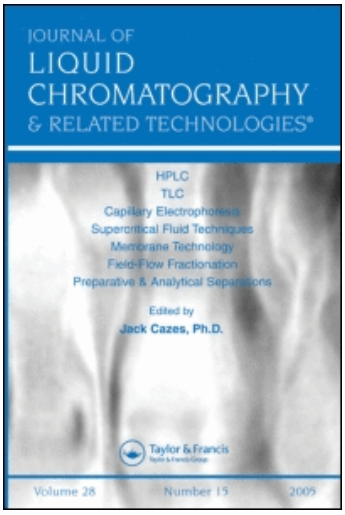

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INFLUENCE OF TEMPERATURE ON ENANTIOSEPARATION EMPLOYING AN AMYLOSE-DERIVATIVE STATIONARY PHASE

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ABSTRACT

In reversed-phase mode, chromatographic retention is investigated thermodynamically for an enantiomeric pair on an amylose-derivative bonded phase. Ehthalpies and entropies of solute transfer (mobile to stationary phase) are calculated from retention data by evaluation of van't Hoff plots.

Conformational change of the stationary phase is observed at about 20°C. The enantioselectivity is exclusively driven by enthalpy above about 20°C, whereas below about 20°C enantioseparation was achieved by the combination of enthalpy and entropy. The inclusion process of the enantiomer, retained stronger on the stationary phase, plays a important role for chiral recognition on the amylose-derived stationary phase.

INTRODUCTION

A wide range of polysaccharide-based chiral stationary phases (CSPs) are now available for the separation of enantiomers by HPLC and they have proved to be very useful in the chromatographic resolution of racemic mixtures.[1,2] In particular, triacetate, tribenzoate, and triphenylcarbamate derivatives of cellulose and amylose derivatives show high abilities to recognize chirality.

An understanding of the recognition of chirality at a molecular level is of great interest and importance in many fields of chemistry and biology. Unfortunately, relatively little is understood about the mechanism of interaction between enantiomers and the chiral stationary phase. In the past few years, several approaches to clarifying the mechanism for the recognition of chirality on CSPs for liquid chromatographies have been attempted by means of chromatography,[3,4,5] NMR spectroscopy,[6] X-ray analysis, and computational methods.[7] In contrast, very few studies on chiral discrimination mechanism at a molecular level have been performed for polymeric CSPs.

Chiral polymers usually have a number of different binding sites with different affinities to enantiomers, and the determination of their exact structures in solution is laborious work. This makes it difficult to evaluate a precise mechanism for the recognition of chirality on the polymeric CSPs. In this study, we attempted to evaluate the thermodynamic parameter of interactions occurring between racemate and the stationary phase in the reversed-phase mode.

Using a racemic mixture, *R*-2-(4-bromo-2-fluorobenzyl)-(1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine-4-spiro-3'-pyrrolidine)-1,2',3,5'-tetrone (Figure 1)[8] and corresponding *S*-enantiomer, as a probe, we attempted to clarify the mechanism of chiral recognition on an amylose derivative stationary phase. The chemical structure of amylose-derivative is depicted in Figure 2.

This paper reports the results of an investigation on the thermodynamic behavior of selected compounds in reversed-phase HPLC employing an amylose tris(3,5-dimethylphenylcarbamate) chiral column. The relative effect of $\Delta\Delta H°$ and $\Delta\Delta S°$ on chiral separation is discussed.

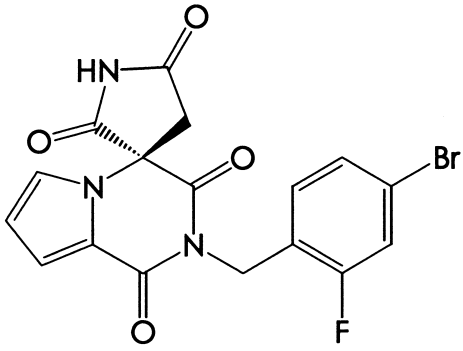

Figure 1. Chemical structure of *R*-2-(4-bromo-2-fluorobenzyl)-(1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine-4-spiro-3'-pyrrolidine)-1,2',3,5'-tetrone.

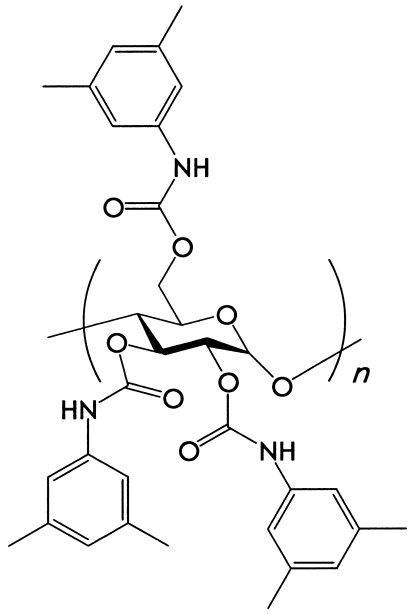

Figure 2. Chemical structure of amylose derivative.

