ELSEVIER

Journal of Chromatography A, 848 (1999) 9–20

JOURNAL OF CHROMATOGRAPHY A

Thermodynamic properties for the solute transfer from the mobile to the stationary phase in reversed phase liquid chromatography obtained by squalane-impregnated C_{18} bonded phase

Choong Sik Lee, Won Jo Cheong*

Department of Chemistry and Center for Chemical Dynamics, Inha University, Incheon 402-751, South Korea

Received 21 January 1999; received in revised form 17 March 1999; accepted 17 March 1999

Abstract

We have devised a reversed phase chromatographic system which secured a simple retention mechanism and showed reproducible solute retention over a long period of time. We used a squalane impregnated C_{18} phase in a thermostated system with extreme care, and obtained retention data of some selected solutes (benzene, toluene, ethylbenzene, phenol, and acetophenone) at 25, 30, 35, 40, 45, and 50°C in aqueous methanol eluents. The van't Hoff plots were nicely linear, thus we calculated dependable enthalpies and entropies of solute transfer from the mobile to the stationary phase based on three independent retention measurements on different days (or weeks). We observed that the solute transfer from the mobile to the stationary phase was enthalpically favorable and entropically unfavorable in general except for highly aqueous mobile phases. The enthalpic contribution to the overall solute transfer free energy was found generally more important than the entropic contribution. The cavity formation effect was found the major factor that governs the solute distribution between the mobile and stationary phases for methanol-rich mobile phases while the hydrophobic effect became significant in highly aqueous mobile phases. Comparison of our data with the literature data leaded to the conclusion that C_{18} stationary phases with a high ligand density generally follow a partition mechanism while a C_{18} phase with a low ligand density follows an adsorption-like mechanism.

Keywords: Solute transfer; Enthalpy; Entropy; Stationary phases, LC; Squalane

1. Introduction

Reversed phase liquid chromatography (RPLC) has been one of the most popular chromatographic tools since the eighties of this century [1]. The retention mechanism is still a topic of controversy, but the partition mechanism seems to be more supported than the adsorption mechanism [2–6].

There have been numerous studies of temperature effects on solute retention in reversed phase liquid chromatography. The linear van't Hoff plots were observed in the typical RPLC systems [7–11]. In these studies, the enthalpies of solute transfer from the mobile phase to the stationary phase (ΔH^{o}) were calculated from the slopes of the van't Hoff plots, but the entropies of solute transfer (ΔS^{o}), in most cases, were not reported owing to the unknown phase ratios of the columns used.

Nonlinear van't Hoff plots were also observed in some studies of temperature effects on solute retention in RPLC [12–20]. Nonlinear van't Hoff plots

*Corresponding author. Fax: +82-032-867-5604.
E-mail address: wjcheong@inha.ac.kr (W.J. Cheong)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00404-5

are often observed when the temperature range is more than 45°C. Some workers [13,14] reported based on the GC techniques that the bending point in the van't Hoff plot is ca 22°C when the C_{18} stationary phase is free of solvent contact. Such phase transition of stationary phase occurs more readily with higher ligand density (over 4 $\mu mol/m^2$) [12]. Cole and Dorsey [21] clearly showed that the phase transition of a stationary phase occurs at 20–30°C when the ligand density is larger than 3.0 $\mu mol/m^2$ and that the phase transition temperature gets higher as the ligand density increases, thus they cleared conflicts in observations of linearity vs. nonlinearity in the van't Hoff plots.

The studies of temperature effects on solute retention in RPLC tend to expand the territory to a variety of systems. Alkaloids of special van't Hoff plot trends [22], nonlinear van't Hoff plots for steroids upon addition of β-cyclodextrin to the mobile phase [23], separation of enantiomers in the chiral columns [24,25], polynuclear aromatic hydrocarbons of various sizes and shapes [26], and ion chromatographic separation [27] are some examples of recent research.

A series of homologous solutes were often used to examine the separation efficiencies of columns and mobile phases, and to study the characteristics of stationary phases in RPLC. Issaq [10] studied enthalpic and entropic contributions to solute transfer using a homologous series of alkylbenzenes, and concluded that the thermodynamic properties were linearly correlated with the solute carbon number regardless of the ligand chain length of stationary phase. Goldberg [28] examined the characteristics of 31 stationary phases and showed that the ligand density was the major factor in improving separation of a homologous series of solutes. Sentell et al. [29] studied the temperature effects on selectivity of the phenylene group repeating solutes (rod-like) and the fused ring repeating solutes (plate-like) in addition to the methylene group repeating solutes (chain-like) and observed that the ability of improving selectivity by temperature variation was in the order of plate> rod>chain.

Yamamoto et al. [9] comparatively evaluated the enthalpy and entropy of solute transfer from the mobile to the stationary phase for a group of purine solutes among various C_{18} columns and found that the thermodynamic retention properties varied by a large extent depending on the choice of C_{18} stationary phase. They also observed that an enthalpy–entropy compensation relationship for the retention of a purine base in the C_{18} columns examined.

Recently, Bell and coworkers [30] studied the influence of temperature on the liquid chromatographic retention of carotenoids for the C_{18}, C_{30} and C_{34} stationary phases and observed linear van't Hoff plots for all the solutes in the C_{18} phase, but a variety of retention behaviors in response to temperature in the C_{30} and C_{34} stationary phases.

McGuffin et al. [31] measured thermodynamic properties for methylene and benzene homologues in reversed phase liquid chromatography using octadecylsilica phases and found that for octadecylsilica phases with low bonding density, ΔH^o was small and relatively unaffected by temperature whereas ΔH^o was markedly affected by temperature for a polymeric octadecylsilica phase with a high bonding density.

Thus, there have been a large amount of studies in temperature effects on solute retention in RPLC. Most of such works were, however, related to rather qualitative discussions such as changes of physical properties of the stationary phase on temperature variation, increasing or decreasing trends of the magnitudes of the thermodynamic properties for a group of solutes, and their comparison among different stationary or mobile phases.

In this study, we have tried to obtain reliable quantitative thermodynamic properties for solute transfer between the mobile and stationary phases by measuring solute retention on a squalane-impregnated C_{18} phase. The squalane impregnated C_{18}phase seems to be regarded as a bulk stationary phase and the phase ratio can be unambiguously evaluated, so the retention will follow a simple partition mechanism to give results of straightforward thermodynamic meaning.

