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RETENTION BEHAVIOUR OF ALKYLBENZENES AS A FUNCTION OF TEMPERATURE AND MOBILE PHASE COMPOSITION IN REVERSED-PHASE CHROMATOGRAPHY

ELI GRUSHKA*

Department of Inorganic and Analytical Chemistry, The Hebrew University, Jerusalem (Israel)

and

HENRI COLIN and GEORGES GUIOCHON*

Laboratoire de Chimie Analytique Physique, École Polytechnique, 91128 Palaiseau Cedex (France)

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SUMMARY

The reversed-phase chromatographic behaviour of a homologous series has been investigated as a function of the mobile phase composition and temperature. In general, it was found that the methylene selectivity decreased with increasing temperature and decreasing amount of water in the mobile phase. The dependence of the calculated data on the correct determination of the hold-up time is discussed. The methylene group contribution to ΔH of transfer was calculated using two different methods and the agreement was excellent. In addition, the agreement with one other reported value was excellent. Using the homologous series data, the solvent strength of the water–methanol mobile phases was calculated at different temperatures. The results were compared with the data obtained on pyrocarbon supports and the agreement was very good. Linearization methods for calculating t_0 are discussed and the difficulties in using such procedures are described.

INTRODUCTION

Although reversed-phase liquid chromatography (RPLC) accounts for a large proportion of high-performance liquid chromatographic (HPLC) separations, there is still uncertainty as to the exact effect of the operating conditions on the retention. One means of studying retention behaviour in RPLC is to examine the capacity ratios, k', of homologous series under various temperature and mobile phase conditions. Surprisingly little work has been carried out with homologous series. Colin and co-workers[1–3] discussed several aspects of selectivity and optimization of separations of such series. Melander *et al.*[4] used *n*-alkylbenzenes in a study of the concomitant dependence of the retention on temperature and mobile phase composition. Vigh and Varga-Puchony[5] investigated some aspects of the temperature dependence of the

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* On sabbatical leave at the École Polytechnique, Palaiseau, France.

capacity ratios of three homologous series. Berendsen *et al.*[6] used homologous series in order to obtain the hold-up time of a chromatographic column.

Homologous series offer the advantage of a simple relationship between the capacity ratios and the carbon numbers of the homologues. Moreover, and perhaps more importantly, the relationships between the chromatographic parameters and the carbon number of the members of the series lend themselves to relatively straightforward analysis. Thus we can write

$$\ln k'_n = n \ln \alpha + \ln \beta \tag{1}$$

where k'_n is the capacity ratio of the nth homologue, α is the selectivity of the system and β is the capacity ratio of the functional group of the series. This equation assumes a constant contribution to the free energy of the homologues with each CH_2 increment in the chain length. Each term in this equation depends, to a lesser or greater extent, on the temperature and on the mobile phase composition, because both capacity ratio and selectivity are thermodynamic quantities. The dependence of k' on the temperature, T, is well known:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \Phi \tag{2}$$

where ΔH and ΔS are the enthalpy and entropy, respectively, associated with the transfer of the solute from the mobile to the stationary phase, R is the gas constant and Φ is a system constant depending on the phase ratio in the column. Eqn. 2 assumes that either the phase ratio does not change significantly with temperature or that ΔH includes contributions due to changes in that ratio. From eqn. 2 and the definition of α, the CH_2 contribution to ΔH and ΔS can be ascertained as

$$\ln \alpha = \ln \left(\frac{k'_n}{k'_{n-1}} \right) = -\frac{\Delta \Delta H}{RT} + \frac{\Delta \Delta S}{R} \tag{3}$$

If the assumption of eqn. 1 is correct, then the enthalpy of the solute transfer should be a linear function of the carbon number, and the slope of the line of ΔH *versus* n should be $\Delta \Delta H$. This can be easily proved as follows. From eqn. 2 we can write

$$\frac{\partial \ln k'_n}{\partial (1/T)} = -\frac{\Delta H_n}{R} \tag{4}$$

where ΔH_n is the enthalpy of transfer of the nth homologue. Substitution in eqn. 1 yields

$$\frac{n \, \partial \ln \alpha}{\partial (1/T)} - \frac{\Delta H_0}{R} = -\frac{\Delta H_n}{R} \tag{5}$$

where ΔH_0 is given by

$$\Delta H_0 = -\frac{\partial \beta}{\partial (1/T)} \tag{6}$$

From eqn. 3 we finally obtain

$$\Delta H_n = n \, \Delta \Delta H + \Delta H_0 \tag{7}$$

It should be mentioned here that one can, and perhaps should, question the validity of eqn. 1[7]. At low carbon numbers the functional group should affect the retention time to a much greater extent than at high carbon numbers. In addition, errors in determining k' values for the lower homologues may affect the linearity of ln k' versus n. On the other hand, at very large n values, it is possible that the contribution of additional CH_2 groups to ΔH becomes less significant than with lower homologues. A typical example of the non-linearity of the ln k' dependence on the homologous series carbon number was shown by Engelhardt and Ahr[8]. Part of this paper will examine this linearity as a function of the temperature and mobile phase composition.

