Influence of temperature on dead volume of ODS columns and on n-alkane retention in high-performance liquid chromatography

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Abstract

Dead volume of ODS columns as determined from the homologous series of n-alkanes with n-pentane or methanol eluent decreases with increasing temperature. This decrease which occurs in addition to the influence of thermal expansion of the eluent, is thought to be caused by thermal expansion of the stationary phase. Dead volume decreases about 2% per 40°C with n-pentane eluent and 5% with methanol eluent. No such decrease is found with a silica column. In addition, there appears to be a slight dependence of exclusion coefficients on temperature.

With n-pentane eluent, net retention of n-alkanes on ODS is very low (on the order of 1% of retention with methanol eluent). Solutes smaller than eluent molecules (butane to methane) exhibit higher retention times than expected from larger solutes (hexane and higher). With methanol eluent, plots of log capacity factor (a) *versus* carbon number for various temperatures and (b) *versus* inverse temperature for various carbon numbers show common intersection points.

1. Introduction

Influence of temperature on retention in various HPLC systems has been dealt with in numerous investigations [1–23 and references cited therein]. Most of this work was concerned with either structural changes in bonded phases, or with determination of enthalpy and entropy contributions to retention. Only little information is available on the effect of temperature on dead volume. An influence of thermal eluent expansion on dead time has been described by Colin et al. [3] and by Krstulovic et al. [10]. Recent data by Bidlingmeyer et al. [24] indicate a decrease of V_m of 5.2% between 45 and 65°C

with uracil as a marker, of 4.3% between 35 and 55°C with acetone as marker, and of 1.5% between 35 and 55°C with ^2H_2O as marker, all at 2 ml/min flow. Laub and Madden [13] calculated V_0 from retention data of the series toluene through butylbenzene and benzyl alcohol through phenylpentanol and found temperature-independent, although strongly scattering V_0 from phenylalkanes, but decreasing values from phenylalkanols, both between 10 and 30°C.

Bidlingmeyers data and some other experimental results [24–28] suggested that dead volume might depend on temperature more strongly than expected from thermal expansion of the mobile phase. A temperature-induced

change of stationary phase volume has been proposed in this context [26–28]. The present work was performed in order to (i) confirm the existence of this effect, (ii) determine its extent and (iii) investigate the possible influence of temperature on exclusion.

2. Experimental

2.1. Apparatus, columns, chemicals

The HPLC apparatus was assembled from commercial components and combined with various custom-made parts. Pumps were either Gynkotek 300C or Knauer 64. For 15–55°C experiments, pumps were thermostated in an air bath at 25 ± 1°C. For all other experiments, pumps operated at ambient temperature. The eluent was continuously kept under helium. Injection valve was a Valco C6W with air actuator which was run on 0.7–0.8 MPa helium via a pilot valve system [29], yielding switching times around 10 ms. The injector valve was immersed into the column thermostat bath and automatically loaded using a Hamilton Microlab P syringe drive. Eluent feed line (2 m × 0.7 mm I.D.) and column were kept in a water–methanol thermostat/cryostat bath (Haake F3) at a ± 0.02°C level for 15–55°C experiments. At low-temperature experiments, control was probably worse, ± 1°C at best. For detection, a Sicon LCD 200 refractive index detector was used. For higher-temperature work, its thermostating connectors were arranged in series with the column thermostat bath. At low temperature, the optical unit was taken out and mounted in an air-tight box flushed with dry nitrogen to prevent moisture condensation. The data system was PE/Nelson 960 and for some work, Shimadzu CR6A, both giving retention times in minutes with three decimals. It was started by a precision switch mounted on the injection valve drive. Further experimental details have been published elsewhere [30,31,54].

Columns (stainless steel, 250×4 mm I.D.) were purchased from Knauer Säulentechnik, Berlin, Germany. Packings were (data on grain and pore size as quoted by manufacturer): (column 1) Eurospher silica 100 Å 5 μm and (column 2) Eurospher ODS 80 Å 5 μm (100 Å base silica) (these two columns had been made as similar as possible, from the same silica batch B911); (column 3) Nucleosil ODS 120 Å 5 μm and (column 4) LiChrospher ODS 100 Å 5 μm.

Eluents methanol (MeOH), n-pentane (nC$_5$) and tetrahydrofuran (THF) were HPLC grade from Riedel-de Haen, Germany. n-Alkanes (Aldrich) were of the highest available grade and used as received.

Reproducibility of data in five repetitive runs was 0.2–0.3% R.S.D. in low-temperature experiments. Accuracy was certainly lower due to imperfections in the cryostating apparatus. Reproducibility at higher temperatures (15–55°C) was 0.1% R.S.D. or better. The elution volume of a polystyrene standard (molecular mass $2.95 \cdot 10^6$) in THF eluent at 25°C was taken as interstitial volume. Total liquid volume was measured either gravimetrically or by injecting C^2H_3OH samples with MeOH eluent. Mobile phase volume in pores was calculated from the difference between total liquid volume and interstitial volume.

3. Data treatment

3.1. Corrections

Retention time data t_R from five runs were fed into a computer. If an outlier (>0.1% R.S.D.) for at least one solute was detected, the run was discarded and another one was started. In low-temperature experiments the R.S.D. limit was correspondingly increased. Outliers occurred sometimes with an insufficient time interval after a new temperature setting. Data were first averaged and pertaining statistical data were calculated (standard deviation and maximum data scatter). Next, averaged data were corrected for thermal expansion or contraction of the eluent and normalized to 25°C. Eq. 1 was used for correction:

$$t_R^*(25°C) = t_R(\tau°C) \cdot \frac{(1 + \gamma \cdot \tau)}{(1 + \gamma \cdot 25)} \tag{1}$$

where $\tau = K - 273 = °C$. This procedure is very similar to that of Colin et al. [3]. For MeOH, $\gamma = 0.001199$ and for nC_5, $\gamma = 0.001608/°C$ were taken [32], which is an approximation because γ is the cubic expansion coefficient at 20°C. Actually, expansion coefficients depend on temperature. Expansion of column tube and silica skeleton were not accounted for because expansion coefficient of stainless steel is very small, and no information is available on the thermal behaviour of silica.

If there were any changes in flow-rate, data were further normalized to a flow-rate of 1 ml/min at 25°C which yielded V_R^*. Finally, effective gross retention volumes V_{ms}^{eff} were obtained by subtracting extra column dead volume (at 25°C) from V_R^*. This proved to be important at the given relatively high precision level.

V^{eff} indicates that these data (effective or accessible volume data) are influenced by exclusion effects and may differ considerably from data solely controlled by the thermodynamic partition coefficient K^{th}. V_{ms}^{eff} values were used for calculation of dead volume and of ln k' specified as from "measured data".

