of pH value, and the enantioselectivities are nearly kept constant with the further increase of pH value from 5 to 7. Therefore, it is concluded that the pH of 5 is suitable for reaction extraction of OBN enantiomers by HP-β-CD.

Influence of HP-β-CD Concentration. The influence of HP-β-CD concentration on distribution behavior of OBN enantiomers was investigated by varying the concentration from 0 to 0.2 mol/L, at pH 5 and 5°C. A comparison of the experimental values with the model predictions of distribution ratio and enantioselectivity is shown in Figure 6. It is also concluded from Figure 6 that, the model predictions are in good agreement with the experiment, as shown by mean relative errors of 0.91% for k_S, 1.37% for k_R, and 0.56% for α.

It is shown from Figure 6a that k_R and k_S are linearly proportional to the concentration of HP-β-CD. It is also

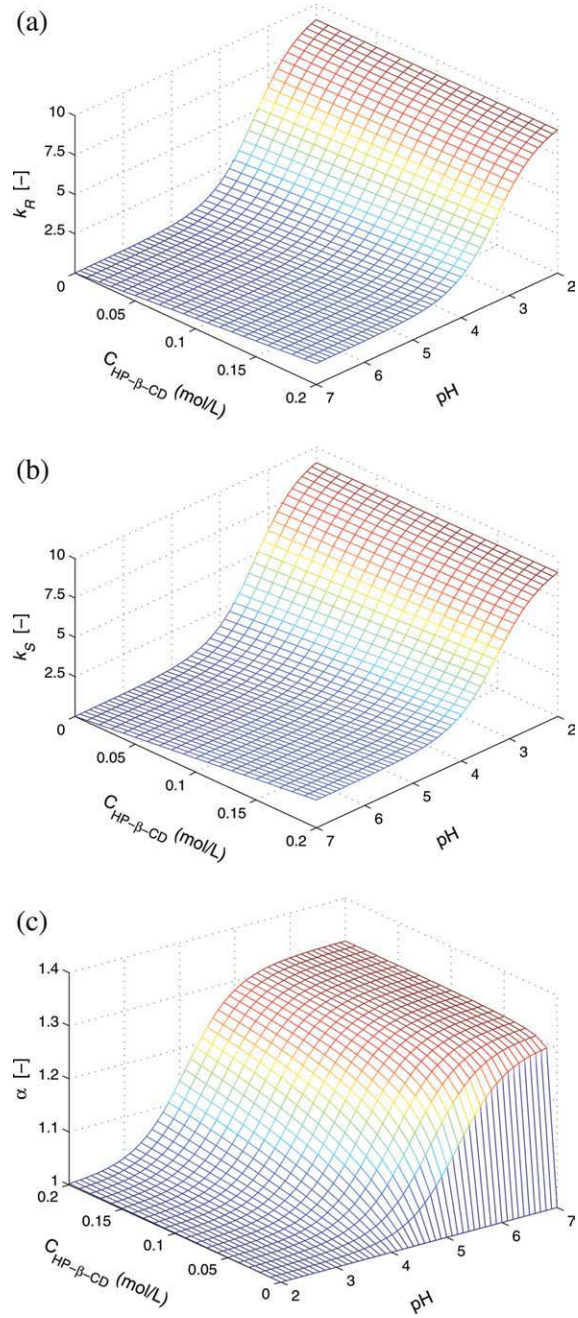

Figure 8. Calculated distribution ratio and enantioselectivity for OBN enantiomers as a function of pH and HP-β-CD concentration.

C_{OBN} = 1.0 mmol/L, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

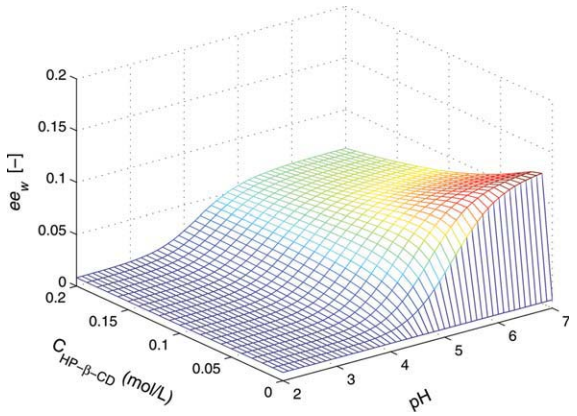

Figure 9. Calculated enantiomeric excess (ee) for OBN as a function of pH and HP-β-CD concentration.