2. Experimental

Methanol and water were of HPLC grade and purchased from Fisher (Pittsburg, PA, USA) and used without further purification. Benzene, toluene, ethylbenzene, phenol, and acetophenone were ob-

tained from Aldrich (Milwaukee, IL, USA) and used as received.

The chromatographic system we used was a Shimadzu (Tokyo, Japan) HPLC system composed of a 10 AD pump, a SCL-10 A system controller, a SIL-10 A autoinjector, a CTO-10 AC column oven, a SPD-10 A UV/VIS detector, and a Chromatopac C-R7 A data system.

The column (4.6 mm I.D.×250 mm) was home-made, and was packed with a squalane impregnated C_{18} stationary phase. The Spherisorb ODS2 (particle diameter: 5 μm) from Phase Separation (Norwalk, CT, USA) was used. The raw C_{18} phase was dried at 90°C for at least 24 hours and weighed. The dried C_{18} phase was immersed in a squalane solution in hexane with stirring for a while. The C_{18} phase was filtered and dried at 90°C for at least 24 hours and weighed. Such a procedure (immersing, drying, and weighing) was repeated until the C_{18} phase gained a 12–15% weight increase. A portion of the squalane impregnated C_{18} phase was measured and suspended in methanol. The stationary phase slurry was quantitatively transferred to the slurry reservoir of the Alltech (Deerfield, IL, USA) slurry packer. After column packing, the residual stationary phase in the reservoir and the transfer tubing was carefully collected in a pre-weighed beaker. The solvent in the beaker was evaporated by gentle heating, and the beaker was placed in a oven at 90°C and dried. The difference between the initial weight of the stationary phase used for the slurry and the weight of the residual stationary phase was adopted as the weight of the stationary phase packed in the column.

The stationary phase volume (1.106 ml) was determined by dividing the total weight of the C_{18} ligands (based on the carbon load data from the manufacturer) and the adsorbed squalane by the density of squalane (0.81) assuming that the density of the squalane adsorbed C_{18} phase can be approximated to be the density of squalane. The mobile phase volume (2.257±0.017 ml) was determined by measuring and averaging the retention volume of a void volume marker (KNO_3) at 35°C. The temperature range we examined was 25–50°C. We observed some variation in flow rate but it was less than 2% over the temperature range and the flow rate at 35°C was about the mean. Thus the phase ratio was found to be 0.490.

The column was placed in the column oven, and its temperature was controlled with an accuracy of ±0.1°C. The transfer tubing (1 m, 1 mm I.D.) between the pump and the injector was water-jacketed, and thermostated water was circulated through the jacket with a Jeio Tech (Seoul, Korea) Excel-18E circulating water bath. The bath temperature was adjusted to the same temperature as that of the column oven. The solvent bottle was placed in the bath and pre-heated before entering the pump, too. The injector was connected to the column via a tubing of very small inner volume (50 cm, 100 μm I.D.). The column was connected to the detector in the same way. Thus the extracolumn void volume (8 μl) was negligible.

The mobile phases used were methanol/water mixtures (10/90, 30/70, 60/40, 70/30, 80/20 v/v%), and the flow rate was fixed at 1.0 ml/min throughout. The capacity factors of the solutes were measured at 25, 30, 35, 40, 45, and 50°C. The wavelength of the detector was set at 254 nm. The sample solution was prepared by dissolving all the solutes in methanol. At each run, the sample was injected 3–4 times to check reproducibility. The reproducibility of capacity factor for repetitive injections was better than 1% for the worst case and better than 0.5% in most cases. Both KNO_3 and uracil were used as the void volume marker. Uracil was added to the sample solution. KNO_3 was dissolved in water and injected alone right before and after 3–4 repetitive sample injections.

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. The long-term reproducibility of k' was better than 2%.

3. Results and discussion

As mentioned before, three independent measurements were made on different days for a given mobile phase and temperature. From the van't Hoff plots ($\ln k'$ vs. $1/T$) of each run, the enthalpy (ΔH°) and entropy (ΔS°) of solute transfer from the mobile to the stationary phase were obtained.

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

In the above equation, φ is the phase ratio. All the van't Hoff plots observed were linear, and the regression correlation coefficients were better than 0.999 in all cases. A typical example of van't Hoff plot is shown in Fig. 1. The averages and standard deviations of the calculated thermodynamic properties based on three independent runs are assembled in Table 1.

We can easily figure out that the thermodynamic properties of nonpolar solutes (benzene, toluene, ethylbenzene) are more reproducible than those of polar solutes (phenol, acetophenone). We can also note that the difference in a thermodynamic property between the two data sets based on the void volume of KNO_3 and uracil, respectively, is greater in water-rich mobile phases than in methanol-rich mobile phases. For example, the enthalpies and entropies of solute transfer based on the two void volume markers for phenol and toluene are comparatively shown in Fig. 2.

Choice of void volume marker is important especially when we consider a shortly retained solute. According to the reviews [32,33] concerning void volume markers in RPLC, they generally use D_2O as the marker of choice, while some people use uracil or KNO_3 as well. We also used D_2O, too, but detection of D_2O peak was frequently hampered by

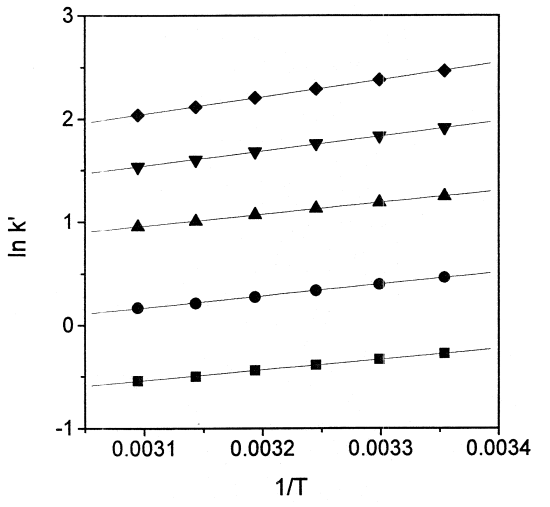

Fig. 1. The van't Hoff plot for the data obtained in 60/40 (v/v%) methanol/water at 25–50°C and based on KNO_3 as the void volume marker. From the top, ethylbenzene (♦), toluene (▼), benzene (▲), acetophenone (●), and phenol (■).

appearance of the system peak nearby. In most cases, KNO_3 proved to show up very close to D_2O. A disadvantage of using KNO_3 is difficulties in dissolving the marker in the sample solution since an organic solvent is usually used to dissolve solutes while KNO_3 shows enough solubility only in water. KNO_3 always showed up a little earlier than uracil and the difference gets larger as the mobile phase composition gets closer to pure water. It is uncertain whether KNO_3 is better than uracil as a void volume marker for our system. Nevertheless, we will primarily consider thermodynamic data based on the void volume of KNO_3 since its value is very close to that of D_2O.

