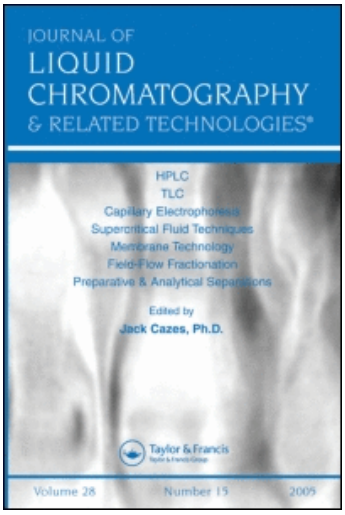

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Kang, Gyoung Won , Ko, Joung Ho and Cheong, Won Jo(2005) 'Thermodynamic Study of Enantioseparation of Arylpropionic Acids with a Chiralcel OJ-H Stationary Phase', Journal of Liquid Chromatography & Related Technologies, 28: 4, 513 — 526
To link to this Article: DOI: 10.1081/JLC-200047202
URL: http://dx.doi.org/10.1081/JLC-200047202

Journal of Liquid Chromatography & Related Technologies®, 28: 513–526, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200047202

Thermodynamic Study of Enantioseparation of Arylpropionic Acids with a Chiralcel OJ-H Stationary Phase

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Abstract: Enantioseparation data of ketoprofen, fenoprofen, and ibuprofen have been obtained and thermodynamically analyzed. The Chiralcel OJ column was used for chiral separation. The mobile phases were 100/0, 95/5, and 90/10 v/v% hexane/2-propanol mixtures with 0.5% acetic acid. The capacity factors of the solutes were measured at 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C. The thermodynamic properties of solute transfer from the mobile to the stationary phase were critically analyzed in comparison with the thermodynamic properties obtained by the Chirex 3001 phase in methanol/water eluents. The well-known enthalpic–entropic compensation is clearly valid for the systems studied in this research. The solute–Chiralcel OJ interactions of *R*- and *S*-ibuprofen were found weaker than those of *R*- and *S*-fenoprofen and ketoprofen, since ibuprofen is much less polar than others and subject to much weaker interactions with the stationary phase. Careful interpretation of the data revealed that the solute–Chiralcel OJ interaction is stronger than the solute–Chirex 3001 interaction. It is likely that the supramolecular structure of the Chiralcel OJ phase with chiral cavities provides more versatile functional and steric environments, to give stronger interactions and better chiral recognition and discrimination. There appeared extrema in the plots of ΔH^0 and ΔS^0 against eluent composition obtained in the Chiralcel OJ system. Adsorption of 2-propanol to multiple sites with different priorities and different steric environments seems to cause such a phenomenon.

Keywords: Thermodynamic properties, enantioseparation, arylpropionic acids, chiralcel OJ phase

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INTRODUCTION

2-Arylpropionic acids are useful anti-inflammatory, antipyretic, and analgesic drugs.[1] The biological activity of pharmaceutical compounds is mostly restricted to one of the enantiomers and there are many examples of the undesired effect of one isomer against the pharmaceutical effect of the other isomer,[2] which prompted development of enantioseparation. Among various chromatographic techniques, high-performance liquid chromatography (HPLC) seems to be the most widely used technique for chiral separation.[3]

Many chiral columns such as Chirex 3005 column (ligand: R-1-naphthylglycine 3,5-dinitrobenzoic acid),[4,5] polysaccharide-based columns,[6–8] and protein-based columns[9–14] have been used for chiral separation of 2-arylpropionic acids and their derivatives. Another trend of enantioseparation of arylpropionic acids is capillary electrophoresis (CE) with chiral selectors such as cyclodextrin added to the mobile phase.[2]

