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Cui, X, Ding, Q, Shan, R-N et al. (2 more authors) (2019) Enantioseparation of flurbiprofen enantiomers using chiral ionic liquids by liquid-liquid extraction. Chirality, 31 (6). pp. 457-467. ISSN 0899-0042

https://doi.org/10.1002/chir.23071

Enantioseparation of Flurbiprofen Enantiomers Using Chiral Ionic Liquids by Liquid-Liquid Extraction

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Abstract

Flurbiprofen is a kind of non-steroidal anti-inflammatory drug which has been widely used in clinic for treatment of rheumatoid arthritis and osteoarthritis. It has been reported that S-flurbiprofen shows good performance on clinic anti-inflammatory treatment, while R-enantiomer almost has no pharmacological activities. It has important practical values to obtain optically pure S-flurbiprofen. In this work, chiral ionic liquids which have good structural designability and chiral recognize ability were selected as the extraction selector by the assistance of quantum chemistry calculations. The distribution behaviors of flurbiprofen enantiomers were investigated in the extraction system, which composed of organic solvent and aqueous phase containing chiral ionic liquid. The results show that maximum enantioselectivity up to 1.20 was attained at pH 2.0, 25 ℃ using 1,2-dichloroethane as organic solvent, 1-butyl-3-methylimidazole L-tryptophan ([Bmim][L-trp]) as chiral selector. The racemic flurbiprofen initial concentration was 0.2 mmol·L^{-1} and [Bmim][L-trp] concentration was 0.02 mol·L^{-1}. Furthermore, the recycle of chiral ionic liquids has been achieved by reverse extraction process of the aqueous phase with chiral selector, which is significant for industrial application of chiral ionic liquids and scale-up of

Key word: flurbiprofen; chiral separation; liquid-liquid extraction; amino acid ionic liquid; reverse extraction

1. Introduction

Flurbiprofen, namely (±)-2-(2-fluoro-4-biphenyl)-propionic acid, is a commonly used non-steroidal anti-inflammatory drug in clinic. It is one of the most effective drugs in this kind, better than aspirin and ibuprofen in anti-inflammatory and analgesic effects. It is mainly used for the treatment of rheumatoid arthritis, osteoarthritis and other diseases as a chiral compound (Figure 1).[1,2] Its S enantiomer has less side effects, smaller dosage requirement and higher pharmacological activity compared with its racemate, while its R enantiomer has little therapeutic effect and presents some toxic side effects to the human body.[3,4] In recent research, it has been found that the R enantiomer has a certain therapeutic effect on Helmer's disease, inhibiting tumor growth and reduction of neuropathic pain.[5-11] However, at present, flurbiprofen is mainly produced and used as racemate. So it is significant to obtain pure enantiomers of flurbiprofen.

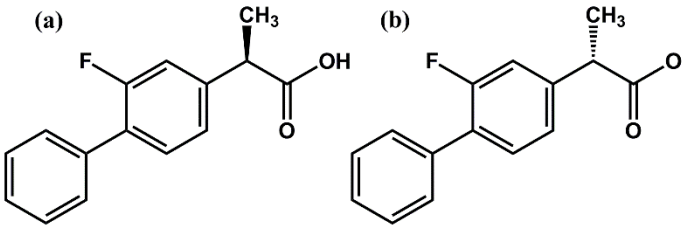

FIGURE 1 The chemical structures of flurbiprofen enantiomers. (a) S-flurbiprofen, (b) R-flurbiprofen.

Now, the artificial monomeric enantiomers of chiral drug are mainly obtained through asymmetric chemical synthesis and racemic mixture resolution. Asymmetric chemical synthesis usually requires more complex synthetic process and higher cost, which is not conducive to industrial production.[12-14] Nevertheless, the resolution of the racemate is relatively simple and it is possible to obtain two enantiomers simultaneously, which need to be considered.[15,16] Most of the resolution methods, such as crystallization,[17-19] kinetic resolution method,[20-22] chromatographic resolution method,[23] membrane separation method,[24] etc., are difficult to achieve industrialization due to low capacity and narrow application range. Chromatography and electrochemical methods are mainly used for the analysis of enantiomers, currently.[25-27] However, chiral liquid-liquid extraction which applies the liquid-liquid extraction technology to racemate separation is the most prospective method to scale-up because of low energy consumption, simple equipment requirements and mild operating conditions.[16,28-33] Tang et al.[34] reported enantioselective partitioning of flurbiprofen enantiomers in a biphasic recognition chiral extraction (BRCE) system combining a hydrophobic L-tartrate in organic phase and hydrophilic-cyclodextrin derivative in aqueous phase, which preferentially recognize R-enantiomer and S-enantiomer, respectively. In these biphasic resolutions, the influence of the concentrations of the extractants and flurbiprofen enantiomers, the types of organic solvents and extractants, pH and temperature on the biphasic recognition process have been investigated and the enantioselectivity is 1.24 in optimized condition at 25 ℃. Chen et al.[35] also developed a method using aqueous two-phase extraction (ATPE) coupled with biphasic recognition chiral extraction to separate flurbiprofen by L-dioctyl tartrate and L-tryptophan and achieved good separation effect. But because of the more complex separation system, longer phase separation time and the use of a certain amount of salts ((NH_4SO_4)) as addictive, the extraction process is not beneficial to subsequent drug separation and the recycled utilization of extractants, which should be mainly considered and improved.