Partial steric exclusion effects have recently been described in detail [25,55]. Although exclusion is not the objective of the present work, it will be necessary to discuss a few related effects in order to explain the general shape of measured retention functions. Exclusion refers to volumes contained in pores, the stationary phase volume V_{stat} and the "stagnant" part of mobile phase, V_{mp}. It is accounted for by the exclusion coefficient K^e:

$$V_{mp}^{eff} = V_{mp}K^e \quad (0 \leqslant K^e \leqslant 1) \tag{2}$$

$$V_{stat}^{eff} = V_{stat}K^e \tag{3}$$

As net retention volume $V_s = V_{stat}K^{th}$, effective net retention volume becomes

$$V_s^{eff} = V_{stat}K^{th}K^e \quad (0 \leqslant V_s^{eff} \leqslant V_s) \tag{4}$$

V_m consists of interstitial volume V_{int} and V_{mp} [56], so effective dead volume is

$$V_m^{eff} = V_{mp}^{eff} + V_{int}$$
$$= V_{mp}K^e + V_{int} \quad (V_{int} \leqslant V_m^{eff} \leqslant V_m) \tag{5}$$

In a system which is known to produce no retention at all, measured elution volumes are effective dead volumes, and all observed changes are solely caused by exclusion effects. Then it is easy to determine exclusion coefficients :

$$K^e = \frac{V_m^{eff} - V_{int}}{V_{mp}} = \frac{V_m^{eff} - V_{int}}{V_m - V_{int}} \tag{6}$$

V_{ms}^{eff}, being the sum of net retention volume and dead volume, can be written as

$$V_{ms}^{eff} = \left(V_{mp} + V_{stat}K^{th}\right)K^e + V_{int}$$
$$= (V_{mp} + V_s)K^e + V_{int} \tag{7}$$

It appears exceedingly difficult to determine K^e in systems producing so much retention that V_s (Eq. 7) is on the order of or greater than V_{mp}. If it is possible to make V_s small enough, Eq. 7 eventually approaches Eq. 5. We have approximated this situation by using n-pentane as eluent.

According to the treatment of Giddings et al. [33], K^e for "capsule shaped" molecules in a system of cylindrical pores, shows an exponential decrease with increasing ratio of solute length to pore diameter. n-Alkanes in non-polar or low-polarity solvents can be regarded as approximately cylindrical or capsule shaped. Their length (or better, mean external length $\bar{L}$ [33]) depends linearly on carbon number n_C. If this dependence is introduced into Giddings et al.'s expression for K^e, a generally non-linear decrease of K^e with increasing n_C is found, with pore diameter D_{pore} as parameter. In the range of small- to medium-size alkanes (up to about C_{30}) and larger pores (100 Å and wider), the decrease does not deviate much from linearity. With decreasing pore diameter, K^e decreases, as expected. Furthermore, curvature of K^e versus n_C functions may become more evident.

Elution functions $V_R^* = f(n_C)$ in systems assumed to have little or perhaps no retention, SiO_2/C_5 and ODS/C_5, are found to vary from practically linear to visibly curved. They can reasonably well be approximated using first- or higher-order polynomials :

$$V_m^{eff} = f(n_C) = \sum_{i=0}^{i=n} a_i n_c^i \qquad (8)$$

Zeroth order coefficient a_0 always gives the maximum dead volume because exclusion vanishes for vanishing solute size,

$$a_0 = V_m = V_{mp} + V_{int} \qquad (9)$$

This equation is used in solving Eq. 6.

As a matter of fact, existence of some retention cannot be ruled out with sufficient certainty in practical systems. Since retention as well as exclusion must vanish for vanishing solute size, we assume that the slope of an exclusion function has to be calculated from the first derivative of the elution function at $n_C = 0$. Then, $V_m^{eff} = a_0 + a_1$ in Eq. 6, a_0 and a_1 being 0th and 1st order coefficients of polynomials used to describe measured elution functions.

Exclusion coefficients calculated from low/no retention systems were tentatively applied without modification to retention data obtained with MeOH eluent in order to calculate exclusion-corrected gross retention volume:

$$V_{ms} = \frac{V_{ms}^{eff} - V_{int}}{K^e} + V_{int} \qquad (10)$$

This procedure could be an oversimplification because the extent of exclusion might be different in solvents having different properties.

3.2. Dead volume

Dead volume calculation was performed by establishing a function which relates V_{ms} of a series member having $n + 1$ carbon atoms to V_{ms} of the preceding one having n carbon atoms:

$$V_{ms}(n + 1) = f[V_{ms}(n)] \qquad (11)$$

This function is empirically found to be linear with correlation coefficient on the order of $r = 0.9998$:

$$V_{ms}(n + 1) = SL \cdot V_{ms}(n) + IN \qquad (12)$$

where SL and IN are slope and intercept, respectively.

While linearity is perfect with exclusion-corrected V_{ms} data, there is a small, but distinctly systematic deviation of non-corrected V_{ms}^{eff} data from linear regression, exceeding experimental data scatter at least by a factor of 10. Using $V_{ms} = V_s + V_m$ and the definition (Eq. 13) of selectivity α:

$$\alpha = V_s(n + 1)/V_s(n) \qquad (13)$$

it follows that

$$SL = \alpha \qquad (14)$$

$$IN = V_m(1 - \alpha) \qquad (15)$$

from which V_m is readily obtained.

This process is basically the same as that of Berendsen et al. [34]. Calculation yields effective V_m^{eff} which decrease with increasing n_C if measured data V_{ms}^{eff} are used, while V_{ms} data (without contribution from exclusion) yield constant V_m [25,54,55].

4. Results

4.1. Silica column, n-pentane eluent

Experimental retention times of n-alkanes C_1–C_{28} at nine temperatures from $-15°C$ to $+25°C$ in $5°C$ steps on an Eurospher silica column (column 1) are given in Table 1. The following is observed:

(1) Retention times t_R are highest at $-15°C$ and lowest at $+25°C$ for each solute.

(2) At each temperature, t_R of C_1–C_{28} decrease with increasing carbon number n_C. As seen from Fig. 1, this decrease is steepest from C_1 to C_2 and then gradually diminishes. Above $n_C = 6$ it seems to be linear, but data quality is not sufficient to unambiguously rule out some curvature.

Fig. 2 shows V_{ms}^{eff}, which is obtained from the above data (without C_1–C_4) by correction for thermal expansion of the eluent, if necessary for deviating flow-rate, and for extra column dead volume. There is only little temperature dependence left. It is observed that expansion-corrected elution volumes V_{ms}^{eff} increase slightly with increasing temperature. If straight regression

Table 1
n-Alkanes on silica with n-pentane eluent

n_C	Experimental retention time (min)								
	−15°C	−10°C	−5°C	0°C	5°C	10°C	15°C	20°C	25°C
1	2.724	2.703	2.692	2.665	2.635	2.621	2.593	2.576	2.555
2	2.673	2.664	2.660	2.625	2.604	2.588	2.568	2.557	2.541
3	2.650	2.642	2.633	2.610	2.590	2.581	2.550	2.540	2.526
4	2.643	2.635	2.623	2.596	2.583	2.557	2.542	2.529	2.512
6	2.632	2.617	2.608	2.582	2.570	2.546	2.533	2.514	2.497
7	2.627	2.613	2.599	2.579	2.565	2.544	2.529	2.509	2.492
9	2.613	2.601	2.587	2.567	2.554	2.532	2.517	2.501	2.482
11	2.599	2.587	2.568	2.553	2.539	2.521	2.505	2.488	2.475
13	2.586	2.570	2.555	2.542	2.527	2.506	2.495	2.480	2.464
15	2.572	2.557	2.543	2.530	2.514	2.495	2.488	2.469	2.459
17	2.566	2.547	2.531	2.515	2.505	2.485	2.477	2.457	2.446
19	2.540	2.528	2.512	2.504	2.488	2.475	2.467	2.448	2.436
22	2.523	2.509	2.494	2.491	2.475	2.458	2.448	2.433	2.419
28	2.521	2.495	2.459	2.454	2.440	2.435	2.421	2.405	2.392