EXPERIMENTAL

Chemical and Reagents

R-2-(4-bromo-2-fluorobenzyl)-(1,2,3,4-tetrahydro-pyrrolo-[1,2-a]pyrazine-4-spiro-3'-pyrrolidine)-1,2',3,5'-tetrone and corresponding *S*-enantiomer were synthesized in the laboratory of Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan).

Ammonium acetate was of reagent grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). HPLC grade acetonitrile was also purchased from Wako Pure Chemical Industries Ltd. The water was deionized prior to usage.

Apparatus

The HPLC system consisted of a solvent delivery system, column oven, variable wavelength detector set at 297 nm, and variable volume injector set at

10 μL (Hitachi model D-7000 system, Hitachi, Tokyo, Japan). Chiralpak AD-RH (150 mm x 4.6 mm i.d., Daicel Chemical Industries, Ltd., Tokyo, Japan) was employed for this study. The flow rate was 0.5 mL/min. Duplicate injection was performed at each column temperature and mobile phase composition.

With each change in column temperature and mobile phase composition, the column was permitted to re-equilibrate by flushing with 15 void volumes of eluent. Enthalpies and entropies of transfer were calculated through linear regression analysis of van't Hoff plots.

RESULTS AND DISCUSSION

The retention of *R*-2-(4-bromo-2-fluorobenzyl)-(1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine-4-spiro-3'-pyrrolidine)-1,2',3,5'-tetrone and corresponding *S*-enantiomer on a amylose tris(3,5-dimethylphenylcarbamate) chiral column was studied to clarify the mechanism for the separation of enantiomers. Elution of enantiomers on an amylose-derivative column is shown in Figure 3. Retention factor can be expressed in terms of standard enthalpies and entropies of transfer from the mobile phase to the stationary phase as the following equation to give the van't Hoff equation:

$$\ln k = \Delta H^\circ / RT + \Delta S^\circ / R + \ln \phi$$

where ΔH^0 and ΔS^0 represent the standard enthalpy change and the standard entropy change of mass transfer from the mobile phase to the stationary phase, is phase ratio, T and R are absolute temperature and gas constant. It is important to note that ΔH^0 value is independent of the phase ratio.

The standard enthalpy change, ΔH^0, can be obtained from the slope of van't Hoff plots. However, if the stationary phase undergoes a change in conformation at a certain temperature (transition temperature), the enthalpy and entropy of the retention process will change, and the non-linear van't Hoff plots will be obtained.

As shown in Figure 4, van't Hoff plots were generated for respective enantiomers. The van't Hoff plots were not linear, suggesting that the conformational change of the stationary phase occurred at about 20°C. This result indicates that there are two mechanisms which control the enantioseparation. One region occurred above about 20°C, and another region occurred below about 20°C.

The calculated thermodynamic parameters are given in Table 1. Enthalpies of transfer are negative, suggesting that transfer of the solute from the mobile phase to the stationary phase is enthalpically favored. This is con-

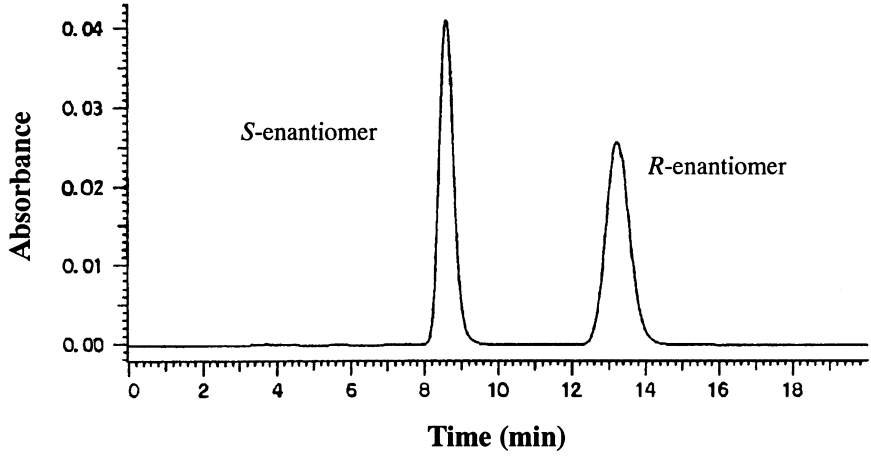

Figure 3. Separation of *R*-2-(4-bromo-2-fluorobenzyl)-(1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine-4-spiro-3'-pyrrolidine)-1,2',3,5'-tetrone and *S*-enantiomer on a chiral column by reversed-phase HPLC. HPLC operating condition: column, Chiralpak AD-RH (150 mm × 4.6 mm I.D.); column temperature, 40°C; mobile phase, 0.01 M acetate buffer (pH 4.7) - acetonitrile (1:1); flow rate, 0.5 mL/min; detection, 297 nm.