In general, the selection of high enantioselectivity chiral selector is the key step of the flurbiprofen enantiomer resolution technology by liquid-liquid extraction.[36] Ionic liquids which are composed of organic cations and inorganic or organic anions, have been extensively applied to electrochemical, chemical catalysis, gas absorption, extraction separation and other aspects recently, based on their negligible vapor pressure, good thermal stability, and adjustable structure.[37-39] Chiral ionic liquid (CIL) which has chiral center in the molecular structure, with the general characteristics of chiral molecules, is an important subclass of ionic liquids with good chiral recognition ability. Recently, the related studies about chiral ionic liquids applied in

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chemical separation process have sustained grown, especially in enantioseparation extraction aspect.[30,40-44] So, it is expected that the introduction of the chiral ionic liquid as a chiral selector into the enantioselective liquid-liquid extraction system of flurbiprofen can achieve a better separation effect.

Quantum chemistry calculations has been proved to be a useful tool to investigate the structure-effective relationship, analyze the interaction relation and further predict the distribution behaviors of ionic liquids in the extraction separation process. Recently, we have successfully applied the quantum chemistry calculations for the guidance of chiral ionic liquids selection in the racemic amlodipine extraction system. The extraction system was consistent with the simulation calculation predictions and obtained favorable separation effect.[30] Based on this work, the preliminary prediction before the extraction experiment for the optical isomer separation systems by the quantum chemistry calculations is feasible and simple, which should be considered for the flurbiprofen extraction process.

In this work, the distribution behavior of flurbiprofen enantiomers is investigated in the chiral liquid-liquid extraction system, which is composed of organic solvent and the methanol aqueous solution containing chiral ionic liquid as an aqueous chiral extractant. The influence of chiral ionic liquid type was first studied with quantum chemistry calculation method by Gauss 16 (Gaussian) software and further verified by extraction experiments, subsequently. The influences of the organic solvent, the concentration of chiral extractant, the concentration of drug enantiomers, the pH of aqueous phase and the temperature on enantioseparation were also optimized. Moreover, a reverse extraction process was also carried out to separate and recover the aqueous phase with chiral ionic liquid, which is conductive to industrial application of chiral ionic liquids.

2. Materials and Methods

2.1 Materials

Flurbifropfen (racemate, purity > 99%) was purchased from J&K Chemical Co. Ltd. (Beijing, China). The standard reference sample of flurbifropfen (racemate, purity > 99.8%) was purchased from National Institutes for Food and Drug Control (Beijing, China). The R-flurbifropfen enantiomer standard sample (purity > 98%) was purchased from J&K Chemical Co. Ltd. (Beijing, China). 1-Butyl-3-methylimidazolium L-tryptophan ([BMIM][L-trp], 97%), 1-butyl-3-methylimidazolium L-phenylalanine ([BMIM][L-phe], 97%), 1-butyl-3-methylimidazolium L-serinate ([BMIM][L-ser], 97%), 1-butyl-3-methylimidazolium L-glutamate ([BMIM][L-glu], 97%) was purchased from Chengjie Chemical Co. Ltd (Shanghai, China). The molecular structures of the above chiral ionic liquids are displayed in Figure 2. 1,2-Dichloroethane (AR, 99%), dichloromethane (AR, 99.5%), methanol (HPLC grade, 99.5%) was purchased from Sinopharm Chemical Reagent Co. Ltd (China). n-Octanol (AR, 99%), n-decanol (AR, 98%), ethanol (HPLC grade, 99.8%), phosphoric acid (H_3PO_4, AR, 85%), sodium dihydrogenphosphate (NaH_2PO_4, AR, 99%), disodium hydrogenphosphate (Na_2HPO_4, AR, 99%), trifluoroacetic acid (TFA, HPLC grade, 99.5%) was purchased from Aladdin Reagent Co. Ltd (Shanghai, China). N-hexane(HPLC grade, 95%) was purchased from Sigma-Aldrich Chemical Co. Ltd. $H_3PO_4/NaH_2PO_4/Na_2HPO_4$ buffer solutions in different pH ranges were prepared by mixing of sodium dihydrogenphosphate, disodium hydrogenphosphate and phosphoric acid solutions (both 0.3 mol/L) in proper proportions, and the pH measurements were performed with a pHs-3C digital pH-meter (LeiCi, Shanghai, China).