Cellulose-based phases have been known to be very versatile chiral phases. The chiral recognition mechanism in these phases is very complicated and has not been thoroughly clarified. Some major factors of chiral recognition in specified systems have been examined. Okamoto et al. proposed that the hydrogen bonding between the solute and the carbamate group of the Chiralcel OC [cellulose *tris*(phenylcarbamate)] and its modified phases play an important role in chiral recognition after observing the effects of electron donating or withdrawing substitutes of the phenyl ring on chiral separation.[15] Similar observation was reported for the Chiralcel OD [cellulose *tris*(3,5-dinitrophenylcarbamate)] and its modified phases.[16] On the other hand, Wainer and Alembik proposed for the Chiralcel OB [cellulose trisbenzoate] that not only hydrogen bonding but also $\pi-\pi$ interactions and dipole–dipole interactions contribute to chiral recognition.[17] Wainer also inferred that, in chiral separation with the Chiralcel OB phase under the normal phase mode (hexane/alcohol), the cosolvent (alcohol) competes with the solute for hydrogen bonding to the chiral stationary phase (CSP), and causes changes of the steric environments of the chiral cavities by adsorbing to the chiral cavities or achiral polar functional groups.[18]

There have been a few chiral separation studies with the Chiralcel OJ phase. Roussel and coworkers studied the retention of various *N*-arylthiazoline-2-(thi)one atropisomers (unable to form hydrogen bond with the Chiralcel OJ phase) by factorial design and lipophilicity approaches, and proposed that the replacement of a hydrogen of the solute by a methyl can result in attractive interaction with the CSP, as well as a strong repulsive interaction, depending upon the precise localization of the change.[19,20] Enantiomeric resolution of theophylline derivatives was carried out in the Chiralcel OD, OC, and OJ phases, and it turned out that the best performance of Chiralcel OD was found with 2-propanol in hexane at higher temperatures,

that of Chiralcel OC with methanol at ambient temperature, and that of Chiralcel OJ with increased modifier concentrations in hexane at increased temperatures.[21] Examination of chiral separation of indandiol in the Chiralcel OJ phase by Ding et al.[22] revealed that hydrogen bonding does not have a gross direct effect on enantiorecognition, but may contribute through its effect on the tertiary structure of the CSP, and that inclusion is the major contributor to enantioselectivity with the size and shape of the probe being the determinant. Tang et al.[23] examined effects of various factors upon chiral separation of fluorenylmethoxycarbonyl-amino acids in the reversed-phase mode, and found that buffer pH was the only factor that caused a significant change in chiral selectivity on Chiralcel OJ, while several factors affected chiral selectivity on Chirex 3005. Chiral separation of fluorene derivatives was comparatively carried out with the CTA-I (cellulose triacetate) and Chiralcel OJ phases, and it was found that the framework and substitution effects do not result in the same response on the two phases, and that the certain areas of the molecule responsible for chiral discrimination are different in the interaction within CTA-I and Chiralcel OJ.[24] Ketoprofen enantiomers were derivatized with 9-aminophenanthrene and analyzed by HPLC on Chiralcel OJ to examine bio-inversion of *R*-ketoprofen to the S-isomer in horse blood.[8] O'Brien et al. carried out an impressive work on elucidating the types of interactions occurring between a diol intermediate and Chiralcel OJ, and showed with rigorous, experimental evidences that the enantioselectivity is entropy-driven at low temperatures and enthalpy-driven at high temperatures, the transition temperature of the stationary phase being the boundary (18°C), and that hydrogen bonding is a primary factor in the separation, causing decreased retention and increased selectivity with increased alcohol modifier concentration.[25]

There has been no thermodynamic study for chiral separation of arylpropionic acids on the Chiralcel OJ phase. We reported thermodynamic enantioseparation data of some arylpropionic acids in the Chirex 3001 column in the reversed-phase mode in the previous study.[26] In this study, we have measured the enantioseparation data of arylpropionic acids in the Chiralcel OJ-H [cellulose *tris*(4-methylbenzoate) chemically bonded to silica] column in the normal phase mode to comparatively analyze the thermodynamic properties.

EXPERIMENTAL

Chemicals

Hexane and 2-propanol were of HPLC grade and purchased from Fisher (Pittsburg, PA, USA) and used without further purification. Racemic ketoprofen, fenoprofen, ibuprofen, *S*-ketoprofen, and *S*-ibuprofen were obtained from Aldrich (Milwaukee, IL, USA) and used as-received.

