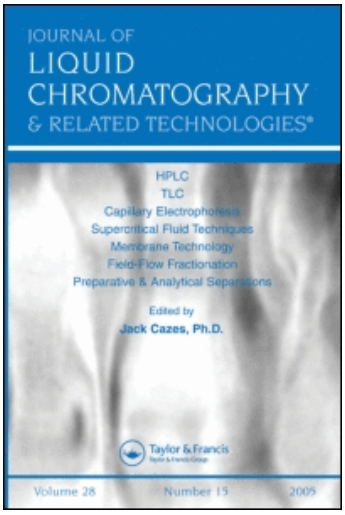

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Thermodynamics and Kinetics of Chiral Separations with β-Cyclodextrin Stationary Phase: II. Effect of Temperature and Pressure

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Abstract: In this study, a series of coumarin-based compounds is separated using a β-cyclodextrin stationary phase with a polar-organic mobile phase. Temperature and pressure are varied in order to observe the effects and determine important thermodynamic and kinetic parameters. Increasing the temperature decreases the retention and chiral selectivity, but increases the mass transfer rates for all chiral compounds. Increasing the pressure decreases the retention, but does not significantly affect the chiral selectivity. Van't Hoff plots of the natural logarithm of retention factor versus inverse temperature are linear with positive slopes, indicating an enthalpically favorable transfer from mobile to stationary phase. The second eluted enantiomer has a more negative change in molar enthalpy than the first, suggesting an enthalpically more favorable transfer. For all compounds, both the differential change in molar enthalpy and the differential change in molar entropy between the two enantiomers are negative. From these values, the compensation temperature is determined and is above ambient temperature, indicating an enthalpy-driven separation. Although all the compounds have similar structures, different compensation temperatures are determined and enthalpy-entropy compensation is not observed. This suggests that the retention mechanism is distinctly different. The change in molar volume is positive, indicating that the compounds occupy more space in the stationary phase than in the mobile phase and that inclusion in the cyclodextrin cavity does not occur to a significant extent. With regard to the kinetic behavior, the rate constants generally increase with increasing retention factor for the coumarin-based solutes. However, the second eluted enantiomer has a surprisingly faster rate constant than the first enantiomer.

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The activation energy is positive, and the second eluted enantiomer always has larger activation energy than the first enantiomer. These thermodynamic and kinetic measurements provide a detailed and comprehensive view of the chiral retention mechanism.

Keywords: Thermodynamics, Kinetics, Chiral separation, β-Cyclodextrin

INTRODUCTION

In liquid chromatography, thermodynamics and kinetics are equally important for any successful separation. Thermodynamic parameters provide an understanding of the energetic interactions between the solute and the mobile and stationary phases, which are intrinsically related to the aspects of retention and selectivity. Kinetic parameters provide an understanding of the rates of mass transport processes, which are intrinsically related to the efficiency or plate height. Both thermodynamic and kinetic information is necessary in order to optimize resolution.[1,2] While many publications are available for complete thermodynamic and kinetic characterization of traditional reversed-phase separations,[3–5] limited information is available for chiral separations. At the present time, chiral separations are still in the era of "trial-and-error"; when one type of chiral stationary phase is not appropriate, another type is tried until a successful separation is achieved. This "trial-and-error" approach is the direct result of lack of knowledge of the chiral separation mechanism. Thermodynamic and kinetic information is necessary to elucidate the retention mechanism for chiral separations.

β-Cyclodextrin, which contains seven glucose units with 35 chiral centers, is one of the most versatile and popular chiral stationary phases (CSPs). Once the stationary phase is chosen, mobile phase composition will have the largest effect on the separation.[6–15] Compared to the large number of studies on mobile phase composition, temperature and pressure are usually neglected. Recently, in order to achieve faster separations, high temperature and high pressure systems are becoming more popular. Thus, a detailed understanding of temperature and pressure effects is warranted.

The earliest studies of the effect of temperature on cyclodextrin CSPs were focused on optimization of the separation process.[10,11] However, temperature studies have also been utilized for thermodynamic characterization. An exceptional example is the detailed study carried out by Cabrera and Lubda.[16] They separated two chiral pharmaceuticals, oxazapam and prominal, on a native β-cyclodextrin CSP in the reversed-phase mode. Whereas a decrease in temperature caused an increase in retention factor for both compounds, it caused different effects on their enantioselectivities. Enantioselectivity was increased by reducing temperature for oxazepam, but by increasing temperature for prominal. These phenomena were related to the entropy-controlled separation for oxazepam and the enthalpy-controlled separation for prominal from the thermodynamic data. Morin et al.[17] also

studied the effect of temperature on a cyclodextrin CSP with a series of six imidazole derives. They observed different van't Hoff plots at different mobile phase pH values, which indicated a change in retention mechanism with the change of pH. However, at fixed pH, an enthalpy-entropy compensation analysis revealed the same retention mechanism for all solutes. From the thermograms and thermogravimetric curves, evidence was presented for a phase transition between the ordered and disordered states of the cyclodextrin CSP at different pH values. In the disordered state, a gain in freedom of the hydroxyl groups on each edge of the cyclodextrin cavity is obtained, with the minimum hydrogen bond formation between them. In contrast, in the ordered state, a loss in freedom of the hydroxyl groups is obtained, with the maximum hydrogen bond formation. As a result, the hydrophobic character, surface tension, and structure of the cyclodextrin cavity are all affected.