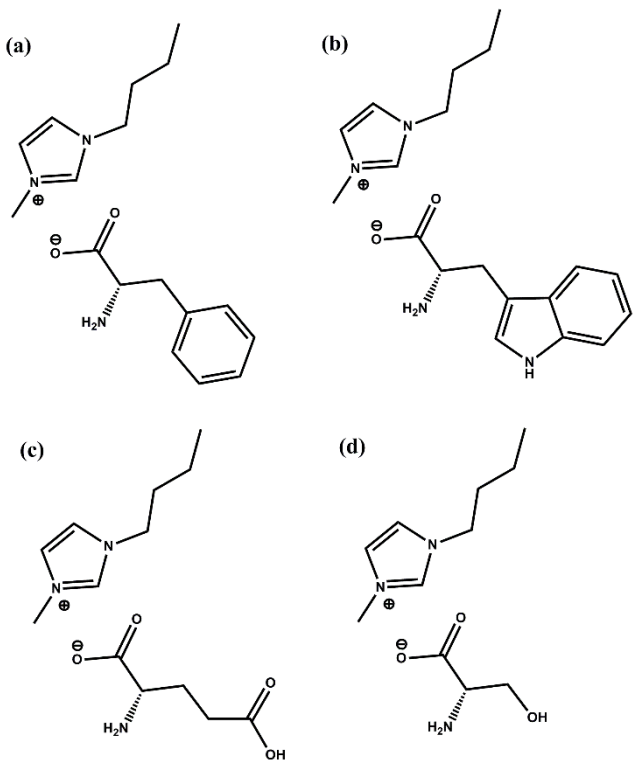

FIGURE 2 The molecular structures of chiral ionic liquids used in this work. (a) [BMIM][L-trp], (b) [BMIM][L-phe], (c) [BMIM][L-glu], (d) [BMIM][L-ser]

2.2 Extraction Experiments

Extraction process

Chiral amino ionic liquid and flurbiprofen racemate dissolved in a certain pH 0.3 mol/L phosphate salt buffer solution. Due to poor solubility of flurbiprofen in water, 10% (v/v) methanol was added to improve its solubility in aqueous phase and it has been reported that the addition of alcohol has no influence on ibuprofen drugs.[45,46] The aqueous phase was mixed with an equal volume of pure organic solvent as the organic phase and shaken for two hours until the phase equilibrium in a water bath at a fixed temperature. After completing phase separation, a small amount of organic phase was taken to determine the concentration of flurbiprofen enantiomers by HPLC. The concentration of flurbiprofen in aqueous phase can then be calculated with mass subtraction method.

Reverse extraction process

The extracted aqueous phase is collected, mixed with an equal volume of dichloromethane, and shaken at 25 °C for 2 hours to perform the reverse extraction process until phase equilibrium. After setting for a while and phase separation, the reverse extracted aqueous phase is re-used for extraction step to achieve the recovery of the aqueous phase containing the ionic liquid without further separation and purification. The schematic representation of the experimental procedure is shown in Figure 3.

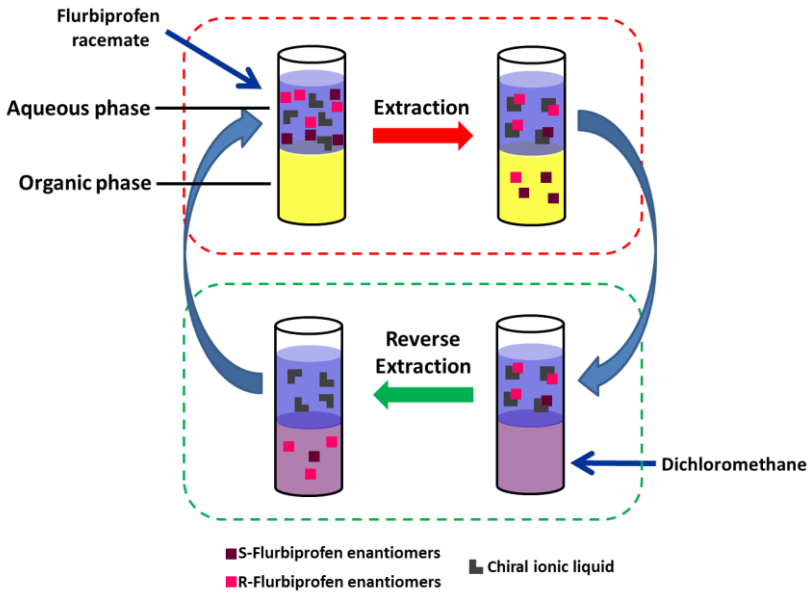

FIGURE 3 Schematic representation of the experimental procedure.

2.3 Analytical Method

The concentration of flurbiprofen enantiomers in organic phase was performed by HPLC using a UV detector (Agilent 1260 Infinity II) at the UV wavelength of 254 nm at 20 °C. The column was CHIRALPAKR IG (5 μm, 4.6 × 250 mm^2). The mobile phase was hexane (containing 0.1% trifluoroacetic acid): ethanol (95:5, v/v) at a flow of 1mL·min^{-1}. The inject volume is 20 μL. The retention time of the R-enantiomer is less than that of the S-enantiomer which was verified by the standard R-flurbiprofen enantiomers and the peaks of R-flurbiprofen and S-flurbiprofen enantiomer were completely separated. The standard curves of both R-flurbiprofen (A = 119.05C+138.68, r = 0.9996, where A (mAu·s) is the peak area and C (μg·mL^{-1}) is the enantiomer concentration) and S-flurbiprofen (A = 156.26C-31.41, r = 0.9992) were used to quantify the enantiomers. The detector responses were linear from 10 μg·mL^{-1} to 200.00 μg·mL^{-1} and the detection limit was 0.06 μg·mL^{-1} for R-flurbiprofen and 0.12 μg·mL^{-1} for S-flurbiprofen. All the extraction processes were repeated at least three times. The standard uncertainties of measurement are $u(T)$ = 0.1 ℃, u(pH) = 0.01. The relative standard uncertainty of enantiomers concentration measurement is u = 0.6%.The reproducibility of distribution coefficient was within 5% and the reproducibility of the calculated enantioselectivity is within ±2%.

