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Enantioseparation of amino acids by co-extractants with di(2-ethylhexyl)phosphoric acid and tartaric acid derivatives

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Abstract—A co-extraction method using di(2-ethylhexyl)phosphoric acid (D2EHPA) with each of the two tartaric acid derivatives, *O*,*O*′-dibenzoyl-(2*S*,3*S*)-tartaric acid [(+)-DBTA] and *O*,*O*′-dibenzoyl-(2*S*,3*S*)-4-toluoyl-tartaric acid [(+)-DTTA] was developed for the enantioseparation of four amino acids: racemic tryptophan (*rac*-Trp), racemic phenylalanine (*rac*-Phe), racemic *p*-hydroxyphenylglycine (*rac*-Hpg) and racemic tyrosine (*rac*-Tyr). The extractants were prepared in various proportions and diluted in *n*-octanol. The influence of the extractant composition, concentration of amino acid enantiomers, molecular structure of the solutes and extractive equilibrium temperature were studied. The maximum enantioselectivities of the four amino acids were 4.02, 1.37, 1.52 and 2.06 for Trp, Phe, Hpg and Tyr, respectively. The effects of the molecular structure of the solutes and amino acids along with the equilibrium temperature on the distribution ratio and enantioselectivity were examined, which is helpful for optimizing the extraction systems and realize the large-scale production of pure enantiomer.

1. Introduction

Amino acids are biologically active compounds. They play an important role in living systems. Most amino acids are chiral. The separation of chiral amino acids is of great importance in many scientific fields, such as pharmaceuticals, food processing, amino acid biochemistry, proteins and related areas, and also asymmetric syntheses in organic chemistry.[1–3] Although previously only natural L-series acids were available, D-amino acids are now becoming increasingly available as a result of being required as component materials of industrial interest.[4]

The enantiomers can be separated after derivatization[5–7] (diastereoisomeric esters or a diastereoisomeric salt) or, in one step, by complex formation.[8–10] The good complex forming abilities of DBTA and DTTA arise from a number of facts.[11–13] The carboxylic acid groups of DBTA and DTTA can donate protons for hydrogen bonding, while they can also behave as a proton acceptor due to the eight oxygen atoms they contain. The benzoyl groups can take part in hydrophobic interactions while the other part of the molecule contains polar hydrophilic groups.[13]

Although DBTA and DTTA are the two most frequently used, widely available and inexpensive acidic resolving agents, they both have a low distribution ratio when extracting amino acids from a water phase to an organic phase. We wondered whether it was possible with co-extractants to facilitate the separation ability of DBTA and DTTA to amino acids? This consideration led us to investigate the application of a co-extraction method in the separation of chiral amino acids. Several papers reported that the extractant of di(2-ethylhexyl)phosphoric acid (D2EHPA) has high extraction capacity to amino acids.[14–17] Recently we found that di(2-ethylhexyl)phosphoric acid (D2EHPA) and *O*,*O*′-dibenzoyl-(2*R*,3*R*)-tartaric acid [(−)-DBTA] can form new complexes, which can be used as chiral selectors for the resolution of amino acid enantiomers and both showed good enantioselectivity and a high distribution ratio.[18]

However, no more tartaric acid derivatives and amino acids have been tested while no discussion of the structures of the amino acids on the chiral resolution has been carried out. Based on previous results, this work presents more extensive results of the co-extraction

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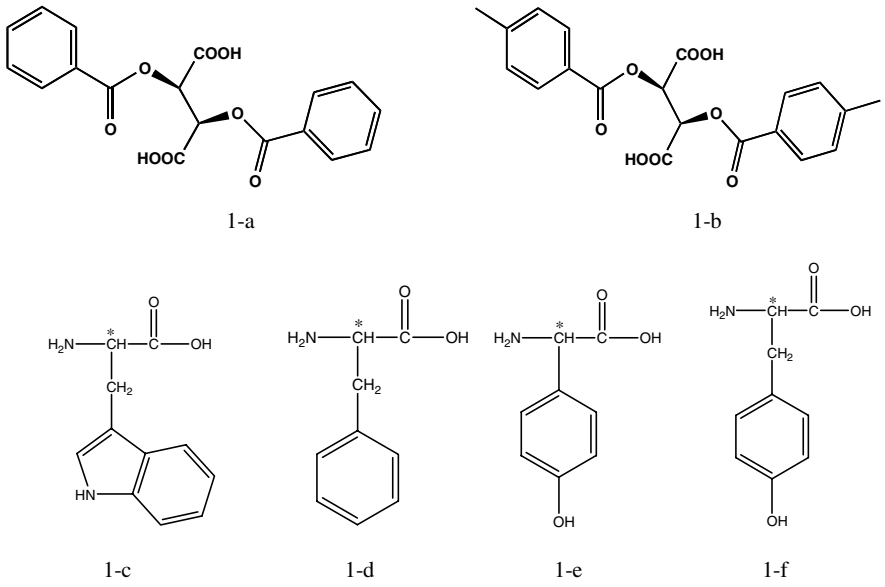


Figure 1. Molecular structure: (a) *O*,*O*′-Dibenzoyl-(2*S*,3*S*)-tartaric acid; (b) *O*,*O*′-Dibenzoyl-(2*S*,3*S*)-4-toluoyl tartaric acid; (c) *rac*-Tryptophan; (d) *rac*-Phenylalanine; (e) *rac*-*p*-Hydroxyphenylglycine; (f) *rac*-Tyrosine.

method of tartaric acid derivatives and D2EHPA. Two more tartaric acid derivatives, *O*,*O*′-dibenzoyl-(2*S*,3*S*)-tartaric acid [(+)-DBTA] (Fig. 1a) and *O*,*O*′-dibenzoyl-(2*S*,3*S*)-4-toluoyl-tartaric acid [(+)-DTTA] (Fig. 1b) as chiral selectors and four racemic amino acids of tryptophan (*rac*-Trp) (Fig. 1c), phenylalanine (*rac*-Phe) (Fig. 1d), *p*-hydroxyphenylglycine (*rac*-Hpg) (Fig. 1e) and tyrosine (*rac*-Tyr) (Fig. 1f) were tested. The influence of molecular structures on enantioseparation ability is discussed.