The effect of pressure on the separation process with cyclodextrin CSPs was investigated by Ringo and Evans. With the positional isomers of nitrophenol as model solutes, a shift in retention with modest pressure change (300 bar) was observed and correlated to the change in partial molar volume for the complexation process.[18] The same method was applied to chiral separations with a few barbiturate compounds.[19–21] Most chiral compounds showed a dependence of retention factor, chiral selectivity, and separation efficiency on pressure. The retention factor dependence reflected the statistically non-zero changes in molar volume during the complexation process. In the reversed-phase mode, the retention factors exhibited an increase or no change with pressure. The corresponding change in molar volume was negative or negligible. However, in the polar-organic mode, the retention factors showed a decrease with pressure for all solutes. The corresponding change in molar volume was positive or negligible. For all mobile phases, the change in molar volume ranged from -12 ± 1.4 to $+17 \pm 5.1\ \mathrm{cm}^3/\mathrm{mol}$. These pressure experiments provided insight into the differential volume of enantiomeric complexes formed with cyclodextrin.

While most of the previous investigations have been concerned only with thermodynamics, Ching et al.[22,23] investigated both thermodynamic and kinetic information on derivatized cyclodextrin stationary phases. The kinetic and equilibrium constants were obtained by statistical moment analysis based on the solid-film, linear driving force model. The overall mass transfer coefficients, bed voidage of the column, axial dispersion coefficients, and equilibrium constants were used to simulate the enantiomeric band profiles. Excellent correspondence between simulation and experiment confirmed the accuracy of the method as well as the results. The overall mass transfer coefficients were determined to be 0.97 and $1.21\ \mathrm{s}^{-1}$ for S- and R-fluoxetine, 1.77 and $14.1\ \mathrm{s}^{-1}$ for RSR- and RRS-nadolol, and $11.2\ \mathrm{s}^{-1}$ for the coeluted SRS- and SSR-nadolol. These kinetic data suggest that mass transfer rates are relatively rapid on derivatized cyclodextrin CSPs.

Most of the previous studies have been performed in the reversed-phase mode and, consequently, limited information is available in the polar-organic

mode. The goals of this research are as follows: 1) to conduct systematic investigations of polar-organic mobile phases, 2) to observe temperature and pressure effects on both thermodynamic and kinetic behavior, 3) to report specific thermodynamic and kinetic parameters, and to use them to elucidate the chiral separation mechanism.

THEORY

The calculation of thermodynamic and kinetic contributions to retention requires a synthesis of traditional thermodynamic and transition state theories.[3–5] These theories will be discussed in the following sections.

Thermodynamics

The thermodynamic parameters describe the path-independent measures of solute transfer from mobile to stationary phase. These parameters are calculated from the retention factor (k)

$$k = \frac{K}{\beta} = \frac{(t_r - t_0)}{t_0} \tag{1}$$

which is related to the equilibrium constant (K) and the volumetric ratio of the mobile and stationary phases (β). The retention factor is calculated experimentally from t_r and t_0, which are the elution times of a retained and nonretained solute, respectively. The ratio of the equilibrium constants or retention factors for two enantiomers defines the chiral selectivity (α)

$$\alpha = \frac{K_2}{K_1} = \frac{k_2}{k_1} \tag{2}$$

where the subscripts 1 and 2 denote the first and second eluted enantiomer, respectively.

The retention factor is related to the changes in molar enthalpy (ΔH) and molar entropy (ΔS) by the van't Hoff equation

$$\ln k = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta \tag{3}$$

where R is the gas constant and T is the absolute temperature. The change in molar enthalpy is determined from the slope of a graph of the natural logarithm of the retention factor versus inverse temperature at constant pressure. The change in molar entropy is contained in the intercept, but cannot be reliably quantitated since the phase ratio (β) is also contained

therein. The chiral selectivity is correspondingly related as

$$\ln \alpha = \frac{-\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \tag{4}$$

Thus, a graph of the natural logarithm of the selectivity versus inverse temperature will yield from the slope and intercept the differential changes in molar enthalpy ($\Delta\Delta H$) and molar entropy ($\Delta\Delta S$), respectively, between the two enantiomers.

From the definition of the molar enthalpy,

$$\ln k = \frac{-(\Delta E + P\,\Delta V)}{RT} + \frac{\Delta S}{R} - \ln \beta \tag{5}$$

which is a function of the molar internal energy (ΔE) and the pressure-volume work ($P\,\Delta V$). The change in molar volume (ΔV) is determined from the slope of a graph of the natural logarithm of the retention factor versus pressure (P) at constant temperature. The chiral selectivity is correspondingly related as

$$\ln \alpha = \frac{-(\Delta\Delta E + P\,\Delta\Delta V)}{RT} + \frac{\Delta\Delta S}{R} \tag{6}$$

Thus, a graph of the natural logarithm of the selectivity versus pressure will yield from the slope the differential change in molar volume ($\Delta\Delta V$) between the two enantiomers.

Enthalpy-Entropy Compensation

To gain a greater understanding of the balance of thermodynamic contributions to solute retention, enthalpy-entropy compensation is very useful. For a pair or series of solutes that obey a linear free energy relationship,[24] there exists a hypothetical temperature at which the relative changes in enthalpy and entropy are balanced and the net change in free energy is zero. By rearrangement of Equation (4), this compensation temperature (T_c) can be expressed as

$$T_c = \frac{\Delta\Delta H}{\Delta\Delta S} \tag{7}$$

At this temperature, the pair or series of solutes would coelute and no separation would be achieved ($\alpha = 1.0$). Hence, it is also called the isoselective or isoenantioselective temperature (T_{iso}) in chiral separations. When the sign of $\Delta\Delta H$ and $\Delta\Delta S$ are the same, the compensation temperature will be positive; when the sign of $\Delta\Delta H$ and $\Delta\Delta S$ are different, the compensation temperature will be negative. If sign is neglected, a compensation temperature that is substantially greater than ambient temperature suggests that the retention

mechanism is enthalpy-dominated, whereas a compensation temperature that is less than ambient temperature is entropy-dominated.

