



ELSEVIER

Journal of Chromatography A, 872 (2000) 49–59

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Low-energy interactions in high-performance liquid chromatography

Y.V. Kazakevich^{a,*}, H.M. McNair^b

^aSeton Hall University, S. Orange, NJ 07079, USA

^bVPI&SU, Blacksburg, VA 24061, USA

Received 13 September 1999; received in revised form 29 October 1999; accepted 15 November 1999

Abstract

Liquid chromatographic systems with very weak excessive analyte–adsorbent interactions have been studied. These systems consisted of a homologous series of *n*-alkanes as both analytes and mobile phases with a C₁₈ reversed-phase adsorbent. A linear decrease of the analyte retention volume with an increase of the number of analyte carbon atoms was found. Corresponding increases of analyte retention with an increase in the number of eluent carbon atoms was also discovered. An explanation of these two effects on the basis of adsorption theory is proposed. A good correlation of column hold-up volume calculated by interpolation of the retention dependencies for above mentioned systems with that measured by the minor disturbance method has been shown. A study of the temperature dependencies of these alkane systems has shown entropy-governed retention dependencies. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Low-energy interactions; Hold-up volume; Temperature dependence; *n*-Alkanes

1. Introduction

The relationship between the chromatographic behavior of the analyte and the thermodynamic parameters of the system (adsorption energy, partitioning constant, etc.) is one of the most interesting areas of chromatographic theory [1–7] and is indispensable for the understanding of many chromatographic effects [5–7].

It has been shown that the theory of excess adsorption from solutions could be used for the description of liquid chromatographic retention assuming an ideal system [8–11].

All theoretical approaches end up with equations relating retention volume with thermodynamic parameters of that system. All these equations contain one important parameter – hold-up volume (V_0).

Discussions regarding both the definition and measurement of the hold-up volume have been very active [5,12–15]. The most appropriate definition (in our opinion) is “Hold-up volume is the total volume of the liquid phase in the column”. This definition is equivalent to “Nothing is Adsorbed” convention introduced by Riedo and Kovats [8]. Application of this definition to the actual measurement of hold-up volume has been previously described [14,15].

In general, a component should elute with the hold-up volume when it has zero excess interaction with the adsorbent surface. This means that it behaves exactly as the eluent (assuming single-component eluent). One question is what kind of adsorption system (or chromatographic system) can show zero excess analyte interactions with the surface and at the same time be close to ideal conditions.

Obviously weaker intermolecular and surface in-

*Corresponding author.

teractions will approximate an ideal system. A system composed of a hydrophobic adsorbent (reversed-phase), hydrophobic analyte and eluent (alkanes) will show weak excessive adsorption and approach ideality. Such a system may be, for example, a reversed-phase (ODS) column with hexane as an eluent and heptane as an analyte.

These types of experiments have already been made [16,17]. Mockel and Dreyer have reported that retention of *n*-alkanes with *n*-pentane as the eluent on a reversed-phase column has a linear dependence on the number of analyte carbon atoms with a negative slope (decreasing retention). The authors ascribe this effect to the size-exclusion of the analytes from the internal pore volume and used their results to calculate the hold-up volume with a correction for this exclusion effect. If there is only a size-exclusion effect, then why is the retention dependence linear with the number of carbon atoms (or essentially molecular mass)? In gel permeation chromatography (GPC) the dependence of $\log(M_r)$ vs. V_r is linear.

We report here the extension of these experiments but with different eluents. We also studied the temperature dependencies from the point of view of excess adsorption and propose a thermodynamic interpretation for these dependencies.

2. Experimental

The experimental system consisted of a Hitachi LC-6200 reciprocating piston pump, an HP Model 1050 autosampler, a Waters R-401 refractive index detector, and an HP Model 3396 computing integrator.

Extracolumn volumes (between injector and detector cell) have been measured by removing the column and connecting the injector line directly to the detector. A 1- μ l volume of a 50 ppm solution of heptane in hexane was injected and extracolumn volume determined to be 23 μ l.

The column used was a Prodigy ODS2 (Phenomenex, Torrance, CA, USA) of 150 \times 4.6 mm, thermostated (25°C) with a water jacket (Alltech, Chicago, IL, USA) and circulating water bath (Brinkmann, NY, USA).

Technical details for the column were supplied by

Phenomenex: adsorbent surface area, $S=310$ m²/g; pore volume, $V_{\text{pore}}=1.17$ ml/g, pore diameter, $d_{\text{pore}}=150$ Å, (manufacturer's data). Our experimental data for V_0 measurements are shown in Fig. 3 and Table 2.