2. Results and discussion

The chromatograms for amino acids are illustrated in Figure 2. According to the suggested mechanism in the co-extraction method for amino acid enantiomers, the extractant composition, concentration of solutes, molecular structure, pH in the aqueous phase and temperature could affect the extraction efficiency. To make the experimental data comparable, the pH in the aqueous phase was fixed at the isoelectric points of each amino acids, and the influence of other factors was studied.

2.1. Influence of co-extractant compositions

The influence of the co-extractant compositions is summarized in Figure 3. When D2EHPA was not added to the extractant ($X = 0$), both (+)-DBTA and (+)-DTTA showed enantioselectivity on *rac*-Trp, *rac*-Phe, *rac*-Hpg and *rac*-Tyr, but with very small distribution ratios. With the increase of the D2EHPA content, the distribution ratios for all of the amino acids were greatly enhanced. Meanwhile, the enantioselectivities all increased before the concentration of D2EHPA was up to 0.15 mol/L. Increasing the concentration of D2EHPA further, the distribution ratios continuously increased, while the enantioselectivities followed an opposite tendency. This is because D2EHPA does not have chiral separation ability. But it has high extraction capacity. So when there is more D2EHPA, the selectivity will be less.

Figure 4 shows the influence of the concentration of [(+)-DBTA or (−)-DTTA] on the extraction efficiency. When no chiral selector was added to the extractant, distribution ratios of *rac*-Trp, *rac*-Phe , *rac*-Hpg and *rac*-Tyr were 4.25, 1.05, 0.06 and 0.30, although no enantioselectivity was found. With an increase of the concentration of (+)-DBTA or (+)-DTTA, the distribution ratios of the amino acids kept to a moderate extent and showed no obvious trend. However, the enantioselectivities increased steadily.

2.2. Influence of the concentration of amino acids

The influence of concentration of the amino acids is shown in Figure 5. The distribution ratios of the D-enantiomer and L-enantiomer are both enhanced upon increasing the initial concentration of the solutes; these results are in accordance with the conclusion reported by previous work.[17] However, the values of enantioselectivities are relatively higher at low concentrations, which indicates a better enantioseparation efficiency at low initial concentrations.

2.3. Influence of the molecular structure of amino acids

The amino acids for the study of the influence of the molecular structure were chosen in such a way that they had the same asymmetric carbon atom and carboxylic side chain but varying one substituted group. The results listed in Table 2 clearly indicate that, when comparing the molecular structure of *rac*-Tyr with *rac*-Phe, replacing a hydrogen atom in the phenyl by a hydrophilic group