The compensation temperature can be determined from the slope of a graph of the change in molar enthalpy versus the change in molar entropy, both derived from Equation (3). However, for statistical reasons, a linear relationship may be observed between ΔH and ΔS even in the absence of enthalpy-entropy compensation.[25] Krug et al.[26] investigated different methods to identify true enthalpy-entropy compensation that is not influenced by statistical artifacts. By means of Equation (3), the free energy at a specific temperature (T) can be related to the free energy at the compensation temperature (T_c) by

$$\Delta G = \Delta H\left(1 - \frac{T}{T_c}\right) + \frac{T\Delta G_{T_c}}{T_c} \tag{8}$$

The retention factor can then be related to the free energy at the compensation temperature by

$$\ln k = \frac{-\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_c}\right) + \frac{\Delta G_{Tc}}{RT_c} - \ln \beta \tag{9}$$

Thus, a graph of the natural logarithm of the retention factor versus the change in molar enthalpy may be used to evaluate enthalpy-entropy compensation. If compensation occurs, this graph will be linear and the slope can be used to calculate the compensation temperature. The compensation temperature may be used to compare the retention mechanisms for different solutes, different mobile phases, or different stationary phases. As discussed by Ranatunga et al.,[27] if the compensation temperatures are identical, then the relative contributions of enthalpy and entropy to the overall free energy are the same for the two systems. However, if the compensation temperatures are different, then the underlying retention mechanism must be distinctly different.

Kinetics

The kinetic parameters describe the path-dependent measures of solute transfer from mobile to stationary phase. During the transfer, the solutes pass through a high-energy transition state (‡) that uniquely characterizes the path-dependent aspects of the retention mechanism. The derivation of the kinetic rate constant has been described elsewhere.[3] The adsorption rate constant for transfer from mobile to stationary phase (k_{sm}) is given by

$$k_{sm} = \frac{2k^2 t_0}{\tau^2} \tag{10}$$

whereas the desorption rate constant for transfer from stationary to mobile phase (k_{ms}) is given by

$$k_{ms} = \frac{k_{sm}}{k} = \frac{2k\,t_0}{\tau^2} \tag{11}$$

where τ represents the exponential contribution to variance that arises from slow mass transfer kinetics. The kinetic rate constants can be expressed by means of the Arrhenius equation

$$\ln k_{sm} = \ln A_{\ddagger m} - \frac{\Delta E_{\ddagger m}}{RT} \tag{12}$$

$$\ln k_{ms} = \ln A_{\ddagger s} - \frac{\Delta E_{\ddagger s}}{RT} \tag{13}$$

where $\Delta E_{\ddagger m}$ and $\Delta E_{\ddagger s}$ are the activation energies from mobile phase to transition state and from stationary phase to transition state, respectively. The activation energy is determined from the slope of a graph of the natural logarithm of the rate constant versus inverse temperature at constant pressure. The intercept contains information about the pre-exponential factor ($A_{\ddagger m}$ and $A_{\ddagger s}$), which is related to the activation entropy ($\Delta S_{\ddagger m}$ and $\Delta S_{\ddagger s}$) according to transition state theory[28]

$$\ln A_{\ddagger m} = \ln\left(\frac{e\,k_B\,T}{h}\right) + \frac{\Delta S_{\ddagger m}}{R} \tag{14}$$

$$\ln A_{\ddagger s} = \ln\left(\frac{e\,k_B\,T}{h}\right) + \frac{\Delta S_{\ddagger s}}{R} \tag{15}$$

where the frequency term includes the exponential constant (e), Boltzmann's constant (k_B), and Planck's constant (h).

EXPERIMENTAL

Chemicals

As depicted in Figure 1 of the previous manuscript,[15] five coumarin-based solutes are chosen based on their structural similarities. Warfarin, coumachlor, coumafuryl, and coumatetralyl are chiral, whereas 4-hydroxycoumarin is achiral. The solutes are obtained from Sigma-Aldrich as solids and are dissolved in high-purity acetonitrile (Burdick and Jackson, Baxter Healthcare) to yield standard solutions at 10^{-4} M. Nitromethane is used as a non-retained marker and is added to each solution at a concentration at 15% (v/v). High-purity acetonitrile and methanol (Burdick and Jackson, Baxter Healthcare), as well as acetic acid and triethylamine (Sigma-Aldrich) are used for mobile phase preparation.

Experimental System

The solutes are separated on a capillary liquid chromatography system. The column is a fused-silica capillary (200 μm i.d., 105 cm, Hewlett-Packard) that is packed via the slurry method and terminated with a quartz wool frit.[29] Columns prepared by this method have very uniform packing across the diameter and along the length. The silica packing (Cyclobond I 2000, Astec) is characterized by a 5.2 μm particle size, 0.89 mL/g pore volume, and 305 m^2/g surface area, reacted with β-cyclodextrin at a bonding density of 0.39 $\mu mol/m^2$.

In the chromatographic system, the mobile phase is delivered by a single-piston reciprocating pump (Model 114 M, Beckman Instruments) operated in the constant-pressure mode at a nominal flow rate of 1.0 μL/min. After injection (Model ECI4W1, Valco Instruments), the sample is split between the column and a fused-silica capillary (100 μm i.d., Polymicro Technologies), resulting in an injection volume of 15 nL. A fused-silica capillary (20 μm i.d., Polymicro Technologies) is attached to the column terminus to serve as a restrictor. By reducing the length of the splitter and restrictor proportionally, the pressure drop along the column is held constant as the average pressure is varied from 700 to 3100 psi ($\pm$15 psi). The column, injector, splitter, and restrictor are housed within a cryogenic oven (Model 3300, Varian Associates) that enables the temperature to be varied from 283 to 333 K ($\pm$0.1 K).

The polyimide coating is removed from the capillary column at lengths of 34.6, 39.8, 63.6, and 69.4 cm to facilitate on-column detection by laser-induced fluorescence. A helium-cadmium laser (Model 3074-20M, Melles Griot) provides excitation at 325 nm. The fluorescence emission is isolated by a 420 nm interference filter (S10-420-F, Corion), and detected by a photomultiplier tube (Model R760, Hamamatsu). The resulting photocurrent is amplified, converted to the digital domain (Model PCI-MIO-16XE-50, National Instruments), and stored by a user-defined program (Labview v5.1, National Instruments).