Retention of a series of *n*-alkanes (from pentane to hexadecane) was measured using hexane, decane, tetradecane and heptadecane as eluents. Flow rates of 1.0 ml/min was used in all experiments and the column was thermostated at 25°C.

The column was equilibrated with each eluent for at least 25 column volumes (~50 min) before making injections. Six replicate injections were made for each analyte, which had been diluted to 0.1% (v/v) in the eluent. Reproducibility of the retention volumes in six repetitive injections ranged from 0.2–0.3% relative standard deviation (RSD).

The effect of column temperature on the retention times of *n*-alkanes was also measured. Column temperatures of 15, 25, 35, 45 and 55°C were investigated using water jacket attached to the circulating water bath. Temperature stability was better than 0.5°C [measured with a Cole-Parmer thermocouple thermometer (0.05°C accuracy). The thermocouple was inserted into the water jacket and wrapped around the column].

3. Results and discussion

Experimental results on the retention of *n*-alkanes eluted with *n*-alkane mobile phases on a reversed-phase column are shown in Fig. 1 and Table 1.

We first highlight the main discrepancies of these experimental results with common chromatographic practice.

As can be seen in Fig. 1, the retention of alkanes decreases linearly with an increase of the number of analyte carbon atoms. This decrease with the increase of analyte molecular mass is usually attributed to a size-exclusion process. As discussed before, classical size-exclusion process usually shows a linear dependence of the retention volume on the logarithm of the analyte molecular mass, rather than linear relationship of the molecular mass itself vs. the retention volume, as it is shown here.

A second unusual effect is the increase of analyte retention with an increase of the eluent molecular

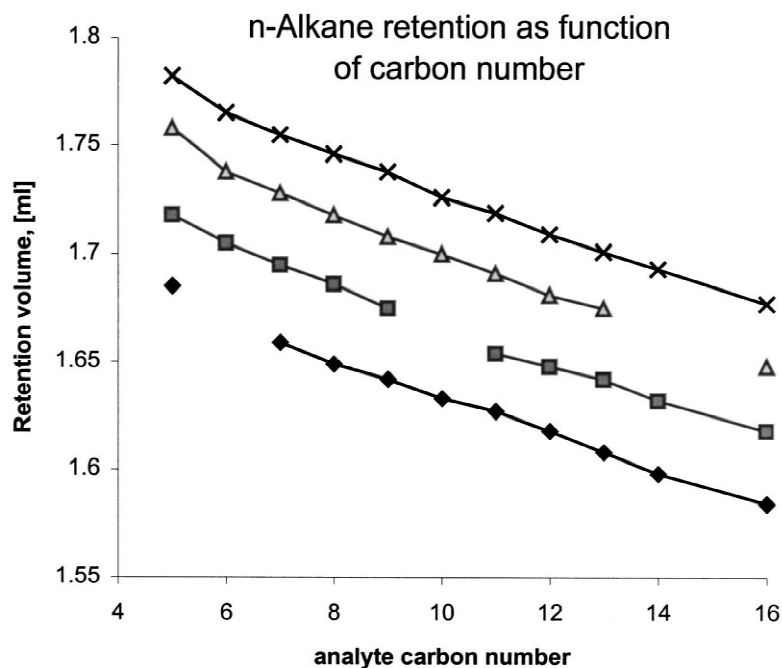


Fig. 1. Dependencies of *n*-alkane retention volumes on the Prodigy-ODS2 column with *n*-alkanes used as eluents (experimental data in Table 1). Eluents: ♦ *n*-hexane; ■ *n*-decane; ▲ *n*-tetradecane; × *n*-heptadecane.

mass (Fig. 1). All analytes eluted with *n*-decane as eluent show higher retention volumes compared to *n*-hexane. Further increase of the analyte retention

could be seen for *n*-tetradecane and *n*-heptadecane used as eluents.

An important point in this discussion is the value

Table 1
Retention of *n*-alkanes on the Prodigy-ODS2 column from different eluents

Analyte, n_c	Experimental retention volumes (ml)				Correlation coefficients
	Eluent (<i>n</i> -alkanes), n_c				
	6	10	14	17	
5	1.685	1.718	1.758	1.782	0.9991
6		1.705	1.738	1.765	0.9997
7	1.659	1.695	1.728	1.755	0.9998
8	1.649	1.686	1.718	1.746	0.9995
9	1.642	1.675	1.708	1.738	0.9991
10	1.636		1.700	1.724	1.0000
11	1.623	1.654	1.691	1.719	0.9990
12	1.618	1.648	1.681	1.709	0.9990
13	1.608	1.642	1.675	1.701	1.0000
14	1.598	1.632		1.693	1.0000
16	1.584	1.618	1.648	1.677	0.9990
Correlation coefficients	0.9955	0.9963	0.9935	0.9955	
Slope ($\mu\text{l}/\text{CH}_2$)	-8.88	-9.08	-9.51	-9.26	