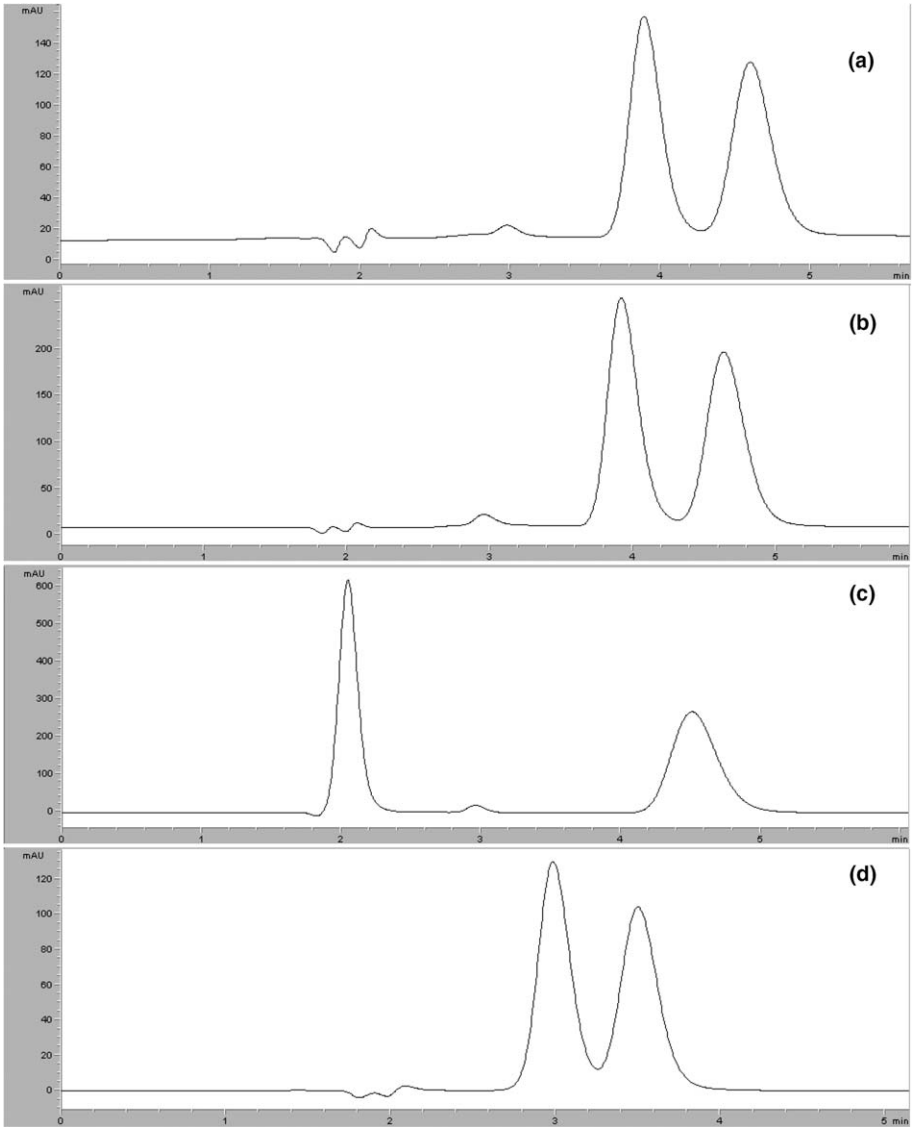


Figure 2. Chromatograms for amino acid enantiomers: (a) *rac*-Trp; (b) *rac*-Phe; (c) *rac*-Hpg; (d) *rac*-Tyr. The retention time of D-enantiomer was less than that of L-enantiomer.

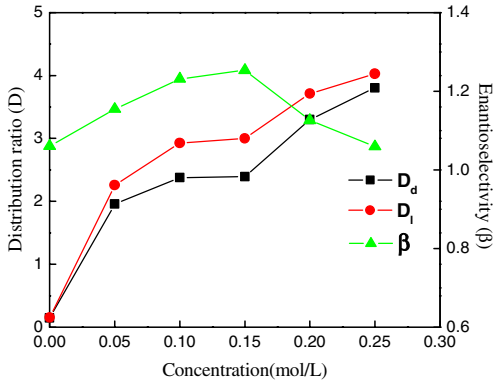
(–OH–) leads to a decrease in the distribution ratio, while compared to that of *rac*-Tyr with *rac*-Hpg, adding a hydrophobic group (–CH_2–) leads to an increase in the distribution ratio. These observations are in accordance with the results shown in Table 1, which was obtained by using *n*-octanol as an extractant. As tryptophan contains a most hydrophobic group (indole), it has the largest distribution ratio among the four studied amino acids.

The influence of the molecular structure of the solutes on the enantioselectivity, however, is more complicated than that on the distribution ratios. As shown in Table 2, the enantioselectivity for Trp is much higher than that for Phe or Tyr. Due to a more prominent influence by the substituted hydroxyl group on the phenyl group, Hpg and Tyr have a greater enantioselectivity than that of Phe while their distribution ratios are opposite as mentioned above. Therefore, the selectivity may not be just explained as an influence of the distribution ratio. More fundamental mechanism should consider the molecular interaction and spatial resistance effect. More detailed studies will be carried out to confirm this observation.

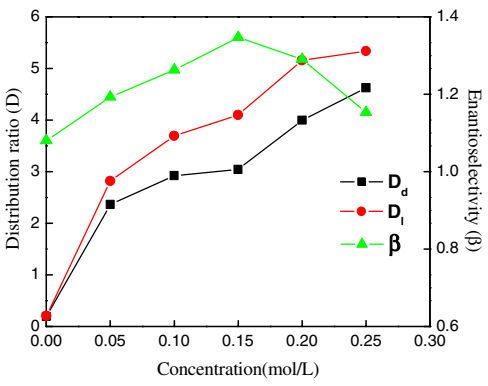
2.4. Influence of temperature

The influence of temperature was partly investigated with *rac*-Trp as the solute. Table 3 shows that higher temperature leads to a decrease in both distribution ratios and enantioselectivities.

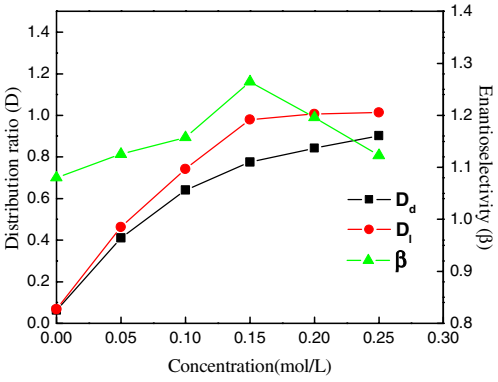
Figure 6 shows the variations of lnD and $\ln\beta$ versus $1/T$. The results can be described as fitting very well with the Van't Hoff model, indicating that the complexes do not change in conformation[19,20] and that enantioselective interactions are unchanged in the temperature range studied.[19]



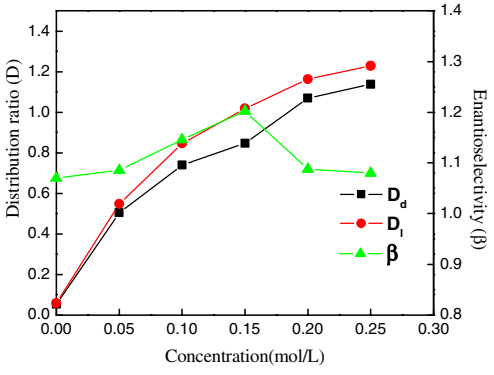
3-a: Solute: 200 ppm *rac*-Trp
Chiral selector: 0.1 mol/L (+)-DBTA