Data Analysis

After data collection, the zone profile for each solute is extracted from the chromatogram. Each profile is fit by nonlinear regression to the exponentially modified Gaussian (EMG) equation by using a commercially available program (Peakfit v4.14, SYSTAT Software). The EMG equation is chosen because the statistics of fit are better than those for any other model that has been demonstrated to have physical meaning. Specifically, for the zone profiles analyzed in this study, the square of the correlation coefficient (R^2) and the F-statistic from the EMG model are higher than those from the Thomas[30] or Giddings[2] models. The residuals from the EMG model are smaller and more random than those from the Giddings or Thomas models as well.

The EMG equation is the convolution of Gaussian and exponential functions, with the resulting form

$$C(t) = \frac{A}{2\tau}\exp\left(\frac{\sigma^2}{2\tau^2} + \frac{t_g - t}{\tau}\right)\left(\operatorname{erf}\left(\frac{t - t_g}{\sqrt{2}\sigma} - \frac{\sigma}{\sqrt{2}\tau}\right) + 1\right) \quad (16)$$

where C(t) is the concentration as a function of time, A is the peak area, t_g is the retention time of the Gaussian component, σ is the standard deviation of the Gaussian component, and τ is the standard deviation of the exponential component. Symmetrical zone broadening, which arises from diffusion and mass transfer processes that are fast relative to the separation time, is quantified by σ. Asymmetrical broadening, which arises from volumetric sources (i.e., injectors, unions, etc.), electronic sources (i.e., amplifiers, etc.), nonlinear isotherms, and diffusion and mass transfer processes that are slow relative to the separation time, is quantified by τ. By careful and deliberate design of the experimental system, the asymmetrical broadening from volumetric and electronic sources can be minimized.[31] The contribution of nonlinear isotherms to asymmetric broadening can be eliminated by injecting solutes at sufficiently low concentrations. Under these conditions, the slow mass transfer rate will dominate the asymmetrical broadening. The resultant fitting parameters are used to calculate thermodynamics and kinetics by Equations (1), (10), and (11), where

$$t_r = t_g + \tau \quad (17)$$

RESULTS AND DISCUSSION

In the previous study,[15] the effect of mobile phase composition on chiral separations with β-cyclodextrin stationary phase was carefully investigated. On the basis of these results, a standard mobile phase of acetonitrile:methanol: acetic acid:triethylamine (99%:1%:0.15%:0.1% (v/v/v/v)) is chosen for the present study.

Thermodynamic Behavior

Retention Factor

Retention factors for the coumarin-based compounds are reported in Table 1. The retention follows the same general order reported previously for coumafuryl, warfarin, coumachlor, coumatetralyl, and 4-hydroxycoumarin.[15] The retention order is greatly affected by the hydrogen bonding abilities. Coumafuryl has the greatest intramolecular hydrogen bonding between the hydroxyl group and the carbonyl group as well as the ether group. Thus, it has the least intermolecular hydrogen bonding with the stationary phase

Table 1. Retention factors for the coumarin-based solutes

	283 K[a]		303 K[a]		700 psi[b]		3100 psi[b]	
Solute	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
Coumafuryl	0.85	1.00	0.59	0.68	0.72	0.84	0.66	0.76
Warfarin	1.07	1.42	0.76	0.96	0.92	1.19	0.85	1.09
Coumachlor	1.07	1.35	0.70	0.86	0.89	1.11	0.80	1.01
Coumatetralyl	3.20	3.39	2.11	2.18	2.60	2.73	2.44	2.55
4-Hydroxy-coumarin	4.89	N/A[c]	4.15	N/A	4.59	N/A	4.29	N/A

[a]Retention factor for the first (k_1) and second (k_2) eluted enantiomers, calculated at P = 1300 psi.

[b]Retention factor for the first (k_1) and second (k_2) eluted enantiomers, calculated at T = 293 K.

[c]Not available (N/A).

and, accordingly, the smallest retention. By replacement of the furan ring on coumafuryl with the benzene ring on warfarin and coumachlor, there is one less intramolecular hydrogen bonding site. Thus, they have slightly larger intermolecular hydrogen bonding with the stationary phase and correspondingly larger retention. By removal of the carbonyl group, coumatetralyl has no intramolecular hydrogen bonding ability and the largest retention among the chiral molecules. Finally, 4-hydroxycoumarin has the largest retention overall because it lacks a bulky substituent at the 3-position and can interact simultaneously through the hydroxyl and carboxyl groups with the cyclodextrin stationary phase.

The effects of temperature and pressure on retention are demonstrated in Table 1. The retention factors for all solutes decrease dramatically with an increase in temperature. With an increase in pressure, the retention factors decrease as well. The detailed reasons for these effects are discussed in the following sections.

Molar Enthalpy

A representative graph of the logarithm of the retention factor versus the inverse temperature (van't Hoff plot) is shown in Figure 1. The graph for each solute is linear ($R^2 = 0.993–0.999$) with a positive slope. A linear graph indicates that the change in molar enthalpy is constant, which suggests that there is no significant change in retention mechanism and no conformational change in the stationary phase over the temperature range of 283–303 K. A positive slope is indicative of a negative change in molar enthalpy, which suggests that the transfer from mobile to stationary phase is an enthalpically favorable process. The change in molar enthalpy is calculated

