Thermodynamic Properties of Enantioseparation of Two N−Carbobenzyloxy−D, L−Amino Acids on Cellulose Tris (4−methylbenzoate) Chiral Stationary Phase

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Abstract

The isocratic retention of enantiomers of N−carbobenzyloxy−D, L−tryptophan and N−carbobenzyloxy−D, L−phenylalanine was studied on cellulose tris (4−methylbenzoate) coated on silica as a chiral stationary phase at different temperatures and with different mobile phase compositions in the reversed−phase mode. The thermodynamic parameters were calculated in order to promote an understanding of the relationship between enthalpy and entropy for enantioseparation in this chromatographic system. The separation was enthalpy−controlled with negative entropic contribution in the HPLC condition studied. The enthalpy−entropy relationship indicates that enthalpic gain/loss for enantioseparation from any change of organic modifier content in the mobile phase is almost cancelled out by the entropic loss/gain.

Keywords: cellulose tris (4−methylbenzoate) chiral stationary phase, enantioseparation, enthalpy−entropy compensation

1. Introduction

The separation and analysis of biologically active chiral compounds continue to be an area of increasing interest in pharmaceutical industry since biological activities may vary dramatically between enantiomers. Through the advances of industrial technologies, direct chiral high−performance liquid chromatographic separation is now well established with many commercially available columns containing chiral stationary phases (CSPs). In the past few years, cellulose−based derivatives have been developed and shown to be exceptionally useful for the separation of enantiomers of biological and pharmaceutical importance. Cellulose tris (4−methylbenzoate) is one of the most recently introduced CSPs (Figure 1).

Although there is now a considerable amount of information regarding interaction between solutes and cellulose−based chiral stationary phases, [1−3] little is known from a view of thermodynamics, especially in the reversed−phase mode. The degree of chiral recognition of enantiomers by a CSP depends strongly on the temperature. The separation factor usually decreases as the temperature increases. Retention data obtained at different tempera-
rameters were determined in order to better understand the thermo-
dynamic driving forces affecting retention and separation of enanti-
omers. These effects on retention in reversed–phase high–performance
ance liquid chromatography have been considered for different
CSPs, including those based on chiral crown ethers, [4, 5] cy-
cloextrinsics, [6, 7] -acid glycoprotein, [8] human serum albumin,
[9] derived aromatic ring, [10–12] derived amylose [13, 14] and
cellulose, [14–19] and chiral macro cyclic antibiotics [20] by many
groups.

Our recent studies, from the thermodynamic point of view, re-
vealed the conformation change of the CSP consisted of amylose
tris (3, 5–dimethylphenylcarbamate) over the temperature range
studied, [13] and unusual behavior of the enthalpy and entropy of
transfer of the solute from the mobile to the CSP of cellulose tris
(3, 5–dimethylphenylcarbamate) as a function of organic modifier
content in the mobile phase, [18] respectively. The aim of the pre-
sent report is to provide the information concerning the relation be-
 tween enthalpy and entropy on the mechanisms involved in the
separation of derived -amino acids with cellulose tris (4–meth-
ylbenzoate) coated on silica as a chiral stationary phase in the re-
versed–phase mode.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and methanol of HPLC grade, phosphoric acid
and sodium dihydrogen phosphate were obtained from Wako Pure
Chemical Industries Ltd. (Osaka, Japan). Water was deionized
prior to usage. Sodium dihydrogen phosphate was dissolved in
deionized water and phosphoric acid was added to make a 0.02–M
phosphate buffer at pH 2.0. The amino acid derivatives investi-
gated were -carbobenzyloxy–, phenylalanine (CBZ–, t–Phe), -car-
benzyloxy–, t–tryptophan (CBZ–, t–Trp) and -carbenzyloxy–, t–tryptophan (CBZ–, t–Trp) (Figure 2) from Tokyo Kasei
Kogyo Co., Ltd. (Tokyo, Japan). Derived amino acids were dis-
solved in acetonitrile at 0.01 mg/mL for chiral HPLC analysis. The
-t–enantiomers were employed for the identification of elution or-
er order of enantiomers.