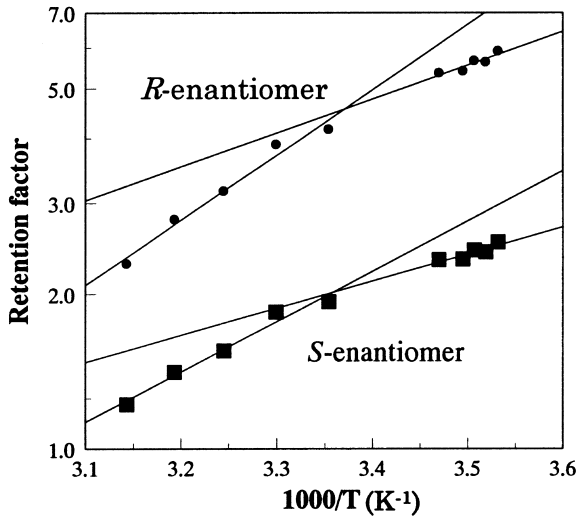

Figure 4. van't Hoff plots for *R*-enantiomer and *S*-enantiomer. For chromatographic conditions, see Figure 3, except for column temperature.

sistent with thermodynamics, because retention on the column in HPLC operation is usually enthalpy controlled. Additionally, the separation factor, α, is obtained from the equation described below:

$$\ln \alpha = \ln (k_R / k_S) = -\Delta(\Delta H^\circ) / RT + \Delta(\Delta S^\circ) / R$$

where k_R and k_S are the retention factor of *R*, *S* - enantiomers, respectively. This relationship is graphically shown in Figure 5. $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ were calculated from chromatographic parameters and summarized in Table 2.

The values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ above about 20°C indicate enantioseparation is enthalpically governed in this temperature range. However, from trends in $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ below about 20°C on this column, the importance of $\Delta\Delta S^\circ$ to enantioseparation is seen to increase with the increase of organic modifier character. Though, it can be seen that in the temperature range of 11°C to 15°C $\Delta\Delta H^\circ$ is of primary importance at relatively low and moderate acetonitrile concentration, at relatively high acetonitrile concentration the contribution of the $\Delta\Delta S^\circ$ surpasses that of $\Delta\Delta H^\circ$ for optical resolution. In this case, a comparison of the magnitudes of thermodynamic characters shows that $\Delta\Delta S^\circ$ is the major contributor to enantioseparation.

Table 1

Enthalpies of Solute Transfer from the Mobile Phase to the Stationary Phase

Column Temperature (C)	Acetonitrile Concentration (%)	$\Delta H^\circ_{S\text{-enantiomer}}$ (kcal/mol)	$\Delta H^\circ_{R\text{-enantiomer}}$ (kcal/mol)
	45	2.58	3.52
11 - 15	50	2.43	3.02
	55	2.09	2.16
	45	4.24	5.54
25 - 45	50	4.48	5.77
	55	3.50	4.66

Mobile phase consisted of acetonitrile and 0.01 *M* acetate buffer, pH4.7. $\Delta H^\circ_{S\text{-enantiomer}}$ and $\Delta H^\circ_{R\text{-enantiomer}}$ are enthalpy of transfer from mobile to stationary phase, respectively.

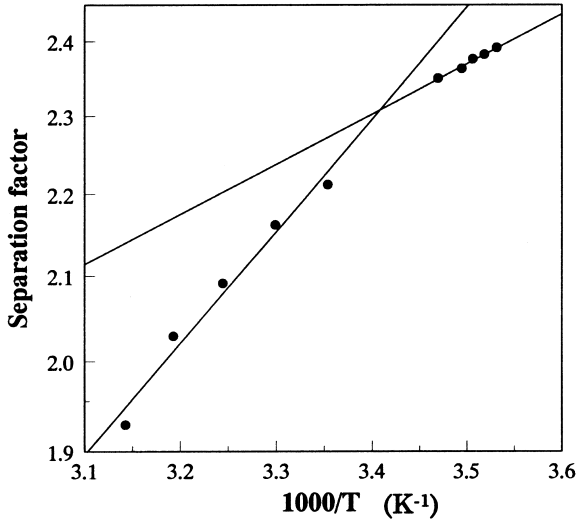

Figure 5. Relation between separation factor and reciprocal absolute temperature. For chromatographic conditions, see Figure 3, except for column temperature.