HPLC

The chromatographic system was a Shimadzu (Tokyo, Japan) HPLC system composed of a 10AD pump, a SCL-10A system controller, a SIL-10A auto-injector, a CTO-10AC column oven, a SPD-10A UV/VIS detector, and a Chromatopac C-R7A data system.

The Chiralcel OJ-H column (4.6 mm I.D. × 250 mm) was purchased from Diacel (Tokyo, Japan). The column was placed in the column oven, and its temperature was controlled with an accuracy of $\pm 0.1^{\circ}$C.

Procedures

The mobile phases used were 90/10, 95/5, and 100/0 hexane/2-propanol mixtures with 0.5% acetic acid. The flow rate was fixed at 1.0 mL/min throughout. The capacity factors of the solutes were measured at 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C. The wavelength of the detector was set at 254 nm. The sample solution was prepared by dissolving the solutes in 2-propanol. At each run, the sample was injected two to three times to check reproducibility. The reproducibility of the capacity factor for repetitive injections was better than 1% for the worst case and better than 0.5% in most cases. The hold-up time was measured with 1,3,5-tertiarybutylbenzene.

The capacity factor data based on three independent measurements on different days (usually different weeks) were used to calculate the thermodynamic properties of solute transfer.

RESULTS AND DISCUSSION

The molecular structures of arylpropionic acids used in this study are shown in Figure 1.

From the van't Hoff plots ($\ln k'$ vs. $1/T$), the enthalpy (ΔH^0) and entropy (ΔS^0) of solute transfer from the mobile phase to the stationary phase can be obtained as follows:

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi \tag{1}$$

In equation (1), ΔH^0 and ΔS^0 are the standard enthalpy and entropy for the solute transfer from the mobile phase to the stationary phase, respectively, φ, the phase ratio, and R, the gas constant. All the van't Hoff plots observed were linear, and the regression correlation coefficients were better than 0.995 in all cases and better than 0.999 in most cases. However, it should be noted that the temperature range of this study is confined to 25–50°C (the practical range of general chromatographic application). For a wider temperature range (5–50°C), the van't Hoff plots were nonlinear with a transition occurring between 18°C and

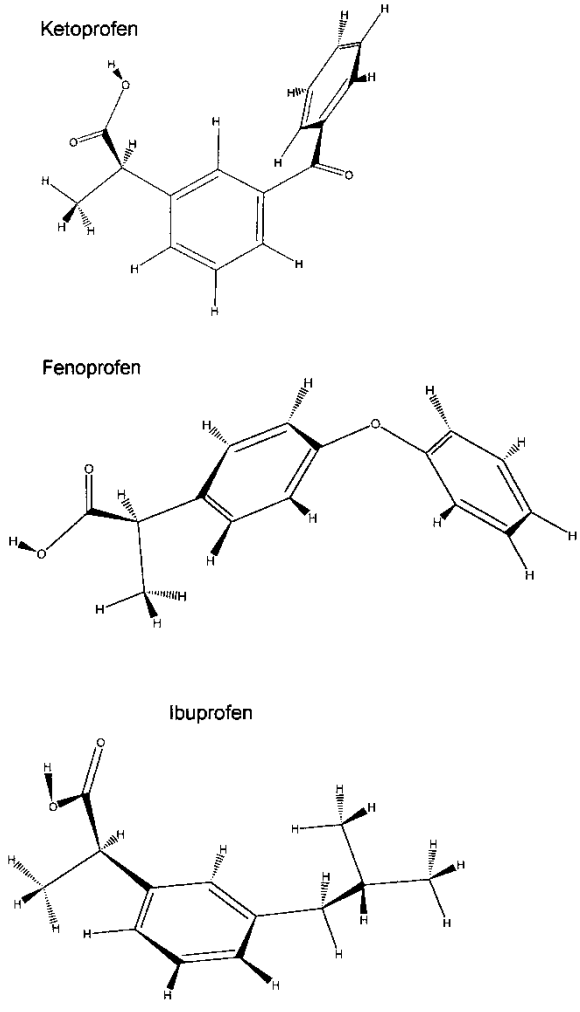

Figure 1. The R-isomer structures of ketoprofen, fenoprofen, and ibuprofen.

