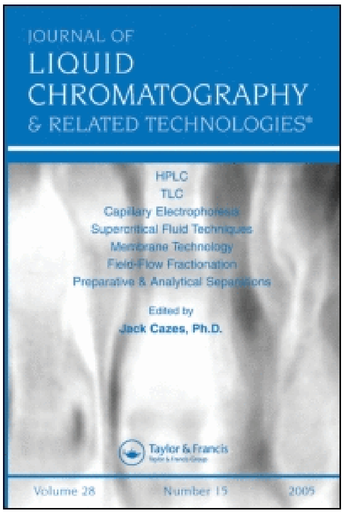

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. VI. ENTHALPY-ENTROPY COMPENSATION: THE COLUMN TEMPERATURE AND MOBILE-PHASE COMPOSITION

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ABSTRACT

The concept of enthalpy-entropy compensation in "non-polar" reverse-phase (ODS) column liquid chromatography is examined in terms of the column temperature (10^{o}-35^{o}C) as well as mobile-phase composition (acidified water/tetrahydrofuran) for the solutes: phenol, p-nitrophenol, 2,4-dinitrophenol, o-chlorophenol, o-nitrophenol, 2,4-dimethylphenol, 2,4-dinitro-o-cresol, 4-chloro-m-cresol, and 2,4-dichlorophenol. Plots of log (solute capacity factor, k') against reciprocal column temperature, T^{-1}, exhibit approximate linearity only over the volume-fraction based mobile-phase compositional range ϕ_{THF} = 0 to 0.2. Substantial deviations occur beyond ϕ_{THF} = 0.3. Plots of ln k' against $-\Delta H^{o}$ (compensation plots) yield a random distribution of data, indicating that what enthalpy-entropy compensation may exist for the systems studied is masked both in terms of temperature and mobile-phase composition. It is concluded that interaction(s) of the solutes with the stationary sorbent are more complex than has heretofore been supposed.

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INTRODUCTION

Column liquid-chromatographic (lc) retentions observed with chemically-bonded ("non-polar reverse-phase", rplc) packings have yet to be rationalized satisfactorily, as described and discussed in the previous papers of this Series (1-5). In essence, there have been postulated over the years at least three types of solute/stationary-phase interactions. In the first, some or other kind of simple partitioning is said to occur (6,7) and, although this view is now recognized to be naive, it nevertheless marks at least a beginning to speculation regarding solute retardation. In the second model, solutes are said to adsorb in classical fashion onto the stationary phase (8-10), the fundamental difference between this and liquid-solid chromatography (lsc) being that, rather than retentions being governed by specific ("polar") forces of adsorption, the solute-rplc stationary phase interactions are taken to be comprised only of non-specific (and comparatively-weak) van der Waals and London dispersive forces. Solubility in the mobile phase is therefore said to govern very largely the solute retentions. The third model proposes that a layer or so of mobile phase (that may or may not differ in composition from the bulk mobile phase) is sorbed onto the surface of the non-polar stationary phase (11). Partitioning of solute molecules is then said to take place between the mobile phase and the component(s) of it that is(are) sorbed on the surface of the stationary phase. At least several workers have also cited some or other kind of mixed sorption/partition mechanism for the rplc systems of their work (12-15; see also Ref. 2).

Each of the retention models pertinent to rplc can in principle be tested in terms of the column temperature. One approach then employed to quantitate the results is based upon so-called enthalpy-entropy compensation of family-related compounds (16,17), wherein the variation of the system free energy with the corresponding enthalpy should be linear if the physicochemical interaction(s) of each compound with the system, that is, the factors that govern the respective thermodynamic equilibra, are intrinsically similar. If such behavior is observed, the process is said to exhibit iso-equilibrium.

In this work, we examine a series of substituted phenolic solutes eluted from a reverse-phase system at several temperatures and over the complete solvent composition range, pure organic additive (here, THF) to pure aqueous phase, with the view of examining the veracity of the compensation concept. We also take the opportunity to explore the likely possibility that there exist multiple retention mechanisms with these systems. In addition, and according to all models currently extant in reverse-phase liquid chromatography, the extent of solute/stationary-phase interaction(s) can be altered also by changing the mobile-phase composition. And, while rigorous thermodynamic relations between the effects of doing so and the system temperature have yet to be formulated, some results suggest that the model of enthalpy-entropy compensation can be applied in these instances as well (16,17). For example, Melander, et al., (18,19) studied the the retentions of a series of arylalkanes, and formulated a relation between solvent composition and temperature on the premise that there exists a linear function between log k' and the volume-fractional composition ϕ of the solvent. This is not infrequently found to be the case although, in some instances, quadratic functions are in fact required. In complete contrast, Laub and Madden (4) showed in a preceding paper of this Series that extension of the Langmuir model of sorption provides an exact and, apparently, universal description of the variation of inverse retentions with mobile-phase composition, irrespective of the manner or extent of curvature in plots either of $(k')^{-1}$ or of $(V_R)^{-1}$ against ϕ. We therefore explore in this work also the variation of retentions as a function of temperature over the complete solvent-compositional range, $\phi = 0$ to 1.