A second aim of this work was to investigate the solvent strength, ε^0, of water–methanol mixtures as a function of temperature and mobile phase composition. The expression given by Snyder[9] for calculating ε^0 in normal-phase adsorption chromatography has already been used in RPLC using carbon adsorbents[1]:

$$\ln\left(\frac{k'_{s_1}}{k'_{s_2}}\right) = a(\varepsilon^0_{s_2} - \varepsilon^0_{s_1}) \tag{8}$$

a is the molecular area of the solute and the subscripts s_1 and s_2 indicate solvents 1 and 2, respectively. If one chooses one mobile phase as a reference solvent, that is, with $\varepsilon^0 = 0$, eqn. 8 provides the means of calculating ε^0 under different conditions. This equation was used to determine solvent strengths when pyrocarbon supports were employed. These packings are known to be more retentive than silica-based reversed phases. Therefore, a comparison between the two types of packing is of interest.

The definition of the solvent strength given in eqn. 8 is perhaps not the best, as it not only characterizes the solvophobic (non-specific) contribution to the retention, but also includes the specific effects that also contribute to solute retention. As these contributions are dependent on the solute, it is incorrect to take them into account in the calculation of ε^0, which must be independent of the solute. We have nevertheless used this approach here to compare carbon surfaces and reversed-phase adsorbents. A different method of evaluating ε^0 in RPLC from the slope of the ln k' versus carbon number plots will be discussed elsewhere[7].

A third aim of the study was to examine the dependence of the capacity ratios, and the related thermodynamic quantities such as ΔH, $\Delta\Delta H$ and $\Delta\Delta S$, on the mobile phase composition at very high concentrations of the organic modifier.

There is a controversy in the literature regarding the dependence of k' on the volume percentage of the modifier. One school of thought claims a linear dependence[10,11], whereas another advocates a quadratic relationship[12,13]. Melander et al.[4] indicated the lack of rigorous theoretical connection between k' and the phase composition. While the quadratic approach[12,13] indicates that deviation from linearity will be more pronounced at high concentrations of the modifier, there are reports[14] of curvatures at low concentrations.

EXPERIMENTAL

Apparatus

The chromatograph consisted of a Waters Assoc. (Milford, MA, U.S.A.)

Model 6000A pump, a Model 440 UV detector, a Model 401 RI detector, a Rheodyne (Berkeley, CA, U.S.A.) Model 7010 injector and a Sefram (Paris, France) Servotrace recorder. The column was 5×0.41 cm I.D. stainless steel packed with Hypersil C-18 $5-\mu m$ particles (Shandon, Runcorn, Great Britain). The column was packed using the balanced-density slurry method. The column, injector, pre-column and a pre-heater capillary tubing were placed in a water jacket, the temperature of which was maintained constant with a Haake (Karlsruhe, G.F.R.) proportional heat controller.

Reagents

Distilled, deionized water was used. Methanol was purchased from Merck (Darmstadt, G.F.R.). ^2H_2O for the determination of t_0 was obtained from CEA (Saclay, France). The alkylbenzenes were obtained from various sources.

Procedures

The temperature range studied was about 30–50°C, measurements being made at approximately 5°C intervals. The column was equilibrated at each new temperature for several hours before data collection. Once a temperature study was finished, a new mobile phase was introduced and a new temperature study was initiated. The mobile phases used were pure methanol and 9:1 and 4:1 methanol–water. The measurements of t_0 were made with methanol spiked with ^2H_2O.

The results were corrected for the extra-column volume of the chromatographic system as follows. The volume of the tubing connecting the column to the detector was calculated to be 4.7 μl. The RI detector has a 15-μl heat exchanger. Hence the total extra-column volume, or the dead volume, of the chromatographic system with the RI detector is 19.7 μl. The t_0 value measured with ^2H_2O and a RI detector was corrected for this volume. The alkylbenzenes were chromatographed using a UV detector. To correct for the difference in the cell volumes of the detectors, benzene was injected into the chromatographic system with both the RI and UV detectors. The retention time was longer when the RI detector was connected to the column. Hence the difference in the measured retention times of benzene with the two detectors gives the difference of their contributions to the retention volume:

$$(t_R^0 + t_{dRI}) - (t_R^0 - t_{dUV}) = t_{dRI} - t_{dUV} \tag{9}$$

where t_R^0 is the retention time due to the column only and t_{dRI} and t_{dUV} are the contributions to the observed retention times due to the dead volume associated with the RI and the UV detector, respectively. This difference, when converted into volume and subtracted from the dead volume of the RI system, gives the extra-column contribution of the UV detector. Using this value, the retention times of the alkylbenzenes were corrected for the extra-column contribution.

RESULTS AND DISCUSSION

Measurements of t_0

The hold-up time, t_0, is a crucial parameter that is needed for the accurate determination of k', especially for early eluting solutes. The correct measurement of t_0

is an intensely active research area[6,15-18], and no general agreement exists. In this work, several methods of obtaining t_0 were examined. The procedure of Neidhart et al.[18], which is based on the temperature dependence of retention, failed to give reasonable volumes. The linearization approach suggested by Berendsen et al.[6] did not give self-consistant results unless critical tests were performed[17]. Although the linearization fits were very good, with correlation coefficients greater than 0.9999, some of the t_0 values obtained were greater than those measured with ^2H_2O and others were lower. A different linearization technique and the problems associated with it will be discussed in another paper[17].