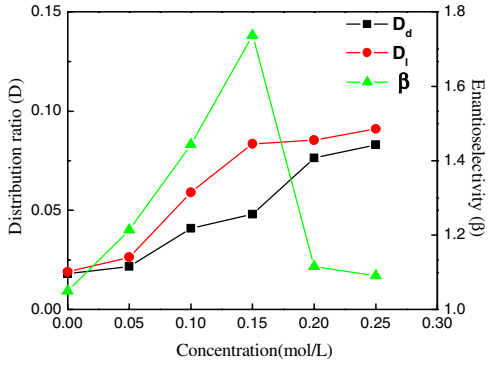
3-b: Solute: 200 ppm *rac*-Trp
Chiral selector: 0.1 mol/L (+)-DTTA



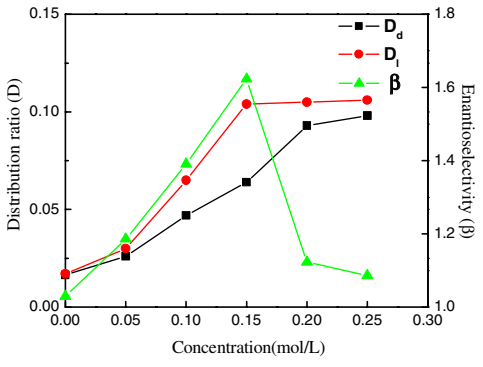
3-c: Solute: 500 ppm *rac*-Phe
Chiral selector: 0.1 mol/L (+)-DBTA



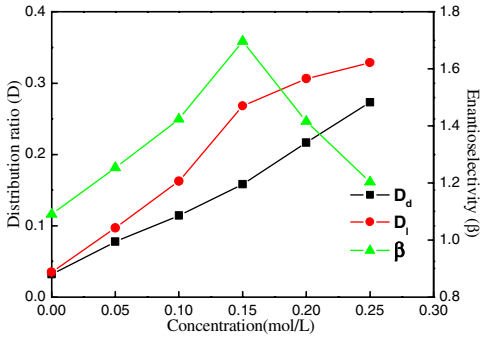
3-d: Solute: 500 ppm *rac*-Phe
Chiral selector: 0.1 mol/L (+)-DTTA



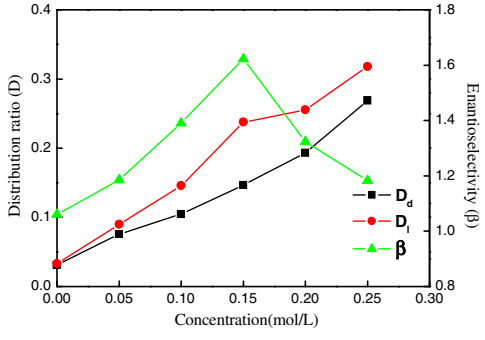
3-e: Solute: 500 ppm *rac*-Hpg
Chiral selector: 0.1 mol/L (+)-DBTA



3-f: Solute: 500 ppm *rac*-Hpg
Chiral selector: 0.1 mol/L (+)-DTTA

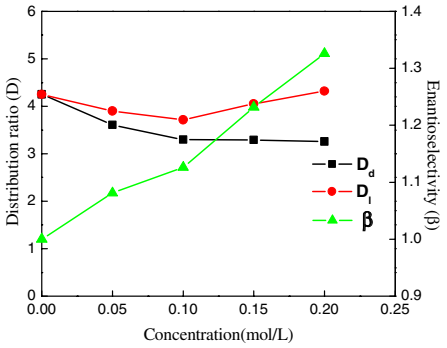


3-g: Solute: 100 ppm *rac*-Tyr
Chiral selector: 0.1 mol/L (+)-DBTA

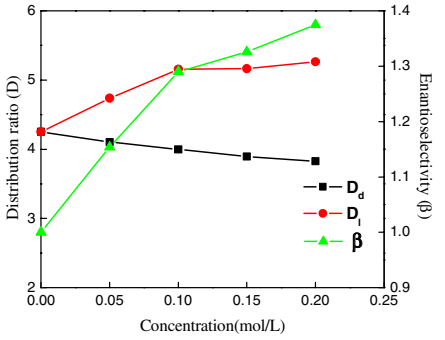


3-h: Solute: 100 ppm *rac*-Tyr
Chiral selector: 0.1 mol/L (+)-DTTA

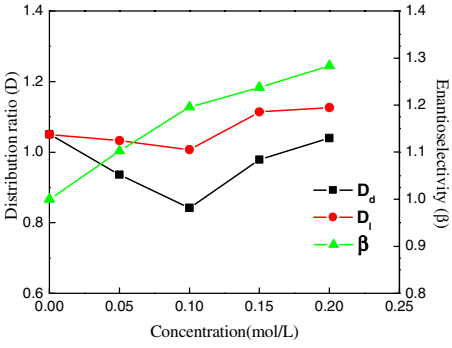
Figure 3. Influence of initial concentration of D2EHPA on the enantioseparation of amino acids.