C_{OBN} = 1.0 mmol/L, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

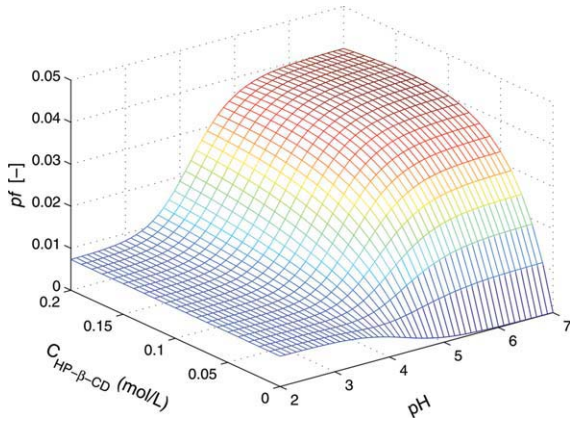

Figure 10. Calculated performance factors as a function of pH and HP-β-CD concentration.

C_{OBN} = 1.0 mmol/L, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed that, with the increase of the concentration of HP-β-CD, the operational enantioselectivity increases rapidly and then increases slightly (in Figure 6b). OBN enantiomers can form complexes with HP-β-CD in aqueous phase. With the increase of HP-β-CD concentration, more complexes are formed in aqueous phase and the distribution ratio consequently increases. Meanwhile HP-β-CD can recognize OBN enantiomers and a relatively higher concentration will enhance the recognition ability. Therefore, enantioselectivity for OBN enantiomers increases with the increase of HP-β-CD concentration.

Influence of Temperature. The influence of temperature on the distribution behavior was investigated in the range of 5–30°C with racemic OBN as the solute. It can be seen from Figure 7 that higher temperature leads to the decrease of distribution ratio and enantioselectivity. A decrease in distribution ratio and enantioselectivities can be explained that the selector-enantiomer interaction weakens with rising temperature and the discrimination ability of the selectors for OBN enantiomers weakens as well, which indicates the chiral extraction is carried out by the formation of two inclusion complexes between OBN enantiomers and HP-β-CD. In addition, the results can be described as fitting very well with the Van't Hoff model, indicating that the complexes do not change in conformation and that enantioselective interactions remain unchanged in the temperature range studied.[41]

Model predictions in the extraction system

Comparison with experimental results indicates the model established is a good means of predicting enantiomer partitioning over a range of experimental conditions and therefore, we used the model to explore the influence of various operating conditions on extraction efficiency in a single stage.

Figures 8a, b, and c show the distribution ratio and enantioselectivity for OBN enantiomers as a function of pH and HP-β-CD concentration, respectively. It can be observed from Figures 8a, b that k_R and k_S follow a similar tendency with the change of pH and HP-β-CD concentration. The decrease of pH and increase of HP-β-CD concentration can lead to the increase of distribution coefficient for the two enantiomers. As shown in Figure 8c, high enantioselectivity is obtained at high pH and HP-β-CD concentration.

Figure 9 shows the enantiomeric excess (ee) for OBN enantiomers in aqueous phase as a function of pH and HP-β-CD concentration. The ee is relatively high at conditions in which pH value is high (>5) but the HP-β-CD concentration is not very high (<0.1 mol/L), which indicates that the increase of HP-β-CD concentration can lead to an increase of enantioselectivity but not always lead to an increase of enantiomeric excess (ee) value.

The opposing trends of the enantiomeric excess and enantioselectivity make it difficult to use Figures 8c and 9 to identify optimal solution conditions for enantiomer resolution. To facilitate optimization of reactive extraction systems, performance factor, pf_i, is introduced, which is defined as the product of the ee in the aqueous phase and the fraction of enantiomer extracted into the aqueous phase. A high performance factor indicates conditions in which the given enantiomer can be purified to high purity with maximum yield.

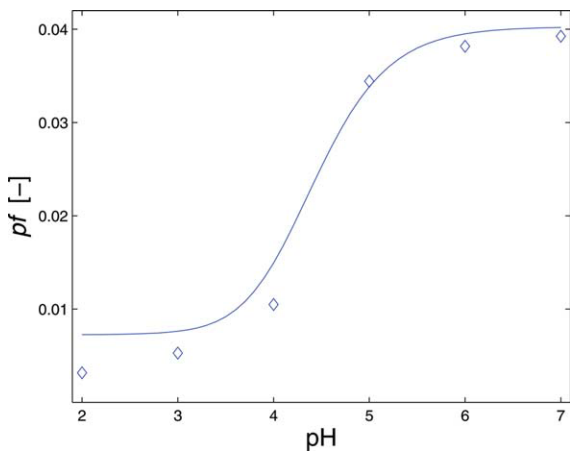

Figure 11. Performance factors as a function of pH.