The retention volumes, as measured with ^2H_2O, were fairly constant, with no noticeable trends under all experimental conditions. The average retention volume of ^2H_2O was 0.525 ml and the standard deviation was 0.0107 ml, which represents a 2 % relative error. In view of the failure of other techniques, this retention volume was used to calculate t_0, and thus the capacity ratios. Moreover, the data obtained allow easy comparisons with other published results. We are well aware of the errors that might be involved when using ^2H_2O-based t_0 values. However, in view of the difficulties of using other methods, we chose a known approach for obtaining t_0. Other parts of the paper will address themselves more specifically to the validity of this approach.

Behaviour of homologous series

Tables I–III show the k' and α values of *n*-alkylbenzenes, starting from toluene (C_1), using three mobile phases, each at five different temperatures. As expected, the capacity ratios and the α values increase with increasing water content in the mobile phase, and decreasing temperature. The data obtained with pure methanol should be used with caution. The k' values are very small and the α values seem to

TABLE I

CAPACITY RATIOS AND α VALUES OF *n*-ALKYLBENZENES AT FIVE TEMPERATURES WITH 100 % METHANOL AS THE MOBILE PHASE

n	T (°C), $1/T$ (°K^{-1})									
	31.8 (3.28·10^{-3})		36.5 (3.23·10^{-3})		40.2 (3.19·10^{-3})		45.8 (3.13·10^{-3})		51.0 (3.085·10^{-3})	
	k'	α	k'	α	k'	α	k'	α	k'	α
C_{12}	1.38		1.28		1.23		1.14		0.97	
		1.17		1.17		1.18		1.18		1.19
C_{11}	1.18		1.10		1.04		0.97		0.83	
		1.21		1.20		1.19		1.19		1.18
C_{10}	0.97		0.92		0.87		0.81		0.71	
		1.18		1.18		1.17		1.19		1.19
C_9	0.82		0.78		0.74		0.68		0.59	
		1.21		1.20		1.18		1.16		1.20
C_8	0.68		0.64		0.63		0.59		0.49	
		1.19		1.19		1.22		1.22		1.24
C_7	0.57		0.54		0.51		0.48		0.39	
		1.16		1.22		1.21		1.19		1.23
C_6	0.49		0.44		0.43		0.40		0.32	
		1.28		1.23		1.20		1.18		1.13
C_5	0.39		0.36		0.35		0.34		0.28	
		1.19		1.17		1.23		1.13		1.38
C_4	0.32		0.31		0.29		0.26		0.21	
		1.20		1.23		1.19		1.18		1.22
C_3	0.27		0.25		0.24		0.22		0.17	
		1.21		1.19		1.27		1.26		1.35
C_2	0.22		0.21		0.20		0.18		0.12	
		1.30		1.23		1.16		1.13		1.22
C_1	0.17		0.17		0.16		0.16		0.10	

TABLE II

CAPACITY RATIOS AND α VALUES OF n-ALKYLBENZENES AT FIVE TEMPERATURES WITH METHANOL–WATER (9:1) AS THE MOBILE PHASE

n	T (°C) [$1/T$ (°K⁻¹)]									
	29.2 (3.3·10⁻³)		35.8 (3.24·10⁻³)		40.8 (3.185·10⁻³)		46.0 (3.13·10⁻³)		50.0 (3.09·10⁻³)	
	k'	α	k'	α	k'	α	k'	α	k'	α
C_{12}	9.01	1.33	7.80	1.31	7.04	1.32	6.15	1.28	5.64	1.27
C_{11}	6.75	1.33	5.96	1.29	5.34	1.31	4.74	1.30	4.43	1.30
C_{10}	5.09	1.33	4.62	1.34	4.08	1.30	3.69	1.29	3.41	1.29
C_9	3.82	1.33	3.45	1.32	3.14	1.29	2.85	1.30	2.64	1.29
C_8	2.88	1.33	2.61	1.33	2.44	1.33	2.19	1.29	2.04	1.28
C_7	2.16	1.35	1.97	1.32	1.84	1.32	1.70	1.31	1.59	1.31
C_6	1.60	1.32	1.49	1.33	1.39	1.31	1.29	1.31	1.22	1.29
C_5	1.22	1.34	1.12	1.33	1.06	1.30	0.99	1.31	0.94	1.29
C_4	0.91	1.37	0.85	1.32	0.82	1.33	0.76	1.32	0.73	1.33
C_3	0.66	1.31	0.64	1.34	0.61	1.34	0.57	1.31	0.55	1.31
C_2	0.58	1.28	0.48	1.30	0.46	1.37	0.44	1.28	0.42	1.24
C_1	0.40		0.37		0.33		0.34		0.34	

be independent of temperature. This could be an artifact of either an error in measuring t_R or the use of a wrong t_0, as will be discussed shortly. The α values were calculated for neighbouring pairs of solutes.

