Liquid Chromatographic Enantiomer Separation of Racemic Amine Using Chiral Crown Ether Stationary Phase

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Abstract

Direct enantioseparation of racemic amine, amino-thiophene-2-yl-acetonitrile (TAN), on chiral crown ether stationary phase [Crownpak CR (+)] is described in this study. The elution behavior and the effect of acid additives on resolution of racemic amine, TAN, is intensely investigated. Moreover, the chiral recognition mechanism in this specific system is proposed based on computational methods with the density functional theory. Diastereomeric complexation of the ammonium ion of racemic amine inside the cavity of chiral crown ether appears essential for the chiral discrimination. The pH of the mobile phase containing acid additives also acts as an important factor for the chiral recognition.

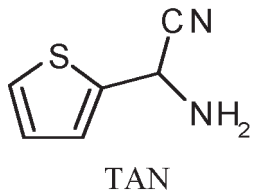
Introduction

Liquid chromatographic (LC) separation of enantiomers on chiral stationary phases (CSPs) has been widely employed for simple and accurate assessment of the enantiomeric purity. Enantiomerically pure compounds can also be prepared by the extension of these separation techniques to the preparative scale (1). Amino-thiophene-2-yl-acetonitrile (TAN), a racemic compound with a primary amino group, is a key intermediate in the synthesis of ethaboxam, a novel aminocarboxamide fungicide (2–4). It is necessary to confirm whether the fungicidal efficacies of each enantiomer of ethaboxam synthesized from TAN are biologically equivalent to that of racemic ethaboxam. Therefore, enantiomer separation of ethaboxam as well as their intermediate (TAN) is an essential requirement for biological screening. Several studies have previously reported on the enantiomer separation using polysaccharide derived CSPs (5–8). Polysaccharide-derived CSPs have been widely adopted in the separation of enantiomers by LC (9,10). These CSPs were initially applied for the enantiomer separation of the racemic compound with a primary amino group, TAN. However, acceptable resolution was hardly achieved on any available kinds of polysaccharide CSPs. Shinbo et al. developed a chiral crown ether consisting of substituted 1,1'-binaphthyl-20-crown-6 (11). This CSP was applied and utilized for the LC resolution of racemic compounds containing a primary amino group. In the late 1980s, this CSP has been commercialized as Crownpak CR (Daicel Chemical Industries, Osaka, Japan). The method of enantiomer separation of racemic amine (TAN) was developed on this commercial Crownpak CR (+). The effect of various acid modifier and chromatographic elution behaviors was investigated. In addition, the elucidation of the chiral recognition mechanism using computational method based on density functional theory (DFT) is proposed.

Experimental

Chemicals and reagents

TAN used in this study was developed and supplied by LG Life Science Ltd. (Daejeon, South Korea) (Figure 1). All solvents used in this study were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ). Water was purified by Milli-Q water purification system (Millipore, Billerica, MA). Perchloric acid and sulfuric acid were purchased from Fluka company (Buchs, Switzerland). Formic acid, acetic acid, trifluoroacetic acid, and trichloroacetic acid were purchased from Aldrich (Milwaukee, WI). Methanesulfonic acid was purchased from Janssen Chimica (Geel, Belgium).



TAN

Figure 1. The structure of racemic amine (TAN) in this study.

Chromatographic conditions

LC was carried out on a Waters Alliance 2690 HPLC system and 996 photodiode array detector (Waters, Bedford, MA). Signs of optical

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Table I. Enantiomer Separation Properties with Various Acid Additives on Crownpack CR (+)

Entry	Acid additives	pH	k_1'*	α†	R_s‡
1	10mM $HClO_4$	2.22	1.47	1.37	0.73
2	10mM CF_3CO_2H	2.25	1.13	1.35	0.60
3	10mM CCl_3CO_2H	2.35	3.52	1.31	0.91
4	10mM CH_3CO_2H	3.15	2.74	1.19	0.42
5	10mM HCO_2H	2.98	1.43	1.23	0.34
6	10mM CH_3SO_3H	2.30	0.88	1.28	0.39
7	10mM H_2SO_4	2.16	0.95	1.26	0.36

* Capacity factor of first eluted enantiomer.
† Separation factor.
‡ Resolution factor.

