Enantioselective Partitioning of Racemic Ibuprofen in a Biphasic Recognition Chiral Extraction System

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Enantioselective partitioning of ibuprofen enantiomers in a biphasic recognition chiral extraction system was studied. A combination of hydrophobic *L*-isobutyl tartrate in organic phase and hydrophilic *β*-cyclodextrin derivative in aqueous phase is necessary to establish a biphasic recognition chiral extraction system. The studies performed involve an enantioselective extraction in a biphasic system, where ibuprofen enantiomers form four complexes with the *β*-cyclodextrin derivative in aqueous phase and the *D*(*L*)-isobutyl tartrate in organic phase, respectively. In these biphasic resolutions, the types and the concentrations of the extractants, pH and temperature all exert a considerable influence on the biphasic recognition process. Good enantioselectivities for ibuprofen enantiomers were obtained at pH≤2.5 and a ratio of 2∶1 of [*L*-isobutyl tartrate] to [HP-*β*-CD]. Biphasic recognition chiral extraction is of strong chiral separation ability, and may be very helpful to optimize the extraction systems and realize the large-scale production of enantiomers.

Keywords *L*-isobutyl tartrate, hydroxypropyl-*β*-cyclodextrin, ibuprofen enantiomer, biphasic recognition chiral extraction

Introduction

Development of new chiral technologies continues to be a very active field of research in the chemical industry.[1] Preparative separation of racemic compounds is an important method of producing single enantiomer drugs.[2,3] Many researchers have attempted the separation of optically active compounds.[4-13] Such chiral separation technologies as crystallization, chromatography, kinetic resolution, *etc*. accelerate researches about chiral compounds, but there still exist some defects for most racemic compounds. Membrane-based approaches will most certainly become very important for continuous operation, but at the moment still suffer from being generally less enantioselective.[14-16]

As a potential large-scale production technique, chiral solvent extraction has attracted the attention of many of researchers to make great efforts in recent years.[4-10] Enantioselectivity (α) is the most important parameter for chiral extraction. For example, for a 99% pure product (R/S=100) about 190 NTU (number of transfer units) are required for an enantioselectivity α of 1.05, but the number decreases to approximately 30 when α increases to a value of 1.20.[17] There are several normal chiral extractants, such as tartaric acid derivatives,[17-19] crown ethers,[20] cholestery *L*-glutamate[21] and so on.[7] However, the enantioselectivities of the chiral extractants are somewhat low, and a large number of transfer units are required in chiral solvent extraction process. Until now, few new types of extractants have been tested to separate enantiomers. To look for new extraction techniques with high enantioselectivity will speed up the application of chiral solvent extraction, and realize large-scale production with low energy cost. More recently, the chiral ligand-exchange concept has been applied to liquid-liquid extraction technology and obtained high enantioselectivities holding advantages over chiral ligand-exchange chromatography for large-scale applications.[5] Tartaric acid derivatives and cyclodextrins are normal selectors for separation of enantiomers. Cyclodextrins have been used for chiral recognition in liquid system,[22] and for extraction of toluene, *o*-xylene from heptane and benzyl alcohol from toluene.[23] Enantioselectivities for some enantiomers have been improved by biphasic recognition chiral extraction (BRCE) in our recent work.[24,25]

This work presents more extensive results of separation of ibuprofen (HA) enantiomers by biphasic recognition chiral extraction, which is commonly used as non-steroidal anti-inflammatory drugs (shown in Figure

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1). Recent studies show that *S*-ibuprofen is the active form both *in vitro* and *in vivo* and is 28 times more physiologically potent than *R*-enantiomer, while *R*-ibuprofen can cause gastrointestinal toxicity, water sodium retention, kidney perfusion and increase the allergic reactions and other side effects. Factors affecting the extraction efficiency were investigated, namely the influence of the types of *β*-CD derivatives, isobutyl tartrate configurations, the concentrations of the extractants, pH and temperature.

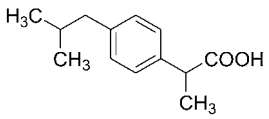

Figure 1 Molecular structure of ibuprofen.