Column 250 × 4 mm Eurospher silica, 100 A; 5 μm (column 1). Eluent nC$_5$, flow 1 ml/min at 25°C. Column temperature as indicated. Refractive index detection. Experimental retention times averaged from five runs.

lines C$_6$–C$_{28}$ are extrapolated to $n_C = 0$, all of them but the −15°C line yield almost identical intercepts of 2.523 ± 0.004 ml. Residual deviations are non-systematic and probably due to data scatter. Regression lines for V_{ms}^{eff} can be described by

$$V_{ms}^{eff}(n_C, \tau) = a_0 + a_1(\tau)n_C$$

$$= 2.523 + (-0.00613 + 6.43 \cdot 10^{-5}\, \tau)n_C \quad (16)$$

The temperature effect is small, but it persists if expansion correction is performed using the complete polynomial expression for expansion coefficient γ, and if thermal change of the column tube volume is accounted for.
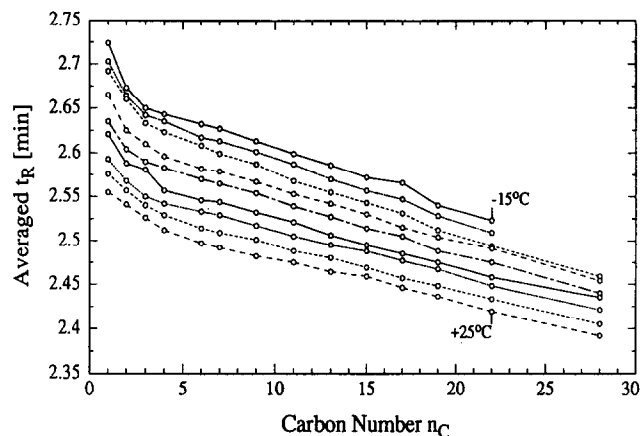

Fig. 1. Experimental retention times, t_R, ot n-alkanes C$_1$–C$_{28}$ at temperatures from −15 to +25°C in 5°C steps. Eurospher silica, 100 Å, 5 μm, 250 × 4 mm (column 1). Eluent n-pentane. Flow 1 ml/min at 25°C. Refractive index detection.
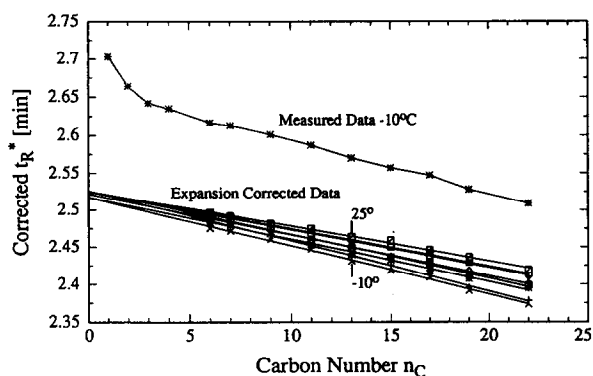

Fig. 2. Expansion-corrected retention times, t_R^*. Same data as in Fig. 1 (without −15° set) but corrected for thermal expansion of eluent, normalized to 25°C. Temperatures from original t_R data set. For comparison, measured t_R (−10°C) trace from Fig. 1 is included.

Liquid volume within the column as determined by weighing with different liquids (n-hexane, dichloromethane, carbon tetrachloride) was 2.516 ml and agreed with n-hexane data in Fig. 2.

Interstitial volume was found to be $V_{int} = 1.253$ ml at 25°C. The volume of mobile phase residing in pores is calculated to be $V_{mp} = 2.523 - 1.253 = 1.27$ ml at 25°C.

If it was assumed that there is no retention ($V_s = 0$), expansion-corrected elution volumes are effective dead volumes $V_{ms}^{eff} = V_m^{eff}$, and Eq. 9 can be used to determine exclusion coefficients:

$$K^e = 1 - (0.00483 - 5.06 \cdot 10^{-5} \, \tau)n_C \qquad (17)$$

According to this calculation, the pore-entering probability is reduced to about 90% for eicosane on the silica column.

In another calculation, C_1–C_4 were included and elution functions were represented by third-order polynomials. Intercept a_0 was found to be 2.566 ml (S.D. = 0.0056 or 0.2% R.S.D.) for all temperatures except $-15°$. For the first-order coefficient, $a_1 = -0.01922 + 1.28 \cdot 10^{-4} \, \tau$ was

obtained. With these figures, exclusion coefficient K^e is

$$K^e = 1 - (0.0146 - 9.74 \cdot 10^{-5} \, \tau)n_C \qquad (17a)$$

From this equation, a pore-entering probability of 75% is calculated for C_{20}.

4.2. ODS columns, n-pentane eluent

Experimental retention times t_R on the Eurospher ODS column (column 2) are given in Table 2. Fig. 3 shows retention behavior to be similar to that on the parent silica column (column 1). There are three notable differences: (1) t_R values are lower on ODS; (2) t_R of C_6–C_{32} are slightly curved, and curvature is more pronounced at lower temperature; and (3) curves run almost parallel to each other, deviations increase for higher n_C and lower temperature.

Temperature dependence is reduced after correction for thermal expansion of the eluent. Before correction, $-10°C$ t_R data were 0.15 ml higher than $+25°C$ data, after correction, the difference between respective V_R^* data is only

Table 2
n-Alkanes on ODS with n-pentane eluent

n_C	Experimental retention time (min)								
	$-15°C$	$-10°C$	$-5°C$	$0°C$	$5°C$	$10°C$	$15°C$	$20°C$	$25°C$
1	2.422	2.369	2.350	2.319	2.300	2.267	2.243	2.227	2.202
2	2.330	2.331	2.312	2.284	2.293	2.242	–	–	–
3	2.310	2.280	2.265	2.241	2.225	2.221	2.179	2.163	2.142
4	2.292	2.263	2.248	2.225	2.209	2.187	2.164	2.147	2.128
6	2.262	2.239	2.221	2.198	2.180	2.152	2.137	2.120	2.098
7	2.250	2.229	2.210	2.186	2.168	2.144	2.126	2.108	2.088
9	2.228	2.208	2.187	2.164	2.144	2.128	2.103	2.087	2.066
11	2.208	2.182	2.168	2.144	2.127	2.107	2.083	2.067	2.048
13	2.191	2.162	2.147	2.125	2.104	2.090	2.066	2.048	2.030
15	2.176	2.143	2.128	2.103	2.085	2.072	2.047	2.030	2.012
17	2.163	2.131	2.112	2.086	2.069	2.053	2.029	2.014	1.997
19	2.152	2.113	2.095	2.068	2.056	2.037	2.014	1.997	1.981
22	2.138	2.102	2.078	2.053	2.039	2.020	1.999	1.984	1.968
24	–	–	–	–	–	–	–	1.961	1.945
28	–	2.072	2.041	2.014	1.988	1.971	1.950	1.933	1.921
32	–	–	2.023	1.983	1.963	1.944	1.924	1.912	1.895