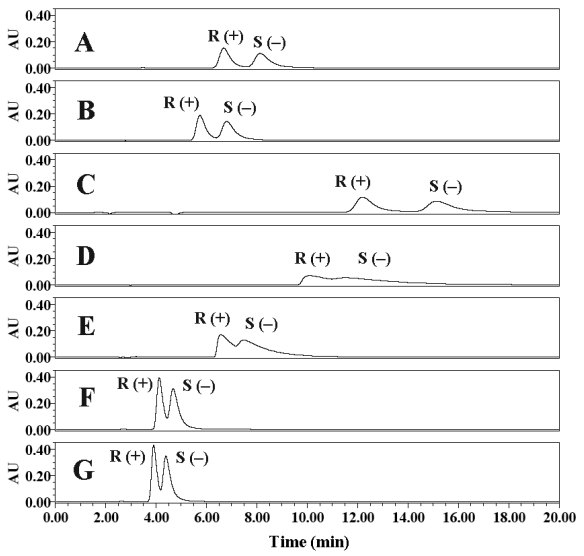

Figure 2. Enantiomer separation of TAN varying acid additives on Crownpak CR (+): 10mM $HClO_4$ (A); 10mM CF_3CO_2H (B); 10mM CCl_3CO_2H (C); 10mM CH_3CO_2H (D); 10mM HCO_2H (E); 10mM CH_3SO_3H (F); and 10mM H_2SO_4 (G).

Table II. Enantiomer Separation Properties with Various Concentrations of Acid Additives

Acid additives	Concentration of acid additives	k_1'*	α†	R_s‡
CH_3SO_3H	10	0.88	1.28	0.39
	20	0.92	1.28	0.47
	50	0.90	1.29	0.56
	100	1.01	1.28	0.62
H_2SO_4	10	0.95	1.26	0.36
	20	0.95	1.26	0.45
	50	0.94	1.26	0.49
	100	0.98	1.24	0.57

* Capacity factor of first eluted enantiomer.
† Separation factor.
‡ Resolution factor.

rotation were determined using a shodex OR-1M optical rotation detector (Showa Denko, Tokyo, Japan). HPLC system control and data processing were performed by Empower software (Build 1154, Waters). The chiral columns used were a commercially available Crownpak CR (+) (150- × 4.6-mm i.d., 10-µm particle size) (Daicel), Chiralcel OD, Chiralcel OB, Chiralpak AD, and Chiralpak AS (250- × 4.6-mm, 10-µm particle size) (Daicel). The detection wavelength was set at 230 nm in all experiments. The eluents used were mixtures of water with various acid additives and concentrations from 10 to 100mM. The injection volume of sample dissolved in water was 10 µL of 1 mg/mL.

Computation details

All calculations were performed with DMol^3 software package (Accelrys, San Diego, CA) (12,13) based on the DFT, utilizing the double numerical with polarization (DNP) basis set. DNP is comparable in size to the commonly used 6-31G** Gaussian basis set. However, the numerical basis set is much more accurate than a Gaussian basis set of the same size (14). The geometry optimizations of Crownpak CR (+) (TAN) and their diastereomeric complexes were performed to calculate

Table III. Enantiomer Separation Properties with Various Eluent Flow Rate

Acid additives	Flow rate (mL/min)	k_1'*	α†	R_s‡
CH_3SO_3H	0.1	0.99	1.29	0.95
	0.2	0.77	1.32	0.81
	0.3	0.97	1.29	0.72
	0.5	1.01	1.28	0.62
H_2SO_4	0.1	0.83	1.28	0.87
	0.2	1.80	1.20	0.73
	0.3	1.04	1.25	0.64
	0.5	0.98	1.24	0.57

* Capacity factor of first eluted enantiomer.
† Separation factor.
‡ Resolution factor.