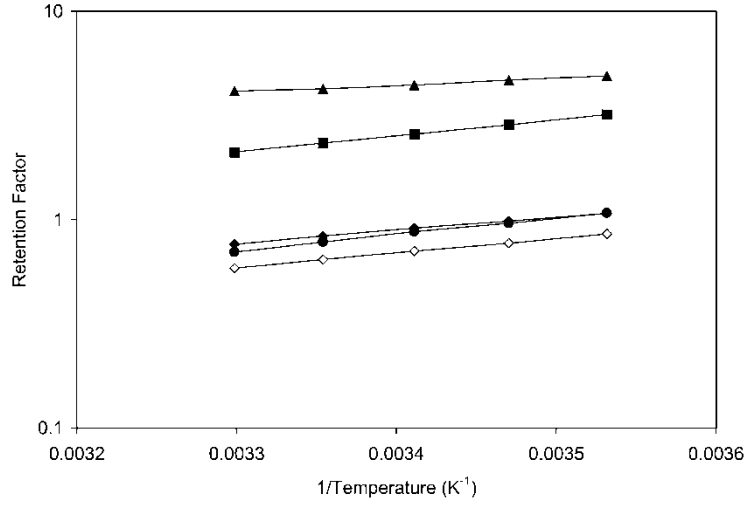

Figure 1. Representative graph of the logarithm of retention factor versus inverse temperature used to calculate the change in molar enthalpy. Mobile phase: acetonitrile:methanol:acetic acid:triethylamine (99%:1%:0.15%:0.1%), 1300 psi. Solutes: first eluted enantiomers of coumafuryl (◇), warfarin (◆), coumachlor (•), coumatetralyl (■), 4-hydroxycoumarin (▲). Other experimental details as given in the text.

from the slope of this graph according to Equation (3), and is reported in Table 2.

Although 4-hydroxycoumarin is the most retained solute, it has the least negative change in molar enthalpy. In other words, the cohesive energy of attraction between the solute and stationary phase is the smallest. If the van't Hoff plots in Figure 1 were extrapolated to calculate the intercepts,

Table 2. Changes in molar enthalpy and molar volume for the coumarin-based solutes

Solute	ΔH_1 (kcal/mol)[a]	ΔH_2 (kcal/mol)[a]	ΔV_1 (cm^3/mol)[b]	ΔV_2 (cm^3/mol)[b]
Coumafuryl	-3.21 ± 0.04	-3.33 ± 0.03	13.8 ± 0.5	15.0 ± 1.3
Warfarin	-2.91 ± 0.07	-3.28 ± 0.07	14.1 ± 1.0	13.4 ± 1.1
Coumachlor	-3.65 ± 0.09	-3.82 ± 0.10	16.0 ± 1.5	15.1 ± 1.5
Coumatetralyl	-3.54 ± 0.02	-3.78 ± 0.02	10.7 ± 1.1	11.2 ± 1.1
4-Hydroxycoumarin	-1.45 ± 0.10		17.6 ± 2.4	

[a]Molar enthalpy for the first (ΔH_1) and second (ΔH_2) eluted enantiomer, calculated at P = 1300 psi.

[b]Molar volume for the first (ΔV_1) and second (ΔV_2) eluted enantiomer, calculated at T = 293 K.

4-hydroxycoumarin would have the largest intercept. A large intercept usually implies that the change in molar entropy for 4-hydroxycoumarin is large or, alternatively, that the phase ratio experienced by 4-hydroxycoumarin is small. 4-Hydroxycoumarin has two sites (hydroxyl and carboxyl) for intermolecular hydrogen bonding with cyclodextrin. Every hydrogen bonding process will displace a solvent molecule from cyclodextrin and introduce a change in molar entropy. Thus, the more interaction sites, the more solvent molecules displaced, and the greater the change in molar entropy. In addition, the smaller size and relatively planar structure of 4-hydroxycoumarin facilitate its binding with cyclodextrin in more possible configurations, which may increase the change in molar entropy as well. The phase ratio experienced by 4-hydroxycoumarin might be different from the other chiral solutes, depending on the interaction sites. For example, if 4-hydroxycoumarin can access the unreacted silanol sites on the silica surface because of its small size, whereas other solutes cannot, then the phase ratio for 4-hydroxycoumarin would be different.

When the changes in molar enthalpy are compared for all chiral solutes in Table 2, no apparent trend can be found. In the reversed-phase separation with an octadecylsilica stationary phase, the change in molar enthalpy is usually more negative as the retention factor increases.[3] However, this trend is not observed for the cyclodextrin stationary phase with our series of compounds. This behavior suggests that the change in molar entropy is an important parameter, such that the trend of molar enthalpy alone is not consistent with the trend of retention. When we carefully consider warfarin and coumachlor, they have notably similar structures and similar retention at ambient temperature. However, their changes in molar enthalpy are surprisingly different, as coumachlor has a more negative change in molar enthalpy than warfarin. Although both compounds have a similar response to mobile phase composition,[15] their differences in the change in molar enthalpy enable their separation by means of a change in temperature. At lower temperature, warfarin elutes before coumachlor, while at higher temperature, the elution order is reversed and coumachlor elutes first. This illustrates the important balance of enthalpic and entropic effects for the separation of these solutes.

When the change in molar enthalpy is compared for the enantiomers of each solute, the second eluted enantiomer always has a more negative change in molar enthalpy than the first enantiomer. This difference indicates that the second enantiomer is enthalpically more favorable in the transfer from mobile to stationary phase. This difference will be discussed in more detail in the second section following.