THEORY

Enthalpy-entropy compensation can be expressed by the relation:

$$\Delta G^o_\theta = \Delta H^o - \theta \, \Delta S^o \tag{1}$$

where ΔG^o_θ denotes the Gibbs free energy of a physicochemical phenomenon at temperature θ, and where ΔH^o and ΔS^o are the corresponding standard-state enthalpy and entropy. When, in the vicinity of θ, changes in ΔH^o are offset by changes in ΔS^o for each member of a family of compounds partaking in a particular chemical equilibrium process, the free energy becomes nearly independent of temperature. That is, when enthalpy-entropy compensation is observed, the values of θ and ΔG^o_θ are invariant; θ is then called the compensation temperature.

Utilizing next the Gibbs relation,

$$\Delta G^o = \Delta H^o - T\Delta S^o \tag{2}$$

we rewrite eqn. 1 to express the free energy change ΔG^o_T pertinent to some fixed temperature T in terms of θ and ΔG^o_θ:

$$\Delta G^o_T = \Delta H^o\left(1 - \frac{T}{\theta}\right) + \Delta G^o_\theta \frac{T}{\theta} \tag{3}$$

We can now relate the Gibbs free energy to terms measured directly in the chromatographic technique:

$$\Delta G^o_T = -RT \ln K_R \tag{4}$$

where K_R is the equilibrium constant (i.e., partition coefficient) for the solute distributed between the mobile and stationary phases at temperature T. Replacing K_R with the capacity factor k' and phase ratio β (the ratio of the volume of mobile phase to that of the stationary phase in the column), $k' = K_R/\beta$, eqn. 4 becomes:

$$\ln(k'\beta) = -\frac{\Delta G^o_T}{RT} \tag{5}$$

Substitution of eqn. 3 into eqn. 5 then provides:

$$\ln k' = -\frac{\Delta H^o}{RT}\left(1 - \frac{T}{\theta}\right) - \frac{\Delta G^o_\theta}{R\theta} - \ln \beta \tag{6}$$

When, in column liquid chromatography, there is a commonality of interaction of family members of compounds (solutes) involved in isothermal sorption from the mobile phase onto the stationary phase then, according to eqn. 6, plots of ln k' against the corresponding enthalpy change for the solutes will be linear when compensation occurs, i.e., when retardation of the solutes by the stationary phase is brought about essentially by the

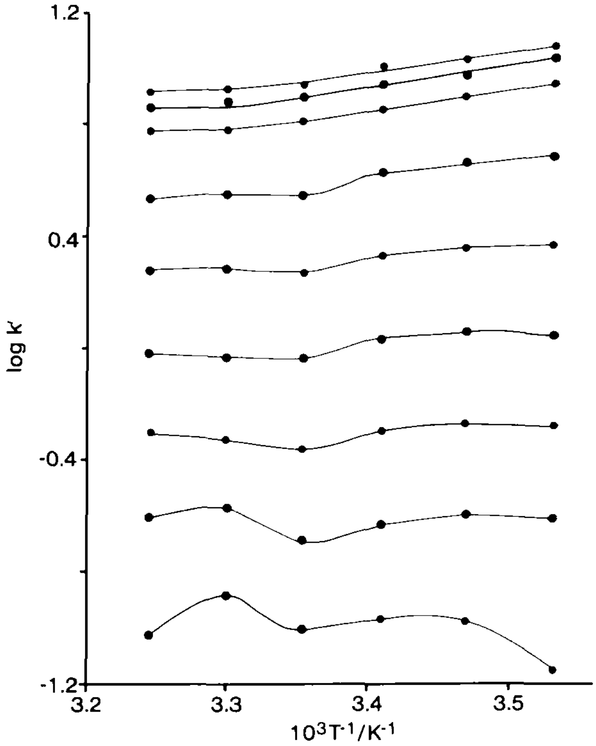

FIGURE 1. Plot of log k' versus 1/T, K^{-1} for phenol solute at various volume fractions ϕ of THF in water + acetic acid (1% v/v). Column: 25 cm x 4.6 mm i.d., 5μ Spherisorb ODS; flow rate: 1 cm^3 min^{-1}; UV detection at 254 nm.