Table IV. Calculated Interaction Energies* between Crownpak CR (+) and TAN

Entry	Absolute configuration of TAN	VWN	PW91	PBE
1		94.46	60.70	59.60
2	R	87.59	59.39	58.34
3		97.34	66.18	64.86
4		90.30	59.73	58.51
5	S	92.20	59.67	58.60
6		98.44	67.18	66.02

* In kcal/mol.

the interaction energies between Crownpak CR (+) and each enantiomer of TAN. Kohn-Sham DFT calculations were performed with three different exchange correlation functionals; namely, local-density approximation (LDA) [Vosko-Wilk-Nusair (15)] and generalized gradient approximations (GGA) [Perdew-Wang 91 (PW91) (16) and Perdew-Burke-Ernzerhof (PBE) (17)], functionals as implemented in DMol^3. The PW91 and PBE functionals have been successfully applied in predicting the interaction energies of hydrogen-bonded complexes (18–20) and pure van der Waals complexes (21).

Results and Discussion

Polysaccharide-derived CSPs, widely adopted in the separation of enantiomers by LC, were initially tested for the enantiomer separation of the racemic amine, TAN. However, acceptable resolution was hardly achieved on any available kinds of polysaccharide CSPs. The best resolution factor, obtained with Chiralpak AS among other polysaccharide CSPs, was no more than 0.40 with the eluent of hexane–ethanol–trifluoroacetic acid of 90:10:0.1 (v/v, %). It was then possible to

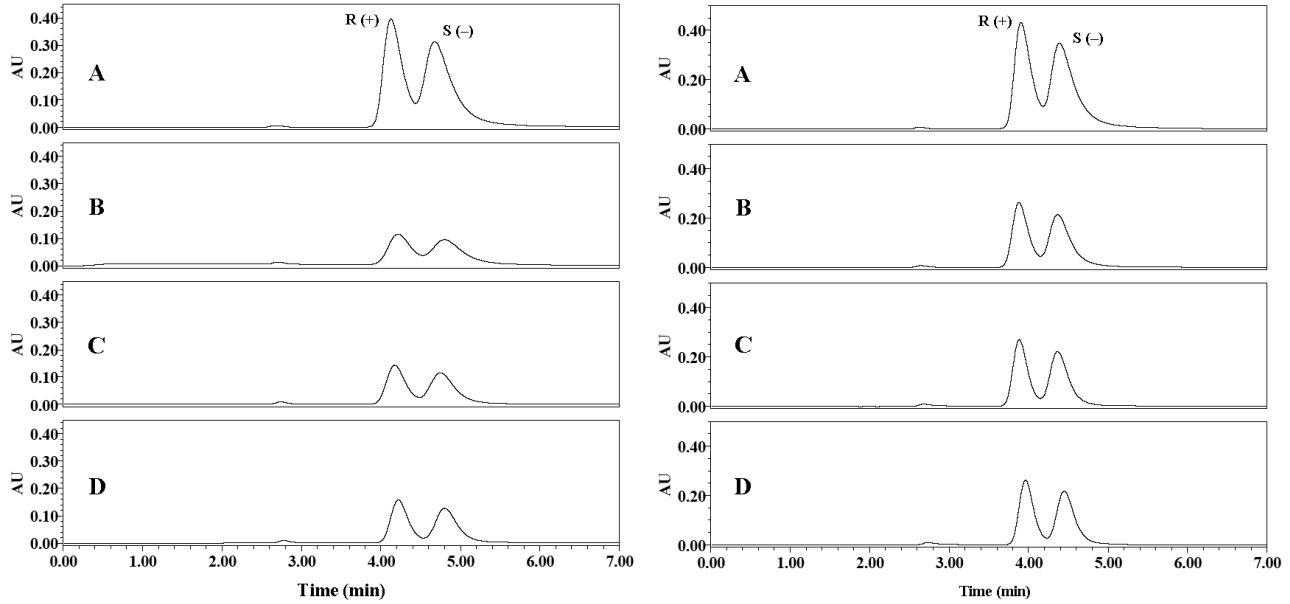

Figure 3. Enantiomer separation of TAN with varying concentrations of acid additives on Crownpak CR (+) CH_3SO_3H (left side) and H_2SO_4 (right side) over the range of 10–100mM concentration: 10mM (A), 20mM (B), 50mM (C), and 100mM (D).