20°C,[25] and the enantioselectivity was found entropy-driven at low temperatures and enthalpy-driven at high temperatures. Thus, our study is limited to the enthalpy-driven case. The averages and standard deviations of the calculated thermodynamic properties based on three independent runs are assembled in Table 1. The peak shape and retention reproducibility of *S*-ibuprofen in 100% hexane (with 0.5% acetic acid) were very poor, as shown in Table 1, and its thermodynamic properties were subject to a high error (Figure 3).

When we consider a pair of enantiomers (A and B) in chiral separation, the solute–solvent interaction in the mobile phase is exactly the same for A

Table 1. The thermodynamic properties of solute transfer from the mobile phase (hexane/2-propanol with 0.5% acetic acid) to the Chiralcel OJ phase based on three independent measurements (Unit: J/mol)

Thermodynamic properties (J/mol) for each solute	Hexane (%)		
	90	95	100
ΔH^0			
Ibuprofen (R-)	−10,158 ± 1,292	−7,033 ± 1,650	−19,875 ± 939
Ibuprofen (S-)	−11,153 ± 1,090	−8,060 ± 651	−21,433 ± 3,198
Fenoprofen (R-)	−14,406 ± 426	−11,496 ± 791	−29,711 ± 673
Fenoprofen (S-)	−17,715 ± 519	−13,673 ± 628	−30,333 ± 415
Ketoprofen (R-)	−16,016 ± 309	−12,475 ± 566	−25,233 ± 70
Ketoprofen (S-)	−19,229 ± 350	−15,332 ± 673	−25,341 ± 196
$T\Delta S^0 + RT\ln\Phi$			
Ibuprofen (R-)	−13,058 ± 1279	−8,402 ± 1,658	−16,724 ± 909
Ibuprofen (S-)	−13,641 ± 1086	−8,957 ± 1,656	−18,673 ± 3,080
Fenoprofen (R-)	−13,394 ± 428	−8,732 ± 808	−22,417 ± 409
Fenoprofen (S-)	−16,161 ± 524	−10,551 ± 646	−22,863 ± 655
Ketoprofen (R-)	−13,954 ± 321	−8,429 ± 572	−15,603 ± 200
Ketoprofen (S-)	−16,322 ± 357	−10,491 ± 671	−15,968 ± 51

and B; thus, the differential thermodynamic property of the pair reflects only the difference of interactions in the CSP between A and B.

$$\ln\alpha = -\frac{\Delta\Delta G^0}{RT} = -\frac{\Delta\Delta H^0}{RT} + \frac{\Delta\Delta S^o}{R} \tag{2}$$

In equation (2), α means k'_A/k'_B, $\Delta\Delta H^0$, $\Delta H^0_A - \Delta H^0_B$, and $\Delta\Delta S^0$, $\Delta S^0_A - \Delta S^0_B$, respectively. In this study, A is S-isomer and B is R-isomer.

Comparison of Enthalpic and Entropic Thermodynamic Properties

The enthalpic and entropic contributions to the free energy of solute transfer from the mobile phase to the Chiralcel OJ phase were computed based on equation (1), and comparatively plotted as a function of mobile phase composition in Figure 2. The phase ratio was not known; thus, it was included in the entropic term ($T\Delta S^0 + RT\ln\varphi$). A unique feature of Figure 2 is the appearance of extrema in both enthalpic and entropic plots. On the other hand, there is no extremum in the plots of $\Delta\Delta H^0$ and $\Delta\Delta S^0$ (Figure 3). The appearance of extrema will be discussed later. The peak shape and retention reproducibility of *S*-ibuprofen in 100% hexane (including 0.5% acetic acid) was very poor, and its $\Delta\Delta H^0$ is subject to a high error (Figure 3). The enthalpic variation trend mimics the entropic variation trend in all cases as shown in Figures 2 and 3; thus, the