Experimental

Materials

The aqueous phase was prepared by dissolving ibuprofen enantiomers (HA) (Zhejiang Juhua Co., Ltd., Zhejiang, China) in 0.1 mol/L NaH_2PO_4/H_3PO_4 buffer solution containing *β*-cyclodextrin derivative. Hydroxypropyl-*β*-cyclodextrin (HP-*β*-CD), hydroxyethyl-*β*-cyclodextrin (HE-*β*-CD), and methyl-*β*-cyclodextrin (Me-*β*-CD) were bought from Xinda Fine Chemical Co. Inc. (Shandong, China). The organic phase was a solution of *D*(*L*)-tartaric acid esters in 1,2-dichloroethane. *D*(*L*)-isobutyl tartrate esters were synthesized as described in the literature,[26] from *D*- and *L*-tartaric acid with a purity＞99.85% (Shanghai Xinpu Chemical Factory, Shanghai, China). All other chemicals are of analytical-reagent grade.

Analytical method

The quantification of HA enantiomers in the aqueous phase was performed by HPLC using a UV detector (Merck, Hitachi, Japan) at a wavelength of 254 nm. The column was chiralcel OJ-RH, with 5 μm particle size of the packing material, 150 mm×4.6 mm i.d. (Hanbon Science & Technology Co., Ltd.). The mobile phase was 0.5 mol/L sodium perchlorate buffer solution (pH＝2.0)/acetonitrile (*V*∶*V*＝65∶35) at a flow rate of 0.5 mL/min. The pH of the aqueous phases was measured with a pH meter (Orion, model 720A, USA). The retention time of *R*-enantiomer is less than that of *S*-enantiomer (Figure 2).

Extraction method

The extraction experiments were performed in 25 mL glass-stoppered tubes. 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 (24 h) at a fixed temperature until the distribution behavior achieved equilibrium. The mixing time was sufficient to reach the equilibrium state. The

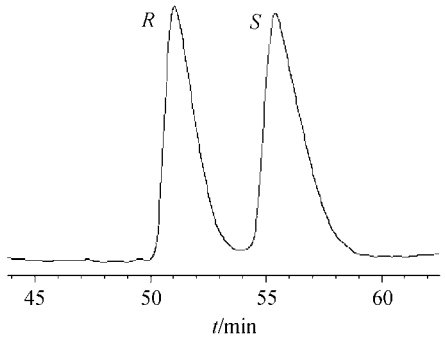

Figure 2 Chromatogram of ibuprofen enantiomers in the aqueous phase.

sample obtained from aqueous phase was analyzed by HPLC. Each experiment was duplicated under identical conditions. Owing to that the change of volume is very small and can be neglected, the concentration of HA in organic phase was calculated by a subtractive method. The distribution ratio of *R*-HA and *S*-HA can be calculated by the following formulas, $k_R = C_{O,R}/C_{W,R}$ and $k_S = C_{O,S}/C_{W,S}$, where $C_{O,R}$ and $C_{W,R}$ represent concentrations of *R*-HA in organic phase and aqueous phase, respectively; $C_{O,S}$ and $C_{W,S}$ represent the concentrations of *S*-HA in organic phase and aqueous phase, respectively. Enantioselectivity $\alpha = k_R/k_S$.

In a BRCE system, hydrophobic *L*-isobutyl tartrate and hydrophilic *β*-cyclodextrin derivative were used as chiral selectors in organic and aqueous phases, respectively, which preferentially recognize *R*-enantiomer and *S*-enantiomer, respectively (in Figure 3). In the biphasic recognition process, the two ibuprofen enantiomers firstly form two diastereomeric complexes with *β*-cyclodextrin derivative in aqueous phase due to such molecular interactions as polarization, induction, electrostatics and hydrogen-bonding interaction. As the two ibuprofen enantiomers are partly soluble in organic phase, the complexation reactions take place in organic phase simultaneously. In the meantime, two dissociation equilibria of the enantiomers exist in aqueous phase.