of the hold-up (void) volume of column used. Three breaks on the curves in Fig. 1 correspond to the elution of an analyte identical to the mobile phase. This peak could not be detected. Theoretically the elution volume of *n*-hexane from *n*-hexane (or *n*-decane from *n*-decane) should give the hold-up volume for the system. Table 2 summarizes hold-up volume values calculated by the interpolation of the alkane retention values of Fig. 1 and Table 1. These interpolated values should theoretically give a true hold-up volume. Table 2 shows that the four interpolated hold-up volumes agree very well (less than 0.2% RSD). The first hold-up volume has been measured using minor disturbance method [12] as an integral average of acetonitrile minor disturbance peaks for water–acetonitrile mixtures through the whole concentration range (0–100%). This is a thermodynamically consistent method for hold-up volume measurements [14]. Derivation of the formula for the calculation of the hold-up volume from the minor disturbance method [15] is based on the assumption that the hold-up volume is the total volume of the liquid phase in the column, both methods show a very good correlation.

3.1. Exclusion

A pure size-exclusion process would force all analytes to elute before the hold-up volume [18]. In our case analytes which have a molecular mass less than the eluent are eluted after the hold-up volume, consequently they have a positive retention which can not be assigned solely to size exclusion. Another argument against size exclusion has been already mentioned. In size-exclusion chromatography (SEC) there is a linear dependence of the retention volume

on the logarithm of molecular mass, not a linear dependence such as shown here (Figs. 1 and 2).

Retention volume dependence on temperature allows calculation of the excess interaction energies of the analyte with the adsorbent surface. In common practice $\ln(k)$ is believed to be a thermodynamic value equal to $\Delta G/RT$ plus some constant. The question is how to consistently calculate the excess interaction energies for both retained and excluded analytes, because the capacity factors for the excluded analytes are negative and the logarithm for them does not exist. Obviously, this definition of $\ln(k)$ does not apply to these systems.

The chromatographic systems studied here are close to ideal. As a first approximation we can use the equation for the excess adsorption isotherm as:

$$\Gamma = \frac{V_0}{S} \cdot \frac{(K-1)x(1-x)}{1+(K-1)x} \quad (1)$$

where Γ is the excess adsorption, V_0 is the total volume of the liquid phase in the adsorption system, S is the adsorbent surface area, K is the adsorption equilibrium constant, x is the analyte equilibrium concentration expressed as mole fraction [19].

Retention volume for the chromatographic system [8,11,20–22] can be expressed in the form:

$$V_R = V_0 + S \frac{d\Gamma}{dc} \quad (2)$$

where V_R is the retention volume, V_0 is the hold-up volume, and $d\Gamma/dc$ is the derivative of the excess adsorption isotherm. The derivative of the excess adsorption isotherm calculated from Eq. (1) would have the form:

$$\frac{d\Gamma}{dc} = \frac{V_0}{S} \cdot \frac{(K-1)[1-2x-(K-1)x^2]}{[1+(K-1)x]^2} \quad (3)$$

Table 2
Hold-up volumes

Technique	Hold-up volume (ml)
Minor disturbance experiments	1.672
Extrapolated from <i>n</i> -alkane retention data for the following eluents:	
<i>n</i> -Hexane	1.670
<i>n</i> -Decane	1.668
<i>n</i> -Tetradecane	1.663
<i>n</i> -Heptadecane	1.664

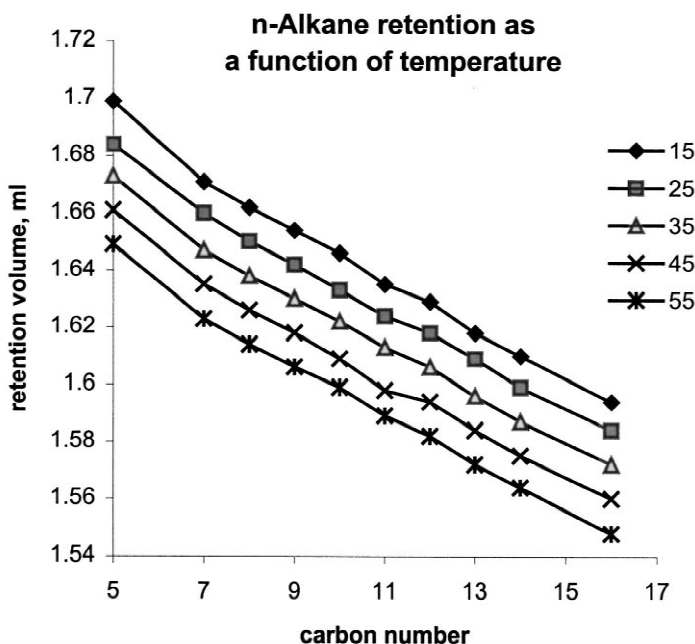


Fig. 2. Retention dependencies of *n*-alkanes eluted with *n*-hexane at different temperatures on the Prodigy-ODS2 column.