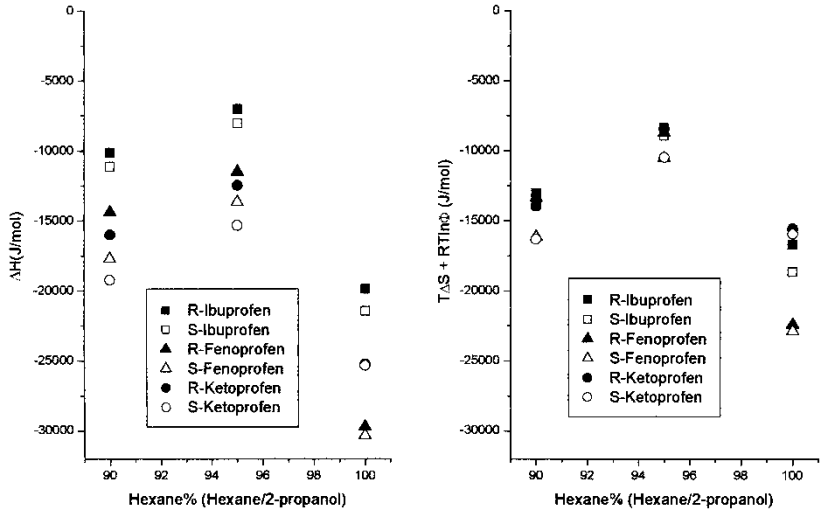

Figure 2. The comparative plots of enthalpic and entropic contributions to the free energy of transfer from the mobile phase to the stationary phase as a function of mobile phase composition.

well-known enthalpic–entropic compensation is clearly valid for the systems considered in this study.

The Effect of Solute Structure

Except for the data of ibuprofen at 100% hexane of low reliability, the absolute values of $\Delta\Delta H^0$ and $\Delta\Delta S^0$ of ibuprofen are much smaller than those of fenoprofen and ketoprofen (Figure 3). The absolute values of solute transfer enthalpies and entropies of ibuprofen enantiomers are also smaller than those of fenoprofen and ketoprofen enantiomers (Figure 2), which indicate that the solute-Chiralcel OJ interactions of *R*- and *S*-ibuprofen are weaker than those of *R*- and *S*-fenoprofen and ketoprofen. Such observations are reasonable, since ibuprofen is much less polar than others (it also has only one phenyl ring, Figure 1) and subject to much weaker interactions with the stationary phase.

Comparison of Trends of Thermodynamic Properties Between the Chirex 3001(MeOH/Water) and Chiralcel OJ (Hexane/2-Propanol) Sytems

ΔH^0 and ΔS^0 (Figure 4), $\Delta\Delta H^0$ and $\Delta\Delta S^0$ (Figure 5), and $\Delta\Delta G^0$ (Figure 6) of fenoprofen enantiomers are compared between the Chirex 3001(MeOH/water)[25] and Chiralcel OJ(hexane/2-propanol) systems to see how the different chiral phases affect the chiral recognition and separation.