Solid lines: model predictions. Symbols: experimental data. C_{OBN} = 1.0 mmol/L, $C_{HP-\beta-CD}$ = 0.1 mol/L, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

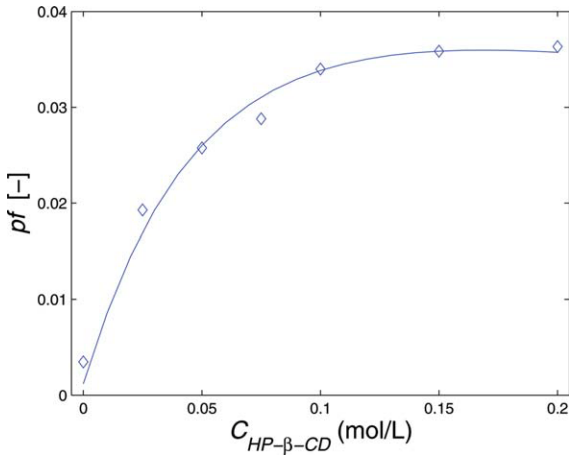

Figure 12. Performance factors as a function of HP-β-CD concentration.

Solid lines: model predictions. Symbols: experimental data. C_{OBN} = 1.0 mmol/L, pH = 5, temperature 5°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The performance factors calculated as a function of pH and HP-β-CD concentration are shown in Figure 10. Figure 10 shows that the performance factor is strongly influenced by pH and HP-β-CD concentration. Relatively high pf_i will be obtained at relatively high pH and HP-β-CD concentration. And there is a plateau in which pH value is above 5 and HP-β-CD concentration is more than 0.1 mol/L. Thus the optimal conditions for reactive extraction of OBN enantiomers will be accomplished for pH of 5 and HP-β-CD concentration of 0.1 mol/L at 5°C.

Experimental performance factors were measured to support the model predictions at solution conditions explored in Figure 10. The experimental values and predictions are plotted in Figures 11 and 12 as a function of pH and HP-β-CD concentration, respectively. It is shown from Figures 11 and 12 that the model predicts experimental results accurately, including the plateaus shown in Figure 10. It is observed from Figure 11 that the performance factors increase with the increase of pH and reach a plateau at pH of 5. It is also observed from Figure 12 that the performance factors increase with the increase of HP-β-CD concentration and reach a plateau at HP-β-CD concentration of 0.1 mol/L. Therefore, these results validate the multiple-chemical equilibria model and its application for extraction system optimization.

Conclusions

Reactive extraction has been carried out for the chiral separation of OBN enantiomers by hydrophilic β-CD derivatives. Experimental results show that the efficiency of extraction is strongly influenced by the process variables such as types of organic solvents and β-CD derivatives, concentration of selector, pH and temperature. Dichloromethane is selected as the suitable solvent and HP-β-CD as the best hydrophilic selector for chiral separation of OBN enantiomers.

A homogeneous aqueous phase complexation model has been developed for reactive extraction of *R*,*S*-OBN with β-CD, involving physical distribution of molecular and ionic OBN, dissociation of ionic OBN and complexation between HP-β-CD and OBN. The experimental data were modeled according the extraction model, and excellent agreement between the model predictions and experimental data was observed. The performance of the extraction process was evaluated using distribution ratio, enantioselectivity, the enantiomeric excess and performance factor to establish the optimum extraction conditions. The best conditions identified involve the use of HP-β-CD concentration of 0.1 mol/L and pH value of 5 at 5°C. The optimal operational enantioselectivity of 1.26 is close to the theoretical maximum of 1.32 and the pf_i is up to 0.036. The presented data indicate that the model is a powerful tool for calculating enantiomer distribution ratios and extraction efficiencies in two-phase chiral extraction systems and full separation of racemic OBN can be carried out by multistage extraction.

Acknowledgments

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Notation

OBN = oxybutynin
CD = HP-β-CD (hydroxypropyl-β-cyclodextrin)
P_0 = physical distribution coefficient for molecular OBN, dimensionless
P_i = physical distribution coefficient for ionic OBN, dimensionless
P_{app} = apparent partition coefficients, dimensionless
k = distribution ratio, dimensionless
K = complexation constants
R = *R*-oxybutynin
S = *S*-oxybutynin
ee = enantiomeric excess, dimensionless
pf_i = performance factor, dimensionless

Greek letters

Φ_i = fraction of the solute i extracted into the aqueous phase, dimensionless
α = enantiomer selectivity, dimensionless