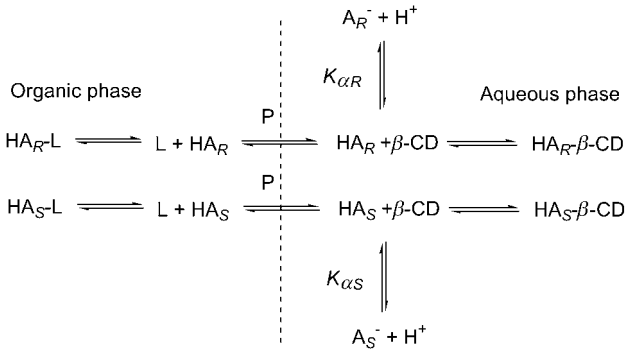

Figure 3 Schematic diagram of the optical resolution of enantiomers by BRCE.

According to the possible mechanism in BRCE for ibuprofen enantiomers, the types of *β*-CD derivatives,

isobutyl tartrate configurations, the concentrations of the extractants, pH and temperature could affect the extraction efficiency.

Results and discussion

Screening of extractants

Distribution ratio and enantioselectivity for ibuprofen enantiomers were investigated in several different chiral extraction systems (Table 1). A number of significant observations may be obtained from Table 1.

It is seen from Table 1 that k_R is always larger than k_S in the extraction systems containing β-CD derivative (HP-β-CD, HE-β-CD or Me-β-CD) in aqueous phase and without tartrate (*L*-isobutyl tartrate or *D*-isobutyl tartrate) in organic phase, which indicates that the three β-CD derivatives preferentially recognize *S*-enantiomer. And HP-β-CD is of the strongest recognition ability among them.

It is also observed that, in BRCE system, α for ibuprofen enantiomers is improved by adding *L*-isobutyl tartrate in organic phase, but it decreases by adding *D*-isobutyl tartrate, indicating that *L*-isobutyl tartrate has stronger recognition ability for *R*-HA than for *S*-HA, and *D*-isobutyl tartrate has reversed recognition ability for them. It is concluded that, in the BRCE system for separation of ibuprofen enantiomers, HP-β-CD and *L*-isobutyl tartrate should be chosen as chiral selectors in the aqueous phase and the organic phase, respectively.

Table 1 k and α of *R*- and *S*-HA in different extraction systems[a]

Extractant	*L*-isobutyl tartrate			*D*-isobutyl tartrate			Non extractant		
	k_R	k_S	α	k_R	k_S	α	k_R	k_S	α
HP-β-CD	5.85	4.39	1.33	6.91	5.81	1.19	4.51	3.76	1.20
Me-β-CD	5.36	4.91	1.09	4.80	5.04	0.95	5.65	5.53	1.02
HE-β-CD	5.33	4.31	1.24	6.45	6.26	1.03	6.60	6.23	1.06

[a] Organic phase: [*L*-isobutyl tartrate]＝0.2 mol/L, [*D*-isobutyl tartrate]＝0.2 mol/L; aqueous phase: [HP-β-CD]＝0.1 mol/L, [HE-β-CD]＝0.1 mol/L, [Me-β-CD]＝0.1 mol/L, [HA]＝10.0 mmol/L, pH 2.5, temperature 5 ℃.

Influence of *L*-isobutyl tartrate concentration

The influence of *L*-isobutyl tartrate concentration on extraction efficiency was studied under condition that the pH value and the concentration of HP-β-CD in the buffer solution were kept constant at 2.5 and 0.1 mol/L, respectively (in Figure 4). When *L*-isobutyl tartrate is not added to the organic phase, HP-β-CD shows the enantioselectivities towards HA enantiomers, but with small distribution ratios. With the increase of *L*-isobutyl tartrate concentration, the distribution ratios are enhanced greatly. Meanwhile, the enantioselectivities all increase before the concentration of *L*-isobutyl tartrate is up to 0.2 mol/L. When the concentration of *L*-isobutyl tartrate is increased further, the distribution ratios continuously increase, while the enantioselectivities follow an opposite tendency. It can be explained by that the larger amount of complex is formed in the organic phase leading to an increase of the distribution ratios, and the enantioselectivities are the results of the cooperation of HP-β-CD and *L*-isobutyl tartrate. It is found that the maximum enantioselectivity is achieved at the ratio of 2∶1 of [*L*-isobutyl tartrate] to [HP-β-CD].