same mechanism, there being differences only in the magnitude ("strength") of the interaction from one solute to the next. Moreover, in such cases, the compensation temperature θ can be evaluated from the slope. Conversely, if a common compensation pattern is not observed, it can then be assumed that there are significant differences in the mechanistic details of the equilibrium reaction from one reactant (sorbate solute) to the next. That is, a significant divergence of iso-equilibrium temperatures can be taken as evidence of fundamental differences in solute-stationary phase interactions within the supposed family of compounds. Compensation data therefore offer some insight, at least with regard to trends, into the kinds

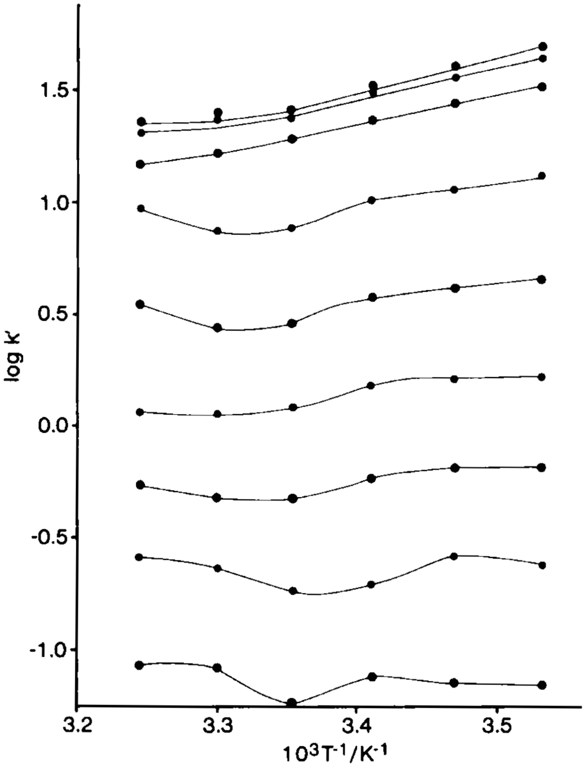

FIGURE 2. As in Figure 1; p-nitrophenol solute.

and magnitudes of solute sorption as the chromatographic conditions (e.g., temperature, mobile phase composition) are varied.

Parenthetically, we note that, while testing for compensation behavior is carried out conventionally with plots of ΔG^o or of ln K_R or ln k' against ΔH^o as described above, it has been pointed out elsewhere that under certain conditions a kind of pseudo-compensation may arise as a result of statistical effects due to errors associated with the determination of the enthalpy (20). Some care must therefore be exercised in the interpretation of compensation data even in those instances where the compensation temperatures derived for families of solutes otherwise appear to be self-consistent.

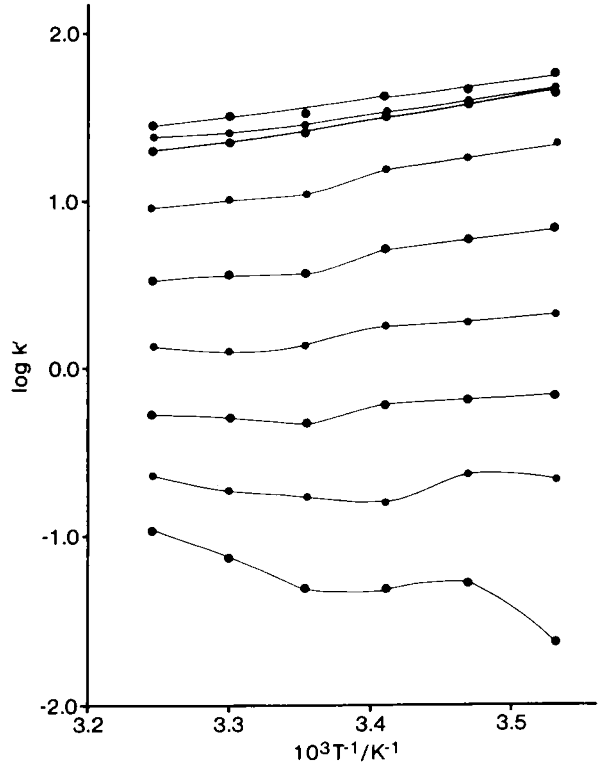

FIGURE 3. As in Figure 1; 2,4-dinitrophenol solute.