Column 250 × 4 mm Eurospher ODS, 80 Å, 5 μm (column 2). Eluent 1 ml/min nC_5 at 25°C. Column temperature as indicated. Refractive index detection. Experimental retention times averaged from five runs.
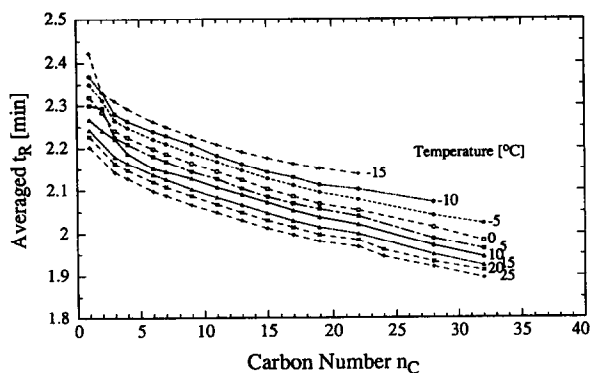
Fig. 3. Experimental retention times, t_R, of n-alkanes C_1–C_{28} (C_{32}) at temperatures from -15 to $+25°C$ in $5°C$ steps. Eurospher ODS, 100 Å, 5 μm, 250 × 4 mm (column 2). Eluent n-pentane. Flow 1 ml/min at 25°C. Refractive index detection.

0.03 ml. It was found that C_1–C_{20} data are well approximated by third-order polynomials of n_C. Because determination of polynomial coefficients is strongly influenced by data scatter, data visibly deviating from smooth curves in Fig. 3 were removed (C_{22} and C_{32}).

Intercept a_0 decreases linearly with increasing temperature:

$$a_0 = 2.247 - 0.0015\tau = 2.247(1 - 0.00067\tau) \quad (18)$$

First-order coefficient a_1 increases slightly with increasing temperature:

$$a_1 = -0.0255 + 1.62 \cdot 10^{-4}\,\tau \quad (18a)$$

Coefficients a_2 and a_3 are small and show severe scatter, but some temperature dependence is recognized:

$$a_2 = 9.54 \cdot 10^{-4} - 1.01 \cdot 10^{-5}\,\tau \quad (18b)$$

$$a_3 = -1.5 \cdot 10^{-5} + 1.7 \cdot 10^{-7}\,\tau \quad (18c)$$

Gravimetrically (2.208 ml at 25°C) and chromatographically determined liquid volume agreed reasonably well. Interstitial volume of this column was $V_{int} = 1.182$ ml, the mobile phase volume within pores $V_{mp} = 1.026$ ml, both at 25°C. The difference in V_{mp} between silica (column 1) and ODS derived therefrom (column 2) is 0.263 ml and probably due to space oc-

cupied by the bonded layer. Interstitial volume is slightly decreased, possibly because of better packing properties of bonded material [35].

Exclusion coefficients were calculated as shown in the data treatment section, using a_0 and a_1 from Eqs. 18 and 18a only.

$$K^e = \frac{a_0 + a_1 - V_{int}}{a_0 - V_{int}}$$

$$= 1 - \frac{0.0255 - 1.62 \cdot 10^{-4}\,\tau}{1.099 - 0.0031\tau}\,n_C \quad (19)$$

This procedure implies the presence of some retention in this system. A rough measure of that retention is given by higher-order coefficients a_2 and a_3.

As seen from Fig. 4, K^e on ODS (solid lines) are lower than on the parent silica (broken lines). This is probably due to narrower pores, but additional entropy effects might be superimposed [36].

Characteristic volume data at 25°C was measured also for the Nucleosil ODS column (column 3) which was later used for experiments with MeOH eluent. Total liquid volume V_{liq} from weighing with MeOH and CH_2Cl_2 (2.028 ml) and from retention of C^2H_3OH in MeOH eluent (2.041 ml) are almost identical. Interstitial volume was determined as described above to be 1.133 ml. Pore volume was calculated as $V_{mp} = 2.028 - 1.133 = 0.895$ ml.
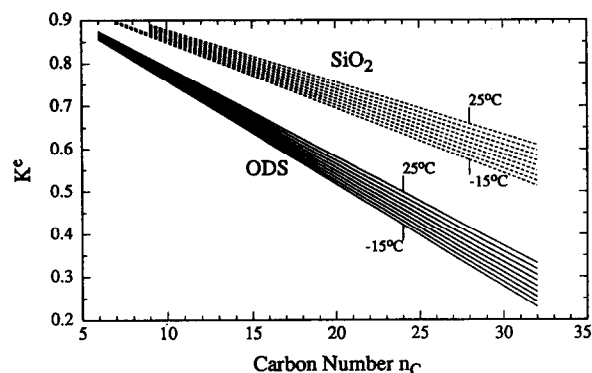

Fig. 4. Dependence of exclusion coefficients K^e on temperature and on solute carbon number for n-alkanes on ODS and on parent silica. Calculation based on data in Tables 1 and 2 (see text).

4.3. ODS columns, MeOH eluent

Retention data for n-alkanes, measured at 25°C on Eurospher ODS (column 2), averaged from 11 runs is shown in Table 3. Relative standard deviation is of the order of 0.05%. More retention data on this column have been published elsewhere [25]. Exclusion coefficients were calculated via Eq. 19 and applied to $V_{ms}^{eff} - V_{int}$ as shown in Eq. 10. Exclusion-corrected V_{ms} are higher than V_{ms}^{eff} (*e.g.* 3.370 *versus* 3.096 for C_6 up to 14.884 *versus* 10.016 for C_{17}) Sub-sets of V_{ms}^{eff} and of V_{ms} were subject to dead volume calculation [25]. With V_{ms}^{eff}, calculated V_m decreases from 1.866 ml for the C_6 to C_1 sub-set almost linearly to 1.634 ml for the C_{12} to C_{17} sub-set. Corrected V_{ms} yield constant $V_m = 1.862$ ml (0.6% R.S.D.). This value was used to calculate ln k' from V_{ms} ($k' = (V_{ms} - V_m)/V_m$) for all n_C, and ln $k' = -1.38596 + 0.19595n_C$ with correlation coefficient $r = 0.9999999$ was found.

We have observed with various ODS columns that changing K^e in Eq. 10 by trial and error until constant V_m is obtained yields the same K^e value as determined from experiments with n-pentane eluent.

The following experiments were performed in

Table 3
n-Alkanes on ODS with methanol eluent

n_C	t_R (min) (average)	S.D. (min)	R.S.D. (%)
6	3.152	0.002	0.08
7	3.384	0.002	0.06
8	3.664	0.002	0.04
9	3.994	0.002	0.05
10	4.388	0.002	0.05
11	4.856	0.002	0.05
12	5.409	0.003	0.05
13	6.065	0.002	0.04
14	6.836	0.003	0.05
15	7.746	0.003	0.04
16	8.815	0.004	0.04
17	10.072	0.006	0.06

Column 250 × 4 mm Eurospher ODS, 80 Å, 5 μm (column 2). Eluent MeOH, flow 1 ml/min at 25°C. Column temperature 25°C. Refractive index detection. Experimental t_R data averaged over 11 runs.

the +15° to +55°C range on a Nucleosil ODS column (column 3) with MeOH eluent and n-alkane solutes C_6–C_{17}. Table 4 gives retention times averaged over five runs. Exclusion coefficients showed slight temperature dependence which was on the same order of magnitude as observed with nC_5 eluent.

