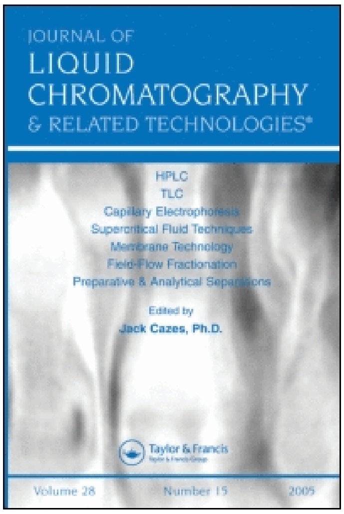

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THE NON-DISPERSIVE FUNCTIONAL GROUP-SOLVENT INTERACTION MONITORED BY HPLC

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ABSTRACT

We have used a squalane adsorbed C_{18} phase as presumably a bulk-like stationary phase to secure a simple partition mechanism for solute retention in reversed phase liquid chromatography, and have evaluated the nondispersive (specific) functional group-solvent interaction separately by measuring the retention data of carefully selected solutes in 60/40, 70/30, and 80/20 (v/v%) methanol/water eluents at 25,30,35,40,45, and 50°C. We have found that the absolute magnitude of the carbonyl group (in acetophenone)-mobile phase specific interaction enthalpy is much greater than that of the hydroxyl group (in phenol)-mobile phase specific interaction enthalpy. If we consider the overall differential solute transfer free energy for a pair of polar and nonpolar solutes of the same size, the entropic contribution is dominant for the BT/phenol pair, and the enthalpic contribution, for the ethylbenzene/acetophenone pair. On the other hand, for a pair of nonpolar solutes, the entropic contribution to the differential free energy of solute transfer is much lower than the enthalpic contribution, and the variation in the differential entropy of solute transfer with respect to mobile phase composition is much smaller than the variation in the differential enthalpy, too.

INTRODUCTION

Chromatography is useful to obtain information on solute-solvent thermodynamic interactions since chromatographic retention data (capacity factor) are well founded on the distribution equilibrium of solutes between the mobile and stationary phases, and the distribution is strictly related to the solute-solvent interactions in both phases.

Gas chromatography (GC) is the method of choice if one likes to make easy measurements of thermodynamic solute-solvent interactions. The solute-solvent interactions in the stationary phase of GC governs the solute retention while the gaseous mobile phase plays only a negligible role, which makes evaluation of solute-phase interactions simple. The stationary phases of GC, however, are viscous bulk-like materials; in general, the solute-solvent (phase) interactions are not a topic of interest.

We are rather interested in the solute-solvent interactions in the mobile phases of liquid chromatography (LC). There are some problems in evaluation of solute-mobile phase interactions from solute retention data in LC.

First of all, the solute-solvent interactions not only in the mobile phase but also in the stationary phase, contribute to the thermodynamic properties of solute retention. Thus, it is complex to get information on solute-solvent interactions in the mobile phase separately. The boundary of stationary phase is not well defined, especially, for the most popular type liquid chromatography (C_{18} RPLC), and; consequently determination of the phase ratio (the volume of stationary phase divided by the volume of mobile phase) is a topic of conflicts. Several different methods are commonly used, to date, to determine the column void volume. Choice of void volume marker is very crucial to evaluation of thermodynamic properties of shortly retained solutes. The retention mechanism of RPLC is also a topic of controversies and may be variant depending upon situations such as temperature and mobile phase composition, etc. It is recommended to set experimental conditions where the solute retention is based on a single mechanism to obtain reliable thermodynamic information on solute-solvent interactions in a mobile phase.

There have been numerous studies on temperature effects on solute retention in RPLC, and many of them were well reviewed in a paper written by Cole and Dorsey.[1] Linear van't Hoff plots were observed in some cases, and nonlinear plots, in other cases. Cole and Dorsey[1] clearly showed that the phase transition of a C_{18} stationary phase occurs at 20-30°C when the ligand density is larger than 3.0 mol/m^2. Temperature effects on solute retention in RPLC for a variety of systems[2-10] have also been recently reported.

Although there have been a large amount of studies about temperature effects on solute retention, not much reliable quantitative thermodynamic data of solute retention exist owing to the difficulties mentioned previously.