The enthalpies and entropies of solute transfer are plotted against mobile phase composition in Fig. 3. Since $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, and ΔH° and ΔS° are both negative as long as the methanol content of the mobile phase is over 30%, the transfer of a solute from the mobile to the stationary phase is enthalpically favorable and entropically unfavorable in general. Especially, ΔH° is of negative value over the whole range of mobile phase composition (methanol 10–80%). The variations in enthalpy of solute transfer for the nonpolar solutes with respect to mobile phase composition are larger than those for polar solutes (Fig. 3).

Such results strongly support the argument that the cavity formation effect is the major factor that governs the solute distribution between the mobile and stationary phases for the system we consider. The stationary phase (squalane impregnated C_{18}) is entirely nonpolar while the mobile phase (methanol/water mixture) is highly polar. Thus, in view of interaction enthalpy between a solute and a solvent, the solute, if polar, will prefer the mobile phase to the stationary phase, or, if nonpolar, will have no particular preference to any phase, which is against the observation of this study that the stationary phase was enthalpically favored by all the solutes.

There should be another enthalpic factor for solute distribution, that is, cavity formation [34,35]. The incorporation of cavity formation concept in reversed phase liquid chromatography was first introduced by Horvath et al. [36,37] when they made use of the Sinanoglu's formulation [38] of cavity formation energy for the solute, the ligand, and the solute–ligand complex to derive equation of solute re-

Table 1
The enthalphies and entrophies with their standard deviations for solute transfer from the mobile phase to the stationary phase based on three independent retention measurements on different days over the temperature range of 25–50°C[a]

ΔH^{o} (J/mol)						
Void volume marker	Solute	Mobile phase composition (methanol%)				
		10	30	60	70	80
KNO_3	phenol	−13336±361	−13603±138	−8550±219	−7522±643	−5629±223
	acetophenone	–	–	−9512±140	−7867±502	−5821±357
	benzene	−8306±32	−11833±66	−9518±105	−7737±314	−5529±90
	toluene	−11084±157	−15060±100	−12037±90	−9799±302	−7259±106
	ethylbenzene	–	–	−13792±94	−11129±320	−8277±51
uracil	phenol	−9663±239	−13005±138	−9224±148	−7003±133	−5342±633
	acetophenone	–	–	−9819±101	−7436±128	−5698±427
	benzene	−4640±87	−11043±31	−9604±43	−7326±81	−5278±224
	toluene	−7465±274	−14262±6	−12071±58	−9431±110	−7067±195
	ethylbenzene	–	–	−13788±68	−10769±155	−8096±129

ΔS^{o} (J/mol·K)						
Void volume marker	Solute	Mobile phase composition (methanol%)				
		10	30	60	70	80
KNO_3	phenol	−19.4±1.2	−29.3±0.9	−25.2±0.8	−25.8±2.1	−23.6±0.7
	acetophenone	–	–	−22.3±0.5	−21.5±1.7	−20.9±1.9
	benzene	9.7±0.2	−9.3±0.2	−15.7±0.4	−14.8±1.1	−12.6±0.3
	toluene	10.9±0.4	−11.5±0.3	−18.6±0.3	−17.1±1.0	−14.8±0.3
	ethylbenzene	–	–	−19.9±0.3	−17.9±1.1	−15.5±0.1
uracil	phenol	−7.9±0.8	−27.7±0.5	−28.2±0.4	−25.2±0.4	−24.1±2.2
	acetophenone	–	–	−23.6±0.3	−20.6±0.4	−19.5±1.5
	benzene	21.5±0.2	−6.8±0.1	−16.1±0.1	−13.7±0.3	−12.2±0.8
	toluene	22.5±0.8	−9.0±0.1	−18.8±0.1	−16.1±0.4	−14.4±0.6
	ethylbenzene	–	–	−19.9±0.2	−16.8±0.5	−15.0±0.4

[a] In the mobile phase of methanol 10% and 30%, only phenol, benzene, and toluene were measured.

tention. Their solvophobic theory was, however, rather based on an adsorption-like retention model. The partition mechanism [2–6] in the octadecyl bonded stationary phase seems to be generally accepted. Recently, Tan and Carr [39,40] reanalyzed solvophobic driving forces in reversed phase liquid chromatography and found that retention on monomeric bonded phases with octyl chains or longer is dominated by a partition mechanism and that an adsorption-like mechanism contributes to retention in monomeric bonded phases with short bonded chains or with low surface coverage density.

If a solute is introduced into a phase, a hole should be formed in the phase to accommodate the solute and the cavity formation is enthalpically endothermic. The cavity formation enthalpy of the mobile phase is much larger than that of the stationary phase because there is only a dispersive interaction in the stationary phase while there exist dipole–dipole and hydrogen bond interactions in addition to the dispersive interaction in the mobile phase. Therefore, the solute prefers the stationary phase to the mobile phase in view of cavity formation enthalpy, which is in agreement with actual observation. Thus we can conclude that the cavity formation effect is dominant compared to the solute–phase interaction effect.

The cavity formation enthalpy gets larger as the mobile phase gets more polar or the solute size gets bigger [34], which is in agreement with the observation that ΔH^{o} values get less negative with increasing methanol content (60–80% range) in the mobile phase and that the slopes in the plot of ΔH^{o} vs.

