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Note

Temperature-induced selectivity in separations by reversed-phase liquid chromatography

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The use of temperature variation as a means of controlling separation in reversed-phase liquid chromatography (LC) systems is receiving increasing attention (*e.g.*, refs. 1-3). Chmielowiec and Sawatzky⁴ have recently reported some interesting data in this connection, for the separation of aromatic hydrocarbons on a C₁₈ column with acetonitrile-water (80:20) as mobile phase. These workers find "... with increase in temperature, retention of more compactly condensed aromatic compounds decreased at a greater rate than retention of less compact compounds". By "more compact compounds", the authors mean fused-ring systems such as benzantracene, while "less compact" compounds refer generally to polyaryls such as *o*-terphenyl. For example, 9,10-diphenylanthracene (less compact) was observed to elute well before 1,2,5,6-dibenzanthracene (more compact) at 20°, whereas this elution order was reversed at 45°. The authors refer to such temperature-dependent selectivity as "entropy dominated".

The purpose of this preliminary communication is to distinguish "regular" from "entropy-dominated" temperature effects, and to suggest that the latter can be correlated with differences in the molecular shape of solute molecules. First, consider the reaction corresponding to transfer of a solute molecule from the mobile to the stationary phase, for which there exists a standard free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0). The dependence of the capacity factor k' on temperature is determined by ΔH^0 :

$$d(\log k')/d(1/T) = \Delta H^0/4.57 \quad (1)$$

Here, T is the absolute temperature (°K) and ΔH^0 is in calories. In many chromatographic systems, it is found that solutes of similar structure have ΔH^0 and ΔS^0 values that are linearly related, with the result that ΔH^0 increases linearly with $\log k'$. If the linear dependence of ΔH^0 on $\log k'$ is exact, then solute retention order will be unchanged as separation temperature is varied. I refer to this as a "regular" temperature behavior.

In gas chromatography it is generally observed that the Kováts retention indices of different compounds do not vary much with temperature. This is equivalent to the same retention order for different compounds as temperature is changed, and is an example of "regular" behavior. Similarly, in the reversed-phase system of

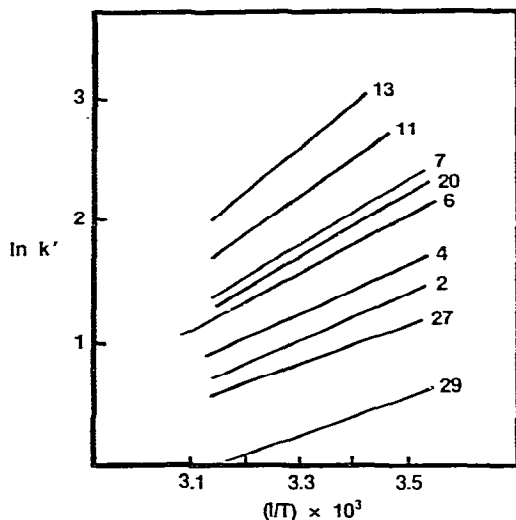


Fig. 1. Dependence of k' on T for unsubstituted fused-ring aromatics in the system of Chmielowiec and Sawatzky⁴. Numbers refer to Table II of ref. 4.

Chmielowiec and Sawatzky⁴, plots of $\log k'$ versus $1/T$ for different "compact" aromatic hydrocarbons show increasing steepness for more strongly retained compounds, as shown in Fig. 1. Again, it can be seen that separation sequence is the same at different temperatures. For the compounds of Fig. 1, it is observed that

$$\Delta H^{\circ} = -0.7 + 5.48 \log k'_{25} \quad (2)$$

within ± 200 cal in ΔH° . Here, k'_{25} refers to k' at 25°C. An equation of the same form as eqn. 2 has been observed to hold for substituted benzenes as solutes, in elution from a C_8 column by different methanol-water mobile phases⁵. A plot of ΔH° for several solutes from that study is shown in Fig. 2, for methanol-water (60:40) and k' at 51°C. Similar examples of "regular" behavior for liquid-solid (alumina) chromatography are described in ref. 6.

What are the consequences of "regular" temperature effects in chromatography? As illustrated by the data of Fig. 1, retention order in "regular" systems cannot be altered by a change in temperature. This means that if $\alpha = 1$ at one value of T , the two solutes will remain unseparated at other temperatures. For compounds that have different k' values at a particular temperature, the separation factor α (and R_s) will decrease with increase in temperature. This suggests the use of lower temperatures for maximum resolution, but this is a poor choice for "regular" systems. The reason is that increased temperature favors maximum column efficiency, and the adverse effect of increased temperature on α can be overcome by decreasing solvent strength (decreasing vol. % organic in the mobile phase, for reversed-phase LC); these trade-offs have been discussed in detail⁵. To conclude, temperature is not an effective parameter for altering α values and maximizing resolution in "regular" systems.