In this study, we have used a squalane adsorbed C_{18} phase, presumably as a bulk-like stationary phase, to secure a simple partition mechanism for solute retention and have evaluated the specific functional group-solvent interaction by measuring the retention data of carefully selected solutes at a certain range of temperature.

THEORY

It is easy to derive the thermodynamic relationship between the capacity factor (k') and temperature (T) as follows:

$$\ln k' = -\Delta H^\circ/(RT) + \Delta S^\circ/R + \ln \varphi \qquad (1)$$

where ΔH° and ΔS° 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. If ΔH° and ΔS° are independent of temperature, the plot of ln k' vs. 1/T (van't Hoff plot) will be linear, and we can compute ΔH° from the slope, and ΔS° from the intercept.

The selectivity, α, of a column for the two solutes A and B is defined as the ratio of the capacity factors.

$$\alpha = k_A'/k_B' \qquad (2)$$

We can easily derive the following equation by writing two equations of (1) type for the solute A and B and by subtracting the equation for the solute B from the equation for the solute A.

$$\ln \alpha = -\Delta\Delta H^\circ/(RT) + \Delta\Delta S^\circ/R \qquad (3)$$

In equation (3), $\Delta\Delta H^\circ$ means $\Delta H^\circ_A - \Delta H^\circ_B$, and $\Delta\Delta S^\circ$, $\Delta S^\circ_A - \Delta S^\circ_B$, respectively.

If we consider a pair of polar and nonpolar solutes which are of the same size and shape except for a polar functional group, the natural logarithm of the selectivity of the two solutes is proportional to the difference between the two solutes in Gibbs free energy of solute transfer from the mobile phase to the stationary phase. If we assume that the stationary phase is nonpolar and capable of only dispersive interactions, the solute-stationary phase interactions are identical for the pair of solutes. Then the natural logarithm of the selectivity corresponds to the difference in solute-mobile phase interactions between the pair of solutes, and consequently to the nondispersive functional group-solvent(mobile phase) interaction, since, the dispersive solute-solvent interactions are identical for molecules of the same size and the nonpolar solute experiences only a dispersive interaction in the mobile phase.

$$\Delta\Delta G^\circ = \Delta H^\circ_{f\text{-}s} - T\Delta S^\circ_{f\text{-}s} \quad (4)$$

$$\ln \alpha = -\Delta H^\circ_{f\text{-}s}/(RT) + \Delta S^\circ_{f\text{-}s} / R \quad (5)$$

$\Delta H^\circ_{f\text{-}s}$ and $-\Delta TS^\circ_{f\text{-}s}$ are the enthalpic and entropic contributions to the Gibbs free energy of nondispersive functional group-solvent interaction in the mobile phase. Here, it is assumed that the cavity formation free energies in a phase are identical for the pair of solutes and their contributions to the overall free energies of solute transfer (ΔG°_A and ΔG°_B) are canceled out when the difference ($\Delta\Delta G^\circ = \Delta G^\circ_A - \Delta G^\circ_B$) is obtained. Thus, we can calculate $\Delta H^\circ_{f\text{-}s}$ and $\Delta S^\circ_{f\text{-}s}$ by plotting ln α against 1/T and measuring the slope and intercept.

EXPERIMENTAL

The selected solutes were benzene, toluene, ethylbenzene, phenol, and acetophenone. They were purchased from Aldrich (Milwaukee, USA) and used without purification. The intrinsic molar volume[11] for benzene, toluene, ethylbenzene, phenol, and acetophenone are 49.1, 59.2, 68.7, 53.6, and 69.0 cm^3/mol according to the literature.[12] The intrinsic molar volume of acetophenone is nearly the same as that of ethylbenzene, thus we can obtain the enthalpy and entropy for the nondispersive carbonyl group-mobile phase interaction by plotting the logarithmic selectivity for ethylbenzene/ acetophenone against reciprocal temperature.

On the other hand, the intrinsic molar volume of phenol is about the middle between those of benzene and toluene, so we defined a hypothetical alkylbenzene whose intrinsic molar volume is exactly the same as that of phenol, and named it BT by assembling the first alphabets of benzene and toluene. Its hypothetical capacity factor was calculated based on the retention data of benzene and toluene under assumption that ln k' is linear with intrinsic molar volume. The enthalpy and entropy for the nondispersive hydroxyl group-mobile phase interaction was obtained from the plot of ln α for BT/phenol vs. 1/T.