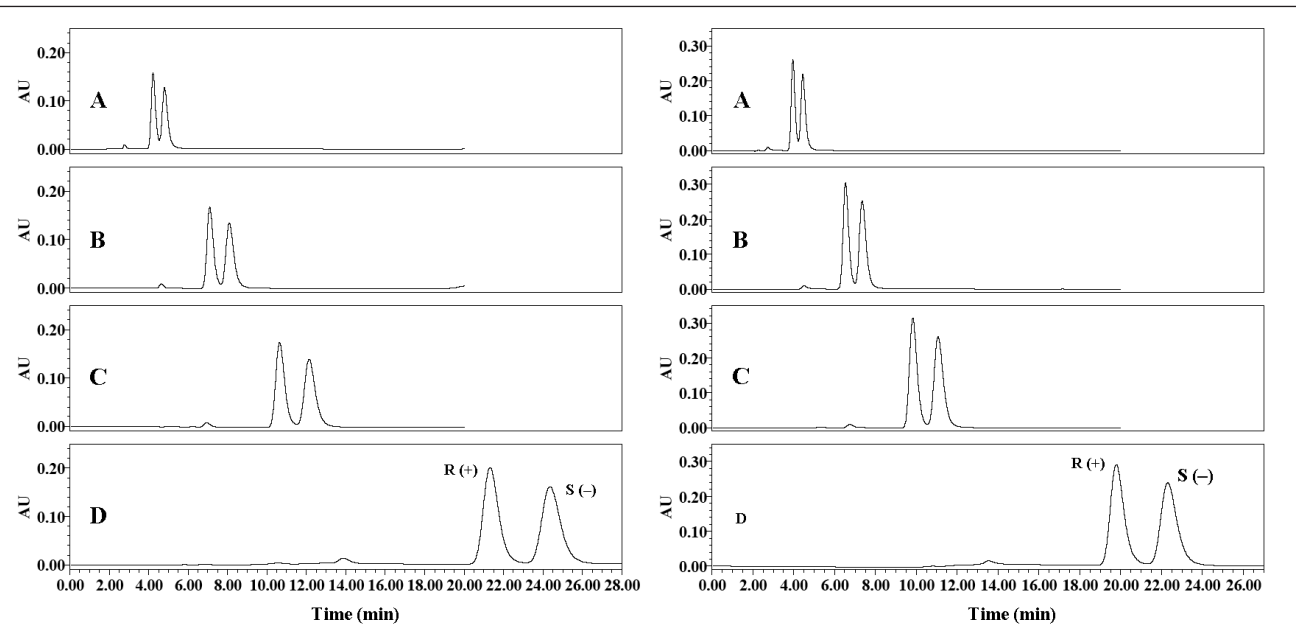

Figure 4. Enantiomer separation of TAN varying flow rate of eluent on Crownpak CR (+) CH_3SO_3H (left side) and H_2SO_4 (right side) over the range of 0.1–0.5 mL/min flow rate of mobile phase: 0.5 mL/min (A), 0.3 mL/min (B), 0.2 mL/min (C), and 0.1 mL/min (D).