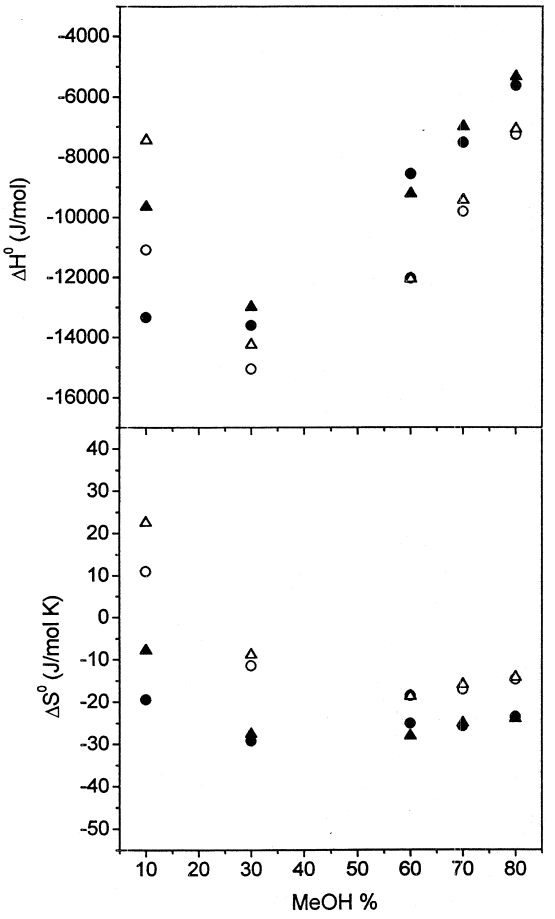

Fig. 2. Comparison of the solute transfer enthalpies and entropies based on KNO_3 (○, ●), and uracil (△, ▲) for phenol (●, ▲) and toluene (○, △).

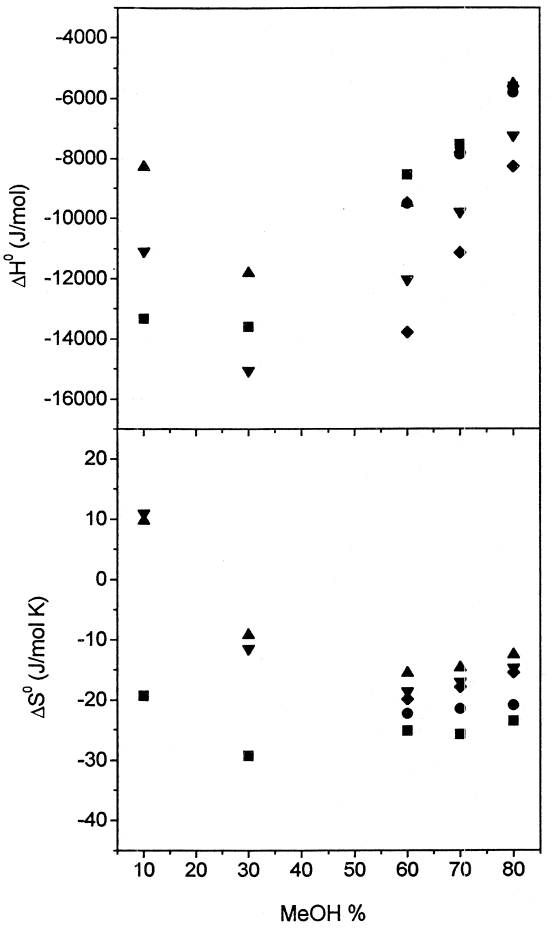

Fig. 3. The plot of solute transfer enthalpies and entropies based on KNO_3 as the void volume marker against methanol volume% of the mobile phase for ethylbenzene (♦), toluene (▼), benzene (▲), acetophenone (●), and phenol (■).

methanol volume fraction are steeper for larger solutes (Fig. 3). On the other hand, the slopes for more polar solutes are less steeper since there exist nondispersive solute–solvent interactions in the mobile phase which partly cancel out the cavity formation enthalpy.

In the composition range of 10–30% methanol, some different trends were observed. If the cavity formation effect were still dominant, ΔH^{o} should have become more negative as methanol content in the mobile phase decreases. The reality is contrary to the hypothesis. Such a phenomenon can be explained by the so called hydrophobic effect [41] caused by restructuring and reinforcement of surrounding water molecules (iceberg formation) when a solute is introduced in a highly aqueous liquid. Water molecules around the solute tend to hold one another more tightly to compensate for the molecular interactions broken by insertion of the solute and such structural reinforcement is strong enough to cancel out the enthalpic energy loss of cavity formation by a large extent especially when the solute is nonpolar and/or the solvent is highly aqueous. We approximately evaluated the hydrophobic interaction enthalpy corresponding to the structural reinforcement of solvent molecules around the inserted solute by assuming that the solute-phase interaction enthalpies are virtually invariant with respect to mobile phase

composition for nonpolar solutes and that the cavity formation enthalpy term is linearly correlated with mobile phase composition. The linearity was found in the composition range of 60–80% methanol where the hydrophobic interaction effect was thought negligible (see Fig. 3). We extrapolated the solute transfer enthalpy in the linear range to the composition of 10% methanol and obtained the hypothetical cavity formation enthalpy term at that composition. We finally obtained the hydrophobic interaction enthalpy by subtracting this value from the solute transfer enthalpy. We found −12 000 J/mol for benzene and −13 000 J/mol for toluene, respectively. The magnitudes are comparable to those of cavity formation enthalpy term in methanol-rich mobile phases and more than a half of the cavity formation energy term in highly aqueous mobile phases.

The entropies of solute transfer from the mobile to the stationary phase are exclusively negative for phenol. The entropies of solute transfer for benzene and toluene are also mostly negative, but the sign is changed to a positive value for highly aqueous solvents (10% methanol, Fig. 3) owing to the hydrophobic effect. The negative sign of entropies of solute transfer can be explained by the following argument. The squalane impregnated C_{18} stationary phase is composed of approximately equal amounts of squalane and octadecyl ligands. The phase is of a narrow thickness and is rather viscous, so a solute in this phase will lose a portion of its freedom (entropy) compared to the solute in the mobile phase. The peaks obtained with the squalane impregnated C_{18} phase were rather broad with some tailing because of slow stationary phase mass transfer as shown in Fig. 4.

The reverse situation applies to a system of a highly aqueous mobile phase. As the water content in the mobile phase increases, the entropically endoergic iceberg formation gets more significant, and transfer of benzene or toluene from the mobile phase of 10/90 (v/v%) methanol/water to the stationary phase is entropically favorable.

The enthalpic and entropic contributions to the overall free energy of solute transfer from the mobile to the stationary phase are comparatively summarized in Table 2. As described before, solute transfer from the mobile to the stationary phase is enthalpically favorable and entropically unfavorable in general except for nonpolar solutes in 10/90 (v/v%) methanol/water. The enthalpic contribution (ΔH^{o}) to the overall solute transfer free energy (ΔG^{o}) is greater than the entropic contribution ($-T\Delta S^{o}$) in general except for polar solutes in methanol-rich mobile phases.