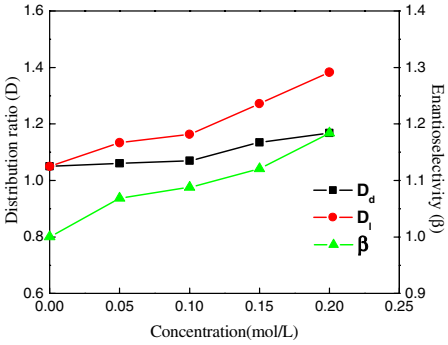
4-a: Solute: 200 ppm *rac*-Trp
Chiral selector: (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



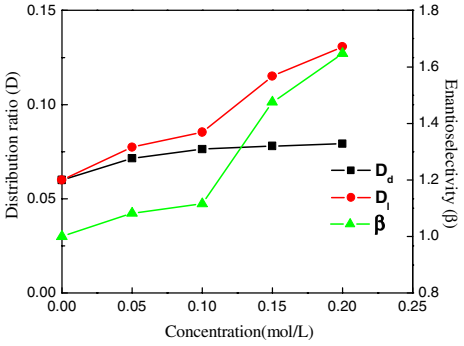
4-b: Solute: 200 ppm *rac*-Trp
Chiral selector: (+)-DTTA
Extractant: 0.2 mol/L D2EHPA



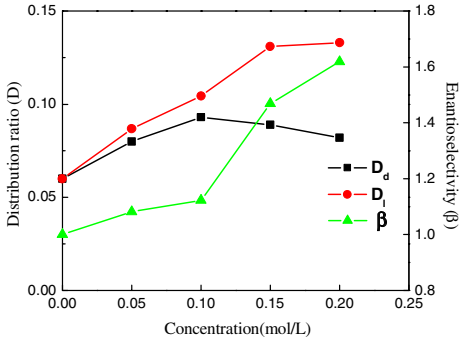
4-c: Solute: 500 ppm *rac*-Phe
Chiral selector: (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



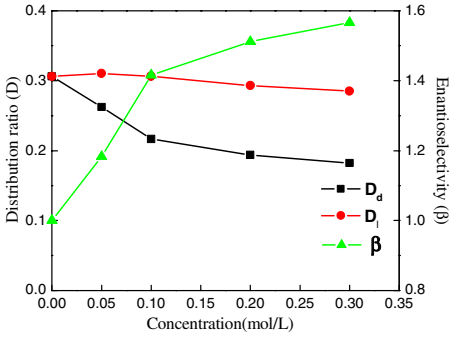
4-d: Solute: 500 ppm *rac*-Phe
Chiral selector: (+)-DTTA
Extractant: 0.2 mol/L D2EHPA



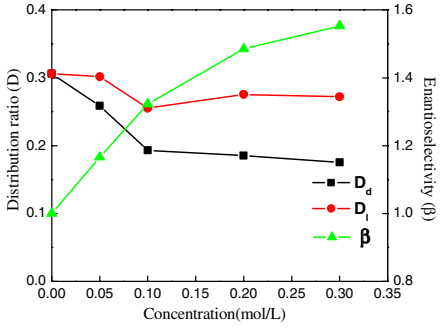
4-e Solute: 500 ppm *rac*-Hpg
Chiral selector: (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



4-f: Solute: 500 ppm *rac*-Hpg
Chiral selector: (+)-DTTA
Extractant: 0.2 mol/L D2EHPA

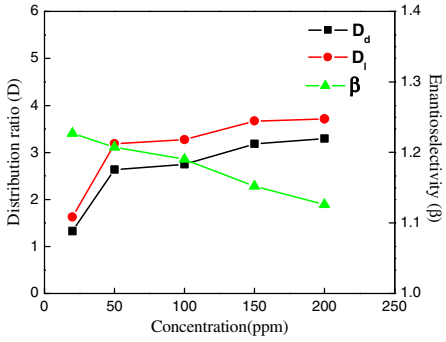


4-g: Solute: 100 ppm *rac*-Tyr
Chiral selector: (+)-DBTA
Extractant: 0.2 mol/L D2EHPA

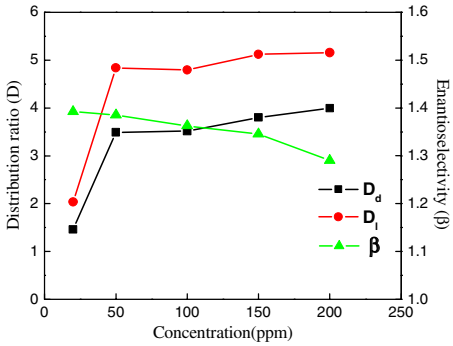


4-h: Solute: 100 ppm *rac*-Tyr
Chiral selector: (+)-DTTA
Extractant: 0.2 mol/L D2EHPA

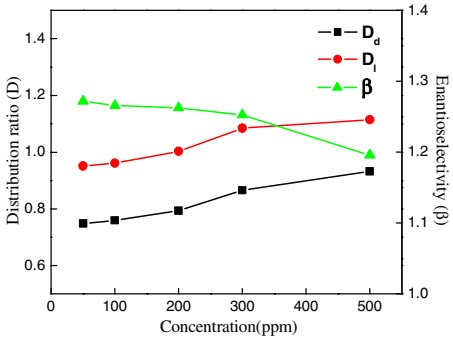
Figure 4. Influence of initial concentration of chiral selectors on the enantioseparation of amino acids.



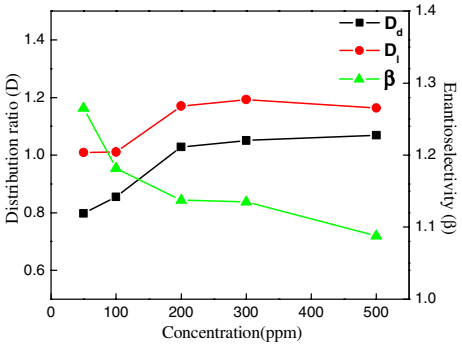
5-a: Solute: *rac*-Trp
Chiral selector: 0.1 mol/L (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