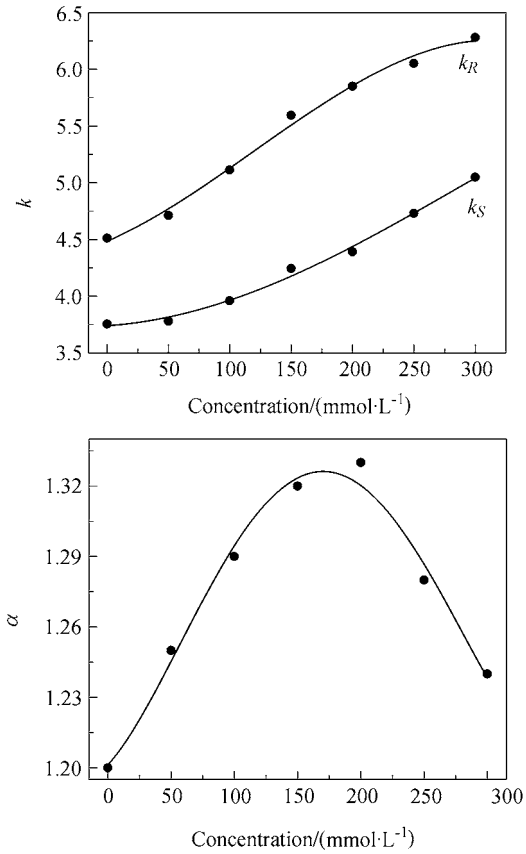

Figure 4 Effect of concention of *L*-isobutyl tartrate on k and α. Aqueous phase: [HP-β-CD]＝0.1 mol/L, [HA]＝10.0 mmol/L, pH 2.5, temperature 5 ℃.

Influence of HP-β-CD concentration

Figure 5 shows the influence of HP-β-CD concentration on distribution behavior of HA enantiomers in a BRCE system. As expected, the following significant conclusions can be seen in this study: (1) the distribution ratios remarkably decrease with the increase of HP-β-CD concentration. (2) the enantioselectivities all increase remarkably before the concentration of HP-β-CD is up to 0.1 mol/L. (3) the distribution ratios and enantioselectivities continuously decrease with a further increase in the concentration of HP-β-CD. (4) the enantioselectivity reaches the maximum at the ratio of 2∶1 of [*L*-isobutyl tartrate] to [HP-β-CD]. It also can be observed that HP-β-CD not only improves the solubility of HA in a buffer solution, but also enhance enantioselectivities of HA enantiomers in a BRCE system. These phenomena can be explained by the differential inclusion of the two enantiomers and the differential behaviour of the two inclusion complexes formed be-

tween the HP-β-CD and the two enantiomers. A good separation was also obtained at the ratio of 2∶1 of [*L*-isobutyl tartrate] to [HP-β-CD], which is in accordance with the above results.

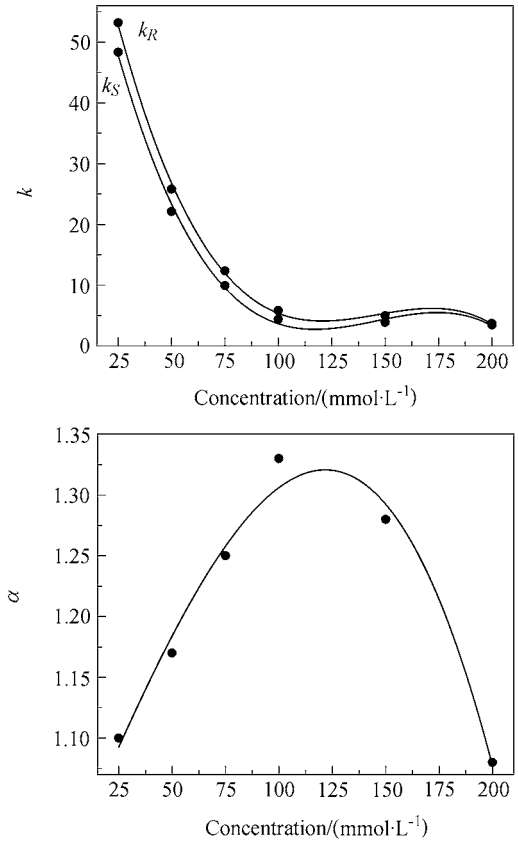

Figure 5 Effect of HP-β-CD concentration on k and α. Organic phase: [*L*-isobutyl tartrate]=0.2 mol/L, [HA]=10.0 mmol/L, pH 2.5, temperature 5 ℃.

