



# A resolution approach of racemic phenylalanine with aqueous two-phase systems of chiral tropine ionic liquids



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## ABSTRACT

Aqueous two-phase systems (ATPS) based on tropine type chiral ionic liquids and inorganic salt solution were designed and prepared for the enantiomeric separation of racemic phenylalanine. The phase behavior of IL-based ATPS was comprehensive investigated, and phase equilibrium data were correlated by Merchuk equation. Various factors were also systematically investigated for their influence on separation efficiency. Under the appropriate conditions (0.13 g/g [C<sub>8</sub>Tropine]pro, 35 mg/g Cu(Ac)<sub>2</sub>, 20 mg/g D,L-phenylalanine, 0.51 g/g H<sub>2</sub>O and 0.30 g/g K<sub>2</sub>HPO<sub>4</sub>), the enantiomeric excess value of phenylalanine in solid phase (mainly containing L-enantiomer) was 65%. Finally, the interaction mechanism was studied via 1D and 2D NMR. The results indicate that D-enantiomer of phenylalanine interacts more strongly with chiral ILs and Cu<sup>2+</sup> based on the chiral ion-pairs space coordination mechanism, which makes it tend to remain in the top IL-rich phase. By contrast, L-enantiomer is transferred into the solid phase. Above chiral ionic liquids aqueous two-phase systems have demonstrated obvious resolution to racemic phenylalanine and could be promising alternative resolution approach for racemic amino acids in aqueous circumstance.

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## 1. Introduction

Enantiomerically pure compounds have attracted increasing attention in the chemical and pharmaceutical industries. In general, the resolution methods of racemic compounds mainly have chromatography [1], kinetic resolution [2], enzymatic resolution [3], molecular imprinting technique [4], enantioselective liquid/solid–liquid extraction [5–7], etc. However, among these methods, such disadvantages as expensive cost, complicated operation, weak generative capacity and requirements of special equipment can limit their further application to various extents. At present, more and more new chiral recognition systems for the separation of enantiomers are expected and are being developed for enantioselective fishing.

In recent years, ionic liquids (ILs) have become one of current research hotspots in many fields. Especially, the widespread attention is focused on applications of ILs in separation, which include metal ions extraction [8], organic and bio-molecules separation [9], and desulfurization of fuels [10]. They have many unique properties, including structural designability, multifunctionality,

excellent thermal stability and negligible vapor pressure. However, research on the chiral ionic liquids in enantioselective separation is still at the initial stage. Compared with traditional ILs, the special function and the structure–activity relationship of chiral ionic liquids need to be found in more examples of successful applications, which would also be helpful to improve the resolution efficiency of related methods and processes.

Aqueous two-phase systems (ATPS) are usually formed by polymer–polymer, polymer–salt and salt–salt combinations in an aqueous solution, which have been widely applied in separation, fraction and biotechnology, and provide an economical, efficient downstream-processing method [11]. In the past, the chiral separation research of ATPS mainly focused on the components comprising polyethylene glycol and inorganic salts (as illustrated in Table 1). However, the common polymer-based ATPS have the limited polarity range and high viscosity [24] and are not favorable for high-efficient mass transfer of phase-forming components [25]. Ionic-liquid-based ATPS has a major benefit of overcoming the limited polarity range of polymer-based ATPS by a proper manipulation of the cation/anion design and their combinations [26]. In 2011, polyethylene glycol-based ATPS containing  $\beta$ -cyclodextrin as chiral selector was reported by Tan et al. [19] for the resolution of racemic mandelic acid with the maximum e.e.% value of 42.13%. Recently, Wu and co-workers [12] first reported imidazolium-based ionic liquid ATPS to separate racemic amino acid with the

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**Table 1**

The typical enantiomer resolution with ATPS published from 1980 to 2015.