Molar Volume

A representative graph of the logarithm of the retention factor versus pressure is shown in Figure 2. The graph for each solute is linear ($R^2 = 0.982 - 0.997$)

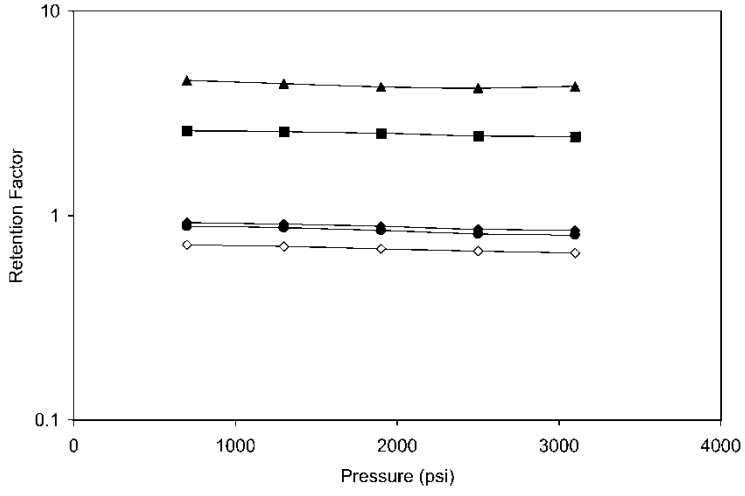

Figure 2. Representative graph of the logarithm of retention factor versus pressure used to calculate the change in molar volume. Mobile phase: acetonitrile:methanol: acetic acid:triethylamine (99%:1%:0.15%:0.1%), 293 K. Solutes: first eluted enantiomers of coumafuryl (◇), warfarin (◆), coumachlor (●), coumatetralyl (■), 4-hydroxycoumarin (▲). Other experimental details as given in the text.

with a negative slope. A negative slope is indicative of a positive change in molar volume, which suggests that the solute occupies more volume in the stationary phase than in the mobile phase. The change in molar volume is calculated from the slope of this graph according to Equation (5), and is reported in Table 2.

In reversed-phase separations with the cyclodextrin stationary phase, the solute is included into the cyclodextrin cavity, which is accompanied by a negative change in molar volume.[20] In the polar-organic mode as described here, the change in molar volume is positive, which is a clear indication that inclusion is not the predominant retention mechanism. However, more detailed information may be gleaned by comparison of the individual values. The change in molar volume is smallest for coumatetralyl, larger and statistically similar for warfarin, coumachlor, and coumafuryl, and largest for 4-hydroxycoumarin. Interestingly, this trend of the change in molar volume is opposite the trend of molar volume itself. The molar volume is smallest for 4-hydroxycoumarin, larger for coumafuryl, coumachlor, and warfarin, and largest for coumatetralyl. Hence, it is possible that a small extent of inclusion does occur for the nonpolar substituent at the chiral carbon. The two large aliphatic rings of coumatetralyl may have the greatest extent of inclusion, the furan and benzene rings of coumafuryl, coumachlor, and warfarin may have a smaller extent of inclusion, and 4-hydroxycoumarin may have no inclusion.

When the changes in molar volume are compared for the enantiomers of each solute, they are statistically similar to each other. This indicates that pressure will have a similar effect on both enantiomers, and chiral selectivity will not be significantly affected by pressure.

Chiral Difference in Molar Enthalpy and Molar Entropy

A representative graph of the logarithm of the chiral selectivity versus the inverse temperature is shown in Figure 3. The graph for each chiral solute is linear ($R^2 = 0.982 - 0.997$) with a positive slope. A positive slope is indicative of a negative differential change in molar enthalpy for the enantiomers.

The differential changes in molar enthalpy and molar entropy are calculated from Equation (4), and are reported in Table 3. Since the mobile phase is an achiral environment, the two enantiomers will have the same molar enthalpy and molar entropy therein. Thus, the differential changes in molar enthalpy and molar entropy solely reflect the chiral interactions with the stationary phase. The differential change in molar enthalpy is negative, which indicates that the second enantiomer is enthalpically more favorable in the stationary phase. The differential change in molar entropy is also negative, which indicates that the second enantiomer is entropically less

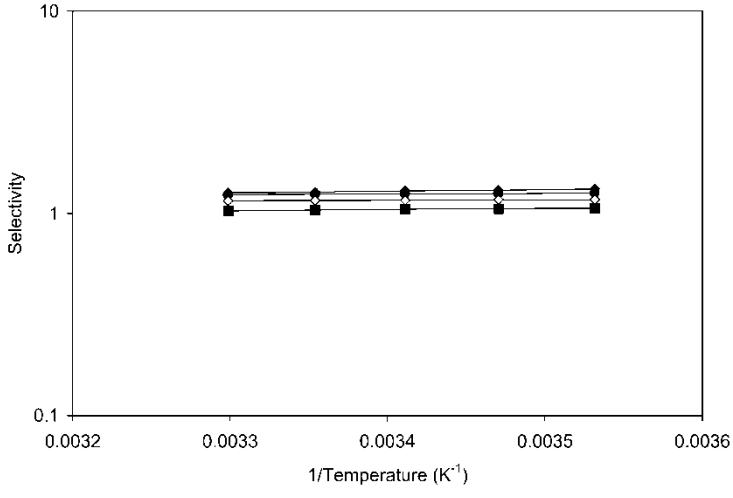

Figure 3. Representative graph of the logarithm of chiral selectivity versus inverse temperature used to calculate the differential changes in molar enthalpy and molar entropy. Mobile phase: acetonitrile:methanol:acetic acid:triethylamine (99%:1%: 0.15%:0.1%), 1300 psi. Solutes: coumafuryl (◇), warfarin (◆), coumachlor (•), coumatetralyl (■). Other experimental details as given in the text.

Table 3. Differential changes in molar enthalpy and molar entropy for the chiral coumarin-based solutes

Solute	$\Delta\Delta H$ (kcal/mol)[a]	$\Delta\Delta S$ (cal/mol K)[a]
Coumafuryl	-0.12 ± 0.01	-0.12 ± 0.02
Warfarin	-0.37 ± 0.00	-0.75 ± 0.01
Coumachlor	-0.17 ± 0.02	-0.14 ± 0.05
Coumatetralyl	-0.23 ± 0.02	-0.71 ± 0.08

[a]Differential changes in molar enthalpy ($\Delta\Delta H$) and molar entropy ($\Delta\Delta S$), calculated at P = 1300 psi.

favorable in the stationary phase. This is the first indication that the chiral separation of the coumarin-based solutes is an enthalpy controlled process.