2.4 Mechanism

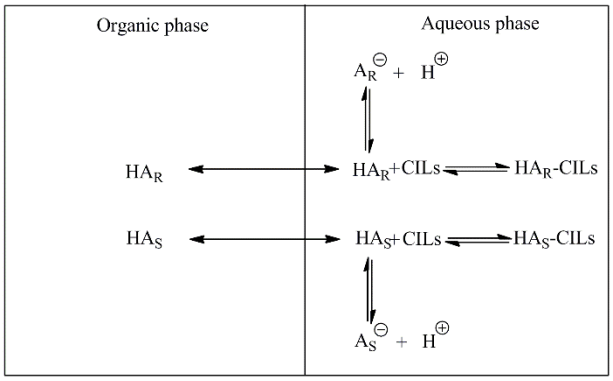

FIGURE 4 The mechanism of flurbiprofen extraction system. HA_R and HA_S represent the R-flurbiprofen and S-flurbiprofen molecules, respectively. A_R^- and A_S^- represent the R-flurbiprofen and S-flurbiprofen ionics, respectively.

The aqueous equilibrium and reaction extraction mechanism is shown in Figure 4. The equilibrium mechanism of the extraction system includes the distribution behaviors of flurbiprofen enantiomer molecules between the two phases, the dissociation equilibrium of flurbiprofen in aqueous phase and the diastereomeric complex equilibrium between the chiral ionic liquids and flurbiprofen enantiomer molecules due to molecular interactions such as hydrogen bond interaction, Van de Waals and electrostatic.

Distribution coefficient (k) and operational enantioselectivity (α) of enantiomers in the equilibrium state are two important parameters to describe the efficiency of the chiral extraction system in this work, which can be calculated from the following equations, respectively.

$$k_S = \frac{C_{S,O}}{C_{S,W}} \tag{1}$$

$$k_R = \frac{C_{R,O}}{C_{R,W}} \tag{2}$$

$$\alpha = \frac{k_S}{k_R} \tag{3}$$

where $C_{S,O}$ and $C_{S,W}$ represent the concentrations of S-flurbiprofen in organic phase and aqueous phase, respectively; $C_{R,O}$ and $C_{R,W}$ represent the concentrations of R-flurbiprofen in organic phase and aqueous phase, respectively; k_S and k_R are the distribution coefficients of S-flurbiprofen and R-flurbiprofen; α is the operational enantioselectivity, while the intrinsic enantioselectivty (α_{int}) is the ratio of the complexation constants, which is the upper limit of α.

3. Result and Discussion

3.1 Quantum chemistry calculation details

Quantum chemistry calculations in this work were performed with Gaussian 16 software. The density functional theory (DFT) and hybrid Becke 3-LeeYang-Parr (B3LYP) exchange-correlation functional with 6–31++G(d,p) basis set were applied to explore the proper geometries without any symmetry constraints. All of the optimizations were performed in vacuo and all optimized initial geometries were locally optimum on the potential energy surface

verified by vibrational frequency calculations. Similar simulation process can be found in our previous work.[30] In brief, firstly, the molecular conformations of S-flurbiprofen, R-flurbiprofen, the anion and cation of chiral ionic liquids were optimized under B3LYP/6-31++G(d,p) level on the basis of the reported conformations[47-49] and selected by their chemical molecular potential energy. Then, the optimization ion pair geometries of chiral ionic liquids were studied by placing the anion and cation in different relative adjacent positions to form different initial geometries, which referred to the electrostatic distribution of optimized individual molecular structure, and optimizing respectively. The geometry with the lowest potential energy was chosen as the optimum conformation. The complexes of chiral ionic liquids and flurbiprofen enantiomers were optimized through similar procedures of ion pairs to obtain the global optimum. [Bmim][L-trp] in this work was chosen as a representative for brief description. The optimized geometries of the diastereomeric complex formed by [Bmim][L-trp] and S-flurbiprofen or R-flurbiprofen at B3LYP/6-31++G(d,p) level are shown in Figure 5. It can be observed that the binding geometries of the two complexes are both affected by C-H…O interactions, distances of which are between 1.5 Å and 3.0 Å, indicating that the chiral recognition ability between [Bmim][L-Trp] and flurbiprofen enantiomers are mainly caused by hydrogen-bonding interactions. Because the number of hydrogen bonds is three in R-flurbiprofen-[Bmim][L-trp] complexion and two in S-flurbiprofen-[Bmim][L-trp] complexion, the former complexion is more stable than the latter.

Subsequently, the complexation energy between chiral ionic liquids and flurbiprofen enantiomers were calculated by Gaussian 16 and corrected by basis set superposition errors (BSSE) and zero-point energies (ZPE). The complexation energies difference (ΔE) of the two optimization geometry was also calculated. The analogous simulation and calculation processes of three other extraction systems containing different chiral ionic liquids were also investigated and the results are shown in Table 1, where ΔE_R and ΔE_S represent for the complexation energy of chiral ionic liquid with R-flurbiprofen and S-flurbiprofen, respectively. ΔE represents for the energy difference which was calculated by subtracting ΔE_R from ΔE_S. It can be seen from Table 1. that the complexation energy of R-flurbiprofen-[Bmim][L-trp] is larger than that of S-flurbiprofen-[Bmim][L-trp], revealing that there exists stronger interaction between R-flurbiprofen and [Bmim][L-trp]. The analogous tendentiousness can be also found in [BMIM][L-glu] and [BMIM][L-phe] extraction system, while [BMIM][L-ser] system exhibits a weak opposite disparity. ΔE presents the discrepancies of interaction energy between two diastereomers formed by flurbiprofen enantiomers and chiral ionic liquid. The larger absolute value of ΔE often corresponds to higher selectivity referring to our previous work. So we guess that the [Bmim][L-trp] with larger ΔE is the better extractant and focus on it to perform the extraction experiments.