EXPERIMENTAL

Solvents. Fisher HPLC-grade tetrahydrofuran (THF) was employed as received, and was the organic modifier in the binary aqueous-organic eluent systems utilized in this work. Doubly-distilled water and reagent-grade acetic acid comprised the aqueous portion.

Mobile-Phase Preparation. Acetic acid at the level of 1% v/v was added to the water component of the mobile phase in order to diminish any interactions between residual unreacted or accessible silanol groups of the reverse-phase packing, as well as to suppress dissociation of the phenols. Solutions of the aqueous-organic blend were then prepared by volume over the complete volume fraction range ϕ = 0 to 1 at intervals of 0.10.

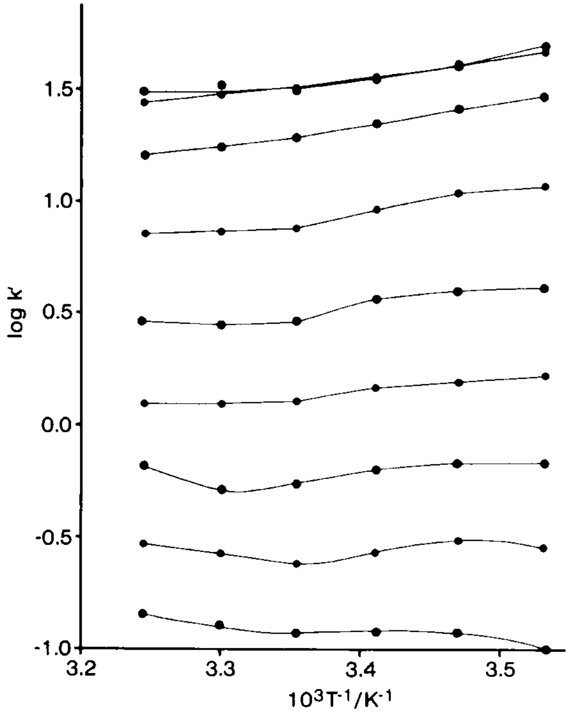

FIGURE 4. As in Figure 1; o-chlorophenol solute.

Solutes. Solutions of phenols in THF comprised the test samples, these being phenol (1), p-nitrophenol (2), 2,4-dinitrophenol (3), o-chlorophenol (4), o-nitrophenol (5), 2,4-dimethylphenol (6), 2,4-dinitro-o-cresol (7), 4-chloro-m-cresol (8), and 2,4-dichlorophenol (9).

Instrumentation. The liquid chromatograph employed in this work was a Varian Model 8500 with a Vari-Chrom variable wavelength UV detector utilized at 254 nm. Injection was via a Valco valve with a 0.2-μl internal sample loop.

Two columns, designated here as A and B, were used during the course of this work. Both were 25 cm x 4.6 mm i.d., and contained 5μ

Spherisorb ODS (Jones Chromatography, Columbus, Ohio). The second column, B, was required due to deterioration of column A over the course of the work. The resultant data and interpretations arising from a change from one presumed-identical column to another are discussed below.

Constant temperature was maintained by enclosing the column in a glass jacket through which was circulated water from a Neslab RTE-8 thermostat. The eluent was brought to the column temperature by immersing a 1-m coil of the solvent line in the bath prior to attachment to the injection valve.

Procedures. The column was allowed to equilibrate with each new mobile phase at a given temperature under constant flow conditions, equilibration being judged satisfactory when the solute retention times remained invariant for at least three sequential injections. Solute retention volumes were then measured over the complete volume fraction range. During the course of each set of measurements, the flow rate was monitored continuously at the detector outlet by means of a graduated and thermostated (25°C) pipet. The column temperature was then incremented by 5°C and the procedure repeated. The range investigated spanned 10° to 35°C. Column A was used from 10° to 20°C, while column B was employed for 25° to 35°C. All the data reported here are average values taken from at least three injections.

RESULTS AND DISCUSSION

Measurement of the Column Dead Volume. The problem of defining and interpreting the column dead volume in hplc is intrinsically difficult, as discussed in the preceding work of this series (5). We chose in this study to employ the method of unretained solutes for dead-volume measurements, which we recognize is not without fault. However, any errors in k' resulting from incorrect values of the dead volume become important only with V_R small; these instances are duly noted below.

We found that all of the solutes studied here exhibited identical retention volumes with 100% THF mobile phase, as shown in Table 1. The average of these at each temperature was therefore taken as the dead volume.