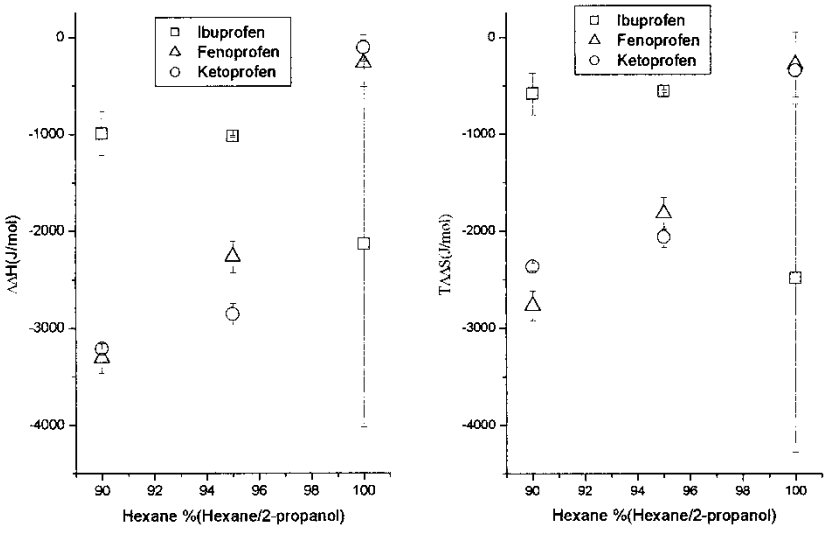

Figure 3. The comparative plots of enthalpic and entropic contributions to the differential free energy of solute transfer between the *R*- and *S*-enantiomers as a function of mobile phase composition.

The variation trends of ΔH^0 and ΔS^0 with mobile phase composition in the previous studies[26–32] were very similar and simple. All the previous studies were done in the reversed-phase mode. A monotonic increase of the absolute ΔH^0 and ΔS^0 with eluent polarity was observed, as far as the water content is low enough to avoid the hydrophobic effect. We proposed that the variations of both ΔH^0 and ΔS^0 with respect to mobile phase composition are primarily governed by the cavity formation in the mobile phase.[26]

However, the normal phase mode was employed in this study, and therefore the cavity formation effect is not a major factor. Nevertheless, it is not straightforward to explain the appearance of extrema.

The absolute magnitudes of ΔH^0 and ΔS^0 of the Chiralcel OJ system are clearly larger than those of the Chirex 3001 system. It may be due to a stronger solute interaction with the Chiralcel OJ than that with the Chirex 3001 or a weaker solute–mobile phase (hexane/2-propanol) interaction in the Chiralcel system than that (methanol/water) in the Chirex 3001 system.

The overall solute–mobile phase interaction should be the sum of the pure exothermic molecular interaction of the solute with the surrounding solvent plus the endoergic solute cavity formation in the mobile phase. The cavity formation energy (endoergic) in hexane/2-propanol is lower than that in methanol/water. The cavity formation effect is dominant in reversed-phase chromatography, whereas it is not in normal-phase chromatography. Therefore, a stronger solute–mobile phase interaction is expected in

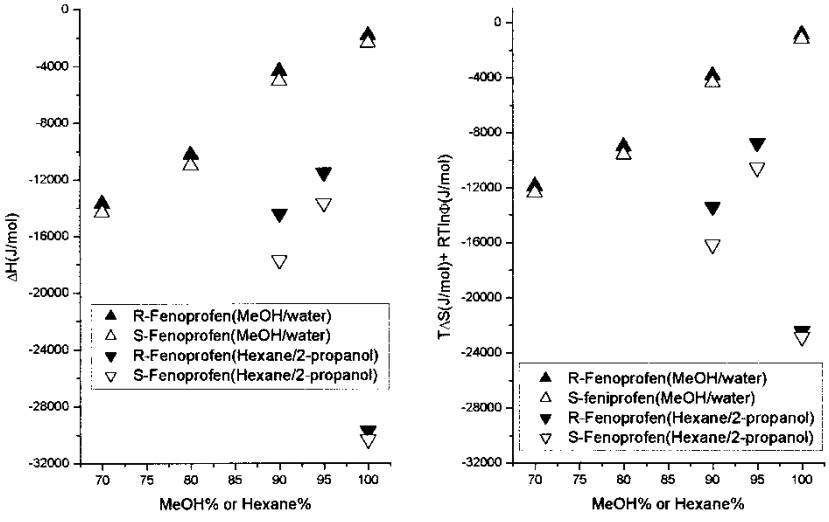

Figure 4. Comparison of the thermodynamic properties of solute transfer (fenoprofen enantiomers) between the two data sets obtained by the Chirex 3001 phase in methanol/water eluents (previous study) and the Chiralcel OJ phase in hexane/2-propanol eluents (this study).

hexane/2-propanol than that in methanol/water if the cavity formation effect is incorporated in the solute–mobile phase interaction.