2.2. Apparatus

HPLC measurements were performed on a Hitachi (Tokyo,
Japan) D–7000 System equipped with two isocratic pumps (L–
7100), variable wavelength detector (L–7420), variable volume in-
jector (L–7200) and column oven (L–7300). CHIRALCEL OJ–RH
(150 mm × 4.6 mm I.D., Daicel Chemical Ltd., Tokyo, Japan),
kindly provided from Fuji Kagaku Yakuhin (Hyogo, Japan), was
employed for analytical separation. The packing material of the
column was a silica–gel support (5 -m) coated with a polymer of
cellulose tris(4–methylbenzoate). The mobile phase consisted of
acetonitrile – 0.02 M phosphate buffer at pH 2.0 (33:67–41:59, v/
v). The column temperature was adjusted at 20–45 ° at a flow–
rate of 1 mL/min, and the injection volume was set at 10 μL. Du-
plicate injections were performed at each column temperature and
mobile phase composition. The elution of amino acid derivatives
was monitored at 220 nm. The chromatographic system was equili-
brated by passing the eluent until a stable baseline signal was ob-
tained. This procedure was always followed when a new mobile
phase or temperature was applied for analysis. The column void
time was determined from the first perturbation of baseline caused
by methanol injection.

3. Results and discussion

Enantiomers of CBZ–, t–Trp and CBZ–, t–Phe were chro-
matographed on CHIRALCEL OJ–RH in the reversed–phase
mode. Acetanilide was used as organic modifier due to the poor du-
rability of CHIRALCEL OJ–RH for pressure. Typical chroma-
tograms are shown in Figure 3. As regards the elution order of
enantiomers, the -enantiomers always eluted before the t–enanti-
omers. In some cases the organic modifier in the mobile phase
plays an important role in the stereo–selectivity by binding to
achiral sites near or at the chiral cavities of CSP, which alters the steric environment of these cavities. [17] However, the chiral separation on this CSP was not affected by varying acetonitrile content in the range tested.

In order to calculate thermodynamic parameters for more understanding of the separation mechanism of enantiomers on this CSP, van’t Hoff plots were constructed. The chromatographic retention, expressed by \( k \), was expressed in terms of the following van’t Hoff equation:

\[
\ln k = -\Delta H^{\circ}/R + \Delta S^{\circ}/R + \ln \varnothing
\]

(1)

In this equation, \( \Delta H^{\circ} \) and \( \Delta S^{\circ} \) are the transfer enthalpy and entropy changes of the enantiomers between the mobile and stationary phases. \( R \) is the gas constant, and \( T \) is the absolute temperature of the chromatographic system. \( \varnothing \) means the phase ratio of the column (volume of the stationary phase divided by the volume of the mobile phase). This expression shows that the plot of \( \ln k \) versus \( 1/T \) should be linear if \( \Delta H^{\circ} \) and \( \Delta S^{\circ} \) are invariant with temperature in the range tested. Furthermore,

\[
\ln \varnothing = \ln (k \, l / k \, d)
= -\Delta \Delta H^{\circ}/R + \Delta \Delta S^{\circ}/R
\]

(2)

where \( k \, l \) and \( k \, d \) are the capacity factors of \( l \)- and \( d \)-enantiomers. \( \Delta \Delta H^{\circ} \) and \( \Delta \Delta S^{\circ} \) represent the differences of the enthalpy and entropy changes of the enantiomers, respectively. In \( \Delta \Delta H^{\circ} \) versus \( 1/T \) (van’t Hoff plot of \( \Delta \Delta S^{\circ} \)) should also be linear if \( \Delta \Delta H^{\circ} \) and \( \Delta \Delta S^{\circ} \) are invariant with temperature in the range tested. Linear and non-linear van’t Hoff plots were reported in the literature in the reversed-phase mode. [13, 18, 21–24] Non-linear van’t Hoff plots may be indicative of a change in the mechanism of retention.

Figure 4 illustrates the representative linear van’t Hoff plots of \( k \) and \( \Delta \Delta S^{\circ} \) with correlation coefficients both of 0.999 for derived amino acids employing the mobile phase, acetonitrile-phosphate buffer of 40:60 (v/v), respectively. These graphs indicate that the mechanisms for retention and separation of phenylalanine- and tryptophan-derivatives on the CSP were not changed in the temperature range studied.

The enthalpic and entropic contributions to the chiral separation of \( d \)- and \( l \)-enantiomers of amino acids were visualized.

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**Figure 3.** Chromatograms of amino acid derivatives. a) : CBZ-\( \alpha \)-, \( l \)-Trp, b) : CBZ-\( \alpha \)-, \( l \)-Phe. Column, CHIRALCEL OJ-RH; mobile phase, acetonitrile-phosphate buffer (40:60, v/v); column temperature, 45 °C; detection, 220 nm; flow-rate, 1 mL/min.