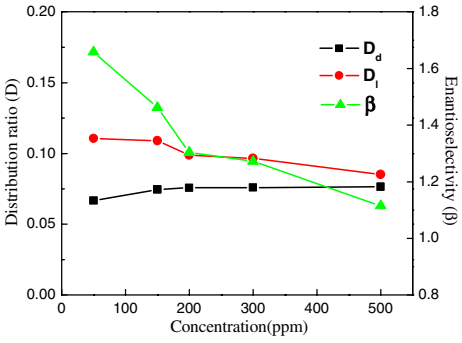
5-b: Solute: *rac*-Trp
Chiral selector: 0.1 mol/L (+)-DTTA
Extractant: 0.2 mol/L D2EHPA



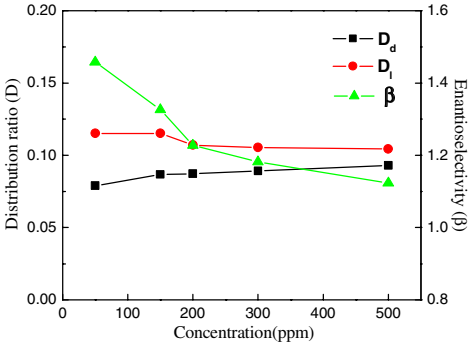
5-c: Solute: *rac*-Phe
Chiral selector: 0.1 mol/L (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



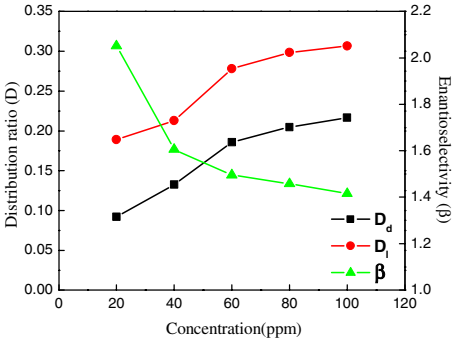
5-d: Solute: *rac*-Phe
Chiral selector: 0.1 mol/L (+)-DTTA
Extractant: 0.2 mol/L D2EHPA



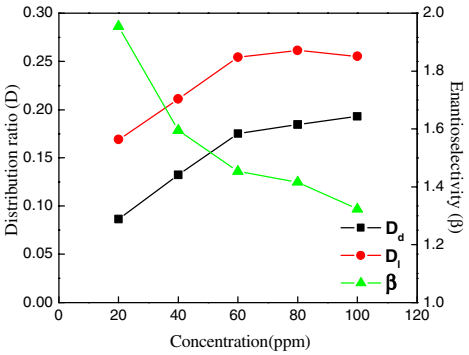
5-e Solute: *rac*-Hpg
Chiral selector: 0.1 mol/L (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



5-f: Solute: *rac*-Hpg
Chiral selector: 0.1 mol/L (+)-DTTA
Extractant: 0.2 mol/L D2EHPA



5-g: Solute: *rac*-Tyr
Chiral selector: 0.1 mol/L (+)-DBTA
Extractant: 0.2 mol/L D2EHPA



5-h: Solute: *rac*-Tyr
Chiral selector: 0.1 mol/L (+)-DTTA
Extractant: 0.2 mol/L D2EHPA

Figure 5. Influence of initial concentration of amino acids.

Table 1. Distribution ratio of the amino acids enantiomers with *n*-octanol as extractant

Amino acids	*rac*-Trp	*rac*-Phe	*rac*-Hpg	*rac*-Tyr
Distribution ratio	0.030	0.025	0.018	0.020

Initial concentration of the amino acids: *rac*-Trp: 50 ppm, *rac*-Phe: 50 ppm, *rac*-Hpg: 50 ppm, *rac*-Tyr: 50 ppm. Temperature: 25 °C.

Table 2. Comparison of enantioselectivities for the resolution of the amino acids

Amino acids	(+)-DBTA			(+)-DTTA		
	D_d	D_l	β	D_d	D_l	β
rac-Trp	2.69	9.65	3.59	2.55	10.26	4.02
rac-Phe	1.53	2.25	1.37	1.68	2.31	1.36
rac-Hpg	0.26	0.40	1.52	0.28	0.40	1.43
rac-Tyr	0.35	0.72	2.06	0.41	0.77	1.89

Initial concentration of the amino acids: *rac*-Trp: 50 ppm, *rac*-Phe: 50 ppm, *rac*-Hpg: 50 ppm, *rac*-Tyr: 50 ppm; (+)-DBTA or (+)-DTTA: 0.3 mol/L; D2EHPA: 0.4 mol/L; temperature: 25 °C.

3. Conclusion

The resolution results of the four amino acids studied, indicate that solvent co-extraction of D2EHPA with (+)-DBTA or (+)-DTTA are well adapted for their enantioseparation.

On the one hand, the good separation of enantiomers of the four amino acids make the extraction method suitable for large-scale production of the pure enantiomer. On the other hand, it opens the way, for searching new extractants or select appropriate enantiomers with both high extractive capacities and enantioselectivities.

4. Experimental

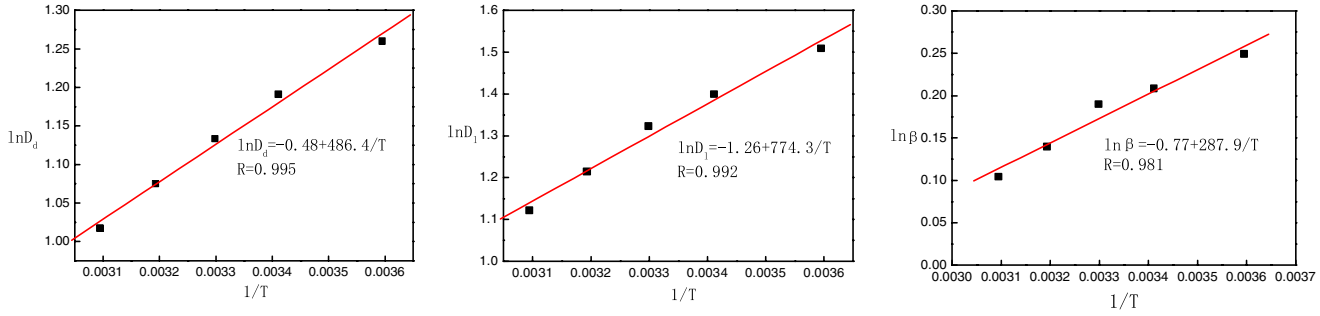
4.1. Chromatography

Chiral chromatography was performed on a HP series 1050 pumping system consisting of a 1050 variable