Subscripts

w = aqueous phase
o = organic phase
int = intrinsic value
op = operational value
0 = initial value

Literature Cited

1. De Camp WH. The FDA perspective on the development of stereoisomers. *Chirality*. 1989;1:2–6.
2. Hutt AJ. Drug chirality: impact on pharmaceutical regulation. *Chirality*. 1991;3:161–164.
3. Gourlay MD, Kendrick J, Leusen FJJ. Predicting the spontaneous chiral resolution by crystallization of a pair of flexible nitroxide radicals. *Cryst Growth Des*. 2008;8:2899–2905.
4. Ward TJ, Baker BA. Chiral separations. *Anal Chem*. 2008;80:4363–4372.
5. Afonso CAM, Crespo JG. Recent advances in chiral resolution through membrane-based approaches. *Angew Chem Int Ed*. 2004;43:5293–5295.
6. Prelog V, Kovakevic M, Egli M. Lipophilic tartaric acid esters as enantioselective ionophores. *Angew Chem Int Ed Engl*. 1989;28:1147–1152.
7. Keurentjes JTF, Nabuurs LWM, Vegter EA. Liquid membrane technology for the separation of racemic mixtures. *J Membr Sci*. 1996;113:351–360.
8. Tang KW, Yi JM, Liu YB, Jiang XY, Pan Y. Enantioselective separation of *R*,*S*-phenylsuccinic acid by biphasic recognition chiral extraction. *Chem Eng Sci*. 2009;64:4081–4088.