Consequently, the solute–Chiralcel OJ interaction should be stronger than the solute–Chirex 3001 interaction. The absolute magnitudes of not only ΔH^0 and ΔS^0 (Figure 4) but also of $\Delta\Delta H^0$, $\Delta\Delta S^0$ (Figure 5), and $\Delta\Delta G^0$ (Figure 6) of the Chiralcel OJ system are clearly larger than those of the Chirex 3001 system, if the data of 100% hexane (low reliability) is excluded. It is conceivable that the supramolecular structure of the Chiralcel OJ phase with chiral cavities, in comparison with the brush structure of the Chirex 3001 phase, provides more versatile functional and steric environments to give stronger interactions and better chiral recognition and discrimination. This study confirms such facts with real quantitative data.

The Effect of Composition of Organic Solvent in the Mobile Phase

Now let us discuss the extrema in the plots of ΔH^0 and ΔS^0 (Figure 2) with respect to mobile phase composition. The meaning of the extrema is that the solute–Chiralcel OJ interaction becomes weaker initially when a small amount of 2-propanol is added to the mobile phase (100% hexane with 0.5% acetic acid), but that the solute–Chiralcel OJ interaction becomes stronger, once the content of 2-propanol passes a certain level. On the other

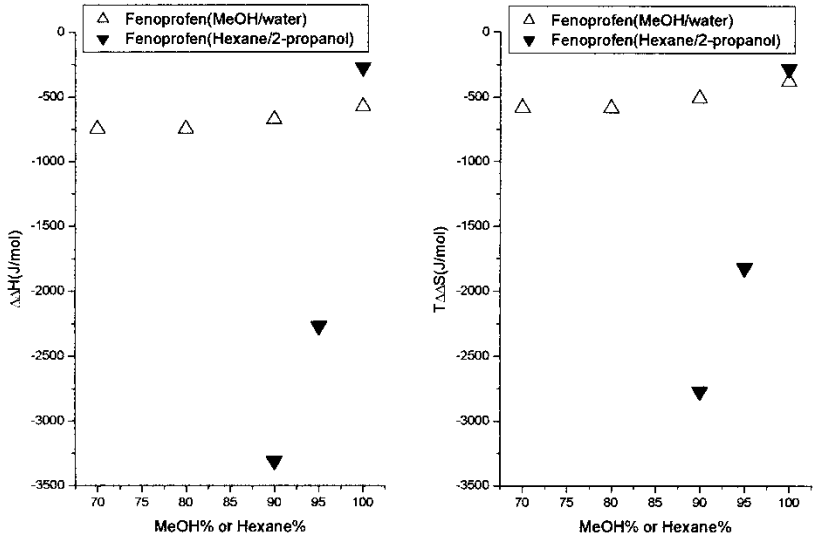

Figure 5. Comparison of $\Delta\Delta H^0$ and $T\Delta\Delta S^0$ between the two data sets obtained by the Chirex 3001 phase in methanol/water eluents (previous study, open symbol) and the Chiralcel OJ phase in hexane/2-propanol eluents (this study, closed symbol).

hand, the absolute magnitudes of $\Delta\Delta H^0$, $\Delta\Delta S^0$ (Figures 3 and 5), and $\Delta\Delta G^0$ (Figure 6) increase monotonously upon addition of 2-propanol.

At this point, we had better discuss the role of 2-propanol in this system. Okamoto et al. reported in their study on chiral separation of mandelic acid on the Chiralcel OD phase that mandelic acid enantiomers eluted and separated in the hexane-based eluent only when both acetic acid and 2-propanol were added.[33] The role of acetic acid was thought to prevent dissociation of solute and to block the residual active sites of silica, and the role of 2-propanol, to compete with the solute for hydrogen bonding to the stationary phase, and cause changes of the steric environments of the chiral cavities by adsorbing to the chiral cavities or achiral polar functional groups.[18] O'Brien et al. argued in their study of chiral separation of diol enantiomers on Chiralcel OJ that there can be a leading interaction based on hydrogen bonding that determines the retention of the enantiomers and a cooperative inclusion type interaction (chiral cavities) that is responsible for enantioselectivity;[25] and the two interactions are affected by addition of modifier. To our understanding, the concept for the role of mobile phase modifier is basically identical for the earlier mentioned two views, that is, retention and selectivity for chiral separation are controlled by adsorption of mobile phase modifier to the stationary phase, although details are different depending on the types of stationary phases and enantiomers.