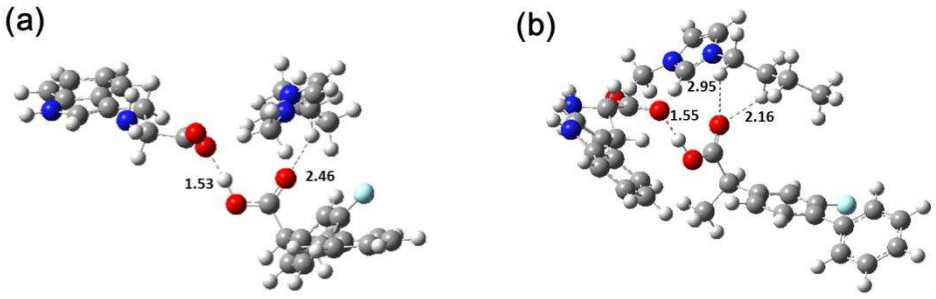

FIGURE 5 Optimazed geometries of S-flurbiprofen-[Bmim][L-trp] and R-flurbiprofen-[Bmim][L-trp] at B3LYP/6-31++G(d,p) level.

TABLE 1 BSSE and ZPE corrected interaction energies calculated on B3LYP/6–31++G(d,p) level.

chiral ionic liquid	ΔE_R	ΔE_S	ΔE

	(kcal·mol^{-1})	(kcal·mol^{-1})	(kcal·mol^{-1})
[BMIM][L-trp]	-23.82	-20.9	2.92
[BMIM][L-glu]	-26.23	-24.6	1.63
[BMIM][L-ser]	-30.08	-31.3	-1.22
[BMIM][L-phe]	-23.55	-20.84	2.71

3.2 Screening of chiral ionic liquids

The 1-butyl-3-methylimidazole was selected as the cation of chiral ionic liquids and the influence of anion was further studied by extraction experiments to verify the quantum chemistry calculation results. Four different kinds of chiral anions including tryptophan, glutamic, phenylalanine and serine determined in the simulation section were used and the results are shown in Table 2. It can be observed that [BMIM][L-glu] existing systems have low enantioselectivety under 1.05 which can't meet the practical application requirements. The lower enantioselectiveties of them correspond to the smaller value of ΔE in the quantum chemistry calculations, referring to previous work.[30,50] When [BMIM][L-ser] was employed as the extractant, an opposite enantioselectivity was obtained, which validated that its $\Delta E_R > \Delta E_S$ and $\Delta E < 0$. Distribution coefficient of R-flurbiprofen is definitely lower than that of S-flurbiprofen in the extraction systems including [Bmim][L-trp] or [Bmim][L-phe] as chiral selector, indicating that the molecular interaction between [Bmim][L-Trp] and R-flurbiprofen is higher than that of S-flurbiprofen, which accords with the quantum chemistry calculation results. The two systems have higher enantioselectivity and well distribution coefficient, probably due to the similar aromatic ring structure in amino acid anion, corresponding to the high ΔE in simulation calculations. Meanwhile, the overall effect of [Bmim][L-trp] system is slightly better than that of [Bmim][L-phe]. Thus, [Bmim][L-Trp] was selected as the chiral selector for the extraction system of flurbiprofen. The relationship between the energies difference (ΔE) of diastereomers pairs and enantioselectivity was displayed in Figure 6. A positive correlation between the differences of complexation energy and enantioselectivities can be found, similar with our previous studies,[30] which reveals the guidance meaning of quantum chemistry calculations results for the chiral extraction process.

TABLE 2 The influence of different CILs on distribution coefficient and enantioselectivity[a]

chiral ionic liquids	k_R	k_S	α
[BMIM][L-trp]	20.69	25.11	1.213
[BMIM][L-glu]	20.46	22.05	1.077
[BMIM][L-ser]	18.80	18.52	0.985
[BMIM][L-phe]	20.23	23.85	1.180

a. the concentration of CILs is 0.02 mol·L^{-1}, the concentration of flurbiprofen is 0.2 mmol·L^{-1}, the organic solvent is 1,2-dichloroethane, pH = 2.0, 25 ℃.

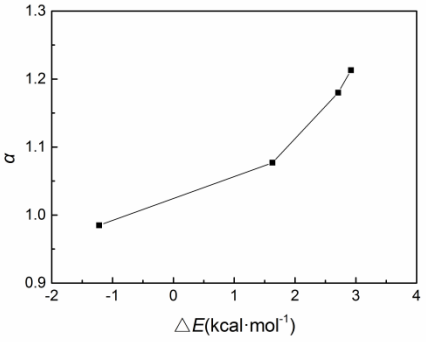

FIGURE 6 The relationship between enantioselectivity and ΔE calculated at B3LYP/6-31++G(d,p) level