Now let us critically compare our data with some relevant literature data. Grushka et al. [7] measured

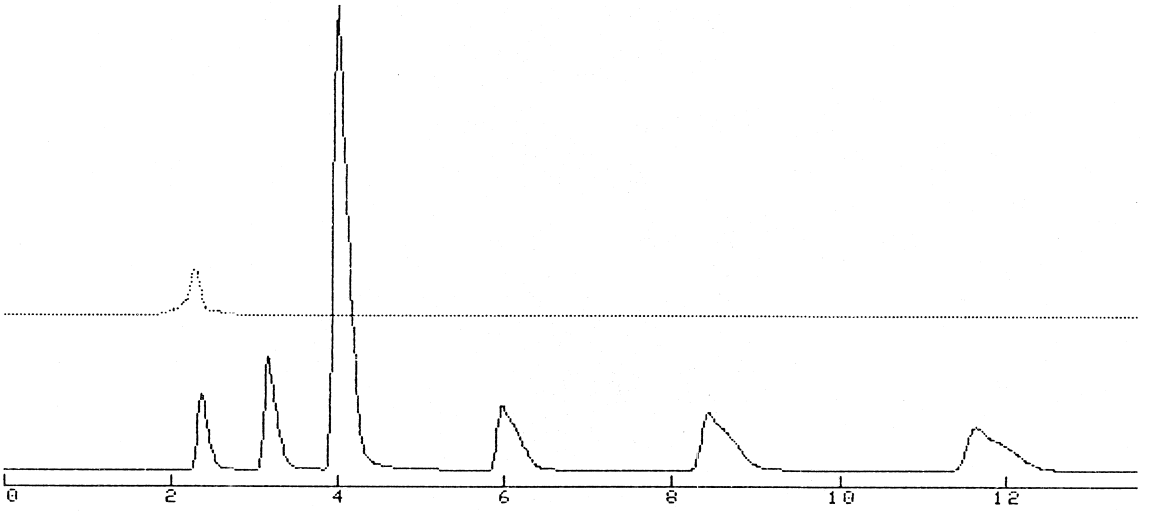

Fig. 4. The chromatograms of KNO_3 (the upper plot), and the sample solutes (the lower plot) obtained in 70/30 (v/v%) methanol/water with a flow rate of 1 ml/min at 25°C. In the lower plot, uracil eluted first, followed by phenol, acetophenone, benzene, toluene, and ethylbenzene.

Table 2
The enthalpic and entropic contributions to the overall solute transfer free energy from the mobile to the stationary phase based on KNO_3 as the void volume marker (unit: kJ/mol)

Mobile phase composition (methanol%)	Thermodynamic properties	Solute				
		phenol	acetophenone	benzene	toluene	ethylbenzene
10%	ΔH°	−13.3	–	−8.3	−11.1	–
	$-T\Delta S^\circ$	5.8	–	−2.9	−3.2	–
	ΔG°	−7.5	–	−11.2	−14.3	–
30%	ΔH°	−13.6	–	−11.8	−15.1	–
	$-T\Delta S^\circ$	8.7	–	2.8	3.4	–
	ΔG°	−4.9	–	−9.0	−11.7	–
60%	ΔH°	−8.6	−9.5	−9.5	−12.0	−13.8
	$-T\Delta S^\circ$	7.5	6.6	4.7	5.5	5.9
	ΔG°	−1.1	−2.9	−4.8	−6.5	−7.9
70%	ΔH°	−7.5	−7.9	−7.7	−9.8	−11.1
	$-T\Delta S^\circ$	7.7	6.4	4.4	5.1	5.3
	ΔG°	0.2	−1.5	−3.3	−4.7	−5.8
80%	ΔH°	−5.6	−5.8	−5.5	−7.3	−8.3
	$-T\Delta S^\circ$	7.0	6.2	3.8	4.4	4.6
	ΔG°	1.4	0.4	−1.7	−2.9	−3.7

ΔH° of ethylbenzene in 80/20 (v/v%) methanol/water using Hypersil C_{18}. Issaq et al. [10] measured ΔH° values for benzene, toluene, and ethylbenzene in 55/45 (v/v%) methanol/water using a C_{18} stationary phase. Martire et al. [42] measured ΔH° and $\Delta S^\circ/R + \ln V_s$ for a homologous series of alkylbenzenes (ethylbenzene to hexylbenzene) in 40–100% methanol and in 30–100% acetonitrile using a C_{18} phase, and found that the changes in ΔH° and ΔS° as a function of volume fraction of organic component were significantly different in the two solvent systems. Miyabe et al. measured ΔH° values for benzene, toluene, and ethylbenzene in 0–100% methanol/water [43] and acetonitrile/water [44] using a stationary phase of large (45 μm) octadecylsilica particles. Comparison of our data with the relevant literature data for nonpolar solutes is shown in Fig. 5. We can note that in the plot of ethylbenzene all the data sets except for Miyabe's are rather in good agreements, while Miyabe's ΔH° values were significantly less negative than others. An interesting point we should note is that Miyabe et al. thought that solute retention took place through adsorption. They used a 45 μm octadecylsilica stationary phase while others used 5 μm C_{18} phases. Miyabe and coworker also observed that the absolute value of ΔH° was smaller in acetonitrile/water systems than in methanol/water systems and con-

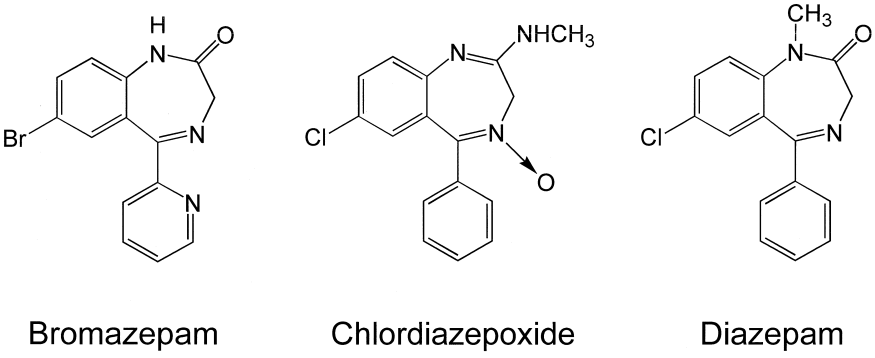

Fig. 5. The structures of the selected benzodiazepins.

cluded that the interaction between ODS ligands and adsorbate molecules was weaker in acetonitrile/water systems than in methanol/water systems.