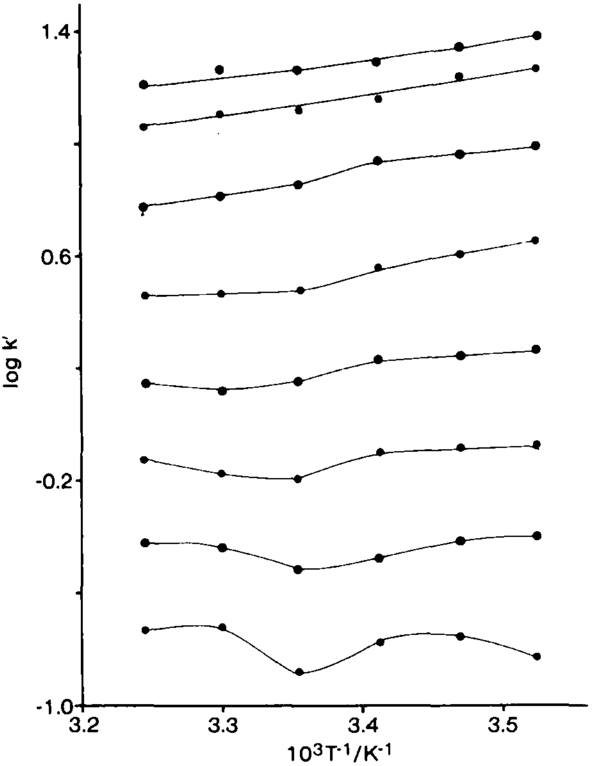

FIGURE 5. As in Figure 1; o-nitrophenol solute.

Variation of Solute Capacity Factors with Temperature. Presented in Figures 1-9 are the plots of log k' versus 1/T for all of the solutes of this work at various volume fractions of THF mobile-phase additive. All exhibit the same general characteristics: at low volume fractions of THF ($\phi < 0.2$) the variation of log k' with inverse temperature is very nearly linear while, at $\phi > 0.3$, substantial deviations from linearity occur. As a result, we are led immediately to say that multiple retention mechanisms are extant in these systems since, if only one retention mechanism were operative at a given value of ϕ, then, according to eqn. 5, the plots would be expected to be linear, the gradient being $-\Delta H^o/R$, i.e., the heat of transfer of the solute from the mobile to the stationary phase. (We are assuming, of

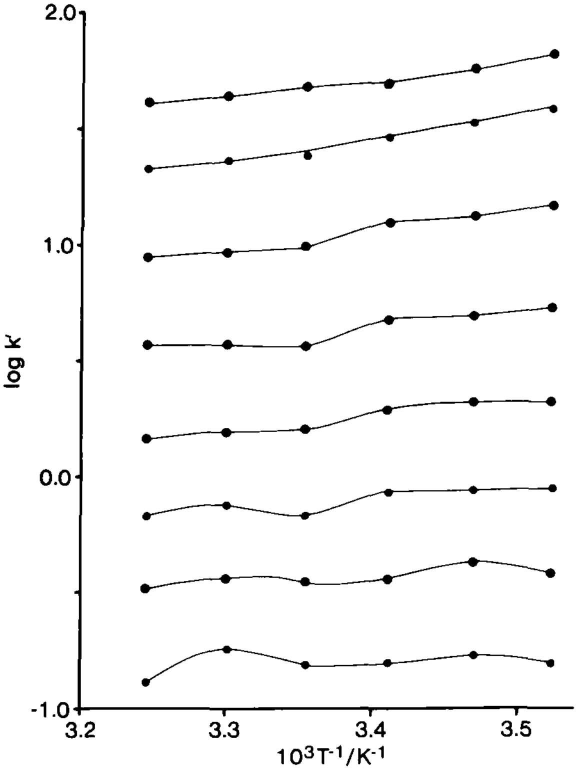

FIGURE 6. As in Figure 1; 2,4-dimethylphenol solute.

course, that the phase ratio β is independent of temperature, which may in fact be naive.)

It might be argued at this point that errors in the dead-volume measurements could be responsible for the observed non-linearity, which would in fact be a valid criticism for small deviations at large ϕ, i.e., small k'. However, the associated errors in V_A are too small to account for the large deviations seen in log k' at, for example, ϕ = 0.3 and 0.4 in Figures 2, 7, 8, and 9.