The linearity of ln k' *versus* carbon number is shown in Table IV. The data in Table IV were obtained using all solutes whose k' values were above 0.40. The linear fit is, as can be seen, very good, especially for data obtained with the two methanol–

TABLE III

CAPACITY RATIOS AND α VALUES OF n-ALKYLBENZENES AT FIVE TEMPERATURES USING METHANOL–WATER (4:1) AS THE MOBILE PHASE

n	T°C [$1/T$ (°K⁻¹)]									
	30.0 (3.3·10⁻³)		35.8 (3.24·10⁻³)		40.2 (3.19·10⁻³)		46.7 (3.13·10⁻³)		51.0 (3.08·10⁻³)	
	k'	α	k'	α	k'	α	k'	α	k'	α
C_{12}	58.16	1.48	48.86	1.47	42.36	1.46	34.23	1.42	29.86	1.41
C_{11}	39.26	1.49	33.30	1.47	29.09	1.45	24.09	1.44	21.10	1.42
C_{10}	26.39	1.48	22.62	1.47	20.04	1.45	16.63	1.39	14.87	1.43
C_9	17.82	1.49	15.39	1.46	13.84	1.47	11.93	1.44	10.41	1.40
C_8	11.93	1.48	10.52	1.46	9.42	1.43	8.27	1.44	7.45	1.44
C_7	8.06	1.48	7.20	1.47	6.56	1.44	5.73	1.45	5.18	1.42
C_6	5.43	1.48	4.88	1.47	4.54	1.46	3.94	1.45	3.66	1.45
C_5	3.65	1.48	3.31	1.47	3.10	1.44	2.72	1.44	2.57	1.41
C_4	2.46	1.51	2.24	1.48	2.15	1.45	1.89	1.45	1.79	1.41
C_3	1.63	1.48	1.52	1.48	1.49	1.46	1.30	1.44	1.27	1.42
C_2	1.10	1.47	1.03	1.35	1.02	1.40	0.90	1.37	0.89	1.39
C_1	0.75		0.76		0.73		0.66		0.64	

TABLE IV

DEPENDENCE OF ln k' ON THE CARBON NUMBER OF ALKYLBENZENES

Mobile phase	T (°C) $[1/T$ (°K^{-1})$]$	Correlation coefficient	Slope	α	Intercept
100% methanol	31.8 (3.28 · 10⁻³)	0.9995	0.1740	1.190	−1.76
	36.5 (3.23 · 10⁻³)	0.9993	0.1763	1.193	−1.851
	40.2 (3.19 · 10⁻³)	0.9993	0.1749	1.191	−1.885
	45.8 (3.13 · 10⁻³)	0.9996	0.1722	1.188	−1.931
	51.0 (3.08 · 10⁻³)	0.9985	0.186	1.205	−2.226
Methanol–water (9:1)	29.2 (3.31 · 10⁻³)	0.99996	0.2879	1.334	−1.252
	35.8 (3.24 · 10⁻³)	0.99994	0.2797	1.323	−1.285
	40.8 (3.18 · 10⁻³)	0.99991	0.2720	1.313	−1.305
	46.0 (3.13 · 10⁻³)	0.99991	0.2647	1.303	−1.341
	50.0 (3.09 · 10⁻³)	0.99987	0.2598	1.297	−1.369
Methanol–water (4:1)	30.0 (3.3 · 10⁻³)	0.99999	0.3963	1.486	−0.688
	35.8 (3.24 · 10⁻³)	0.99999	0.3854	1.470	−0.733
	40.2 (3.19 · 10⁻³)	0.99999	0.3722	1.451	−0.724
	46.7 (3.13 · 10⁻³)	0.99992	0.3630	1.438	−0.80
	51.0 (3.08 · 10⁻³)	0.99998	0.3507	1.420	−0.8096

water mobile phases. The values of α, which are calculated from the slope of the lines according to eqn. 1, are the average selectivities for each experimental set of experiments at a given temperature and mobile phase composition. Table IV shows clearly the decrease in α as the temperature increases and as the water content decreases. The difficulties in measuring the α values with 100% methanol should again be noted. The dependence of α on the mobile phase composition is far greater than that on temperature.

The intercept ln β, as seen from eqn. 1. is the natural logarithm of k'_0, that is, of a hypothetical C_6H_5 moiety. Ln β is, of course, a function of the mobile phase and the temperature. In this work, β increases with increasing water content and decreasing temperature.

Homologous series have some unique properties[19,20], one of which is the intersection, at an imaginary carbon number, of the ln k' versus n plots corresponding to different mobile phase compositions. If eqn. 1 is correct, then the interaction indices equation developed by Jandera et al.[13] can be used to determine the intersection points. Using their terminology it can be shown that