After correction of experimental data for expansion and exclusion, V_{ms} values were used to calculate the dead volume at each temperature. Fig. 5 shows a remarkable dependence of V_m on temperature, V_m being high at 15°C and low at 55°C. This dependence is more pronounced than in Eq. 18. It seems that a second-order polynomial (solid line) fits data better than a first-order one (broken line):

$$V_m = 2.054 - 0.0033\tau + 1.96 \cdot 10^{-5}\tau^2 \qquad (20)$$

It is seen that $V_m = 1.983$ ml at 25°C is slightly lower than V_{liq}.

Similar effects have been found with a variety of ODS columns [26–28]. LiChrospher ODS (column 4) with MeOH eluent is shown as an example in Fig. 6. The nC_{10} trace (squares) was calculated from experimental data, which yield V_m^{eff} depending on both n_C and temperature. After correction for thermal expansion, the slope is somewhat reduced ($\blacklozenge$). Triangles show the temperature dependence of V_m as determined with C^2H_3OH as marker. It has been shown that CD_3OH indicates the total eluent volume in the column, immobilized fraction in the stationary phase included [37]. Temperature dependence of this "dead volume" disappears upon correction for thermal expansion ($\bigcirc$), as expected from earlier work [10]. The volume found with C^2H_3OH is generally greater than V_m from homologous series. With H_2O as marker, no temperature dependence was seen (asterisks).

From exclusion corrected V_{ms} and temperature dependent V_m (Eq. 20), ln k' displayed in Fig. 7 were calculated. Ln k' increase linearly over carbon number n_C for each temperature:

$$\ln k'(n_C, \tau) = a(\tau) + b(\tau)n_C \qquad (21)$$

Linearity of ln k' over n_C is further documented in Table 5 which shows intercepts a and

Table 4
n-Alkanes on ODS with methanol eluent

Table 4 (continued)

n_C	T (K)	t_R (min) (average)	S.D. (min)	Δ_{max} (min)	n_C	T (K)	t_R (min) (average)	S.D. (min)	Δ_{max} (min)
6	288	3.307	0.0021	0.007	6	308	3.067	0.0004	0.001
7		3.557	0.0018	0.005	7		3.253	0.0004	0.001
8		3.864	0.0026	0.007	8		3.477	0.0008	0.002
9		4.239	0.0026	0.008	9		3.740	0.0004	0.001
10		4.697	0.0011	0.003	10		4.053	0.001	0.003
11		5.251	0.0023	0.007	11		4.423	0.0004	0.001
12		5.924	0.0016	0.005	12		4.857	0.0004	0.001
13		6.735	0.0059	0.019	13		5.366	0.001	0.002
14		7.712	0.0016	0.005	14		5.964	0.0012	0.003
15		8.889	0.0043	0.010	15		6.661	0.003	0.007
16		10.295	0.0027	0.008	16		7.471	0.0028	0.007
17		11.979	0.0059	0.017	17		8.421	0.0035	0.010
6	293	3.238	0.0019	0.005	6	313	3.013	0.0004	0.001
7		3.470	0.0021	0.006	7		3.187	0.0005	0.001
8		3.753	0.0012	0.003	8		3.395	0.0004	0.001
9		4.093	0.0026	0.008	9		3.638	0.0004	0.001
10		4.507	0.0022	0.007	10		3.926	0.001	0.002
11		5.005	0.0026	0.007	11		4.263	0.0004	0.001
12		5.603	0.0027	0.008	12		4.657	0.0008	0.002
13		6.319	0.0022	0.006	13		5.116	0.001	0.002
14		7.173	0.0024	0.006	14		5.650	0.0004	0.001
15		8.194	0.0022	0.006	15		6.272	0.001	0.003
16		9.403	0.0043	0.010	16		6.994	0.0022	0.006
17		10.839	0.0056	0.015	17		7.825	0.0032	0.008
6	298	3.180	0.0013	0.004	6	318	2.965	0.0018	0.005
7		3.396	0.001	0.002	7		3.128	0.0019	0.005
8		3.656	0.001	0.002	8		3.321	0.0017	0.005
9		3.969	0.001	0.002	9		3.545	0.0021	0.006
10		4.345	0.0013	0.004	10		3.809	0.0016	0.004
11		4.795	0.0015	0.004	11		4.117	0.0018	0.005
12		5.331	0.001	0.002	12		4.473	0.0019	0.005
13		5.968	0.0005	0.001	13		4.886	0.0034	0.010
14		6.724	0.001	0.002	14		5.367	0.0016	0.005
15		7.618	0.0012	0.003	15		5.921	0.0017	0.005
16		8.673	0.0019	0.005	16		6.560	0.0008	0.002
17		9.915	0.0018	0.004	17		7.294	0.0030	0.008
6	303	3.121	0.0013	0.003	6	323	2.916	0.001	0.002
7		3.321	0.0017	0.005	7		3.068	0.0004	0.001
8		3.562	0.0011	0.003	8		3.247	0.0005	0.001
9		3.850	0.0013	0.004	9		3.455	0.0004	0.001
10		4.190	0.0011	0.003	10		3.696	0.0034	0.009
11		4.599	0.0012	0.003	11		3.978	0.0004	0.001
12		5.081	0.0018	0.005	12		4.303	0.0005	0.001
13		5.650	0.0013	0.004	13		4.676	0.001	0.002
14		6.320	0.0016	0.005	14		5.107	0.0004	0.001
15		7.109	0.0017	0.005	15		5.603	0.0012	0.003
16		8.033	0.0016	0.005	16		6.170	0.0008	0.002
17		9.115	0.0022	0.005	17		6.821	0.0017	0.005

Table 4 (continued)

n_C	T (K)	t_R (min) (average)	S.D. (min)	Δ_{max} (min)
6	328	2.868	0.0008	0.002
7		3.012	0.0005	0.001
8		3.178	0.0005	0.001
9		3.371	0.001	0.002
10		3.592	0.0005	0.001
11		3.850	0.0013	0.004
12		4.145	0.0013	0.004
13		4.483	0.0008	0.002
14		4.871	0.0019	0.005
15		5.315	0.0015	0.004
16		5.821	0.0012	0.003
17		6.397	0.001	0.003

Column 250 × 4 mm Nucleosil ODS, 120 A; 5 μm (column 3). Eluent 1 ml/min MeOH at 25°C. Column temperature as indicated. Refractive index detection. t_R, raw data, averaged from five runs, includes extra column dead time. S.D. in five runs. Δ_{max} data span, max–min value in five runs.

slopes b of the respective regression lines, together with correlation coefficients which are high, of the order of $r = 0.999999$.

Regression lines for ln k' have been extrapolated to $n_C = 0$. It is seen from Fig. 7 that ln k' intersect and become independent of temperature at a hypothetical carbon number of ca. 4.