| Year | ATPS                                                                                       | Chiral selector                 | Racemic compound                               | Ref. |
|------|--------------------------------------------------------------------------------------------|---------------------------------|------------------------------------------------|------|
| 2015 | Imidazole type ionic liquid + inorganic + water                                            | Imidazole type ionic liquid     | Amino acids enantiomers                        | [12] |
|      | C <sub>2</sub> H <sub>5</sub> OH + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + water | HP- $\beta$ -CD                 | Phenylsuccinic acid enantiomers                | [13] |
| 2013 | C <sub>2</sub> H <sub>5</sub> OH + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + water | $\beta$ -CD                     | Tryptophan enantiomers                         | [14] |
|      | PEG400 + Na <sub>2</sub> HPO <sub>4</sub> + water                                          | Microbial cells                 | (S)-(4-Chlorophenyl)-(pyridin-2-yl) methanol   | [15] |
| 2012 | Triton-114 + NaCl + water                                                                  | Cu <sub>2</sub> - $\beta$ -CD   | Mandelic acid enantiomers                      | [16] |
|      | PEG + inorganic + water                                                                    | Cu <sub>2</sub> - $\beta$ -CD   | $\alpha$ -Cyclohexyl-mandelic-acid enantiomers | [17] |
|      | PEG + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + water                              | $\beta$ -CD, HP- $\beta$ -CD    | Phenylalanine enantiomers                      | [18] |
|      | PEG + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + water                              | $\beta$ -CD                     | Mandelic acid enantiomers                      | [19] |
| 2011 | C <sub>2</sub> H <sub>5</sub> OH + inorganic + water                                       | HP- $\beta$ -CD                 | $\alpha$ -Cyclohexyl-mandelic acids            | [20] |
| 1998 | PEG + Na <sub>2</sub> HPO <sub>4</sub> + water                                             | Bovine serum albumin            | D,L-Kynurenine                                 | [21] |
| 1991 | PEG + Dextran 40                                                                           | Bovine serum albumin, Ovomuroid | Ofloxacin                                      | [22] |
| 1980 | PEG400 + K <sub>2</sub> HPO <sub>4</sub> /KH <sub>2</sub> PO <sub>4</sub> + water          | Immobilization of acylase       | N-acetyl-DL-methionine                         | [23] |

maximum e.e.% value reaching 53%. Up to now, research of chiral ionic liquids in aqueous two-phase systems used for enantioselective resolution has rarely been reported. Herein the ATPS composed of new chiral ILs and inorganic salt are considered by researchers of this article for enantioseparation.

Recently, a series of chiral ionic liquids with tropine cation nuclear were synthesized by us for the first time. The objective of this study is to explore the feasibility of using ATPS based on these new chiral ILs and inorganic salt solution to separate racemic amino acids via specific cooperative effects. Various conditions were investigated for their effects on the phase behavior of related aqueous two-phase systems. Then phase equilibrium data were correlated by Merchuk equation. In addition, the effects on the separation efficiency of racemic phenylalanine, including the alkyl chain length of cations of ionic liquids, the amount of racemic phenylalanine, the amount of copper acetate and water together with the amount and type of salts were carefully investigated. Furthermore, one-dimensional Proton Nuclear Magnetic Resonance (<sup>1</sup>H NMR) and Nuclear Overhauser Enhancement Spectroscopy (NOESY) were used to study the interaction mechanism of chiral separation. The chiral ionic liquids aqueous two-phase systems demonstrated obvious selectivity for the enantiomers of racemic phenylalanine and could be promising alternative resolution approach for racemic amino acids in aqueous circumstance.