$$I_X^* C_X^* = 2 I_{H_2O} C_{CH_3OH} \tag{10}$$

where I_X^* is the interaction index of the imaginary solute where the lines intersect, C_X^* is a coefficient characterizing the ability of these solute molecules to undergo polar interactions with each other, I_{H_2O} is the interaction index of water and C_{CH_3OH} is analogous to C_X^* but for the mobile phase (methanol). It should be noted that eqn. 10 assumes that the dependence of ln k' on the mobile phase composition is linear. Further ramifications of eqn. 10 are discussed in a thesis[19]. Fig. 1 shows an example of
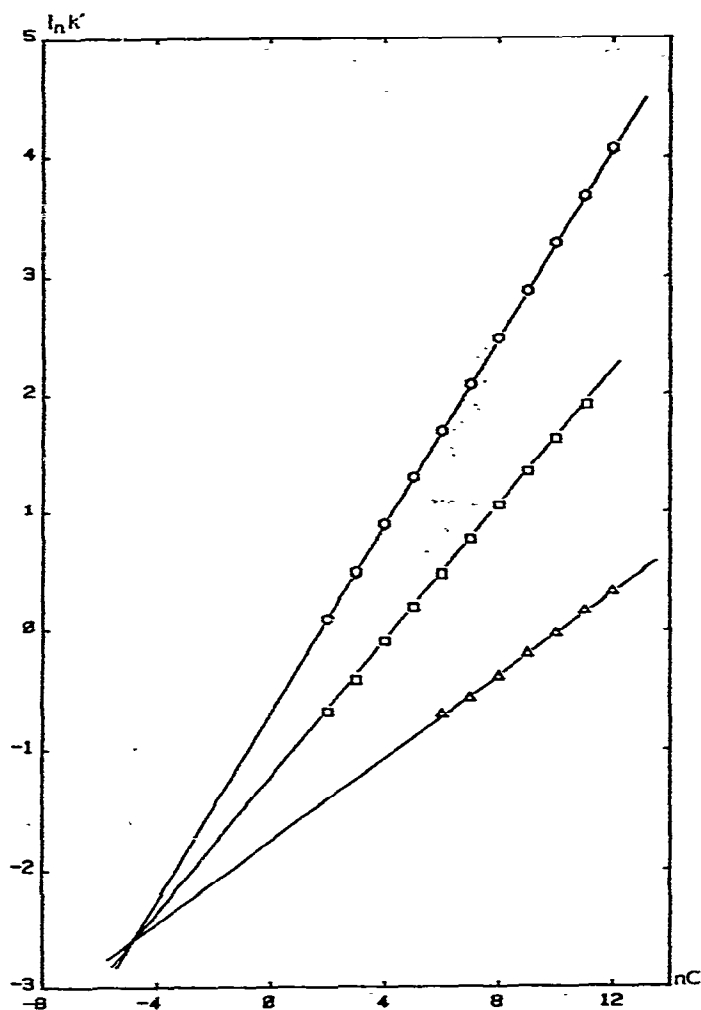
Fig. 1. Ln k' versus carbon number plots showing a common intersection point. Temperature: ca. 30°C. Mobile phase: O, methanol–water (4:1); □, methanol–water (9:1); △, 100% methanol.

plots of ln k' versus n and the common intersection point. The physical significance of this point is not clear, but its existence can be taken as an indicator of the linearity of the dependence of ln k' on the mobile phase composition. Fig. 2 shows some plots of ln k' versus the volume percentage of methanol. As can be seen, a linear dependence is strongly suggested. It is, of course, meaningless to fit three points to a straight line. However, the behaviour depicted in Fig. 2 does not show deviation from linearity. It should be mentioned that ln β should also be a linear function of the mobile phase composition. In fact, as shown in Fig. 3, linearity exists at low but not at high temperatures. The reason for the deviation from linearity is probably due to errors in determining t_0 accurately. The error in β is related to the error in t_0 by:
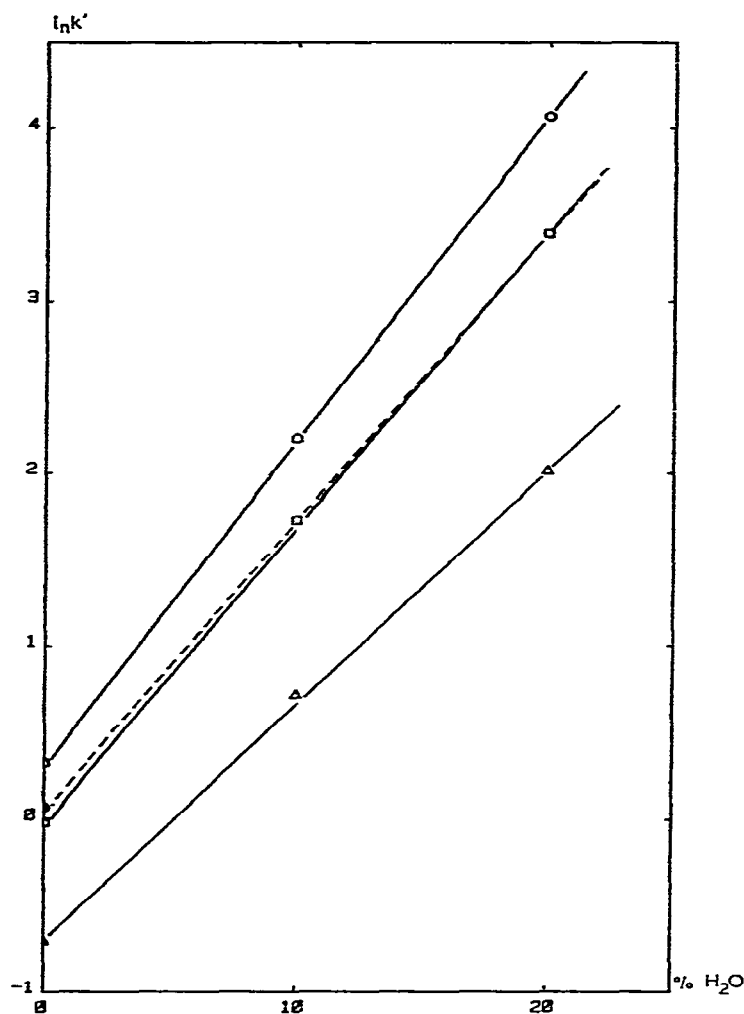
Fig. 2. Ln k' *versus* percentage of methanol. O, C_{12} at *ca.* 36°C; □, C_{12} at *ca.* 51°C; △, C_8 at *ca.* 51°C. The broken line is the result of the extrapolation described in the text.