In Fig. 8, ln k' values from Fig. 7 have been plotted over $1000 \cdot (1/T)$. Again, plots are linear. There is a small irregularity in the vicinity of 40°C. If ln $k'/(1/T)$ values are extrapolated to
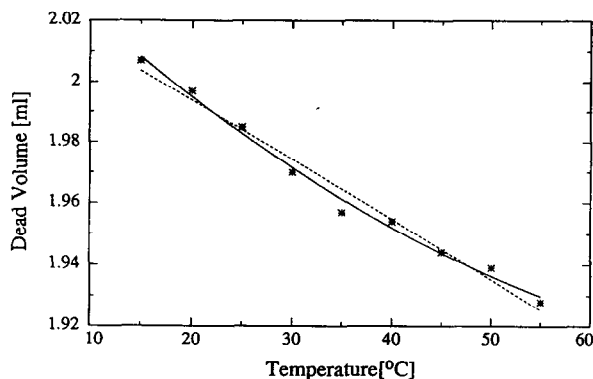

Fig. 5. Dead volume calculated from n-alkane retention data in Table 4 (Nucleosil ODS/MeOH, column 3) corrected for thermal expansion and partial exclusion, as function of temperature.
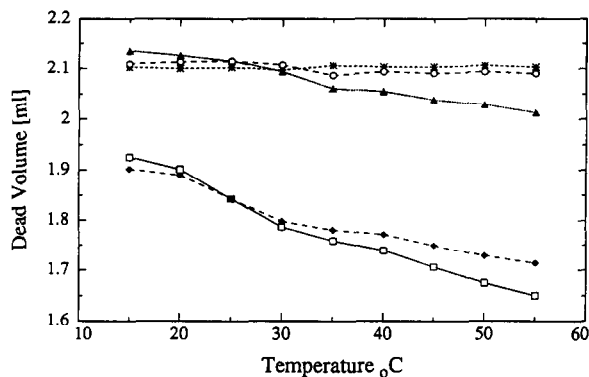

Fig. 6. Dead volume determination on LiChrospher ODS, 100 Å, 5 μm, 250 × 4 mm (column 4) at various temperatures. Eluent methanol. Flow 1 ml/min. $\square$ = solute n-decane; $\blacklozenge$ = same data, but corrected for thermal expansion; $\blacktriangle$ = solute C^2H_3OH; $\bigcirc$ = same data, but corrected for thermal expansion; $*$ = solute H_2O.

higher temperatures, all intersect at $(1/T) = 0.00197$ or $\tau = 235°C$.

Slopes b are linear over $1/T$ with high correlation coefficients. Intercepts a show moderate deviations from linearity if plotted either over $1/T$ or over T, so the actual temperature dependence of a cannot be determined from these data.

5. Discussion

5.1. Silica column, n-pentane eluent

The elution function of n-alkanes on this silica column is strongly curved in the C_1–C_6 range and almost linear for longer-chain solutes. Actually, a certain degree of non-linearity is anticipated as outlined in the data treatment section. This packing material, however, has nominally 100 Å pores and a relatively wide pore size distribution [25], so that curvature is likely to be small and obscured by data scatter.

It is not quite clear whether smallest solutes C_1–C_4 should be included in the evaluation of exclusion coefficients or not. On one hand, the elution function of alkanes longer than eluent molecules looks fairly linear which suggests that the onset of higher retention times at C_4 may
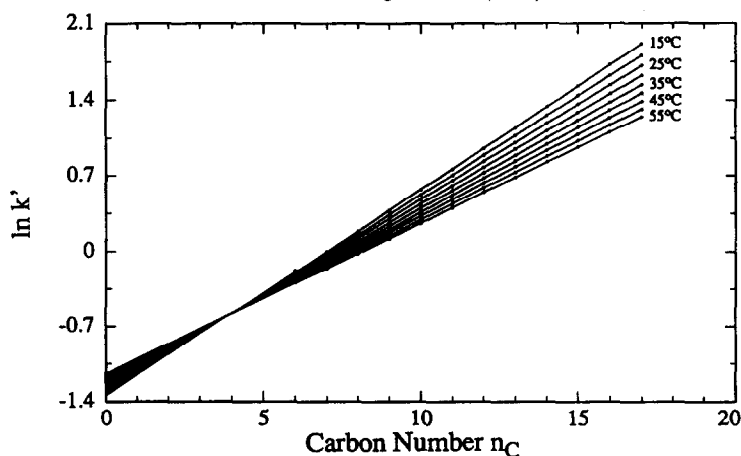
Fig. 7. Logarithm of capacity factor, ln k', as function of alkane carbon number n_C at various temperatures. Data (dots) derived from Table 4 (Nucleosil ODS/MeOH, column 3) corrected for thermal expansion and partial exclusion. Regression lines extrapolated to $n_C = 0$.

Table 5
Parameters of ln $k' = a + bn_C$ with correlation coefficient r

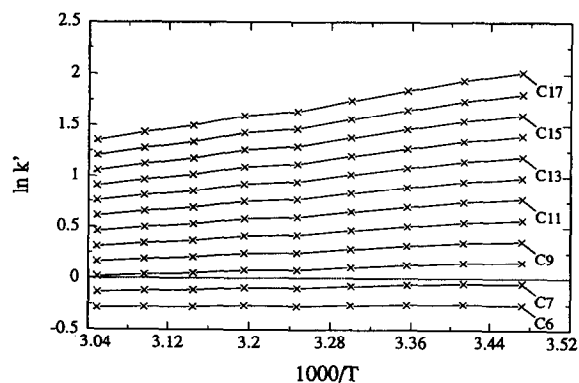| Temperature (°C)	a	b	r
15	−1.49856	0.20735	0.99999987
20	−1.43564	0.19791	0.99999995
25	−1.39008	0.18977	0.99999997
30	−1.34988	0.18206	0.99999998
35	−1.31337	0.17501	0.99999991
40	−1.27102	0.16809	0.99999994
45	−1.23861	0.16153	0.99999991
50	−1.20492	0.15521	0.99999991
55	−1.168	0.14896	0.99999987

Calculation based on data from Table 4.

Fig. 8. Data from Fig. 7, plotted as function of $1000/T$ (Van 't Hoff plot) for carbon numbers 6–17.

indicate an extra effect, and C_1 to C_4 should be omitted. On the other hand, molecular cross-section of C_5 and longer alkanes is practically the same as that of C_1. Consequently, alkanes with $n_C > 1$ can enter the same pores as C_1, even if entering probability decreases. Very high concentration of eluent C_5 probably overrides lowered entering probability so that the eluent can effectively use all pores being accessible to C_1. It is mainly for this reason that we prefer to include the smallest solutes in K^e determination. For this purpose, the elution function is described by a third-order polynomial.

Intercept a_0 of third-order polynomials at $n_C = 0$ does not depend on temperature if expansion-corrected data are used. Because retention (if there is any) as well as exclusion must vanish for vanishing molecular dimensions, this intercept can be interpreted as the maximum possible dead volume, which is the sum of V_{int} and V_{mp}.

Results obtained in the SiO_2/nC_5 system suggest that elution volumes of n-alkanes are primarily controlled by exclusion. Temperature dependence of expansion-corrected data is small and in "wrong" order, *i.e.*, higher retention times are found with higher temperature. We assume that ΔH is very small. This may be interpreted using Snyder's displacement model of adsorption chromatography [38,39], in this particular case for non-polar, non-localizing solutes and eluent molecules. Prior to sorption of a

longer-chain alkane, the silica surface is covered with nC_5. Because interaction of alkanes with any surface is purely dispersive, there is no preferred orientation of in-plane sorbed molecules. If an alkane molecule is to get into contact with the silica surface, it has to displace an adequate number of nC_5 molecules. Consequently, net enthalpy of interaction of n-alkane solutes with silica is expected to be low. Existing differences between (entropic) activity coefficients of C_6 to C_{17} in nC_5 eluent are assumed to be widely cancelled by similar effects in the sorbed monolayer [38,39], so they are not likely to influence retention significantly.