Table 2

Thermodynamic Parameters for Enantioseparation on an Amylose-Derivatized Column

Column Temperature (C)	Acetonitrile Concentration (%)	ΔΔH° (kcal/mol)	TΔΔS° (kcal/mol)
	45	**1.09**	**0.64**
11- 15	50	0.59	0.11
	55	0.07	0.43
	45	1.30	0.85
25 - 45	50	1.30	0.83
	55	1.16	0.70

Mobile phase consisted of acetonitrile and 0.01 *M* acetate buffer, pH4.7. ΔΔ*H*° and ΔΔS° represent the differences of enthalpy and entropy between enantiomers concerning transfer from the mobile to stationary phase. TΔΔS° is evaluated at 298 K.

Further analysis reveals that while $\Delta\Delta H°$ was increased, the $\Delta\Delta S°$ term was proportionally increasing. This phenomenon is well known as entropy - enthalpy compensation and is illustrated graphically in Figure 6.

This trend in $\Delta\Delta H°$ and $\Delta\Delta S°$ indicates, that, in the relatively lower temperature range the loss of interaction between the enantiomers and the stationary phase is balanced by a relative increase in the space available for *R*-enantiomer, retained stronger on the column, when it enters the stationary phase. Conformational changes of the stationary phase, indicated by non-linear van't Hoff plots, can be accompanied by the differences in solute adsorption / desorption rates.

Rizzi showed that acetyl-cellulose has two types of adsorption and desorption sites which differ in adsorption and desorption rate.[9] The slow adsorption and desorption sites bind through an inclusion process, being critically important to chiral recognition. This process results in chromatographic peak broadening and makes plate height reduced. Therefore, an increase in height equivalent to a theoretical plate (HETP) is employed as a probe of sluggish mass transfer.

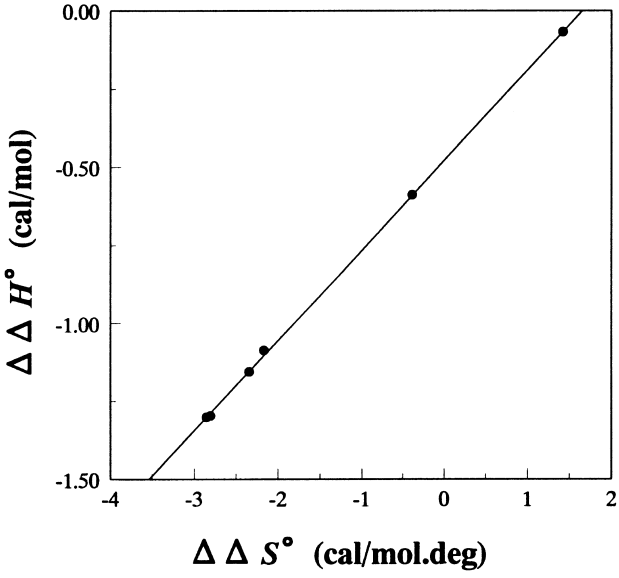

Figure 6. Entropy - enthalpy compensation for the separation of *R*-enantiomer and *S*-enantiomer on AD-RH.

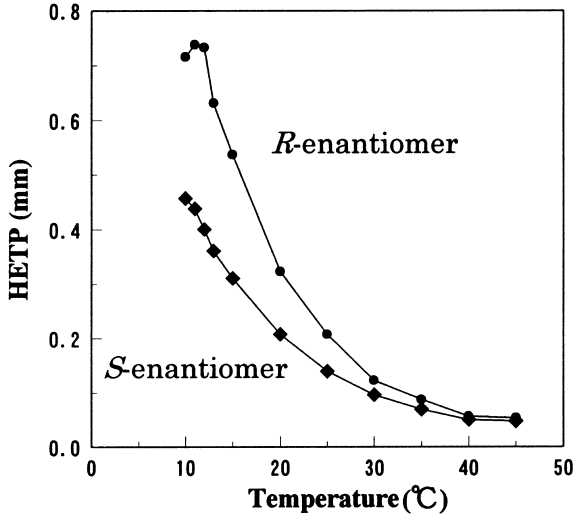

Figure 7. Influence of temperature on the height equivalent to a theoretical plate (HETP). For chromatographic conditions, see Figure 3, except for column temperature.

As shown in Figure 7, for *R*-enantiomer, HETP remains relatively large at temperature up to 15°C, after which a sharp decrease in HETP was observed in the region of 20 ~ 45°C. One can understand that *R*-enantiomer is retained on the stationary phase by inclusion process. At low temperature, the mass transfer of *R*-enantiomer is sluggish due to inclusion into chiral cavities.

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