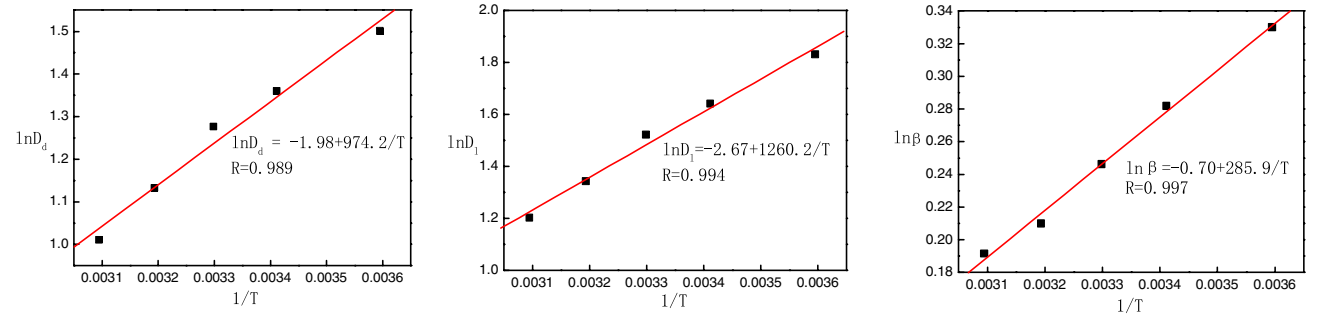
Table 3. Influence of temperature on the enantioseparation of Trp enantiomers

Temp. (°C)	(+)-DBTA			(+)-DTTA		
	D_d	D_l	β	D_d	D_l	β
5	3.525	4.522	1.283	4.485	6.239	1.391
20	3.290	4.052	1.232	3.894	5.162	1.326
30	3.106	3.755	1.209	3.582	4.582	1.279
40	2.930	3.369	1.150	3.102	3.827	1.234
50	2.766	3.070	1.110	2.746	3.326	1.211

Initial concentration of the amino acids: *rac*-Trp: 200 ppm; (+)-DBTA or (+)-DTTA: 0.15 mol/L; D2EHPA: 0.2 mol/L.



6-a: Solute: 200ppm *rac*- Trp; Chiral selector: 0.15 mol/L (+)-DBTA; Extractant: 0.2 mol/L D2EHPA



6-b: Solute: 200ppm *rac*-Trp; Chiral selector: 0.15 mol/L (+)-DTTA; Extractant: 0.2 mol/L D2EHPA

Figure 6. Influence of temperature on the enantioseparation of amino acids.

wavelength detector and an Agilent chemistation. The chromatographic system was equipped with HP 1050 injectors in 20 μL loops. The column was CROWNPAK CR(+), 5 μm particle size of the Packing Material, 150 mm × 4 mm I.D. (DAICELCHEMICAL INDUSTRIES Ltd.). The mobile phase was prepared by dissolving 16.3 g of commercially available 70% perchloric acid with distilled water to 1 L (pH = 2.0). The sample was detected with UV 200 nm and a flow rate of 1 mL/min. The retention time of the D-enantiomer was less than that of L-enantiomer.

4.2. Chemicals and materials

O,*O*′-Dibenzoyl-(2*S*,3*S*)-tartaric acid and *O*,*O*′-dibenzoyl-(2*S*,3*S*)-4-toluoyl-tartaric acid was purchased from Lingxing & Co. Inc. (Zhejiang, China). Di(2-ethylhexyl)phosphoric acid was obtained from Jinke Institute (Tianjin, China). *rac*-Tryptophan (*rac*-Trp), *rac*-phenylalanine (*rac*-Phe), *rac*-*p*-hydroxyphenylglycine (*rac*-Hpg) and *rac*-Tyrosine (*rac*-Tyr) were bought from Aoboxing & Co. Inc. (Beijing, China). *n*-Octanol was obtained from Yili & Co. Inc. (Beijing, China), and perchloric acid purchased from ACROS ORGANICS (New Jersey, USA). All chemicals were of analytical-reagent grade.

4.3. Extraction experiment

Figure 7 shows the possible enantioseparation mechanism with co-extractants of D2EHPA and DBTA or DTTA, which has been described elsewhere.[18] The aqueous phase was prepared by dissolving the amino acids in water. The pH values were adjusted with phosphate salt buffer solutions. (+)-DBTA, (+)-DTTA or D2EHPA were used as the extractants and *n*-octanol as the diluent. Equal volumes (each 1.5 mL) of the organic and aqueous phase were placed in a 5 mL glass-stoppered tube together, and shaken sufficiently (2 h) before being kept in a water bath (24 h) at a fixed temperature. After phase separation, the concentration was measured by HPLC. The total amount of each enantiomer existing in the organic and aqueous phases after extracting was consistent with their initial amount included in the aqueous phase, which proved that the amino acids were not decomposed by bacterium. Each experiment was duplicated under identical conditions and the standard deviation is in the range of 2%. If the extraction of amino acid enantiomers with *n*-octanol is negligible, the distribution ratio (D) and enantioselectivity (β) at equilibrium as the evaluated parameters are defined by

$$D_{d(l)} = \frac{\text{mass concentration (ppm) of D(L-) amino acid in the organic phase}}{\text{mass concentration (ppm) of D(L-) amino acid in the aqueous phase}} \quad (1)$$

$$\beta = \frac{\text{distribution ratio of L-enantiomer}}{\text{distribution ratio of D-enantiomer}} \quad (2)$$

The extractant could extract the L-enantiomer preferentially in all the enantioseparation experiments, and the D-enantiomer in the raffinate phase was in excess.