Overall ΔG^{sorp} is therefore expected to be quite small but not necessarily zero. After exclusion-correction according to Eq. 10 using K^e from Eq. 17a, V_{ms} was obtained. Temperature dependence of V_{int} was accounted for in this calculation. Net retention volume V_s was calculated by subtracting $V_m = a_0$ from V_{ms}. Resulting V_s values are small, increasing almost linearly from ca. 0 for smaller solutes to ca. 0.3 ml for C_{22}. These values are so small that, regarding inaccuracy of data and uncertainty of the exclusion model, no further comments can be made.

The slight temperature dependence of slope in Eq. 16 suggests K^e to be weakly temperature dependent (see Fig. 4). The reason for this effect is not really clear, but we assume that temperature has a slight influence on exclusion via temperature-dependent diffusion of molecules from mobile phase stream paths to pores. The increase of influence of temperature on K^e with increasing solute size seems to support this hypothesis.

5.2. ODS columns, n-pentane eluent

Exclusion behavior of n-alkanes in the ODS/nC_5 system is expected to be similar to that in SiO_2/nC_5 systems. It has been shown [25] that pore size distribution of an ODS packing differs from that of parent silica mainly in the most -abundant pore width which is shifted to smaller values. In addition, some pores might get plugged, thus becoming inaccessible to solutes and eluent.

The shift of pore diameters to smaller values

must necessarily cause a stronger dependence of exclusion on solute size [33], without changing general characteristics of the exclusion function. Stronger exclusion is expressed in a lower value of K^e, causing a decrease of V_R^* as compared to parent silica, which is seen in experimental results. This suggests that elution functions observed with alkanes in the ODS/nC_5 system may be mainly governed by exclusion, but it seems realistic to assume that thermodynamic effects could be involved, to a certain extent [36].

In order to find exclusion functions, the same formalism as for SiO_2/C_5 was used, because there is no reason to assume that exclusion behaviour should be principally different. Consequently, a linear function of n_C was obtained. Temperature dependence of K^e is practically the same as in the SiO_2/nC_5 system which may indicate that this effect originates in the mobile phase.

Contrary to the SiO_2/nC_5 system, V_m depend on temperature with ODS/nC_5. The fact that dead volume of an ODS column depends on temperature is remarkable and needs explanation. As the only perceivable difference between ODS and SiO_2 columns used in these experiments is the presence of the bonded layer, we assume that the stationary phase expands with increasing temperature. A wetted ODS phase should be regarded as a non-isotropic liquid, the maximum layer thickness of which will probably be on the order of C_{18} chain length in all-trans conformation. Bonded chains are likely to serve as "backbone" of the stationary phase, and sorbed eluent molecules sit in between chains. Depending on eluent polarity and temperature, bonded chain conformation will vary. At low eluent polarity and higher temperature, chains tend to become more mobile and to expand [40], thereby increasing stationary phase volume. Since the total volume available for stationary plus mobile phase varies only very slightly with temperature, an increase of V_{stat} will necessarily cause a decrease of V_m.

Exclusion-corrected V_{ms} were used to calculate V_s, in the same way as shown for SiO_2/nC_5, the only difference being temperature-dependent V_m. Again, V_s values are unusually low. They range from ca. 0 for C_1–C_6 to ca. 1 ml for C_{32}.

The increase is close to exponential. There is no information about the origin of this retention.

5.3. ODS columns, MeOH eluent

It has been shown in the Results section that ln k' values are strictly linear over n_C in this system at all investigated temperatures. Furthermore, ln k' values increase linearly over $1/T$ for each solute C_6 through C_{17}. The small irregularity observed in Fig. 8 is probably caused by slightly deviating temperatures. It might, however, as well be comparable to effects observed by Tchapla *et al.* [16] and by Cole and co-workers [17,18]. The latter researchers investigated a wide temperature range and found nonlinear Van 't Hoff plots in various systems.

The most remarkable finding seems to be that, also in this much different system, dead volume decreases with increasing temperature. Although data precision is high, it is still not sufficient to exactly determine the functional relation between V_m and temperature. The actual dependence is probably a relatively complicated function of T, due to anisotropy of the stationary phase. It has to be pointed out that temperature dependence of dead volume in the ODS/MeOH system is more pronounced than in the ODS/nC$_5$ system. This may indicate that the observed effect is not simply expansion of a common liquid, but mainly controlled by bonded chains stretching. Bonded chains are likely to be more compressed in MeOH environment than in nC$_5$, and the effect of temperature is expected to be stronger.

The linear dependence of ln k' on n_C (Fig. 7 and Table 5) can be described as shown in Eq. 21. This implies that each methylene group in the alkyl chain contributes a constant, additive increment, b, to ln k'. In terms of sorption free energy, this ln k' incrementation may be written as follows

$$\ln k'(n_C, T) = -\frac{\Delta G^{sorp}}{R} \cdot \frac{1}{T} + \ln \phi(T)$$
$$= -\frac{\Delta G_r^{sorb}}{R} \cdot \frac{1}{T} - n_C \cdot \frac{\Delta G_2^{sorp}}{R} \cdot \frac{1}{T}$$
$$+ \ln \phi(T) \tag{22}$$

where ΔG_r^{sorp} and ΔG_2^{sorp} are (formal) increments of terminal groups (two hydrogen atoms) and of a CH$_2$ group, respectively, to ΔG^{sorp}, and $\phi(T)$ is the phase volume ratio V_{stat}/V_m which does not depend on n_C as data are exclusion-corrected. Eq. 22 is a description of experimental facts using thermodynamic terms, rather than a consequence of thermodynamics. A similar formalism has been used by several other authors [7,11].

It is very unlikely that ΔG^{sorp} depends linearly on n_C without ΔH^{sorp} and ΔS^{sorp} showing the same behavior. It was therefore assumed that ΔH^{sorp} and ΔS^{sorp} can be split into methylene and residual hydrogen contributions as was done with ΔG^{sorp}. This yields

$$\ln k'(n_C, T) = -\frac{\Delta H_r^{sorb}}{R} \cdot \frac{1}{T} - n_C \cdot \frac{\Delta H_2^{sorp}}{R} \cdot \frac{1}{T}$$
$$+ \frac{\Delta S_r^{sorb}}{R} + n_C \cdot \frac{\Delta S_2^{sorp}}{R} + \ln \phi(T) \tag{23}$$

Eq. 23 interprets slope b and intercept a of Eq. 21 in terms of thermodynamic quantities:

$$b = -\frac{\Delta H_2^{sorp}}{R} \cdot \frac{1}{T} + \frac{\Delta S_2^{sorp}}{R} \tag{24}$$

$$a = -\frac{\Delta H_r^{sorp}}{R} \cdot \frac{1}{T} + \frac{\Delta S_r^{sorp}}{R} + \ln \phi(T) \tag{25}$$
$$\quad \text{I} \qquad\qquad \text{II} \qquad \text{III}$$

Slope b (Eq. 24) as function of $1/T$ yields another linear relation with $-\Delta H_2^{sorp}/R$ as slope and $\Delta S_2^{sorp}/R$ as intercept. Linearity indicates that ΔH_2^{sorp} and ΔS_2^{sorp} are constant in the investigated temperature range:

$$\Delta H_2^{sorp} = -1142 \text{ J mol}^{-1} \tag{26}$$

$$\Delta S_2^{sorp} = -2.24 \text{ J K}^{-1} \text{ mol}^{-1} \tag{27}$$

Negative ΔH^{sorp} shows that retention of *n*-alkanes is enthalpy driven in the ODS/MeOH system. Negative sorption entropy probably indicates an origin in the sorbed state, where solutes loose most of their degrees of freedom of molecular motion. A (positive) entropy contribution from collapsing cavities seems to be less important in this system [41].