3.3 Influence of organic solvents

Several solvents commonly used in chiral extraction process were employed in this work and the influence of different solvents on the distribution behavior of flurbiprofen enantiomers was determined with 0.02 mol·L^{-1} [Bmim][L-Trp] in aqueous phase at pH 2.0, 25 ℃, as shown in Table 3. There is almost no enantioselectivity when n-hexane is used as solvent, and the distribution coefficient is relatively low. When dichloromethane is used as the organic solvent, the enantioselectivity of flurbiprofen enantiomers is 1.21, but the distribution coefficient reach up to around 40, which is too large to extract. However, dichloromethane may be used as the organic solvent in the

reverse extraction step. The extraction selector [Bmim][L-Trp] can slightly dissolve in n-decanol and n-octanol, which causes the decrease of the distribution coefficients of the both enantiomers and an unsatisfactory selectivity. With comprehensive consideration of selectivity and distribution coefficient, 1,2-dichloroethane is an appropriate organic solvent which shows high enantioselectivity and proper distribution coefficient, compared with all other hydrophobic organic solvents used in the experiment.

TABLE 3 The influence of solvents on distribution coefficient and enantioselectivity[a]

solvents	k_R	k_S	α
dichloroethane	20.03	24.09	1.203
n-hexane	1.152	1.168	1.013
n-decanol	15.48	16.89	1.091
dichloromethane	36.06	42.11	1.167
n-octanol	10.52	10.87	1.033

a. the concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, the concentration of flurbiprofen is 0.2 mmol·L^{-1}, pH = 2.0, 25 ℃.

3.4 Influence of pH

pH is an important factor that affects the state of flurbiprofen molecules in the aqueous phase to further influence on the distribution behaviors in extraction system. The distribution coefficients and enantioselectivity of flurbiprofen enantiomers were studied in extraction system with aqueous phase containing 0.3 mol·L^{-1} phosphate salt buffers with pH ranging from 2 to 8. Obviously, it can be seen from Figure 7 that the distribution coefficients of both enantiomers and enantioselectivity were strongly affected by the aqueous phase pH and decreased with the increase of pH sharply.

Since the flurbiprofen is a weak acid drug with the pKa of 4.24, a large amount of flurbiprofen molecules changed into flurbiprofen ions as the pH increases, which caused the increase of the solubility of flurbiprofen in aqueous phase and the significant decrease of distribution coefficient. Meanwhile, the chiral ionic liquids have stronger interaction with the molecular flurbiprofen compared with flurbiprofen ions. The interactions between flurbiprofen enantiomer and the chiral selector decreased as pH increasing, resulting in a decrease of the separation factor. Therefore, the extraction process should be kept at lower pH. During this experiment, the optimal pH of the aqueous phase was fixed on 2.0.

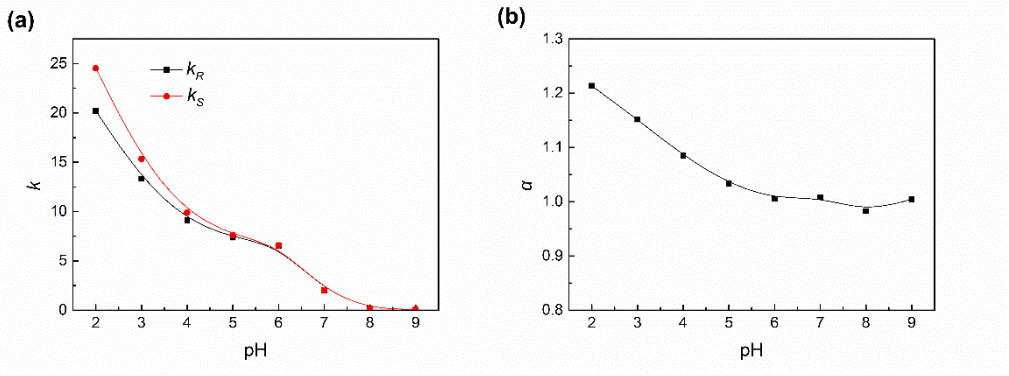

FIGURE 7 The influence of pH on distribution coefficients and enantioselectivity. The concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, the concentration of flurbiprofen is 0.2 mmol·L^{-1}, 25 ℃.

3.5 Influence of [Bmim][L-Trp] concentration

The effect of concentrations of chiral selector [Bmim][L-Trp] on distribution coefficients and enantioselectivity of flurbiprofen was

investigated at pH 2.0, 25 ℃ and the result are shown in Figure 8. The distribution coefficients of both enantiomers obviously decreased with the increase of the concentration of [Bmim][L-Trp], because the higher concentration of [Bmim][L-Trp] leads to more guest-host compounds generated between [Bmim][L-Trp] and flurbiprofen molecules which cause higher concentration of flurbiprofen in aqueous phase. k_S is greater than k_R in the whole experimental concentration range, indicating that the interaction strength of R-flurbiprofen is always higher than that of S enantiomer. It can also be seen that enantioselectivity increased with the concentration of [Bmim][L-Trp] in low [Bmim][L-Trp] concentration range and reach the top at the concentration of [Bmim][L-Trp] 0.02 mol·L^{-1}. With the further increase of [Bmim][L-Trp] concentration, the enantioselectivity shows an opposite tendency, which can be explained by the enhanced non-selective recognition interactions between [Bmim][L-Trp] and flurbiprofen. Besides, it is noted that the concentration of chiral ionic liquids used is much less than that of the traditional extractants such as tartaric ester and β-cyclodextrin derivatives which could contribute to the cost reduction in the industrial application.