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**Figure 4.** Temperature dependence of retention and enantioseparation of derived amino acids on a CSP. Column, CHIRALCEL OJ-RH; mobile phase, acetonitrile-phosphate buffer (40:60); detection, 220 nm; flow-rate, 1 mL/min. a) van’t Hoff plots for retention: \( \varnothing \), CBZ-\( l \)-Trp; \( \square \), CBZ-\( \alpha \)-Trp ; \( \triangle \), CBZ-\( l \)-Phe ; \( \vartriangle \), CBZ-\( \alpha \)-Phe. b) van’t Hoff plot for Enantioseparation : \( \varnothing \), CBZ-\( \alpha \), \( l \)-Trp ; \( \square \), CBZ-\( \alpha \), \( l \)-Phe.
in Figure 5. On the basis of thermodynamic data, Figure 5 clearly shows that the chiral separation of CBZ−D, l−Trp and CBZ−D, l−Phe on cellulose tris (4−methylbenzoate) column are exclusively dominated by enthalpy with negative entropic contribution. With an increase of acetonitrile content in the mobile phase, the $\Delta\Delta\Delta H$ values increased, reached maximum values at about 38% of acetonitrile, and decreased. This trend was cancelled out by the $T\Delta\Delta S$ values, resulting in the constant $\Delta\Delta G$ values. This is the reason for constant separation factors for respective racemates.

Using these data sets for chiral separation, the differences of entropy are plotted against the differences of enthalpy to generate straight lines with correlation coefficients of more than 0.999, as shown in Figure 6. As the magnitude of the enthalpy for enantio−separation decreases, an offsetting increase in the entropy occurs. A relationship of this type is referred to as enthalpy−entropy compensation. [25, 26] For these straight lines the resulting change in entropic term is proportional to the accompanying change in enthalpy as described in the following equation 3:

$$ T\Delta(A\Delta S) = \Delta T\Delta(A\Delta H) $$

where $\Delta$ means the slope of this straight line. The slope ( $\Delta$ ) is a quantitative measure of the entropic canceling of the enthalpic gain for enantio−separation. Integration of equation 3 gives equation 4, where $T\Delta S$ refers to the value at $\Delta\Delta H=0$ (intercept) of Figure 6.

$$ T\Delta\Delta S = \Delta\Delta\Delta H − T\Delta\Delta S $$

Enantiomers are solvated identically in the mobile phase, but would release a different number of solvent molecules when they associate with the CSP. Therefore, this contribution to entropy would not be identical for both enantiomers. Since $\Delta\Delta S$ values are negative, l−enantiomers have fewer degrees of freedom on the CSP. In other words, l−enantiomers are held at more points or are less able to move or rotate in the CSP. Figure 6 indicates that as far as the intercept of the ordinate is positive, that is indeed the case of CBZ−D, l−Phe and CBZ−D, l−Trp with this CSP under the HPLC condition studied, the enantioseparation can take place even in the absence of enthalpic gain ($\Delta\Delta\Delta H$). This result suggests that the enantioseparation without enthalpic contribution would be achieved by the difference in desolvation from each enantiomer adsorbed through achiral contribution in the chiral cavity.

Differential form of Gibbs free energy function for interaction of an enantiomeric pair with the CSP is the following:

$$ \Delta(\Delta G) = \Delta(\Delta H) − T\Delta(\Delta S) $$

Inserting equation 3 in equation 5, equation 6 is obtained.

$$ \Delta(\Delta G) = (1−\Delta)\Delta(\Delta H) $$

Equation 6 indicates that only (1− $\Delta$) proportion of the increment in enthalpy ($\Delta\Delta\Delta H$) contributes toward raising the enantio−separation ($\Delta\Delta\Delta G$).

As can be seen from Figure 6, both linear plots give large slopes ( $\Delta$ : 0.98 for CBZ−D, l−Trp, 1.03 for CBZ−D, l−Phe). These slopes (approximately 1) indicate that the enthalpic gain or loss arising from any change caused by mobile phase composition is...
not reflected on the chiral separation on cellulose tris (4-methylbenzoate) CSP in this chromatographic system. In brief, $\Delta \Delta G$ is kept constant by the compensation of $\Delta \Delta H$ and $T \Delta \Delta S$ under the HPLC condition studied.

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References