As noted in the Experimental Section, the columns were switched half-way through the temperature range, and it might be said that this could be responsible for the upward shift seen in log k' on passing from 1/T = 3.354×10^{-3} to 3.411×10^{-3} K^{-1}. However, as will be shown below, a mixed retention mechanism is indicated irrespective of the manner of consideration of the data sets.

Enthalpy-Entropy Compensation. We next examined the capacity-factor data with the view of establishing, first, whether there was compensation exhibited by the various solutes at a given solvent composition (ϕ constant) and, secondly, whether any one solute exhibited compensation as ϕ was varied. To eliminate the possibility that the use of different columns introduced systematic errors, compensation plots were constructed for each compound, where the capacity factors used were those corresponding to the "harmonic mean" of the temperature range (i.e., 15°C for column A and 30°C for column B).

TABLE 1

Retention Volumes V_R/cm^3 at 10° to 35°C; 100% THF Mobile Phase

	Temperature					
Solute[a]	10.0[b]	15.0[b]	20.0[b]	25.0[c]	30.0[c]	35.0[c]
1	2.15	2.15	2.13	2.10	2.26	2.14
2	2.15	2.05	2.06	2.21	2.14	2.04
3	2.21	2.08	2.07	2.20	2.14	2.11
4	2.17	2.10	2.10	2.20	2.12	2.16
5	2.20	2.13	2.13	2.21	2.15	2.20
6	2.19	2.12	2.07	2.24	2.16	2.08
7	2.19	2.14	2.09	2.23	2.14	2.07
8	2.17	2.12	2.07	2.20	2.14	2.10
9	2.17	2.11	2.07	2.23	2.17	2.12
ave.	2.17	2.11	2.09	2.22	2.14	2.12

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[a] see Experimental Section

[b] column A

[c] column B

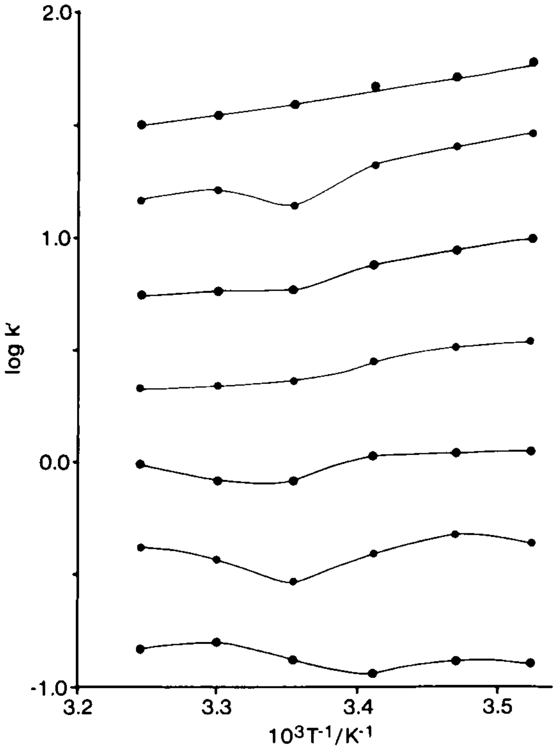

FIGURE 7. As in Figure 1; 2,4-dinitro-o-cresol solute.

The compensation plots are shown in Figures 10 (column A; 15°C) and 11 (column B; 30°C) for all solutes over the volume fraction range $\phi = 0$ to 0.3, i.e., the volume-fraction range over which it might be alleged that the van't Hoff plots exhibit at least approximate linearity. It is clear that the best that can be said of the data is that they lie within the first quadrant, that is, the random distribution of points indicates that if there is enthalpy-entropy compensation for these systems, it is effectively masked.

In order to provide inspection of the data for what might be considered a more closely-defined "family" of compounds, we show in Figures 12 and 13 plots of ln k' against $-\Delta H^o$ (Figure 12: column A, T = 15°; Figure