## 2. Experimental

### 2.1. Chemicals

The following chemicals and reagents were purchased from Kelong Chemical Co., Ltd. (Chengdu, China): methanol, ethanol, dichloromethane, ethyl acetate, acetonitrile, copper sulfate, potassium carbonate, potassium phosphate, dipotassium hydrogen phosphate, copper acetate, acetic acid, ammonia, ethyl bromide, *n*-propyl bromide and *n*-butyl bromide. Moreover, tropine, L-proline and D,L-phenylalanine were supplied by Huawen Chemical Co., Ltd. (Zhengzhou, China). *n*-Amyl bromide, *n*-hexyl bromide, *n*-heptyl bromide and *n*-octyl bromide were obtained from Xinhua Active Material Institute (Changzhou, China). All of the above solvents and chemicals are of analytical grade or above. Deionized water was obtained with the ultra-pure water system (0.4-mm filter) manufactured by Millipore (Bedford, MA).

### 2.2. Apparatus

A Shimadzu LC-20AT high performance liquid chromatography (Kyoto, Japan) consisting of PD-M20A detector and Class VP chromatography workstation was used for the analysis and quantitation of the racemic amino acids. A Welchrom-C18 column

(250 mm × 4.6 mm, 5  $\mu$ m) was used as the HPLC analytical column. An HC-2062 high-speed centrifuge was provided by Zhongke Zhongjia Science Instrument Co., Ltd. (Hefei, China). <sup>1</sup>H NMR and NOESY spectra were obtained by a Bruker AV II-400 NMR and an AV II-600 NMR spectrometer (Fällanden, Switzerland).

### 2.3. Synthesis of chiral ionic liquids

#### 2.3.1. Synthesis of [C<sub>n</sub>Tropine]Br

Ionic liquids of [C<sub>n</sub>Tropine]Br (*n* = 2, 3, 4, 5, 6, 7, 8) were prepared and purified on the basis of imidazolium ionic liquids [27]. To briefly summarize, the reactions of tropine and alkyl bromide (such as ethyl bromide, *n*-propyl bromide, *n*-butyl bromide, *n*-amyl bromide, *n*-hexyl bromide, *n*-heptyl bromide and *n*-octyl bromide) were refluxed in appropriate solvent under certain temperature and duration with continuous magnetic stirring. Then the residual solvents were removed under vacuum, and the resulting products were washed with appropriate solvents and were dried for 24 h. Table 2 shows the reaction conditions of the synthesis of [C<sub>n</sub>Tropine]Br.

#### 2.3.2. Synthesis of [C<sub>n</sub>Tropine]pro

Firstly, the aqueous [C<sub>n</sub>Tropine]Br (*n* = 2, 3, 4, 5, 6, 7, 8) solution was loaded on a column of strongly basic styrene 201\*7 type anion exchange resin (0.315–1.25 mm, Kelong Chemical Co., Ltd., Chengdu, China) and eluted. The reaction progress was monitored by concentrated AgNO<sub>3</sub> aqueous solution. After the ion exchange between Br<sup>−</sup> and OH<sup>−</sup>, no precipitation of AgBr was found with an addition of a few drops of AgNO<sub>3</sub> solution. Secondly, equimolar L-proline was added to the prepared aqueous solution of [C<sub>n</sub>Tropine]OH, and the reaction mixture was stirred magnetically for 12 h at room temperature. Then water was removed under vacuum and the resulting products were dried for 24 h. The yield, specific rotation and optical purity of [C<sub>n</sub>Tropine]pro ionic liquids were shown in Table 3. All of the chiral ILs have been identified via spectral techniques (their <sup>1</sup>H NMR spectra as shown in Fig. S1). The synthetic route of ionic liquids was shown in Fig. S2(a).

### 2.4. Preparation and measurement of ATPS

The binodal curves were determined through the cloud point titration method in a jacketed glass vessel containing a magnetic stirrer at *T* = 293 K and atmospheric pressure [28]. A certain concentrations of inorganic salt aqueous solution and IL aqueous solution were prepared and used to investigate phase behaviors. Repetitive dropwise addition of the aqueous inorganic salt solution to the aqueous solution of IL was kept on until the appearance of a cloudy solution, followed by the dropwise addition of ultrapure water until the detection of a monophasic region (clear and limpid

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