Enthalpy-Entropy Compensation

A graph of the natural logarithm of the retention factor versus the change in molar enthalpy is shown in Figure 4. Each point represents one of the coumarin-based solutes. The scattering of these points clearly shows that enthalpy-entropy compensation is not achieved. Different retention

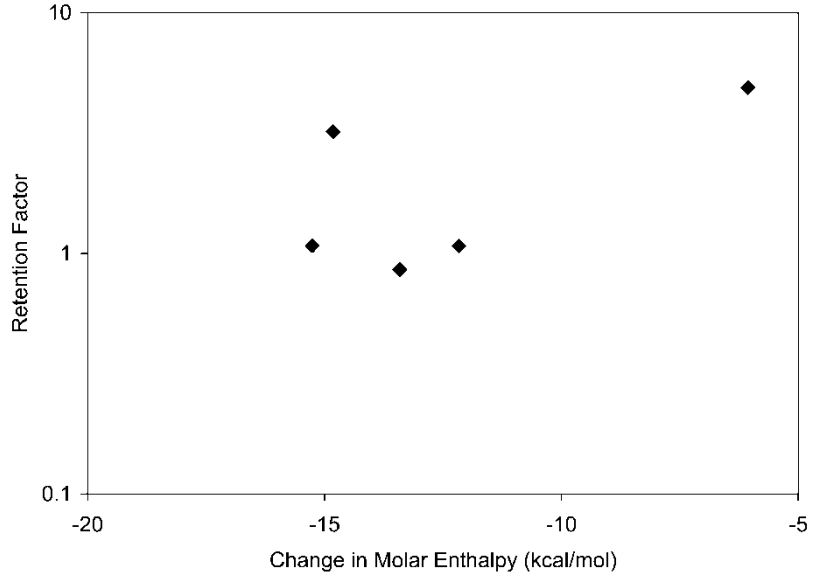

Figure 4. Representative graph of the logarithm of retention factor versus change in molar enthalpy used to observe enthalpy-entropy compensation. Mobile phase: acetonitrile:methanol:acetic acid:triethylamine (99%:1%:0.15%:0.1%), 1300 psi.

mechanisms are, thus, responsible for the retention of the individual solutes. The three points on the right side of the graph may possibly be considered as linear ($R^2 = 0.988$), which suggests that the retention mechanisms for 4-hydroxycoumarin, coumafuryl, and coumachlor may be similar.

The isoenantioselective temperature, T_{iso}, is calculated from Equation (7), and is reported in Table 4. All values are higher than ambient temperature, which suggests that the separation of the chiral solutes is an enthalpy-dominated process. The different values of T_{iso} for all solutes is another indication that enthalpy-entropy compensation does not occur. But the T_{iso} values are similar for coumafuryl and coumachlor, and for warfarin and coumatetralyl. This suggests that the retention mechanism may be similar for these pairs of solutes. The T_{iso} values for coumafuryl and coumachlor are significantly greater than those for warfarin and coumatetralyl. For coumafuryl and coumachlor, $\Delta\Delta H$ mainly controls their chiral separation. But for warfarin and coumatetralyl, $\Delta\Delta S$ has more responsibility for their chiral separation.

Kinetic Behavior

While thermodynamics can elucidate the steady-state information concerned with the change between the initial (mobile phase) and final (stationary phase) states, it does not fully explain the retention mechanism. By considering a transition state, the pseudo-first-order rate constants, activation energies, and activation volumes can be calculated with the equations developed in the Theory section. These values help to quantify the kinetic aspects of mass transfer between the mobile and stationary phases as a function of solute structure. These data provide information about the retention mechanism that would not be available from thermodynamic data alone.

Rate Constants

Representative kinetic rate constants, including the adsorption rate constant k_{sm} and desorption rate constant k_{ms}, are summarized in Table 5. As noted previously,[15] 4-hydroxycoumarin has notably smaller kinetic rate constants than

Table 4. Isoenantioselective temperature (T_{iso}) for the chiral coumarin-based solutes

Solute	T_{iso} (K)
Coumafuryl	1000 ± 170
Warfarin	491 ± 4
Coumachlor	1200 ± 460
Coumatetralyl	320 ± 46

Table 5. Kinetic rate constants for the coumarin-based solutes

Solute	k_{sm1} $(s^{-1})^a$	k_{sm2} $(s^{-1})^a$	k_{ms1} $(s^{-1})^a$	k_{ms2} $(s^{-1})^a$
Coumafuryl	11	12	13	12
Warfarin	8	11	8	8
Coumachlor	8	10	7	7
Coumatetralyl	7	13	2	4
4-Hydroxycoumarin	0.23		0.05	

[a]Rate constants for transfer of the first and second eluted enantiomers from mobile to stationary phase (k_{sm1}, k_{ms2}) and from stationary to mobile phase (k_{ms1}, k_{ms2}), calculated at T = 283 K and P = 1300 psi.

the other chiral solutes owing to its small size and two sites for simultaneous hydrogen bonding with the stationary phase. When the chiral solutes are compared, the kinetic rate constants decrease with an increase in the retention factor. However, this trend is not conserved for each chiral pair, as the second eluted enantiomer always has a surprisingly larger mass transfer rate constant than the first enantiomer. This variation may be attributed to the different kinetic contributions from chiral and achiral selective sites. The second enantiomer must have greater interactions with chiral sites so these sites must, necessarily, have faster mass transfer kinetics. The kinetic rate constants for both enantiomers generally increase with an increase in temperature.