Methanol and water were of HPLC grade and purchased from Fisher (Pittsburgh, USA) and used without further purification. The mobile phase used was methanol/water mixtures(60/40, 70/30, 80/20 v/v %) and the flow rate was

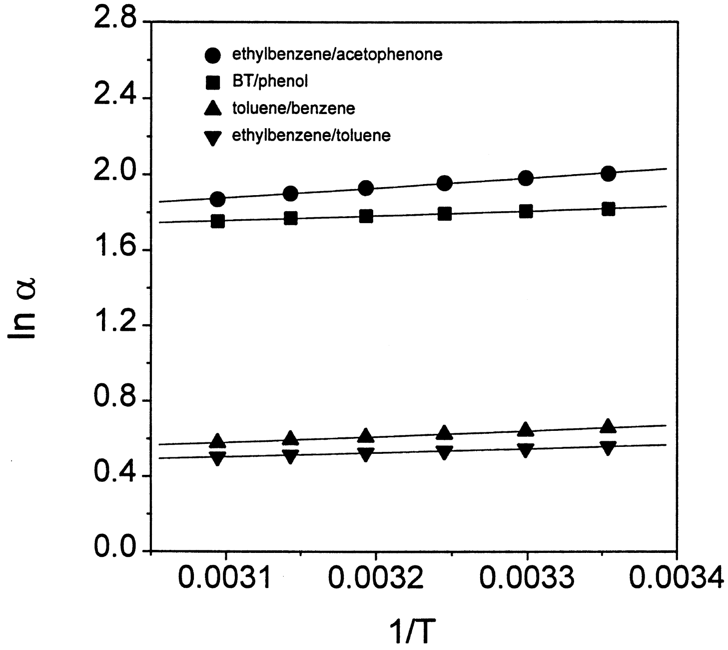

Figure 1. The plot of ln α vs. 1/T for the data obtained in 70/30 (v/v %) methanol/water at 25-50°C and based on KNO_3 as the void volume marker.

fixed at 1.0mL/min throughout. The solute retention data were collected at 25, 30, 35, 40, 45, and 50°C. Both KNO_3 and uracil were used as the void volume marker. Three independent measurements on different days were made to calculate the thermodynamic properties. The long-term reproducibility of k' was better than 2%.

The Shimadzu (Tokyo, Japan) 10AD HPLC system composed of a pump, a system controller, an autoinjector, a column oven, a UV/VIS detector, and a data system was used to collect retention data. The detector was set at 254 nm. The column temperature was carefully controlled with an accuracy of 0.1°C.

The column was packed with a squalane adsorbed Spherisorb ODS2 phase (5μm) from Phase Separation, Inc. (Norwalk, USA). More details of preparation of the squalane adsorbed C_{18} phase and the chromatographic system can be found elsewhere.[13]

Table 1

The Differential Solute Transfer Enthalphies and Entrophies for the Solute Pairs and Their Standard Deviations Based on Three Independent Measurements on Different Days

		Mobile Phase					
		Methanol 60%		Methanol 70%		Methanol 80%	
Void Volume Marker	Solute Pair (A vs. B)	$\Delta\Delta H°$(J/mol) ($\Delta H°_A$-$\Delta H°_B$)	$\Delta\Delta S°$(J/molK) ($\Delta S°_A$-$\Delta S°_B$)	$\Delta\Delta H°$(J/mol) ($\Delta H°_A$-$\Delta H°_B$)	$\Delta\Delta S°$(J/molK) ($\Delta S°_A$-$\Delta S°_B$)	$\Delta\Delta H°$(J/mol) $\Delta H°_A$-$\Delta H°_B$)	$\Delta\Delta S°$(J/molK) ($\Delta S°_A$-$\Delta S°_B$)
KNO_3	BT* vs. Phenol	-2090 ± 122	8.24 ± 0.44	-1133 ± 337	10.00 ± 1.07	-703 ± 221	9.91 ± 0.62
	Ethylbenzene vs. Acetophenone	-4280 ± 63	2.38 ± 0.22	-3262 ± 203	3.61 ± 0.62	-2348 ± 205	4.09 ± 0.61
	Toluene vs. Benzene	-2519 ± 38	-2.97 ± 0.13	-2061 ± 29	-2.37 ± 0.09	-1729 ± 63	-2.16 ± 0.19
	Ethylbenzene vs. Toluene	-1755 ± 22	-1.24 ± 0.07	-1330 ± 36	-0.77 ± 0.11	-1018 ± 56	-0.66 ± 0.19