obtain better resolution of the racemic amine (TAN) with chiral crown ether CSP, especially with the commercially available Crownpak CR (+). The effect of acid additive on the enantioselectivity was investigated with two inorganic acids and five organic acids such as perchloric, sulfuric, formic, acetic, trifluoroacetic, trichloroacetic, and methanesulfonic acids. The chromatographic results of enantioseparation of racemic amine (TAN) with seven different acid additives are summarized in Table I. Among the acids used, the best enantiomer separation was obtained using trichloroacetic acid (entry 3 in Table I). It has been observed that an increase in the lipophilicity of the acid anion leads to elongated retention times caused by relatively poor solvation effect of lipophilic anion by solvent molecules (11). Acetic acid additive containing lipophilic anion, however, showed deteriorated resolution. Methanesulfonic acid, on the other hand, gives slightly shorter retention times than sulfuric acid, although it has more lipophilic anion property than that of sulfuric acid. It appears that the pH of the mobile phase has an affect more on the retention time than the anion lipophilic property. The pH with methanesulfonic acid additive was 2.30, slightly higher than pH 2.16 with sulfuric acid. The behavior of retention times with perchloric acid and methanesulfonic acid could be understood by the same reason. Perchloric acid showed lower pH than methanesulfonic acid. Therefore, the racemic amine was more retained with the use of perchloric acid additive. Figure 2 shows a chromatogram of the enantiomer separation with various acid additives. Although trichloroacetic acid provided the best peak resolution, peak broadening was not suitable (Figure 2C). The separation condition was then improved the by focusing on methanesulfonic and sulfuric acid, because of the relatively sharp peak shape. Increased concentration of acid additives in the mobile phase provided better resolution with slightly delayed retention times (Table II and Figure 3). In the case of methanesulfonic acid, resolution factors varying from 0.39 to 0.62 were observed with concentrations ranging from 10 to 100mM. The effect of the flow rate of mobile phase on enantiomer separation was also considered. Among the flow rates varying from 0.1 to 0.5 mL/min, the best and most satisfactory enantiomer separation was obtained with 0.1 mL/min of flow rate (Table III and Figure 4). The S-(–) form of racemic amine (TAN) was eluted later than the R-(+) form, and a consistent elution order of enantiomer was observed on Crownpak CR (+).

In order to further clarify the results of chromatographic behavior, computational studies were performed on the chiral separation system. The PBE-optimized structures of Crownpak CR (+) and (S)-TAN are shown in Figure 5A (see page 3A). The two naphthalene rings are attached with the 76.8° dihedral angle, and two phenyl rings are placed above and below the 20-crown-6 ring (C_2 symmetry). It was reported that the dihedral angle of two naphthalene rings created a chiral barrier, and the phenyl ring played an important role in a chiral recognition (22). In order to determine the interaction site of Crownpak CR (+), we obtained the electrostatic potential maps of the Crownpak CR (+) and (S)-TAN, which is shown in Figure 5B (see page 3A). Dark regions of the Crownpak CR (+) represent negative electrostatic potential associated with the six oxygen atoms of the crown ether ring. Also, dark regions of (S)-TAN represent positive electrostatic potential associated with the ammonium group. The hydrogen bondings between the ammonium group and the oxygen atoms of 20-crown-6 are the main interactions in the diastereomeric complexes of the Crownpak CR(+) and (R/S)-TAN (Figure 5C, see page 3A). The optimized structures and the calculated interaction energies of three possible complexes are shown for each (R) and (S)-TAN, in Table IV and Figure 6 (see page 3A), respectively. The entry 3 and 6 complexes are the most stable forms in the diastereomeric complexes of (R) and (S)-TAN, respectively. The calculated LDA interaction energies are larger than the GGA ones by approximately 30 kcal/mol, which is consistent with the known overestimation of LDA trend of binding energies (23). It should be noted that the interaction energies of complex entry 6 is larger than complex entry 3 by 1.0–1.2 kcal/mol at the DFT levels of theory considered here. This implies that the (S)-TAN was more retained in the Crownpak CR (+) chiral stationary phase in LC. The thiophene ring of (S)-TAN is placed toward the phenyl-ring in the entry 6 complex, as shown in Figure 6B (see page 3A). In the structure, there are weak interactions between the π-systems of thiophene and phenyl rings. On the other hand, the thiophene ring of (R)-TAN is placed on the opposite side of phenyl ring in the entry 3 complexes. We believe that the weak π-interaction between (S)-TAN and Crownpak CR (+) accounts for the chiral recognition mechanism of crown ether stationary phase.

Conclusion

The enantiomer separation of racemic amine mixture (TAN), a key intermediate of novel aminocarboxamide fungicide, was successfully demonstrated. The effect of acid additives in the mobile phase on the enantioselectivity was investigated with seven different acids. The pH of the mobile phase containing acid additives acts as an important factor for the chiral recognition. Computational study shows that diastereomeric complexation of the ammonium ion of racemic amine inside the cavity of chiral crown ether is essential for chiral discrimination.

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Manuscript received December 9, 2004;
revision received September 14, 2005.