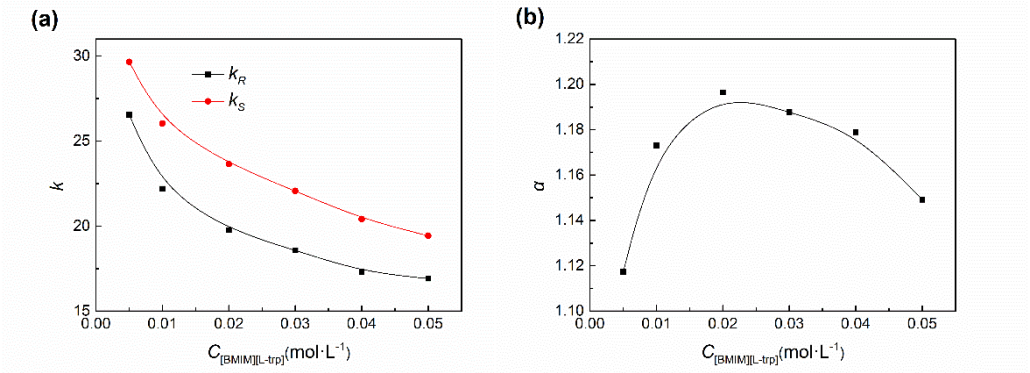

FIGURE 8 The influence of concentration of [Bmim][L-Trp] on distribution coefficients and enantioselectivity. The concentration of flurbiprofen is 0.2 mmol·L^{-1}, pH = 2.0, 25 ℃.

3.6 Influence of flurbiprofen concentration

The initial concentration of flurbiprofen racemates has effect on the concentration of complex formed by chiral selector and flurbiprofen, which can further affect the extraction process. Figure 9 shows the influence of initial flurbiprofen concentration on distribution coefficients and enantioselectivity at the concentration ranging from 0.08 to 0.4 mmol·L^{-1} (from 20 to 100 μg·mL^{-1}). Clear tendencies were observed from Figure 9 that the distribution coefficients increased with the initial concentration and the enantioselectivity followed the opposite.

The similar phenomenon were observed by Tang when hydrophilic-cyclodextrin derivative was used as the aqueous phase extractants.[34] That can be explained by that along with the increase of initial concentration, larger amounts of flurbiprofen molecules in aqueous phase are directly extracted into the organic phase without being selected, while the drugs molecules actually interacted with [Bmim][L-Trp] in the aqueous phase is limited, due to the lower solubility of flurbiprofen in aqueous phase. The gradually increase of the flurbiprofen molecules in organic phase causes the rise of distribution coefficients and the existence of more non-selective extraction leads to the decline of enantioselectivity. So the lower initial concentration is beneficial for higher enantioselectivity and 0.2 mmol·L^{-1} is selected as the suitable concentration in comprehensive consideration of extraction capacity.

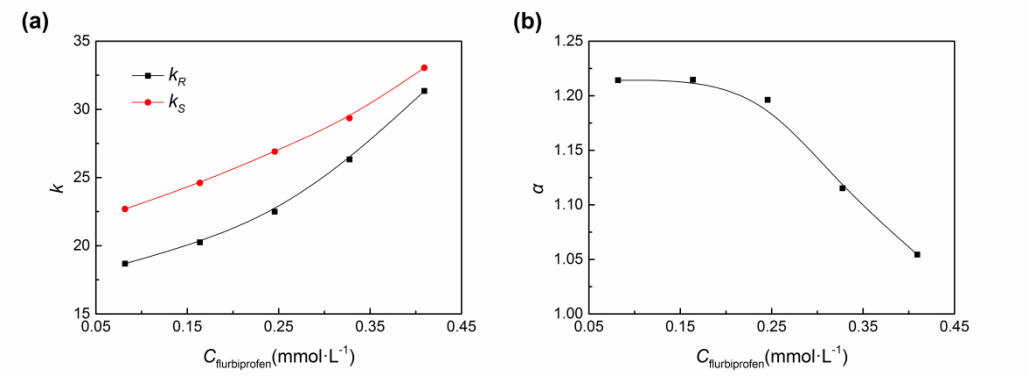

FIGURE 9 The influence of concentration of initial flurbiprofen racemates on distribution coefficients and enantioselectivity. The concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, pH = 2.0, 25 ℃.

3.7 Influence of temperature

Temperature is an important factor in the liquid-liquid extraction process. In general, lower temperature is preferred to obtain better separation efficiency because both the selector-enantiomer interaction and discrimination ability of the selector for enantiomers usually weaken at a higher temperature.[28,29,34,51] The conventional chiral drug extraction systems containing chiral selectors, like cyclodextrin derivative and tartaric acid derivative, exhibited a poor enantioselectivity, below 1.12, at room temperature (25 ˚C).

In this work, the influence of temperature on extraction system was studied from 5 to 55 ℃ at 10 ℃ interval approximately and the results were shown in Figure 10. With the increase of temperature, the distribution coefficients of both enantiomers increase gradually and rise sharply when temperature is higher than 35 ℃ owing to the non-selective physical distributions. The enantioselectivity declines slightly with the increase of temperature from 0 to 25 ℃ and remains 1.21 at 25 ℃ because of the outstanding recognition ability of [Bmim][L-Trp]. When the temperature continues to increase above 35 ℃, an increase of enantioselectivity has been observed due to the too large distribution coefficients and lower drug concentrations in aqueous phase which has an adverse impact on extraction process. Overall, the extraction performance near room temperature is improved by [Bmim][L-Trp] and decreases slightly compared with that at frigid temperature. Taking into account of the energy conservation and cost saving in practical industrial application, 25 ℃ is considered as a suitable extraction temperature.