Influence of pH

Each ibuprofen enantiomer has one carboxylic group and one aromatic group. One dissociation equilibrium exists in aqueous solutions:

$$HA \xleftrightarrow{K_a} A^- + H^- \quad (1)$$

The dissociation constant for Eq. 1 can be described by

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad (2)$$

where HA and A^- are neutral molecule and anion of *R*(*S*)-ibuprofen, respectively. In aqueous solution, HA exists in two states of neutral molecule and anion.

Therefore, there exists influence of pH on distribution behavior of HA enantiomers in a BRCE system. To better understand the effect of pH on the distribution behavior, distribution ratios and enantioselectivities were studied in the BRCE systems with 0.2 mol/L *L*-isobutyl tartrate in 1,2-dichloroethane solution and 0.1 mol/L HP-β-CD in 0.1 mol/L NaH_2PO_4/H_3PO_4 buffer solution at different pH values (Figure 6). It is seen from Figure 6 that the influence of pH on distribution behavior is notable, and the distribution ratios and enantioselectivities all greatly decrease with the increase of pH.

The possible reasons for these may be that the amount of ionic HA (A^-) increases with the rise of the pH, but molecular HA decreases with the rise of the pH value. At pH≤2.5, HA mainly exists in molecular state, but at high pH it mainly exists in ionic state. HP-β-CD and *L*-isobutyl tartrate mainly have chiral recognition ability and affinity for molecular HA, but not for ionic HA. Ionic HA only exists in aqueous phase. The concentration of complexes formed by *L*-isobutyl tartrate and enantiomers decreased with the increase of the pH. As a result, k_R, k_S and α greatly decrease with the rise of the pH. So it should be kept at low pH value to carry out the extraction process.

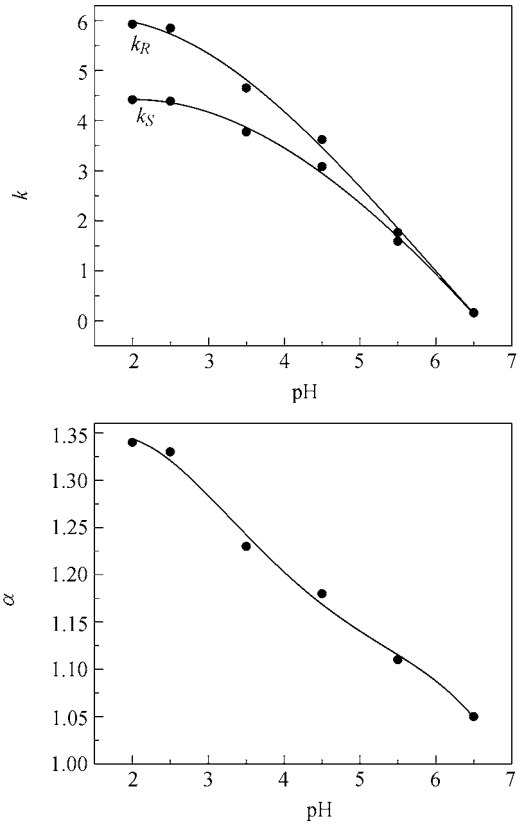

Figure 6 Effect of pH on k and α. Organic phase: [*L*-isobutyl tartrate]=0.2 mol/L, aqueous phase: [HP-β-CD]=0.1 mol/L, [HA]=10.0 mmol/L, temperature 5 ℃.

Influence of temperature

The influence of temperature on the distribution behavior of HA was investigated in the range of 5—30 ℃. A peculiar effect is observed from Table 2 that higher temperature leads to an increase in distribution ratios but a decrease in enantioselectivities. The fact that an increasing distribution ratio is obtained indicates that

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the non-selective physical partitioning is increased with temperature and CD complexation decreased with temperature. A decrease in enantioselectivities can be explained by that the selector-enantiomer interaction weakens with temperature and the discrimination ability of the selectors for HA enantiomers weakens as well.