Intercept a (Eq. 25) cannot be evaluated in

this simple way because it contains the phase ratio term. While term I in Eq. 25 is a function of $1/T$, term III has probably a different temperature dependence. From an approach relating reversed-phase retention to molecular surface area [42,43] it can be concluded that the sum of terms I and II of Eq. 25 is approximately equal to b. Then

$$\ln \phi \approx a - b \qquad (28)$$

From data in Table 5 it is seen that, with this approximation, phase ratio ϕ varies between 0.18 at 15°C and 0.27 at 55°C. With these figures, V_{stat} is calculated to increase from 0.37 ml at 15°C to 0.49 ml at 55°C. The corresponding formal expansion coefficient of the stationary phase is 0.0094 per °C, which is distinctly higher than with a real liquid.

The common intersection point (CIP) of $\ln k'/(1/T)$ seems to be similar to the ones reported by Tchapla et al. [16]. It means that at hypothetical temperature T_x, all n-alkanes C_6–C_{17} would have the same retention, which can occur only if n_C-dependent terms of Eq. 23 cancel at this temperature:

$$\Delta H_2^{sorp} = \Delta S_2^{sorp} \, T_x \qquad (29)$$

$$T_x = 509 \text{ K}$$

Eq. 29 is an example of linear free energy relationships in which common intersection points may be encountered and related to entropy–enthalpy compensation effects. The above calculated T_x is called compensation temperature [2,14,42–52].

The CIP of $\ln k'/n_C$ functions at different temperatures is rather unexpected. It says that at hypothetical carbon number n_x, retention becomes temperature-independent. This, in turn, requires the sum of temperature-dependent terms to be constant at n_x

$$\ln k' = -\frac{\Delta H_x^{sorp}}{RT} + \frac{\Delta S_x^{sorp}}{R} + \ln \phi(T)$$

$$= \text{constant} \qquad (30)$$

where H_x^{sorb} and ΔS_x^{sorp} are the enthalpy and entropy of sorption of a n-alkane having n_x carbon atoms.

The entropy term of Eq. 30 is negative and temperature-independent. The enthalpy term is positive and decreases with increasing temperature. The phase ratio term is negative and becomes less negative with increasing T. In order to keep $\ln k'$ constant when T changes, an alteration of the ΔH term appears to be compensated by an opposite change of the $\ln \phi$ term. This result is surprising as it relates phase ratio to heat of sorption or vice versa.

6. Conclusions

Experiments have shown that there may be two temperature effects which have not been observed so far:

Temperature dependence of expansion-corrected retention data in the SiO_2/nC_5 system suggests a slight influence of temperature on exclusion, possibly through temperature dependence of diffusion. Similar dependence of K^e on temperature was found in ODS/nC_5 and $ODS/MeOH$ systems.

Maximum dead volume (for $n_C \to 0$) in ODS/nC_5 and $ODS/MeOH$ systems depends on temperature, even if data are corrected for thermal expansion. No such dependence is found in a silica/nC_5 system. The observed decrease of V_m with increasing temperature is thought to be caused by an increase of stationary phase volume. As a consequence, phase ratio ϕ must necessarily increase with increasing temperature. The usual way to determine heat of sorption from the slope of $\ln k'$ over $1/T$ does obviously not yield ΔH proper, but the sum of ΔH and the temperature change of $\ln \phi$, instead. This problem has been mentioned earlier by Grushka et al. [11].

If C^2H_3OH is used as dead volume marker, total liquid volume in the column is found. This value does not depend on temperature in excess of the thermal expansion effect.

n-Alkanes in SiO_2/nC_5 and ODS/nC_5 systems have little retention, and retention times are mainly controlled by exclusion effects. Small solutes CH_4 to C_4H_{10} have higher retention

times than expected from larger homologues, probably due to easier access to narrow pores.

In ODS/MeOH systems, temperature has its strongest influence in the sorption enthalpy term. The occurrence of a common intersection point in the dependence of ln k' on $1/T$ for a set of n-alkanes requires description of ln k' by a relation like Eq. 23, in which compensation of terms can occur. This equation relates constant and additive increments of thermodynamic quantities to a temperature-independent molecular property, in this case to carbon number n_C. Basically, other properties proportional to carbon number may be chosen, as is frequently done with molecular surface area (e.g. refs. 42–49,53), which, in turn, is related to cavity energy. Eq. 23 is another example of frequently observed linear free energy relationships in which common intersection points can occur, due to entropy–enthalpy compensation effects [2,14,49–52]. There appears to be another common intersection point in the dependence of ln k' on n_C for different temperatures. This phenomenon leads to a relation between phase ratio and heat of sorption. Its origin is unclear and requires further investigation at a higher data precision level.

7. Symbols and abbreviations

a_i	polynomial coefficient of order i
a	intercept of linear dependence of ln k' on n_C
b	slope of linear dependence of ln k' on n_C
D_{pore}	pore diameter
ΔG^{sorp}	free energy of sorption (standard conditions)
ΔG_r^{sorp}	sorption free energy increment of terminal groups
ΔG_2^{sorp}	sorption free energy increment of 1 CH_2 group
ΔH^{sorp}	sorption enthalpy (standard conditions), same subscripts as with ΔG^{sorp}
IN	intercept of $V_{ms}(n+1)/V_{ms}(n)$ function
k'	capacity factor V_s/V_m
K^e	exclusion coefficient
K^{th}	thermodynamic partition coefficient
$\bar{L}$	mean external length of molecule
n_C	carbon number of molecule
ODS	octadecyl silica
r	correlation coefficient
R	gas constant (8.314 J K^{-1} mol^{-1})
R.S.D.	relative standard deviation in %
ΔS^{sorp}	sorption entropy (standard conditions), same subscripts as with ΔG^{sorp}
S.D.	standard deviation based on $(n-1)$
SL	slope of $V_{ms}(n+1)/V_{ms}(n)$ function
t_R	experimental retention time
t_R^*	t_R corrected for thermal expansion
T	temperature in K
V_0	dead volume, non-specified
V_R^*	experimental retention volume, corrected for thermal expansion
V_{int}	interstitial volume
V_{liq}	total liquid volume in column
V_m	dead volume (maximum value)
V_m^{eff}	effective (accessible) part of V_m
V_{mp}	volume of mobile phase in pores
V_{mp}^{eff}	effective (accessible) part of V_{mp}
V_{ms}	gross retention volume
V_{ms}^{eff}	effective gross retention volume
V_s	net retention volume
V_s^{eff}	effective net retention volume
V_{stat}	volume of stationary phase
V_{stat}^{eff}	effective (accessible) part of V_{stat}
x	(subscript) refers to conditions at common intersection point
α	selectivity (relative retention), here $V_s(n+1)/V_s(n)$
γ	cubic coefficient of thermal expansion
τ	temperature in°C
ϕ	V_{stat}/V_m = phase ratio

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