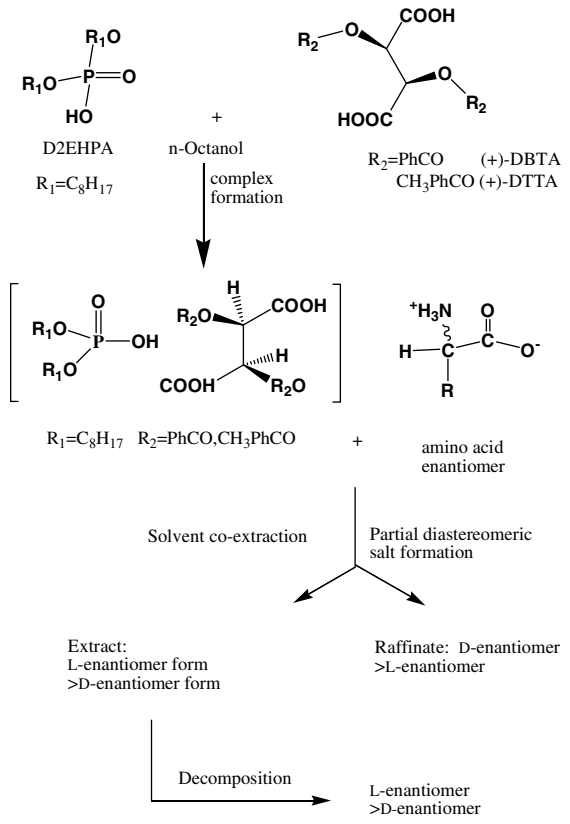


Figure 7. Enantioseparation mechanism of co-extractant extraction.

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References

1. Li, S.; Purdy, W. C. *J. Chromatogr.* **1991**, *543*, 105.
2. Chang, S. C.; Wang, L. R.; Armstrong, D. W. *J. Liq. Chromatogr.* **1992**, *15*, 1411.
3. Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. *J. Chromatogr. A* **1996**, *731*, 123.
4. Crosby, J. *Tetrahedron* **1991**, *47*, 4789.
5. Aboul-Enein, H.; Hefnawy, M. M.; Ehmer, P. B.; Hartmann, R. W. *J. Sep. Sci.* **2003**, *26*, 1455–1458.
6. Bortolini, Olga; Fantin, Giancarlo; Fogagnolo, Marco *Chirality* **2005**, *17*, 121–130.
7. Danel, Cécile; Foulon, Catherine; Park, Chang; Yous, Saïd; Bonte, Jean-Paul; Vaccher, Claude *J. Sep. Sci.* **2005**, *28*, 428–434.
8. Demirel, Nadir; Bulut, Yasemin; Hosgören, Hail. *Chirality* **2004**, *16*, 347–350.
9. Kahle, Claudia; Holzgrabe, Ulrike. *Chirality* **2004**, *16*, 509–515.

10. Chen, Zilin; Uchiyama, Katsumi; Hobo, Toshiyuki. *Electrophoresis* **2001**, *22*, 2136–2142.
11. Kmecz, Ildikó; Simándi, Béla; Székely, Edit; Fogassy, Elemér. *Tetrahedron: Asymmetry* **2004**, *15*, 1841–1845.
12. Kassai, Csaba; Bálint, József; Juvancz, Zoltán; Fogassy, Elemér; Kozma, Dávid. *Synth. Commun.* **2001**, *31*, 1715–1719.
13. Kassai, Csaba; Juvancz, Zoltán; Bálint, József; Fogassy, Elemér; Kozma, Dávid. *Tetrahedron* **2000**, *56*, 8355–8359.
14. Liu, Y. S.; Dai, Y. Y.; Wang, J. D. *Sep. Sci. Technol.* **1999**, *34*, 2165–2176.
15. Liu, Y. S.; Dai, Y. Y.; Wang, J. D. *Sep. Sci. Technol.* **2000**, *35*, 1439–1454.
16. Juang, Ruey-Shin; Wang, Yu-Yin. *J. Membr. Sci.* **2002**, *207*, 241–252.
17. Kelly, N. A.; Lukhezo, M.; Reuben, B. G.; Dunne, L. J.; Verrall, M. S. *J. Chem. Technol. Biotechnol.* **1998**, *72*, 347–355.
18. Tan, B.; Luo, G. S.; Qi, X.; Wang, J. D. *Sep. Purif. Technol.*, in press.
19. O'Brien, T.; Crocker, L.; Thompson, R.; Thompson, K.; Toma, P. H.; Conlon, D. A.; Feibush, B.; Moeder, C.; Bicker, G.; Grinberg, N. *Anal. Chem.* **1997**, *69*, 1999–2007.
20. Kazusaki, M.; Kawabata, H.; Matsukura, H. *J. Liq. Chrom. Rel. Technol.* **2000**, *23*, 2937–2946.