Figure 7 shows the variations of ln k and ln α versus 1/T. 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.[27,28]

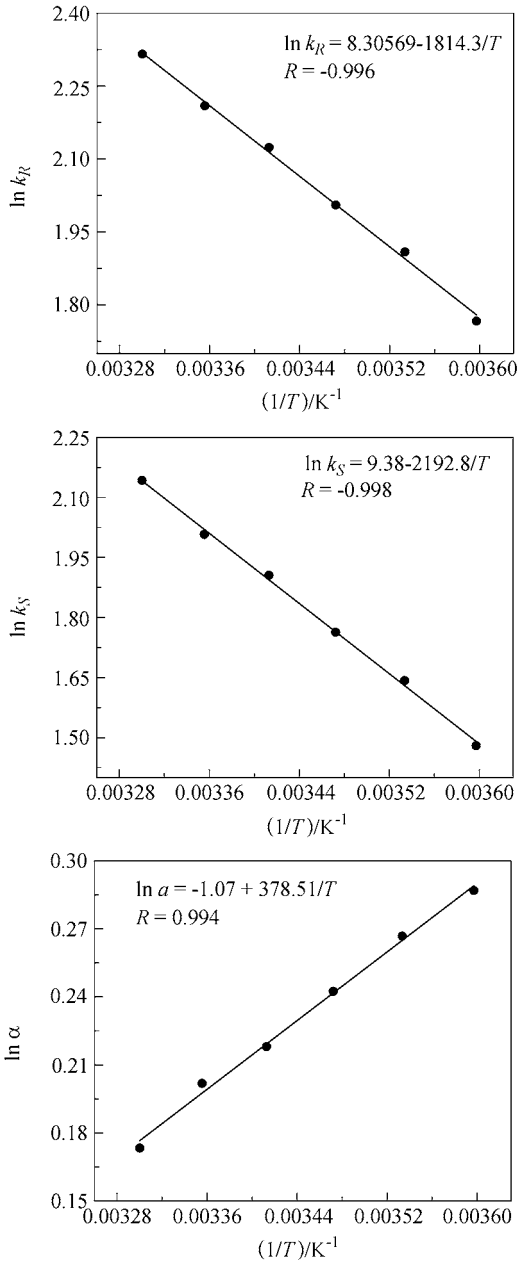

Figure 7 Influence of temperature on k and α. Organic phase: [*L*-isobutyl tartrate]=0.2 mol/L, aqueous phase: [HP-β-CD]=0.1 mol/L, [HA]=10.0 mmol/L, pH 2.5.

Table 2 Influence of temperature on k and α[a]

Temperature/℃	k_R	k_S	α
5	5.85	4.39	1.33
10	6.75	5.17	1.31
15	7.75	5.83	1.33
20	8.36	6.72	1.24
25	9.11	7.44	1.22
30	10.13	8.52	1.19

[a] Organic phase: [*L*-isobutyl tartrate]=0.2 mol/L, aqueous phase: [HP-β-CD]=0.1 mol/L, [HA]=10.0 mmol/L, pH 2.5.

Conclusion

Liquid-liquid extraction proved to be a promising alternative for chiral separation. However, a high enantioselectivity was required in order to optimize the extraction process. Based on our previous work, enantioselective partitioning of racemic ibuprofen was investigated in a BRCE system containing hydrophobic *L*-isobutyl tartrate in organic phase and hydrophilic HP-β-CD in aqueous phase, which could preferentially recognize *R*-HA and *S*-HA, respectively. The influences of such factors as the types of β-CD derivatives, isobutyl tartrate configurations, the concentrations of the extractants, pH and temperature were examined.

It was found that the enantioselectivities in a BRCE system were greatly improved due to the utilization of the cooperations of the separation forces of *L*-isobutyl tartrate and HP-β-CD. HP-β-CD has the strongest recognition ability among HP-β-CD, HE-β-CD, and Me-β-CD. A high enantioseparation efficiency was obtained at low pH and a ratio of 2∶1 of [*L*-isobutyl tartrate] to [HP-β-CD].

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