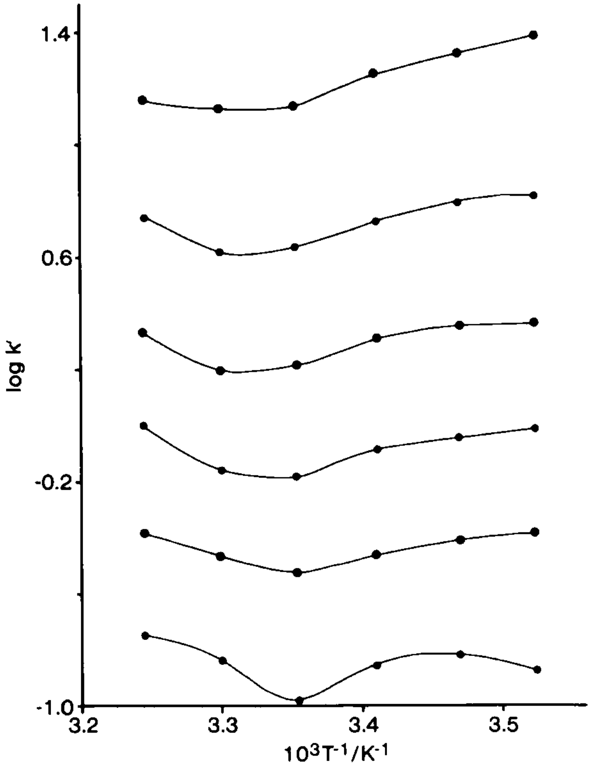

FIGURE 8. As in Figure 1; 4-chloro-m-cresol solute.

13: column B, T = 30°C), again over the range ϕ = 0 to 0.3, for solutes 1-3 and 5 (phenol, p-nitrophenol, 2,4-dinitrophenol, and o-nitrophenol). The distribution of points is once more found to be random, indicating that the retention mechanism(s) vary as the solvent composition is changed even when allowance is made for potential discrepancies arising from the use of two columns. Moreover, it would be difficult to envisage a more closely-related group of solutes for purposes of testing supposed enthalpy-entropy compensation with reverse-phase systems.

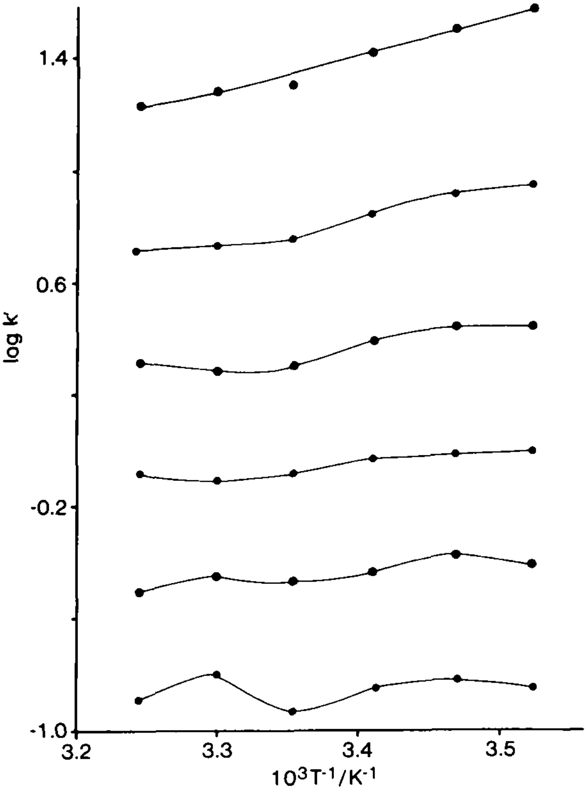

FIGURE 9. As in Figure 1; 2,4-dichlorophenol solute.

SUMMARY AND CONCLUSIONS

There is little doubt that the elution of polar solutes from reverse-phase columns is governed by mechanisms that are both composition- and temperature-dependent. At low volume fractions of THF ($\phi < 0.2$) a near-linear dependence of log k' on 1/T is observed for some of the solutes that might at first glance be taken as evidence for a single retention mechanism. However, enthalpy-entropy compensation plots show no correlation for the solutes as a group, which indicates more than one mode of retention

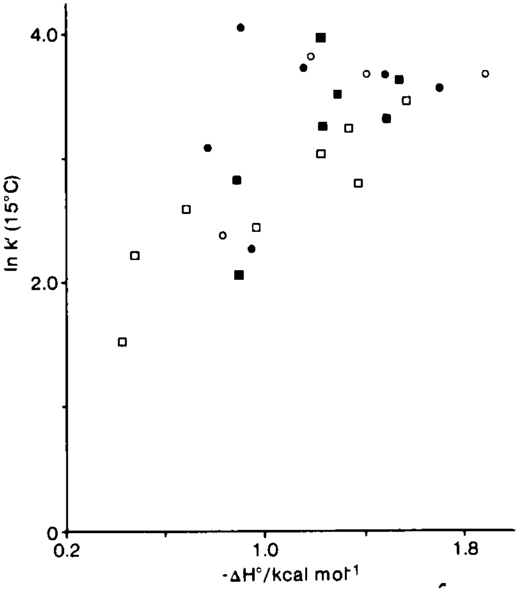

FIGURE 10. Plots of ln k' versus $-\Delta H^{o}$ for solutes 1-9 with column A at 15°C over the volume fraction range ϕ = 0 to 0.3. Enthalpies calculated from the slopes of Figures 1-9.