9. Tang KW, Song LT, Liu YB, Jiang XY, Pan Y. Separation of flurbiprofen enantiomers by biphasic recognition chiral extraction. *Chem Eng J*. 2010;158:411–417.
10. Tan B, Luo GS, Wang JD. Extractive separation of amino acid enantiomers with co-extractants of tartaric acid derivative and Aliquat-336. *Sep Purif Technol*. 2007;53:330–336.
11. Jiao FP, Chen XQ, Hu WG, Ning FR, Huang KL. Enantioselective extraction of mandelic acid enantiomers by L-dipentyl tartrate and beta-cyclodextrin as binary chiral selectors. *Chem Pap* 2007;61:326–328.
12. Colera M, Costero AM, Gaviña P, Gil, S. Synthesis of chiral 18-crown-6 ethers containing lipophilic chains and their enantiomeric recognition of chiral ammonium picrates. *Tetrahedron: Asymmetry*. 2005;16:2673–2679.
13. Pietraszkiewicz M, Koźbia M, Pietraszkiewicz O. Chiral discrimination of amino acids and their potassium or sodium salts by optically active crown ether derived from *D*-mannose. *J Membr Sci*. 1998;138:109–113.
14. Steensma M, Kuipers NJM, de Haan AB, Kwant G. Identification of enantioselective extractants for chiral separation of amines and aminoalcohols. *Chirality*. 2006;18:314–328.
15. Steensma M, Kuipers NJM, de Haan AB, Kwant G. Modelling and experimental evaluation of reaction kinetics in reactive extraction for chiral separation of amines, amino acids and aminoalcohols. *Chem Eng Sci*. 2007;62:1395–1407.
16. Steensma M, Kuipers NJM, de Haan AB, Kwant G. Analysis and optimization of enantioselective extraction in a multi-product environment with a multistage equilibrium model. *Chem Eng Proc*. 2007;46:996–1005.
17. Hallett AJ, Kwant GJ, de Vries JG. Continuous separation of racemic 3,5-dinitrobenzoyl-amino acids in a centrifugal contact separator with the aid of cinchona-based chiral host compounds. *Chem Eur J*. 2009;15:2111–2120.
18. Schuur B, Winkelman JGM, de Vries JG, Heeres HJ. 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*. 2010;65:4682–4690.
19. Schuur B, Floure J, Hallett AJ, Winkelman JGM, de Vries JG, Heeres HJ. Continuous chiral separation of amino acid derivatives by enantioselective liquid-liquid extraction in centrifugal contactor separators. *Org Process Res Dev*. 2008;12:950–955.
20. Schuur B, Hallett AJ, Winkelman JGM, de Vries JG, Heeres HJ. Scalable enantioseparation of amino acid derivatives using continuous liquid-liquid Extraction in a cascade of centrifugal contactor separators. *Org Process Res Dev*. 2009;13:911–914.
21. Dimitrova P, Bart HJ. Extraction of amino acid enantiomers with microemulsions. *Chem Eng Technol*. 2009;32:1527–1534.
22. Kocabas E, Karakucuk A, Sirit A, Yilmaz M. Synthesis of new chiral calix[4]arene diamide derivatives for liquid phase extraction of α-amino acid methylesters. *Tetrahedron: Asymmetry*. 2006;17:1514–1520.
23. Tang L, Choi S, Nandhakumar R, Park H, Chung H, Chin J, Kim KM. Reactive extraction of enantiomers of 1,2-amino alcohols via stereoselective thermodynamic and kinetic processes. *J Org Chem*. 2008;73:5996–5999.
24. Verkuijl BJV, Minnaard AJ, de Vries JG, Feringa BL. Chiral separation of underivatized amino acids by reactive extraction with palladium-BINAP complexes. *J Org Chem*. 2009;74:6526–6533.
25. Verkuijl BJV, Schuur B, Minnaard AJ, de Vries JG, Feringa BL. Chiral separation of substituted phenylalanine analogues using chiral palladium phosphine complexes with enantioselective liquid–liquid extraction. *Org Biomol Chem*. 2010;8:3045–3054.
26. Koska J, Haynes CA. Modelling multiple chemical equilbria in chiral partition systems. *Chem Eng Sci*. 2001;56:5853–5864.
27. Viegas RMC, Afonso CAM, Crespo JG, Coelhoso IM. Modelling of the enantio-selective extraction of propranolol in a biphasic system. *Sep Purif Technol*. 2007;53:224–234.
28. Steensma M, Kuipers NJM, de Haan AB, Kwant G. Influence of process parameters on extraction equilibria for the chiral separation of amines and amino-alcohols with a chiral crown ether. *J Chem Technol Biotechnol*. 2006;81:588–597.
29. Schuur B, Winkelmam JGM, Heeres HJ. Equilibrium studies on enantioselective liquid-liquid amino acid extraction using a cinchona alkaloid extractant. *Ind Eng Chem Res*. 2008;47:10027–10033.
30. Donze C, Coleman AW. β-CD inclusion complexes: Relative selectivity of terpene and aromatic guest molecules studied by competitive inclusion experiments. *J Incl Phenom Macrocycl Chem*. 1993;16:1–15.
31. Rekharsky MV, Inoue Y. Complexation thermodynamics of cyclodextrins. *Chem Rev*. 1998;98:1875–1918.
32. Liu Y, You CC, Wada T, Inoue Y. Molecular recognition studies on supramolecular systems. 22. Size, shape, and chiral recognition of aliphatic alcohols by organoselenium-modified cyclodextrins. *J Org Chem*. 1999;64:3630–3634.
33. Zhou S, Ouyang J, Baeyens WRG, Zhao H, Yang Y. Chiral separation of four fluoroquinolone compounds using capillary electrophoresis with hydroxypropyl-β-cyclodextrin as chiral selector. *J Chromatogr A*. 2006;1130:296–301.
34. Bechet I, Paques P, Fillet M, Hubert P, Crommen, J. Chiral separation of basic drugs by capillary zone electrophoresis with cyclodextrin additives *Electrophoresis*. 1994;15:818–823.
35. Ameyibor E, Stewart JT. Enantiomeric HPLC separation of selected chiral drugs using native and derivatized β-cyclodextrins as chiral mobile phase additives. *J Liq Chromatogr Relat Technol*. 1997;20:855–869.
36. Meindersma GW, van Schoonhoven T, Kuzmanovic B, de Haan AB. Extraction of toluene, *o*-xylene from heptane and benzyl alcohol from toluene with aqueous cyclodextrins. *Chem Eng Process*. 2006;45:175–183.
37. Recuero V, Ferrero M, Goror-Fernández V, Brieva R, Gotor V. Enzymatic resolution of hindered cyanohydrins, key precursors of muscarinic receptor antagonists. *Tetrahedron: Asymmetry*. 2007;18:994–1002.
38. Walker TA. The ciral separation of oxybutnin enantiomers using an ovomucoid column. *J Liq Chromatogr Relat Technol*. 2000;23:841–853.
39. Miyamoto E, Demizu Y, Murata Y, Yamada Y, Kawashima S, Kontani H, Sakai T. High-performance liquid chromatographic preparation of oxybutynin enantiomers on a chiral stationary phase. *J Chromatogr A*. 1993;653:135–137.
40. Higuchi T, Connors KA. Phase solubility techniques. *Adv Anal Chem Instr*. 1965;4:117–212.
41. O'Brien T, Crocker L, Thompson R, Thompson K, Toma PH, Conlon DA, Feibush B, Moeder C, Bicker G, Grinberg N. Mechanistic aspects of chiral discrimination on modified cellulose. *Anal Chem*. 1997;69:1999–2007.

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