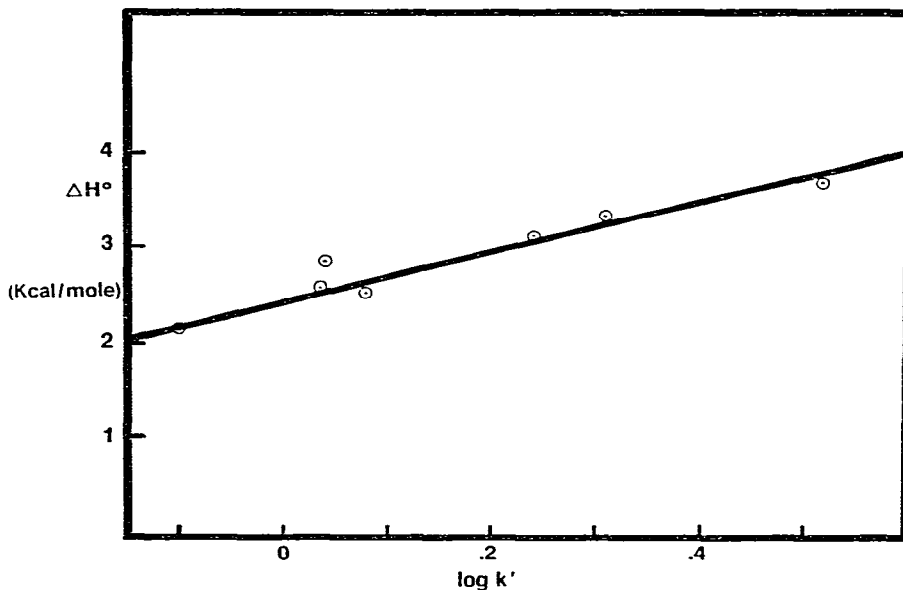


Fig. 2. Dependence of ΔH° on $\log k'$ for substituted benzene derivatives in reversed-phase system of Grant *et al.*⁵. k' values for methanol-water (60:40) at 51 °C.

On the other hand, we know that chromatographic systems are often not "regular", as in the case of "compact" *versus* "less compact" aromatic hydrocarbons⁴. Therefore a better understanding of when an LC system will behave "irregularly" can be of practical value, since in these cases we can exploit temperature variation to maximize separation selectivity. Fundamental considerations suggest the failure of relationships such as eqn. 1 for two possible reasons (*e.g.*, ref. 7): (1) retention of solute molecules in the stationary (or mobile) phase by more than one mechanism (or as a result of very different types of interactions involving a single solute molecule); (2) marked difference in the molecular shapes of two solutes with similar retention in a particular LC system. Examples of the first effect are often found in ion-exchange or ion-pair systems (*e.g.*, ref. 8), because molecules are held in the stationary phase as a result of both physical (Van der Waals) and chemical (ion-exchange) interactions. These two interactions will each be governed by equations similar to eqn. 1, but their composite effect will not be so related.

For reversed-phase LC systems, where non-ionizable solutes are being separated, the dual-mechanism effect will generally not be observed. In this case, one would expect differences in solute molecular shape to be the major contributor to "irregular" temperature behavior. It is of interest in this connection to compare the reported data for a reversed-phase LC system⁴ with similar effects that were observed by Hively and Hinton⁹ in the gas chromatography (GC) separation of hydrocarbons on a squalane stationary phase. The latter workers reported data for a large number of lower-boiling hydrocarbons, in terms of the change in Kováts retention index with temperature. To summarize their results, increasing retention at higher temperatures was generally associated with solutes of decreased molecular diameter. Thus, acetylenes such as 3-hexyne are more rigid and "straighter" and therefore longer than the

corresponding *n*-alkane. Their relative retention at higher temperatures decreases, relative to other alkanes. Branching of an *n*-alkane leads to progressively increased retention at higher temperatures, since molecular compactness increases with increased branching (and maximum molecular diameter decreases). Cyclization leads to even greater compactness, and maximum changes in relative retention with temperature. These effects noted by Hively and Hinton⁹ are summarized with the representative data below:

Solute type	Change in retention index/°C
Alkyne*	-0.08
<i>n</i> -Alkane	0.00
Branched alkane**	
1 branch	0.02-0.07
2 branch	0.02-0.20
3 branch	0.11-0.20
4 branch	0.20
Cyclo C ₆ ***	0.23-0.25

* 3-Hexyne.