For nonpolar solutes, the difference in solute–solvent interactions between the mobile and stationary phases is negligible, and ΔH^{o} is mainly dependent on the cavity formation effects. Since in an adsorption mechanism the solute does not penetrate into the stationary phase but partly remains in the mobile phase when it transfers from the mobile phase to the stationary phase ΔH^{o} will be less negative in an adsorption-like mechanism than in a partition-like mechanism. Miyabe's ΔH^{o} values for benzene were also less negative than ours (Fig. 5). We guess they used a C_{18} phase with a low ligand density to secure an adsorption mechanism on purpose. As mentioned before, Carr et al. [40] showed that retention on monomeric bonded phases with a high ligand density is dominated by a partition mechanism, and retention on bonded phases with a low ligand density, by an adsorption-like mechanism. Thus it seems that all the stationary phases considered in this comparison except for Miyabe's worked through a partition mechanism.

We searched for literature data for phenol and acetophenone but failed. Instead we will critically compare Guillaume's ΔH^{o} and ΔS^{o} for benzodiazepins [45] with our phenol and acetophenone data. Guillaume and coworker observed that the benzodiazepins of almost the same molecular volumes could be distinguished into three groups according to the variation of ΔH^{o} vs. solvent composition. For the first group, the absolute value of ΔH^{o} decreased with methanol content in the solvent increasing. For the second group, ΔH^{o} did not vary with respect to methanol composition. For the third group, the absolute ΔH^{o} values increased with methanol composition. We selected a typical solute for each group. Molecular structures of the three selected solutes are shown in Fig. 6. The ΔH^{o} data of the three com-

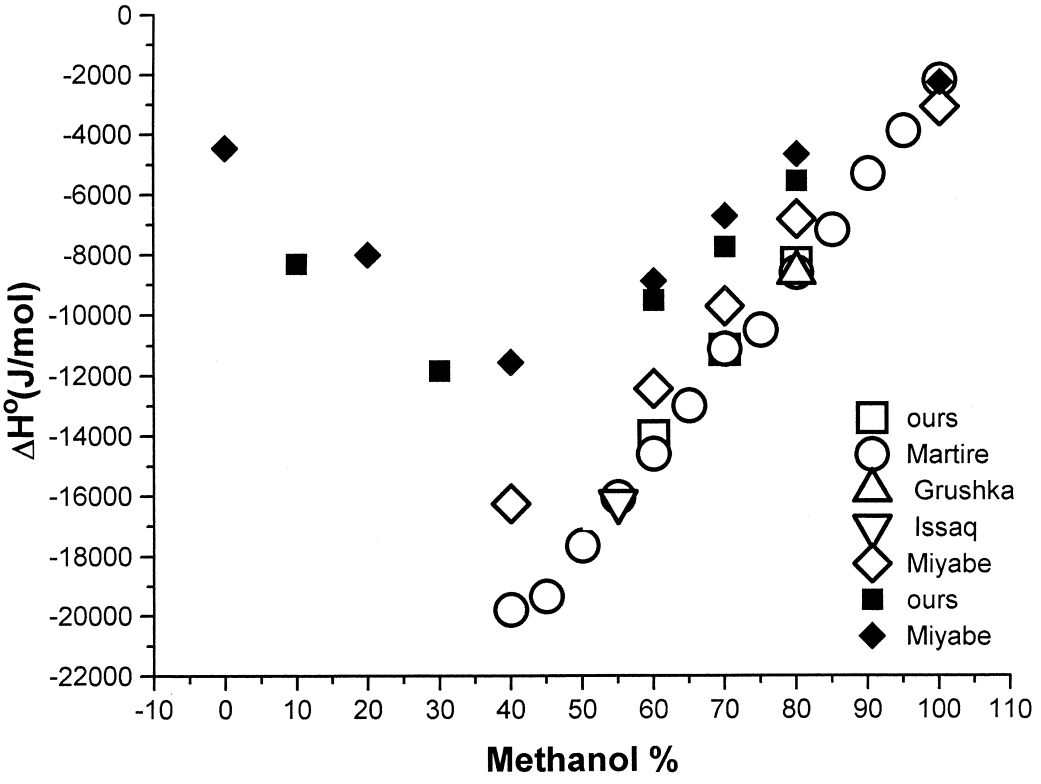

Fig. 6. Comparison of literature ΔH^{o} data with ours for nonpolar solutes. Open symbols are for ethylbenzene, and closed symbols, for benzene.

pounds and our ΔH^{o} data of phenol and acetophenone are comparatively shown with respect to methanol composition in Fig. 7. As mentioned before, the predominating cavity formation effect gets more significant with water content increasing (methanol content decreasing), thus ΔH^{o} values for phenol and acetophenone get more negative with decreasing methanol content. Bromazepam (most polar of the three benzodiazepins) follows such a trend (Fig. 7) for the composition range of 50–80% methanol. Diazepam, however, shows a contrary trend: ΔH^{o} gets less negative with decreasing methanol composition. Chlordiazepoxide, on the other hand, shows an invariant ΔH^{o}.

We propose a hypothesis concerning the hydrophobic effect to explain such phenomena. The solutes have a large nonpolar portion that penetrates into the C_{18} stationary phase when it transfers from the mobile phase to the stationary phase, and the hydrophobic effect is quite significant even in solvents of a rather high methanol content. Trends of ΔS^{o} against methanol composition strongly support the hypothesis. Trends of ΔS^{o} vs. methanol composition for the three benzodiazepins and phenol, acetophenone, and toluene are compared in Fig. 8. Positively shifting trends of ΔS^{o} with decreasing methanol composition in highly aqueous solvents are characteristics of the hydrophobic effect. The benzodiazepins with a large nonpolar part (at least two phenyl rings) show such trends for the range of 50–80% methanol, while phenol and toluene show the hydrophobic effect only for 10–30% methanol (Fig. 8). Explanation of the phenomena shown in Fig. 7 can be completed if we include the cavity formation effect as well. The variation trends of ΔH^{o} are the results of the combined effects of hydrophobic interaction and cavity formation. The two factors exert the contrary effects each other. For diazepam, the hydrophobic effect is dominant (a very high slope in Fig. 8) and overrides the cavity

