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# Specific cooperative effect for the enantiomeric separation of amino acids using aqueous two-phase systems with task-specific ionic liquids

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ABSTRACT

Aqueous two-phase systems (ATPS) based on hydrophilic ionic liquid (IL) and inorganic salt solution were designed and prepared for the enantiomeric separation of racemic amino acids. Two different kinds of hydrophilic ionic liquids (IL-1 and IL-2) containing functional groups were synthesized to separate racemic amino acids. Preliminary experiments showed that D-enantiomer of amino acids cooperatively interacted with ILs, which pushed D-enantiomer to remain in the bottom IL-rich phase. By contrast, L-enantiomer was transferred into the top $Na_2SO_4$-rich phase. The enantioselectivity of IL-1 was better than that of IL-2 because of their different intermolecular interactions. Various factors influencing separation efficiency were also systematically investigated including extraction time, IL volume and temperature. Furthermore, the mechanism was studied by $^1H$ NMR and DFT calculations, which showed that the hydrogen bond between the carboxylate and amide groups and the resonance-assisted hydrogen bond between amino and hydroxyl groups conditioned the movement between the residues and ILs. Finally, IL-1 was validated with other general amino acids by the same procedures based on ATPS.



## 1. Introduction

The increasing demand for enantiomerically pure compounds for chemical and pharmaceutical industries leads to an increasing interest in such compounds. In general, the production of enantiopure compounds is accessible by means of asymmetric catalysis [1,2], enantioselective liquid/solid–liquid extraction [3–5], crystallization [6], membrane-based separation [7,8] and chromatographic separation [9,10]. In the past decade, a metal ion modified with a chiral ligand is usually employed as a chiral host for the enantioselective recognition and separation of racemic mixtures, because such species can provide simultaneous binding between chiral host and analytes [11]. However, the use of volatile organic solvents as the extraction/reaction solvents and harsh conditions to release enantiomers are the major drawbacks of this approach [12]. Therefore, to avoid the above drawbacks, in this study, we direct our attention to aqueous two-phase systems for the separation of enantiomers with the use of task-specific ionic liquids as an enantioselective fishing.

Ionic liquids (ILs) known as salts with low melting temperatures are composed of organic cations and inorganic or organic anions. To date, hundreds of ILs have been synthesized by different resolutions, and interchanging their counterions may require up to $10^{18}$ possible combinations [13]. ILs have attracted much attention as green alternatives to environmentally unfriendly solvents because of their advantages of non-volatility, non-flammability, and thermal stability. At present, ILs have been used in a number of applications such as organic synthesis, separation science, spectroscopy, and electrochemistry [5,14–19]. Especially, ILs could possess variable polarities by changing their chain length, which contribute to their extensive application in the field of extraction [15]. Moreover, one of the most recent developments of ILs is to seek appropriate task-specific ionic liquids [20], and expanding their particular ability used in the field of separation. Task-specific ionic liquids inherit all the advantages of traditional ILs and show more significant function, which can contribute to improving the separation efficiency.

Aqueous two-phase systems (ATPS) or aqueous biphasic systems composed of two immiscible phases are clean alternatives for traditional organic–water solvent extraction systems, though both solutes are soluble in water. Since Albertsson [21] first applied ATPS in traditional extraction, ATPS have been mainly applied to

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separation, fraction and biotechnology. In the past, the research of ATPS mainly focused on the components comprising polyethylene glycol and inorganic salts [22]. However, with the development of ILs, Rogers and co-workers [23] first demonstrated that the addition of inorganic salt could lead to phase separation. That is, inorganic salt and hydrophilic IL were incompatible with water. After this proof of principle, several studies on ATPS based on the IL + inorganic salt + $H_2O$ have been explored in the extraction of alkaloids, amino acids, aromatic hydrocarbons, etc. [24,25]. Previous works, the correct choices of the anion/cation of the functional ILs and the salting-out species are the key factors for the extraction efficiency [26,27]. Thus, there is potential to hypothesize and design new task-specific ILs, as both solvent and substrate, containing special functional groups for the enantiomeric separation of racemic mixtures. To the best of our knowledge, no report has been made on the enantiomeric separation of the racemic amino acids with ATPS comprising the functional IL and inorganic salt.

In summary, the objective of this study is to explore the feasibility of using ATPS based on hydrophilic ionic liquid (IL-1 and IL-2) and inorganic salt ($Na_2SO_4$) solution to separate racemic amino acids via specific cooperative effect. Unlike the traditional liquid–liquid extraction, racemic amino acids could be completely mixed and experience interactions with ILs before the formation of two phases. In addition, amino acids and ILs can be easily recovered from the higher or lower phases after separation process of ATPS. The effects to the separation efficiency of racemic amino acids, including extraction time, IL volume and temperature, were carefully investigated. Furthermore, $^1$H NMR and DFT calculations were used to study the mechanism of this method.