$$\Delta\beta = -\frac{\alpha^{-n}t_R}{t_0^2} \cdot \Delta t_0 \tag{11}$$

Thus, the error in t_0 is particularly important when the retention times of the members of the homologous series are low, as is the case at high temperatures and low water contents. Of course, an error in measuring accurately t_R, which is significant at low retention times, will also result in an incorrect value for ln β.

Thermodynamics of homologous series

ΔH of transfer is easily obtained from the dependence of ln k' on $1/T$. Table V

TABLE V

DATA FROM THE LEAST-SQUARES FIT OF ln k' VERSUS $1/T$

| n	100% Methanol			Methanol-water (9:1)			Methanol-water (4:1)		
	Correlation coefficient	ΔH (kcal/mole)	Intercept	Correlation coefficient	ΔH (kcal/mole)	Intercept	Correlation coefficient	ΔH (kcal/mole)	Intercept
C_{12}	0.983	−3.26	−5.06	0.999	−4.39	−5.11	0.999	−6.21	−6.24
C_{11}	0.983	−3.40	−5.44	0.999	−3.97	−4.70	0.999	−5.76	−5.88
C_{10}	0.981	−3.14	−5.20	0.996	−3.83	−4.73	0.999	−5.36	−5.83
C_9	0.979	−3.23	−5.58	0.998	−3.46	−4.42	0.998	−4.88	−5.22
C_8	0.949	−3.17	−5.58	0.995	−3.22	−4.31	0.999	−4.34	−4.73
C_7	0.953	−3.52	−6.34	0.999	−2.83	−3.94	0.999	−4.08	−4.69
C_6	0.954	−3.99	−7.27	0.998	−2.56	−3.78	0.994	−3.83	−4.66
C_5	—	—	—	0.999	−2.39	−3.79	0.998	−3.41	−4.37
C_4	—	—	—	0.995	−2.07	−3.54	0.995	−2.99	−4.07
C_3	—	—	—	0.983	−1.85	−3.48	0.982	−2.46	−3.59
C_2	—	—	—	0.999	−1.82	−3.71	0.973	−2.06	−3.32
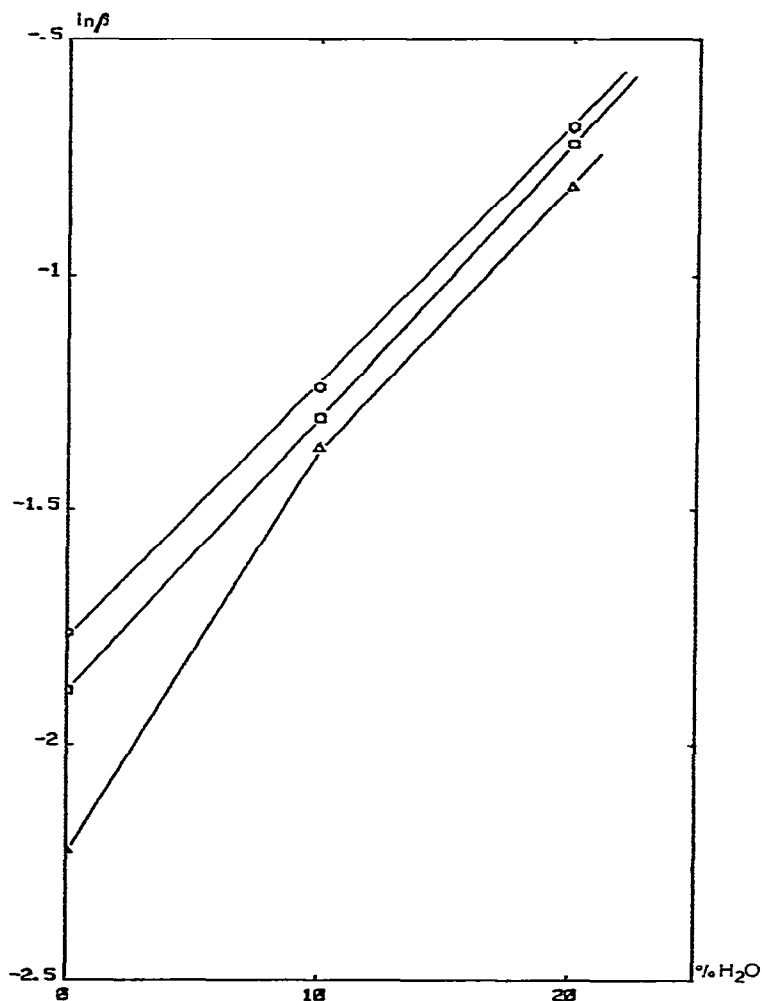
Fig. 3. Ln β *versus* percentage of water at different temperatures: O, 30°C; □, 40°C; △, 50°C.

shows ΔH values for the systems studied here. The difficulties in deducing useful information from the 100% methanol system are again self-evident.