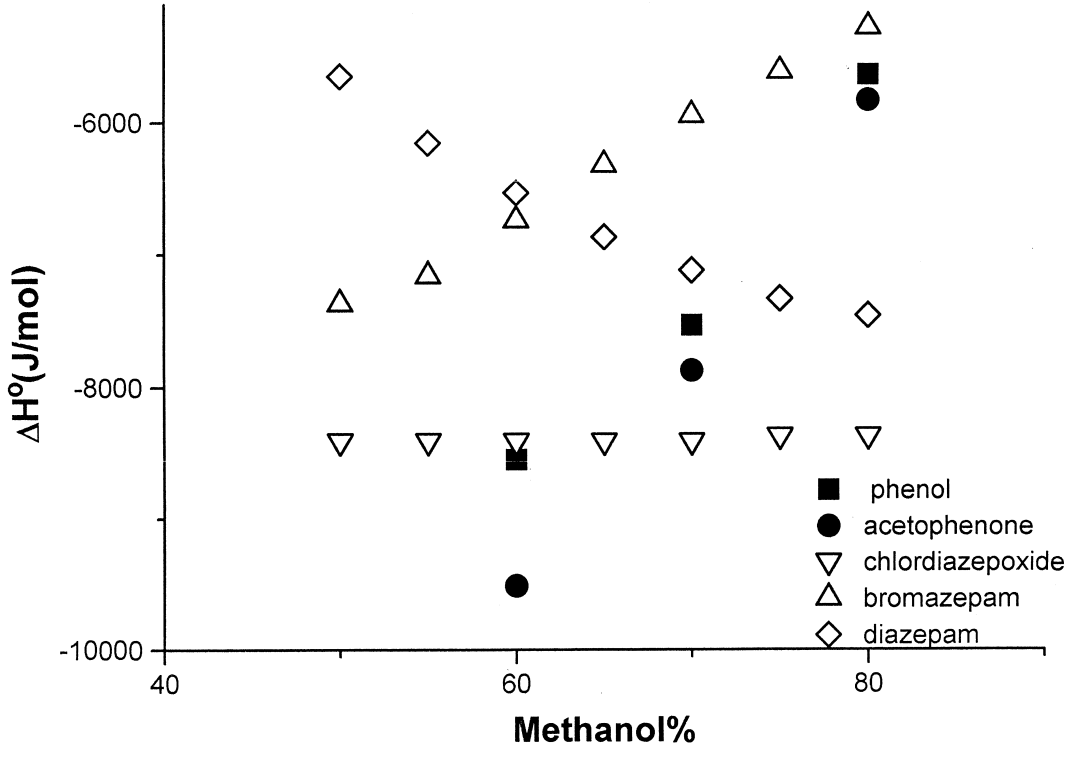

Fig. 7. Comparison of literature ΔH^{o} data with ours for polar solutes. Our data are represented by closed symbols, and the literature data, by open symbols.

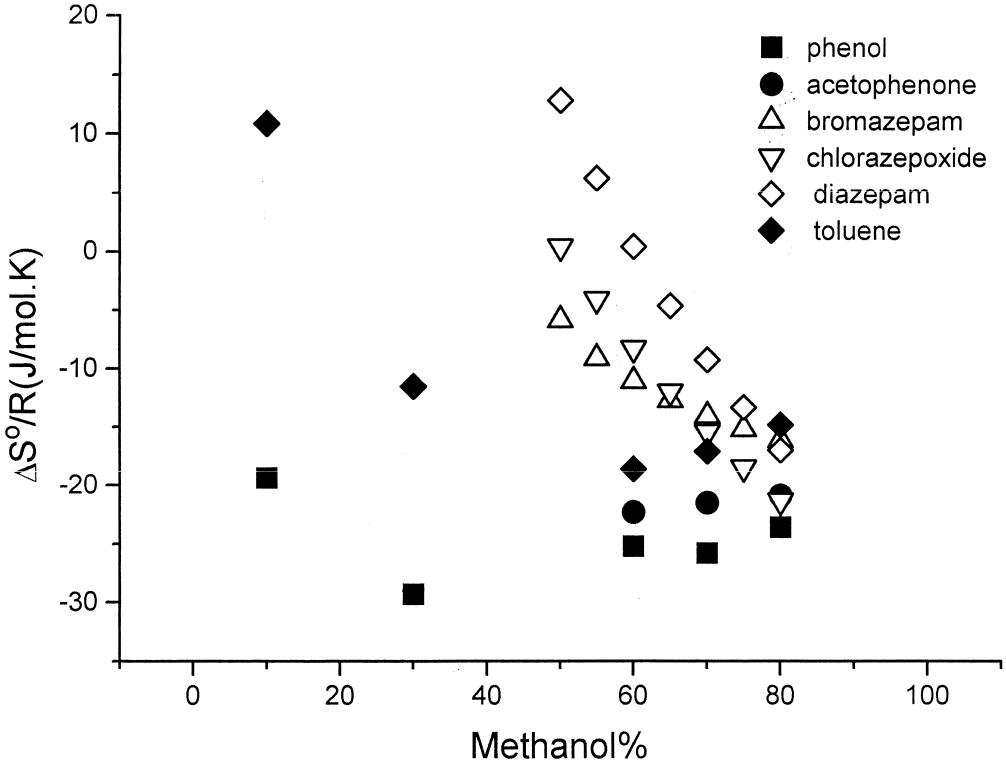

Fig. 8. Comparison of literature ΔS° data with ours. Our data are represented by closed symbols, and the literature data, by open symbols.

formation effect, thus ΔH° shifts negatively with increasing methanol content. For bromazepam (the most polar among the benzodiazepins), the cavity formation effect seems more significant than the hydrophobic effect (the lowest slope among the benzodiazepins in Fig. 8), and ΔH° gets more negative with decreasing methanol composition. For chlordiazepoxide, the hydrophobic and cavity formation effects are virtually balanced, and ΔH° shows no variation.

The specific solute–solvent interactions owing to the polar solute functional groups may play a role, too. However, the hydrophobic effect, the cavity formation effect, and the solute–solvent interaction effect are likely to intermingle to some extent, and the situation gets more perplexing by the possibility that large molecules with several polar functional groups may follow a partial adsorption mechanism. For example, let us consider the case of bromazepam. Since it is quite polar, the specific solute–solvent interaction in the mobile phase should be rather strong, and will be stronger in a more polar solvent, thus ΔH° should have become less negative with increase of solvent polarity (decrease of methanol content) if the specific solute–solvent interaction effect were predominating. As shown in Fig. 7, the contrary phenomenon is observed. Such a large molecule with a few polar functional groups may let its nonpolar portion penetrate into the stationary phase, but its polar portion is left in the mobile phase. In such a case, the specific solute–solvent interaction does not change despite the solute transfer, and the solute–solvent interaction effect on ΔH° will be negligible.