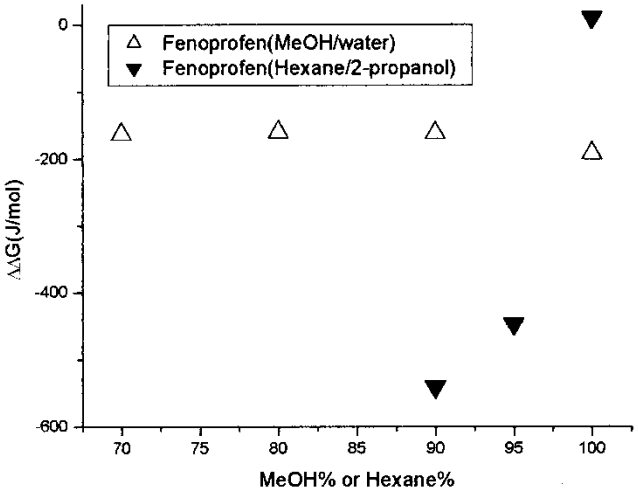

Figure 6. Comparison of $\Delta\Delta G^0$ between the two data sets obtained by the Chirex 3001 phase in methanol/water eluents (previous study, open symbol) and the Chiralcel OJ phase in hexane/2-propanol eluents (this study, closed symbol).

Thus, based on the observations of this study, 2-propanol adsorbs to the Chiralcel OJ phase to weaken the solute–Chiralcel OJ interaction until a certain level of 2-propanol is added in the mobile phase; some change occurs when the 2-propanol content passes such a level to cause strengthening the solute–Chiralcel OJ interaction again, while the ability of chiral discrimination between the R- and S-isomers keeps on improving over the whole range of 2-propanol addition. These phenomena are, of course, likely to occur for some specific enantiomers that satisfy the requirements of the following explanation. A possible explanation is adsorption of 2-propanol to multiple sites with different priorities and different steric environments. Adsorption of 2-propanol to the site of higher priority is accompanied by weakening of the solute–Chiralcel OJ interaction with increased chiral discrimination owing to different steric environments induced by adsorption, but after the saturation of the first site, adsorption of 2-propanol to the next site causes strengthening of the solute–Chiralcel OJ interaction by enabling extra interaction, for example, the interaction between the solute and adsorbed 2-propanol, which causes even more increased chiral discrimination. A study to get proof for such a hypothesis is under way.

CONCLUSIONS

Enantioseparation data of ketoprofen, fenoprofen, and ibuprofen on the Chiralcel OJ phase under the normal-phase mode have been obtained and

thermodynamically analyzed. It has been found that the well-known enthalpic–entropic compensation is valid for the systems considered in this study. The solute–Chiralcel OJ interactions of *R*- and *S*-ibuprofen were weaker than those of *R*- and *S*-fenoprofen and ketoprofen. The solute–Chiralcel OJ interaction was stronger than the solute–Chirex 3001 interaction. The supramolecular structure of the Chiralcel OJ phase with chiral cavities seems to provide more versatile functional and steric environments to give stronger interactions and better chiral recognition and discrimination. The appearance of extrema in the plots of ΔH^0 and ΔS^0 in the Chiralcel OJ system seems to be due to adsorption of 2-propanol to multiple sites with different priorities and different steric environments.

ACKNOWLEDGMENT

This work was supported by the Korea Research Foundation grant (KRF-2003-015-C00417).

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Received July 14, 2004
Accepted October 13, 2004
Manuscript 6459