** C₈ alkanes.

*** Cyclohexane, benzene, etc.

It is of interest to compare the similar "irregularity" of the system of Chmielowiec and Sawatzky⁴. We can do this by comparing values of ΔH^0 from eqn. 2 with experimental values, as summarized in Table I for different groups of solutes. We see in Table I a regular decrease in ΔH^0 versus the calculated ("regular") value with increased crowding of aryl groups within the solute molecule. Chmielowiec and Sawatzky⁴ refer to the polyaryls as less "compact", but this

TABLE I

RELATIVE "IRREGULARITY" OF DIFFERENT SOLUTES IN THE SYSTEM OF CHMIELOWIEC AND SAWATZKY⁴ AS MEASURED BY EXPERIMENTAL VERSUS CALCULATED (EQN. 2) ΔH^0 VALUES

Solute or solute class*	ΔH^0 (expt.) - ΔH^0 (calc) (kcal/mole)	Example
Fused-ring, unsubstituted hydrocarbons (2,4,6,7,11,13,20,27)	0.00 ± 0.20 (1 SD)	Chrysene
Same, alkyl substituted (9,10**,15,18,18a,19)	-0.50 ± 0.22	4-Methylpyrene
Unhindered diaryls (21,22,25,28)	-0.37 ± 0.23	2-Phenylnaphthalene
Unhindered triaryl (3)	-1.51	<i>m</i> -Terphenyl
Unhindered tetraaryl (8)	-2.30	1,3,5-Triphenyl benzene
Moderately hindered diaryls (5,17,26)	-0.93 ± 0.18	1-Phenylnaphthalene
Moderately hindered triaryl (1)	-1.80	<i>o</i> -Terphenyl
Strongly hindered diaryl (23)	-1.78	9-Phenylanthracene
Strongly hindered triaryl (12)	-3.14	9,10-Diphenyl anthracene

* Numbers in parentheses from Table II of ref. 4.

** Dialkyl, counted twice.

definition seems somewhat ambiguous — at least in terms of explaining the data of Table I. Thus the compound *p*-terphenyl is not included among the solutes of Table I, is obviously rather “uncompact”, but has a ΔH^0 value that is only -0.5 kcal/mole less than calculated from eqn. 2. In terms of the argument of “compactness” as an explanation of “irregularity”, *p*-terphenyl should have been more “irregular” than *m*-terphenyl, which has a ΔH^0 value that is -1.5 kcal/mole less than calculated.

I believe the “irregularity” of the system of Chmielowiec and Sawatzky⁴ is essentially similar to that of the GC system of Hively and Hinton⁹. This is not surprising, since the stationary phase is basically an aliphatic hydrocarbon in each case, and it can be argued that entropy effects in the stationary phase would be dominant in each system. If this is the case, then the common denominator for “irregularity” seems to be the relative departure from a flat, straight molecule on the one hand, *versus* a bulky three-dimensional or spherical shape on the other hand. Thus the polyaryls become less flat and become more compact or spherical with increasing intramolecular crowding. This effect is offset in the case of the *p*-polyphenyls by the linear extension of the molecule. In short, it appears that increasing solute retention at higher temperatures (relative to other solutes) is favored by a more compact, near-spherical configuration of the molecule; *i.e.* the opposite of the view advanced by Chmielowiec and Sawatzky⁴.

If this generalization is correct, then the study of Hively and Hinton⁹ for GC retention can serve as a guide for “irregular” temperature effects in reversed-phase LC. That is, preferential retention at higher temperatures will be favored by the following structural changes:

- (i) increased branching of alkyl substituents;
 - (ii) increased cyclization of the molecule, whether alicyclic or aromatic rings are involved;
 - (iii) decreasing overall length in the long dimension; *e.g.*, triphenylene should be increasingly retained at higher temperatures relative to naphthacene.
- Additionally, the data of ref. 4 suggest other factors that will provide increased relative retention at higher temperatures:
- (a) aryl substitution onto an aromatic ring, *versus* fused-ring substitution; crowded aryl groups will be even more effective;
 - (b) out-of-plane substituents in molecules such as cyclohexane derivatives, steroids, etc.

When dealing with the reversed-phase LC separation of samples that incorporate the above structural features, variation of the separation temperature should be explored as a means for the unique control of selectivity and retention order.

Horváth and co-workers¹⁰ have recently discussed the behavior of “regular” reversed-phase systems in terms of temperature effects, referring to such systems as “enthalpy–entropy compensated”. They have also noted an additional source of “irregularity” in the case of polyethyleneoxide oligomers: conformational changes in the oligomeric molecule as a function of structure and chromatographic conditions¹¹.

Finally, it should be noted that entropy effects as a result of change in solute shape are very much dependent upon the relative “order” within the solvent phase. Thus differences can be expected between pure hydrocarbons as stationary phase

(e.g., squalane, less "ordered") and a C₁₈ bonded-phase (more "ordered"). Further differences would be expected between a C₁₈ phase and the more highly ordered surface of graphite as stationary phase.

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