Activation Energy

A representative graph of the logarithm of the desorption rate constant versus the inverse temperature is illustrated in Figure 5. The rate constant for 4-hydroxycoumarin is relatively constant with temperature, so it is not included in the graph. The graph for each chiral solute is linear ($R^2 = 0.942 - 0.999$) with a negative slope, which indicates a positive activation energy. The activation energy is calculated from the slope of this graph according to Equations (12) and (13), and is shown in Table 6. The activation energy for the transfer from stationary phase to transition state ($\Delta E_{\ddagger s}$) is larger than that from mobile phase to transition state ($\Delta E_{\ddagger m}$). These data indicate that it is easier for the solutes to enter the stationary phase than to exit. A comparison between the two enantiomers of each solute demonstrates that the second eluted enantiomer always has a larger activation energy than the first enantiomer. Since the rate constant is also consistently larger for the second enantiomer (Table 5), this implies that the pre-exponential factor in Equations (12) and (13) must be significantly greater for the second enantiomer. According to transition state theory, the pre-exponential factor is related to the activation entropy as shown in

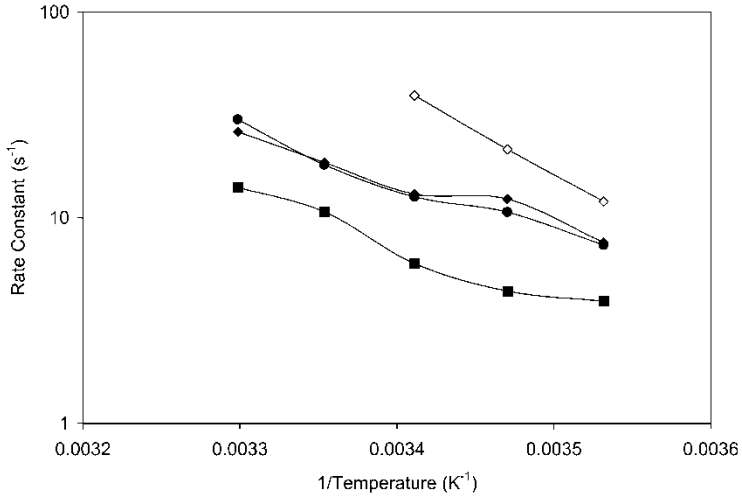

Figure 5. Representative graph of the logarithm of rate constant (k_{ms}) versus inverse temperature used to calculate the activation energy. Mobile phase: acetonitrile:methanol:acetic acid:triethylamine (99%:1%:0.15:0.1%), 1300 psi. Solutes: first eluted enantiomers of coumafuryl (◇), warfarin (◆), coumachlor (•), coumatetralyl (■). Other experimental details as given in the text.

Equations (14) and (15). Hence, the activation entropy must be significantly larger for the second eluted enantiomer. Additional information about the activation entropy for the coumarin-based solutes may be gained by extrapolation of Figure 5 to determine the intercept. It is apparent that the activation entropy is largest for coumafuryl, similar for warfarin and coumachlor, and smallest for coumatetralyl.

Table 6. Activation energy for the coumarin-based solutes

Solute	$\Delta E_{\ddagger m1}$ (kcal/mol)[a]	$\Delta E_{\ddagger m2}$ (kcal/mol)[a]	$\Delta E_{\ddagger s1}$ (kcal/mol)[a]	$\Delta E_{\ddagger s2}$ (kcal/mol)[a]
Coumafuryl	N/A[b]	16.3 ± 0.4	N/A	19.6 ± 0.4
Warfarin	3.6 ± 0.8	6.6 ± 1.1	6.5 ± 0.8	9.9 ± 1.1
Coumachlor	4.5 ± 0.8	7.6 ± 1.1	8.0 ± 0.9	11.4 ± 1.2
Coumatetralyl	5.3 ± 1.9	7.9 ± 1.6	8.9 ± 2.0	11.7 ± 1.6
4-Hydroxycoumarin	N/A		N/A	

[a]Activation energies for transfer of the first and second eluted enantiomers from mobile phase to transition state ($\Delta E_{\ddagger m1}$, $\Delta E_{\ddagger m2}$) and from stationary phase to transition state ($\Delta E_{\ddagger s1}$, $\Delta E_{\ddagger s2}$), calculated at P = 1300 psi.

[b]Not available (N/A).

SUMMARY

In this study, the thermodynamic and kinetic behavior is examined with the β-cyclodextrin stationary phase in the polar-organic mode. In order to understand the implications of these results, it is helpful to follow the thermodynamic and kinetic behavior of a pair of enantiomers. Warfarin can be used as an example here. As the two warfarin enantiomers transfer from the mobile to stationary phase, they start in the mobile phase at the same energy level. Both enantiomers need to pass through a high-energy transition state. The transition state for the first eluted enantiomer requires an activation energy of 3.6 kcal/mol, whereas the second enantiomer requires an activation energy of 6.6 kcal/mol. After the transition state, the first enantiomer reaches the stationary phase with a net decrease in molar enthalpy of 2.91 kcal/mol and a net increase in molar volume of 14.1 cm^3/mol. This transfer occurs at a rate constant of 18 s^{-1}. The second enantiomer reaches the stationary phase with a net decrease in molar enthalpy of 3.28 kcal/mol and a net increase in molar volume of 13.4 cm^3/mol. This transfer occurs at a rate constant of 26 s^{-1}. The second enantiomer has a higher activation energy but a lower final energy in the stationary phase than the first enantiomer. At the same time, the second enantiomer has a larger rate constant than the first enantiomer. For both enantiomers, the molar volume is increased as they transfer from mobile to stationary phase. This positive change in molar volume suggests that inclusion in the cyclodextrin cavity is not the predominant retention mechanism in the polar-organic mobile phase.

For the coumarin-based solutes, the trend of the change in molar enthalpy does not always match the trend of retention. Apparently, the change in molar entropy plays an important role in the separation of some solutes, most notably 4-hydroxycoumarin, coumafuryl, and coumachlor. All the separations are enthalpically controlled, but enthalpy-entropy compensation is not observed among all solutes. These thermodynamic and kinetic studies provide a detailed and comprehensive view of the chiral retention mechanism.

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