Uracil	BT vs. Phenol	-1477 ± 107	10.92 ± 0.34	-1260 ± 68	10.41 ± 0.22	-734 ± 466	10.94 ± 1.54
	Ethylbenzene vs. Acetophenone	-3968 ± 34	3.73 ± 0.13	-3333 ± 29	3.80 ± 0.09	-2399 ± 333	4.50 ± 1.09
	Toluene vs. Benzene	-2467 ± 24	2.72 ± 0.07	-2105 ± 36	-2.42 ± 0.12	-1788 ± 75	-2.22 ± 0.25
	Ethylbenzene vs. Toluene	-1716 ± 16	-1.08 ± 0.05	-1337 ± 46	-0.74 ± 0.15	-1030 ± 68	-0.62 ± 0.23

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* The hypothetical alkylbenzene which has the same intrinsic molar volume as that of phenol. Its capacity factor was estimated by assuming that ln k' is a linear function of intrinsic molar volume for alkylbenzenes.

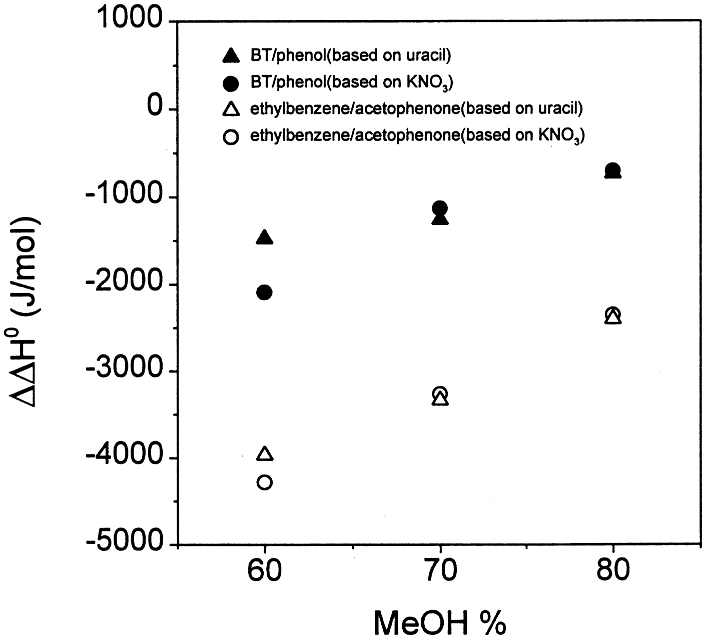

Figure 2. Comparison of the differential solute transfer enthalpies based on KNO$_3$ (circles), and uracil (triangles) for the BT/phenol pair (closed symbols) and the ethylbenzene/acetophenone pair (open symbols).

RESULTS AND DISCUSSION

All the van't Hoff plots we observed were linear, and the correlation coefficients were better than 0.995. Thus, the squalane adsorbed C_{18} phase proved to work through a simple partition mechanism. A typical example of the plot of ln α vs. 1/T is shown in Figure 1. The differential solute transfer enthalpies and entropies ($\Delta\Delta H°$ and $\Delta\Delta S°$) for the solute pairs considered in this study based on three independent measurements are assembled with their standard deviations in Table 1.