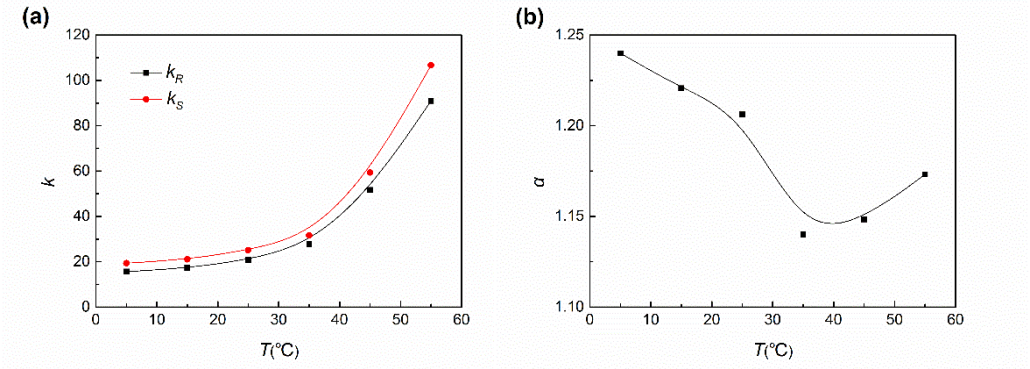

FIGURE 10 The influence of temperature on distribution coefficients and enantioselectivity. The concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, the concentration of flurbiprofen is 0.2 mmol·L^{-1}, pH = 2.0.

3.8 Recycling of [Bmim][L-trp]

The reuse of ionic liquids is crucial for developing an economic and environmentally extraction separation process. In this work, a reverse extraction process was developed to recycle the [Bmim][L-trp] by collecting and reusing the whole aqueous phase after extraction without further separation and purification. Dichloromethane was chosen as the appropriate organic solvents in the reverse extraction process because of the higher distribution coefficients compared with dichloroethane and consequently higher reverse extraction rate. The results of HPLC analysis show that the concentration of flurbiprofen in the aqueous phase is below 0.3 μg·mL^{-1} after the reverse extraction and the reverse extraction rate is up to 98%. The performance of the reused [Bmim][L-trp] containing aqueous phase within 5 cycles is shown in Figure 11. Compared with the initial extraction process, the distribution coefficients obtained by the reverse extraction process remain stable and increase slightly after fifth reuse. Meanwhile, the enantioselectivity declines a little and remains above 1.20, indicating that the regenerated aqueous phase is saturated and stable, which has continuous and efficient separation ability. And there is almost no loss of [Bmim][L-trp].

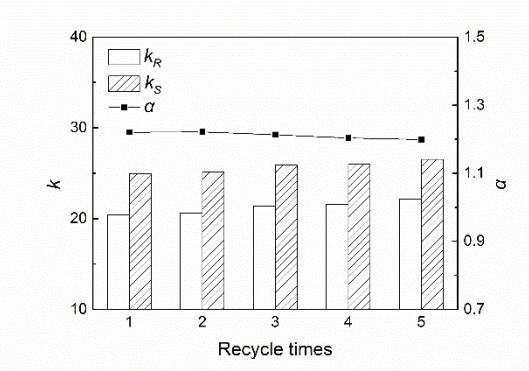

FIGURE 11 The distribution coefficients and enantioselectivity of flurbiprofen in different recycles. The concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, the concentration of flurbiprofen is 0.2 mmol·L^{-1}, pH = 2.0, 25 ℃.

4. Conclusion

In this work, a two phase liquid-liquid extraction system containing chiral ionic liquid in aqueous phase was proposed for the enantiosepration of flurbiprofen. The screening of the type of amino acid ionic liquids as extractant was performed by the assistance of quantum chemical calculation and was further verified through extraction experiments of different biphasic systems. [Bmim][L-Trp] was selected as the suitable chiral extractant. The influence of organic solvents, concentration of chiral extractants, initial concentration of flurbiprofen, pH values, and temperature on the extraction process were also investigated. Under the optimized condition, when dichloroethane is used as organic solvent, the concentration of [Bmim][L-Trp] is 0.02 mol·L^{-1}, the initial concentration of flurbiprofen is 0.2 mmol·L^{-1}, the buffer pH is 2.0 and temperature is 25 ℃, the distribution coefficients of R-flurbiprofen and S-flurbiprofen are 21.1 and 25.5, respectively. The enantioselectivity can reach to 1.24 at 5 ℃ and remain above 1.20 near room temperature, exhibiting good separation effect on flurbiprofen enantiomers. Moreover, the recycle of aqueous phase was performed through a reverse extraction process to achieve the reuse of chiral ionic liquids. And the experiment data show that the regenerated aqueous phase still remains good separation ability. Thus, we successfully developed an enantioseparation system for flurbiprofen and the results can provide references for the further application of chiral ionic liquids in extraction.

Acknowledgement

The authors are grateful for the financial support from National Natural Science Foundation of China (Grant No. 21576231) to this work.

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