## 2. Experimental

### 2.1. Chemicals

For the purpose of this investigation, all chemicals involved in this study were of analytical reagent grade at least, while reverse osmosis Milli-Q water (18 MΩ, Millipore, Bedford, MA, USA) was used throughout. Methanol used for HPLC analysis was of chromatographic grade and purchased from Merck, Darmstadt, Germany. The inorganic salts $Na_2SO_4$ and $KPF_6$ were bought from Sinopharm Group Co. Ltd. (Shanghai, China). L-phenylalanine (L-Phe), D-phenylalanine (D-Phe), Racemic phenylalanine (D, L-Phe), racemic isoleucine (D, L-Ile), racemic threonine (D, L-Thr), racemic glutamime (D, L-Gln), racemic tyrosine (D, L-Tyr), racemic tryptophan (D, L-Try), racemic serine (D, L-Ser) and racemic aspartic acid (D, L-Asp) were purchased from Aladdin Industrial Inc. (Shanghai, China).

2-Chloroacetic acid, 3-chloropropylisocyanate, *N*-methylimidazole, dichloromethane, triethylamine, thionyl chloride, *N*,*N*-dimethylformamide and (R)-2-amino-1-butanol prepared to synthesize the designed functional ionic liquid were purchased from Aladdin Industrial Inc. (Shanghai, China).

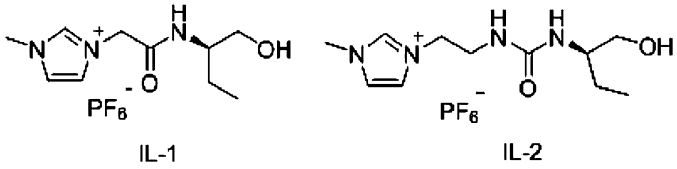


**Fig. 1.** Schematic presentation for the structures of IL-1 and IL-2.

### 2.2. Apparatus

An Agilent 1100 liquid chromatograph consisting of a G1311A Quat-Pump, a G1322 degasser, a G1314A variable-wavelength detector, a model 7725i injection valve with a 20-μL loop, and an Agilent ChemStation for data treatment, were used for the analysis and quantification of the racemic amino acids. A Sigma–Aldrich CHIROBIOTIC column (250 mm × 4.6 mm I.D., 5 μm, 120 Å) was used as the LC analytical column. A constant temperature oscillator was purchased from Tensuc Inc. (Shanghai, China). $^1$H and $^{13}$C NMR spectra were obtained by a Bruker Advance 400 NMR spectrometer.

### 2.3. Preparation of ILs

The structures of the IL-1 and IL-2 are shown in Fig. 1. Both these structures were acquired through a three-step process, and the schematic is shown in Fig. 2.

#### 2.3.1. The compound IL-1

Step 1: 1.2 eq. chloroacetyl chloride was added dropwise to (R)-2-amino-1-butanol in an ice bath. An appropriate amount of dichloromethane and 1.5 eq. triethylamine were chosen as the solvents. The final mixture was stirred vigorously at room temperature overnight. After completion of the reaction, the organic layer was separated, washed with dilute HCl (5%) and dried by anhydrous $Na_2SO_4$.

Step 2: *N*-Methylimidazole was added to 2 in a small excess molar ratio to allow the complete reaction of 2. Acetonitrile was chosen as the solvent, and the mixture was refluxed at 358 K for 24 h, followed by the removal of acetonitrile under a vacuum to generate 3.

Step 3: $KPF_6$ was added to 3 in the same molar ratio and acetonitrile was chosen as solvent. The mixture was refluxed at 358 K for 24 h, followed by filtration. The filtrate was dried under a vacuum to generate pure IL-1. The purity was characterized by $^1$H NMR: (400 MHz, DMSO-$d_6$) $\delta$ ppm 8.96 (1 H, d, $J$ = 3.6 Hz), 7.60–7.67 (2 H, m), 4.65 (1 H, s), 4.16–4.30 (1 H, m), 4.00 (1 H, m), 3.22–3.43 (5 H, m), 3.01 (1 H, m), and 0.79–0.91 (3 H, m). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 157.9, 136.7, 123.1, 60.1, 52.5, 50.1, 49.5, 42.0, 35.5, 24.8,

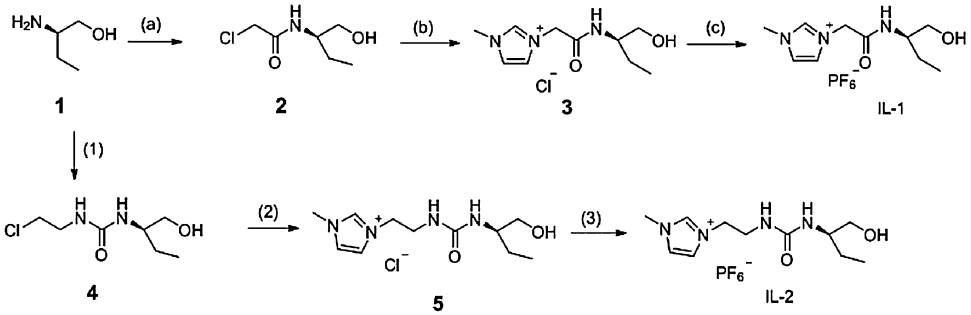


**Fig. 2.** Reaction schematic of the synthesis of IL-1 and IL-2. (a) Chloroacetyl chloride, triethylamine; (b) *N*-Methylimidazole; (c) $KPF_6$; (1) Chloroacetyl chloride, triethylamine; (2) *N*-Methylimidazole; (3) $KPF_6$.