As mentioned before, the $\Delta\Delta H°$ value for the BT/phenol pair corresponds to the enthalpy of stabilization owing to the specific (nondispersive) interaction between the hydroxyl group and the mobile phase, and the $\Delta\Delta H°$ value for the ethylbenzene/acetophenone pair, the enthalpy of stabilization between the carbonyl group and the mobile phase. The meaning of $\Delta\Delta S°$ is rather complex. It is the sum of the solute entropy change and the solvent entropy change upon occurrence of the solute-solvent interaction, and maybe a delicate function of

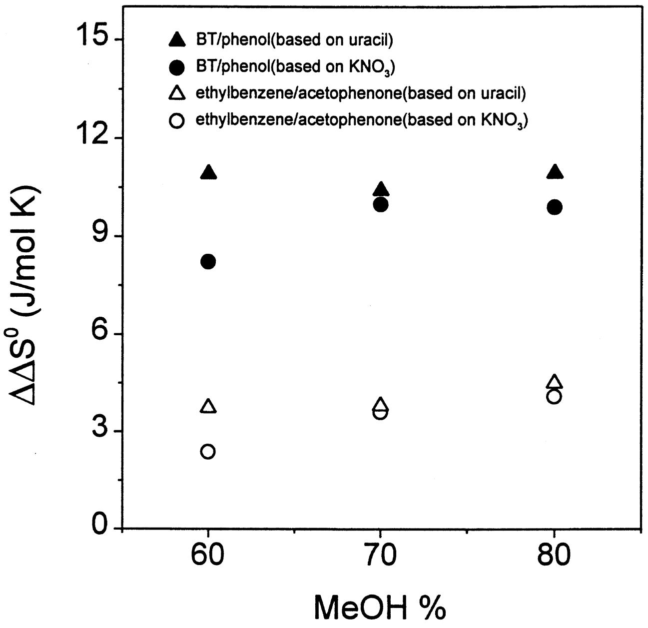

Figure 3. Comparison of the differential solute transfer entropies based on KNO_3 (circles), and uracil (triangles) for the BT/phenol pair (closed symbols) and the ethylbenzene pair (open symbols).

some entangled factors such as the solute size relative to the solvent, the solute shape, the type and position of the functional group, the type and strength of the specific solute-solvent interaction, and the type of solvent and its own intermolecular interactions.

The differential enthalpies and entropies of solute transfer for the solute pairs as a function of mobile phase composition are comparatively plotted in Figure 2 and 3, respectively. As shown in Table 1, Figure 2, and 3, the differential thermodynamic properties based on the two void volume markers are roughly close in general, despite an accidental occurrence of scattering in 60/40 (v/v%) methanol/water for the BT/phenol and ethylbenzene/acetophenone pairs. On the other hand, the differences in the differential thermodynamic properties between the data based on KNO_3 and uracil for the toluene/benzene and ethylbenzene/toluene pairs are very small (Table 1). In view of reproducibilities (in Table 1) based on three independent measurements, the values for the toluene/benzene and ethylbenzene/toluene pairs are much better than those for the BT/phenol and ethylbenzene/acetophenone pairs.

Phenol and acetophenone elute much earlier than benzene, toluene, and ethylbenzene. Therefore, the differences between the data based on two different void volume markers are much higher for the polar solutes (especially for phenol) than for the nonpolar solutes, and measurements of capacity factors are also subject to higher errors for phenol and acetophenone. Conclusively phenol-or-acetophenone related thermodynamic properties are less accurate.

One thing we should note is the relative strengths of the nondispersive functional group-mobile phase interaction enthalpies. The absolute magnitude for the carbonyl group-solvent interaction is much larger than that for the hydroxyl group-solvent interaction (Figure 2). In ordinary situations, the hydroxyl group-aqueous methanol interaction will be stronger than the carbonyl group-aqueous methanol interaction since the hydroxy group can be either a hydrogen bond donor or an acceptor while the carbonyl group can be only an acceptor. However, we should consider another factor--the position of a functional group, in other words, spatial accessibility. The O atom of phenol is directly attached to the phenyl ring, while there is a carbon atom between the phenyl ring and the O atom in acetophenone. It is striking that the existence of a C atom could cause such dramatically increased specific interaction although there is no persuasive reasoning otherwise.

$\Delta\Delta S°$ is a quite complicated function of many unpredictable factors as we mentioned before. It is positive for the BT/phenol and ethylbenzene /acetophenone pairs, and negative for the toluene/benzene and ethylbenzene/toluene pairs. The enthalpic and entropic contributions to the overall differential solute transfer free energy between a pair of solutes averaged for the two data sets based on KNO_3 and uracil, are summarized in Table 2.