These difficulties are related to t_0, as will be demonstrated shortly. With the other two mobile phases, the magnitude of ΔH increases with increasing carbon number and increasing water content of the mobile phase. The interpretation of these observations is simple. As the solute becomes bulkier it is energetically more favourable for it to be in the stationary phase. Likewise, as the water content of the mobile phase increases, the solutes, owing to increased solvophobic interactions, prefer the stationary phase.

The trend of the intercepts of the lines of ln k' *versus* $1/T$ is similar to that of ΔH, *i.e.*, an increase with increasing carbon number and water content. As the intercept is $\Delta S/R + \Phi$, it is difficult to give an exact interpretation of its behaviour. The phase ratio, and its dependence on the mobile phase and temperature, is not known.

TABLE VI

$\Delta\Delta H$ AND $\Delta\Delta S$ VALUES OF THE METHYLENE GROUPS IN ALKYLBENZENES

Method	Methanol–water mobile phase	Correlation coefficient	$\Delta\Delta H$ (cal/mole)	$\Delta\Delta S$ (e.u./mole)	ΔH^o (cal/mole)
From ln α	9:1	0.9991	−268	−0.314	—
versus $1/T$	4:1	0.993	−396	−0.521	—
From ΔH versus	9:1	0.994	−267	—	1076
carbon number	4:1	0.998	−405	—	1291

However, it seems that the trend is for increasing ΔS with bulkier solutes and higher water contents.

As discussed in the introduction, the dependence of ln α on $1/T$ yields the methylene contribution to ΔH and ΔS, that is, $\Delta\Delta H$ and $\Delta\Delta S$. Table VI shows $\Delta\Delta H$ and $\Delta\Delta S$ values and the correlation coefficient of the least-squares fit of ln α to $1/T$. Also shown in Table VI are $\Delta\Delta H$ and ΔH_0 values obtained from the relationship between ΔH and the carbon number. The agreement of the $\Delta\Delta H$ values is excellent. In the present experiments, increasing the water content of the mobile phase by 10% causes a 130–140 cal/mole change in $\Delta\Delta H$ and about a 0.2 e.u./mole change in $\Delta\Delta S$. It would be of great interest to extend this study to other mobile phase compositions in order to ascertain whether the changes in $\Delta\Delta H$ and $\Delta\Delta S$ are linearly related to the water content of the mobile phase.

Vigh and Varga-Puchony[5] have studied the retention behaviour of homologous n-alkanols and the 2-n-alkanone and n-alkanal derivatives of dinitrophenylhydrazones (DNPH). In the latter two homologous series, the mobile phase was methanol–water (4:1). It is thus of interest to compare the results of their work and our results. The magnitudes of ΔH are about the same in the two studies. For example, ΔH for dodecylbenzene is 6.21 kcal/mole in methanol–water (4:1), whereas for C_{12} alkanol DNPH it is 6.68 kcal/mole. The small difference between the two values is probably due to the different functional groups of the two solutes. In both our work and that of Vigh and Varga-Puchony[5], the structural increment in the series is the methylene group and the $\Delta\Delta H$ values should agree. In this work, the value of $\Delta\Delta H$ is about 400 cal/mole, whereas ref. 5 gives values of 419 cal/mole for the alkanol DNPHs and 402 cal/mole for the alkanone DNPH. This agreement is very good, again pointing to the feasibility of using HPLC to determine thermodynamic quantities.

The agreement between the $\Delta\Delta H$ values in the two studies means that the selectivities, but not necessarily the retention times, of the classes of compounds reported should be very close. A comparison of the α values shows that such is the case (note that the present data are in terms of natural logarithms whereas those in ref. 5 are in common logarithms). The capacity ratios, however, are different in the two studies, as would be expected. Similar results were recently obtained by Melander and Horváth[21].

The data in this work can also be compared with those obtained by Colin et al.[1] on pyrocarbon-containing adsorbents. From Figs. 1 and 2 in ref. 1, it is clear that the capacity ratios and ΔH values of the alkylbenzenes are much larger on the pyrocar-

bon support. This, of course, is in agreement with the fact that pyrocarbon supports develop much stronger dispersive interactions than the conventional C_{18} bonded phases.