Thus, we have designed a particular chromatographic system in order to obtain quantitative thermodynamic data, and have shown that our system provides reproducible results over a long period of time. We believe that the thermodynamic data reported in this study are dependable enough to be

used for development or validation of theories on solution thermodynamics. Our future study will focus on such work.

4. Conclusions

The solute retention on the squalane impregnated C_{18} phase was simple and reproducible for a long period of time. We have been able to obtain dependable thermodynamic properties of solute transfer by measurements over a range of temperature based on the carefully designed chromatographic system including the squalane impregnated C_{18} phase. The plot of ln k' vs. $1/T$ was exclusively linear for our system. The transfer of a solute from the mobile phase to the stationary phase is enthalpically favorable and entropically unfavorable in general. The enthalpic contribution is more significant than the entropic contribution in general. The cavity formation effect has proven to be the major factor that governs the solute distribution between the mobile and stationary phases except for the cases of highly aqueous mobile phases. The hydrophobic effect is negligible in methanol-rich mobile phases. In highly aqueous mobile phases, the hydrophobic effect becomes significant, being comparable to the cavity formation effect.

Acknowledgements

This work was supported by the 1998 Inha University Fund.

References

[1] R.E. Majors, LC·GC 6 (1988) 298.
[2] D.E. Martire, R.E. Bohem, J. Phys. Chem. 87 (1983) 1045.
[3] K.A. Dill, Phys. Chem. 91 (1987) 1980.
[4] K.A. Dill, J. Naghizard, J.A. Marqusee, Annu. Rev. Phys. Chem. 39 (1988) 425.
[5] J.G. Dorsey, K.A. Dill, Chem. Rev. 89 (1989) 331.
[6] P.T. Ying, J.G. Dorsey, K.A. Dill, Anal. Chem. 61 (1989) 2540.
[7] E. Grushka, H. Colin, G. Guichon, J. Chromatogr. 248 (1982) 325.
[8] A. Tchapla, S. Heron, H. Colin, G. Guichon, Anal. Chem. 60 (1988) 1443.
[9] F.M. Yamamoto, S. Rokushika, H. Hatano, J. Chromatogr. Sci. 27 (1989) 704.
[10] H.J. Issaq, M. Jaroniec, J. Liq. Chromatogr. 12 (1989) 2067.
[11] L.C. Sander, L.R. Field, Anal. Chem. 52 (1980) 2009.
[12] W.E. Hammers, P.B.A. Verschoor, J. Chromatogr. 282 (1983) 41.
[13] D. Morel, J. Serpinet, J. Chromatogr. 200 (1980) 95.
[14] D. Morel, J. Serpinet, J. Chromatogr. 214 (1981) 202.
[15] D. Morel, J. Serpinet, J. Chromatogr. 248 (1982) 231.
[16] J.C. vanMitenburg, W.E. Hammers, J. Chromatogr. 268 (1983) 147.
[17] Z. Kessaissia, E. Papier, J.B. Donnet, J. Colloid Interface Sci. 79 (1981) 257.
[18] R.K. Gilpin, J.A. Squires, J. Chromatogr. Sci. 19 (1981) 195.
[19] S.S. Yang, R.K. Gilpin, J. Chromatogr. 449 (1988) 115.
[20] D. Morel, J. Serpinet, G. Untz, Chromatographia 18 (1984) 611.
[21] L.A. Cole, J.G. Dorsey, Anal. Chem. 64 (1992) 1317.
[22] L. Han, W. Linert, V. Gutmann, J. Chromatogr. Sci. 30 (1992) 142.
[23] H. Lamparczyk, P.K. Zarzycki, J. Pharmac. Biomed. Anal. 13 (1995) 543.
[24] B. Sellergren, K.J. Shea, J. Chromatogr. A 690 (1995) 29.
[25] R.K. Gilpin, S.E. Ehtesham, R.B. Gregory, Anal. Chem. 63 (1991) 2825.
[26] M. Makela, L. Pyy, J. Chromatogr. A 699 (1995) 49.
[27] M.C. Gennaro, D. Giacosa, C. Abrigo, E. Marengo, J. Chromatogr. Sci. 33 (1995) 260.
[28] A.P. Goldberg, Anal. Chem. 54 (1982) 342.
[29] K.B. Sentell, N.I. Ryan, A.N. Henderson, Anal. Chim. Acta 307 (1995) 203.
[30] C.M. Bell, L.C. Sander, S.A. Wide, J. Chromatogr. A 757 (1997) 29.
[31] V.L. McGuffin, S. Chen, J. Chromatogr. A 762 (1997) 35.
[32] R.J. Smith, C.S. Nieass, M.S. Wainwright, J. Liq. Chromatogr. 9 (1986) 1387.
[33] R.A. Djerki, R.J. Laub, J. Liq. Chromatogr. 10 (1987) 1749.
[34] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.M. Taft, M.H. Abraham, Anal. Chem. 57 (1985) 2971.
[35] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Dohert, M.J. Kamlet, Chromatographia 25 (1988) 373.
[36] C. Horvath, W. Melander, J. Chromatogr. Sci. 15 (1977) 393.
[37] C. Horvath, W. Melander, I. Molnar, J. Chromatogr. 125 (1976) 129.
[38] O. Sinanoglu, Chem. Phys. Lett. 81 (1981) 188.
[39] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, J. Chromatogr. A 656 (1993) 113.
[40] L.C. Tan, P.W. Carr, J. Chromatogr. A 775 (1997) 1.
[41] H.S. Frank, M.W. Evans, J. Chem. Phys. 13 (1945) 507.
[42] A. Alvarez–Zepeda, B.N. Barman, D.E. Martire, Anal. Chem. 64 (1992) 1978.
[43] K. Miyabe, M. Suzuki, AIChE J. 41 (1995) 548.
[44] K. Miyabe, S. Takeuchi, Anal. Chem. 69 (1997) 2567.
[45] Y. Guillaume, C. Guinchard, J. Liq. Chromatogr. 17 (1994) 2807.