According to Table 2, the entropic contribution to the overall differential solute transfer free energy is dominant for the BT/phenol pair while the enthalpic contribution is dominant for the ethylbenzene/acetophenone pair. The overall differential solute transfer free energies are comparable for the two pairs. The positive $\Delta\Delta S°$ implies that when a solute transfers from the polar mobile phase to the nonpolar stationary phase, a nonpolar solute loses less entropy than a polar solute. In other words, a nonpolar solute gains less entropy than a polar solute when it moves from the stationary phase to the mobile phase. The stationary phase is viscous, so the entropy of a solute in the stationary phase is lower than the solute entropy in the mobile phase.

But why does a nonpolar solute gain less entropy than a polar solute of the same size when it transfers to the polar mobile phase? The entropy of a solute gets higher when it gets more freedom. A solute will get more freedom if it is placed in a phase where it can move more freely. In a polar mobile phase, a polar solute may move more freely owing to the specific interaction than a

Table 2

The Enthalpic and Entropic Contributions to the Overall Differential Solute Transfer Free Energy for the Solute Pairs Averaged for the Two Data Sets Based on KNO_3 and Uracil*

Solute Pair (A vs. B)	**Mobile Phase Methanol 60%**			**Methanol 70%**			**Methanol 80%**		
	ΔΔH°	**-TΔΔS°**	**ΔΔG°**	**ΔΔH°**	**-TΔΔS°**	**ΔΔG°**	**ΔΔH°**	**-TΔΔS°**	**ΔΔG°**
BT[a] vs. Phenol	-1783	-2952	-4735	-1197	-3146	-4343	-719	-3214	-3933
Ethylbenzene vs. Acetophenone	-4124	-943	-5067	-3298	-1143	-4441	-2374	-1325	-3699
Toluene vs. Benzene	-2493	878	-1615	-2083	739	-1344	-1759	675	-1084
Ethylbenzene vs. Toluene	-1736	357	-1379	-1334	234	-1100	-1024	197	-827

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* unit: J/mol.

[a] The hypothetical alkylbenzene which has the same intrinsic molar volume as that phenol.

nonpolar solute. The polar functional group will be helpful for the solute's penetrating through the polar mobile phase. It is uncertain why the entropic effect in the differential solute transfer free energy for the BT/phenol pair is much more significant than that for the ethylbenzene/acetophenone pair. We only guess that the solute size and/or shape might play an important role here. The variation in ΔΔS° depending on mobile phase composition is much less significant than that in ΔΔH° as we note in Table 1 and Figure 2, and 3.

The ΔΔH° values for the toluene/benzene and ethylbenzene/acetophenone pairs do not reflect specific solute-solvent interactions. In these cases, ΔH° is the sum of the differential cavity formation enthalpy and the differential dispersive solute-solvent interaction enthalpy between the mobile and stationary phases for each solute; and ΔΔH°, the difference in ΔH°'s between a pair of solutes. There is no specific solute-solvent interactions other than the dispersive interaction. Since there is not much difference in the dispersive solute-solvent interaction between the mobile and stationary phases when the solute is nonpolar, while the difference in the cavity formation enthalpy between the mobile (polar) and stationary (nonpolar) phases is large, the cavity formation effect is the major contributor to ΔΔH°. As we note in Table 1 and 2, the entropic contribution (-TΔΔS°) to the differential free energy of solute transfer (ΔΔG°) for a pair of nonpolar solutes is much lower than the enthalpic contribution (H°), and the variation in ΔΔS° depending upon mobile phase composition is also small.

CONCLUSIONS

The squalane adsorbed C_{18} phase proved to work through a simple partition mechanism. The absolute magnitude of the specific interaction between the carbonyl group of acetophenone and the aqueous methanol is much larger than that between the hydroxyl group of phenol and the same solvent. In view of the overall differential solute transfer free energy between a pair of solutes, the entropic contribution is dominant for the BT/phenol pair, and the enthalpic contribution, for the ethylbenzene/acetophenone pair. When a pair of solutes of the same size transfer from the nonpolar stationary phase to the polar mobile phase, the nonpolar solute gains less entropy than the polar solute. The variation in ΔΔS° depending upon mobile phase composition is much less significant than that in ΔΔH°.

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