As mentioned previously, the relatively uniform and high values of ΔH obtained with the pure methanol mobile phase are thought to be due to errors in measuring t_0. Small errors in t_0 can lead to large errors in ΔH, as will now be illustrated. To a good approximation[1], ΔH, in the case of water–methanol mobile phase, can be assumed to be a linear function of the water content. Thus, the data for 9:1 and 4:1 methanol–water can be extrapolated to pure methanol. For example, with dodecylbenzene, the extrapolated ΔH is 2.57 kcal/mole. Using the van 't Hoff equation and assuming that the retention time of this solute at 31.8°C with pure methanol as the mobile phase is correct, the capacity ratio at 51°C can be calculated. The calculated value is 1.07, whereas the measured value is 0.99. This is an error of about 8.5%. The error in t_0 is even smaller, 3.5%. However, the error in ΔH is large, being about 30%. Even more significant is the fact that, when the extrapolated k' is used in Fig. 2, the line thus obtained is not different from the original one. This last point is extremely important: small changes in t_0 can significantly alter the results derived from chromatographic data.

Solvent strength

Colin et al.[1] have measured the solvent strength of methanol–water mixtures using pyrocarbon adsorbents. It would be of interest to compare the solvent strength of the same mobile phases when using a conventional C_{18} bonded phase. With the aid of eqn. 8 and the assumptions leading to it[1], ε^0 can be calculated as a function of the mobile phase composition and the temperature. In Table VII the results of such calculations are shown. In one instance, pure methanol was taken as the reference mobile phase, that is, its ε^0 was arbitrarily set equal to zero at all temperatures. From Table VII, ε^0 seems to be linearly dependent on the water content for the limited range of mobile phases studied here. It decreases with increasing amounts of water. As the temperature increases the solvent strength decreases. These findings are in agreement with the results of Colin et al.[1]. Moreover, if the ε^0 values in Table VII are compared

TABLE VII

CALCULATED ε^0 VALUES FOR THE MOBILE PHASES USED AS A FUNCTION OF TEMPERATURE

Reference mobile phase	Methanol–water mobile phase	Values calculated from	30°C	35°C	40°C	45°C	50°C
100% methanol	9:1	Dodecylbenzene	−0.046	−0.0449	−0.0434	−0.0419	−0.0437
	4:1		−0.0929	−0.0904	−0.0879	−0.0845	−0.0851
	9:1	Decylbenzene	−0.0458	−0.0447	−0.0427	−0.0414	−0.0435
	4:1		−0.0913	−0.0887	−0.0867	−0.0835	−0.0842
Methanol–water (9:1)	4:1	Dodecylbenzene	−0.0463	−0.0455	−0.0445	−0.0426	−0.0414
	4:1	Decylbenzene	−0.0455	−0.0439	−0.0440	−0.0416	−0.0406

with Fig. 7 in ref. 1, it is seen that the solvent strengths with the two stationary phases are in close agreement.

As it should be, ε^0 seems to be independent of the solutes (in the homologous series) that were used in the calculations. Both dodecylbenzene and the decylbenzene gave self-consistent results including the anomaly at 50°C. The reason for the increase in ε^0 at 50°C is not clear. It might, however, be related to the difficulties in determining accurately k' values with pure methanol as the mobile phase. The results obtained when the methanol–water (9:1) mobile phase was used as the reference tend to substentiate this argument. When ε^0 is calculated for the methanol–water (4:1) mobile phase the results show a continuous decrease in ε^0 with increasing temperature in all instances but one, namely at 40°C, when decylbenzene was used for the calculation. However, this seems to be a random error which did not occur if other solutes were used for determining ε^0.

Linearization of retention and t_0 determination

The importance and difficulties of accurately measuring t_0 are discussed throughout this paper. It has been suggested[6] that homologous series may be ideally suited for the determination of the hold-up time via some sort of a linearization procedure. The argument goes that the t_0 that will yield the best fit of ln k' to the carbon number of the homologues is the dead time. Valid as this idea might be, it must be applied with caution as erroneous results might be obtained[17]. Two examples will illustrate this point. We have linearized the data obtained with the methanol–water (9:1) mobile phase at 40.8°C using the solutes from pentylbenzene to dodecylbenzene. The best fit is obtained with $t_0 = 0.480$ min, with a correlation coefficient of 0.99995. If butylbenzene is included in the linearization, the resultant t_0 is 0.504 min with the same correlation coefficient. The change in the slope of the two lines is minor: 1.30 compared with 1.31. However, the change in ln β is large: -0.489 compared with -0.534. A different example is that of the data obtained with the methanol–water (4:1) mobile phase at 51°C. If the linearization procedure includes octylbenzene to dodecylbenzene, the t_0 obtained is 0.62 min with a correlation coefficient of 0.99997. If heptylbenzene is also included, t_0 is reduced to 0.476, and the correlation coefficient remains the same. Again, while ln α does not change much, ln β is different in the two instances. It is thus seen that while the slope, that is ln α, is not a strong function of t_0, ln β depends greatly on the value of the hold-up time. These findings mean that an omission, or inclusion, of experimental points, either by choice or by design, can change what is thought to be t_0 if a convergence test has not been carried out[17]. This is a crucial point which, to the best of our knowledge, has not been discussed in the literature. One possible explanation might be that ln k' for homologous series is not linearly related to the carbon number. In addition, alternative procedures based on more rigorous linearization methods are being studied.

The discussion in this paper is based on t_0 measured from the retention of ^2H_2O. We feel that this is not a bad approximation of the hold-up time, as will be demonstrated in a forthcoming paper[17]. In any event, data based on ^2H_2O allow comparison with other published results.

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