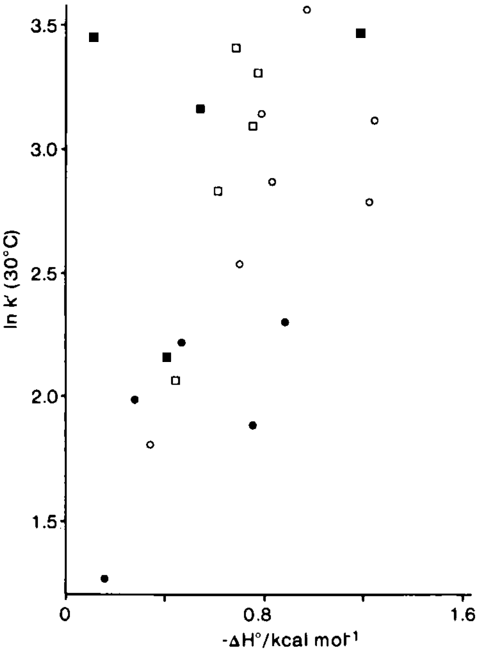

FIGURE 11. As in Figure 10, except data from column B at 30°C.

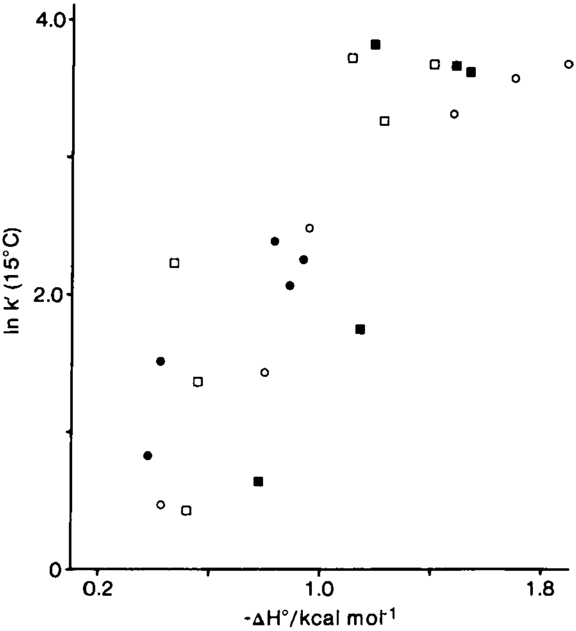

FIGURE 12. Plots of ln k' versus $-\Delta H^o$ for solutes 1-3 and 5 with column A over the volume fraction range ϕ = 0 to 0.3. "Harmonic mean" temperature T = 15°C. Enthalpies calculated from the slopes of Figures 1-3 and 5.

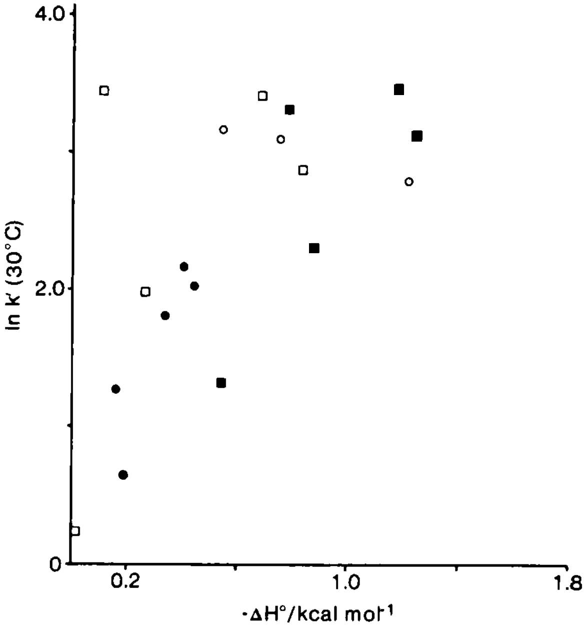

FIGURE 13. As in Figure 12, except data from column B at the "harmonic mean" temperature T of 30°C.

is extant. It was also found that enthalpy-entropy compensation did not correlate with mobile-phase composition; the retention mechanism(s) must therefore be mobile-phase dependent as well.

ACKNOWLEDGMENT

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