In high-performance liquid chromatography (HPLC) we deal with very low concentrations, so we have to consider the limit when x approaches 0, which can be written as:

$$\lim_{x \rightarrow 0} \frac{d\Gamma}{dx} = \frac{V_0}{S}(K - 1) \quad (4)$$

From Eqs. (2) and (4) we can express the thermodynamic equilibrium constant as a function of V_R , and V_0 :

$$K = \frac{V_R}{V_0} \quad (5)$$

The ratio of the retention volume to the hold-up volume represents the adsorption equilibrium constant. The retention factor (capacity factor) in its classical form, $k = (V_R - V_0)/V_0$ is related to the equilibrium constant (from Eq. (5)) as:

$$K = k + 1 \quad (6)$$

The retention factor for a “non-retained” component is equal to 0, so in this case the adsorption equilibrium constant K is equal to 1. This means that excess free Gibbs energy for a “non-retained”

component is equal to 0. If the excess free Gibbs energy is negative for some component, then the equilibrium constant will be less than 1 but larger than 0, which means repulsion of the analyte from the adsorbent surface. For this situation the retention factor will be negative. Experimentally this phenomenon can be demonstrated by the retention of benzoic acid from a non-buffered acetonitrile–water mixture on a reversed-phase column. Benzoic acid is ionized and highly solvated with water molecules at these conditions, and it can not therefore interact with the hydrophobic adsorbent surface. Its excess free Gibbs energy will be negative, the equilibrium constant is less than 1, and its retention factor is negative (it elutes before the hold-up volume). In our suggested approach a regular thermodynamic description could still be applied to that system:

$$\begin{aligned} \ln(K) &= \ln(V_R) - \ln(V_0) = \frac{\Delta(\Delta G)}{RT} \\ &= -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \end{aligned} \quad (7)$$

Because V_R/V_0 is always positive, we do not have a problem with the nonexistent logarithm of the negative k values.

Table 3
Retention volumes of *n*-alkane homologous series from *n*-hexane (eluent) on the Prodigy-ODS2 column at different temperatures

n_c	Temperature (°C)				
	15	25	35	45	55
5	1.699	1.684	1.673	1.661	1.649
7	1.671	1.660	1.647	1.635	1.623
8	1.662	1.650	1.638	1.626	1.614
9	1.654	1.642	1.63	1.618	1.606
10	1.646	1.633	1.622	1.609	1.599
11	1.635	1.624	1.613	1.598	1.589
12	1.629	1.618	1.606	1.594	1.582
13	1.618	1.609	1.596	1.584	1.572
14	1.610	1.599	1.587	1.575	1.564
16	1.594	1.584	1.572	1.560	1.548

Expression (6) can be used for an explanation of the observed linear dependencies of V_R vs. the analyte carbon number (Figs. 1 and 2). The systems studied have very low excess interaction energy, and the retention factors are very close to zero. For *n*-alkanes the interaction energy is additive for the number of CH_2 groups. Accounting for this we can rewrite Eq. (7) using expression (6) and the assumption of additivity:

$$\begin{aligned} \ln(K) &= \ln(k + 1) \\ &= n \frac{\Delta(\Delta G_{\text{CH}_2})}{RT} \Rightarrow \lim_{k \rightarrow 0} [\ln(k + 1)] \approx k \\ &= n \frac{\Delta(\Delta G_{\text{CH}_2})}{RT} \end{aligned} \quad (8)$$

Eq. (8) represents a linear dependence of the retention factor or retention volume on the number of analyte carbon atoms. The experimentally observed negative slope of these curves suggests a negative excess interaction of CH_2 groups with the adsorbent surface.

Dependence of the hold-up volume on the temperature has been calculated by interpolation of the data from Table 3 (see Fig. 3).

The slope of the hold-up volume temperature dependence ($1.2 \mu\text{l}/^\circ\text{C}$) has been calculated from the retention of *n*-alkanes on a reversed-phase column. This slope shows a good correlation with the slope calculated from the excess adsorption isotherms of acetonitrile from water on reversed-phase columns measured at different temperatures ($1.1 \mu\text{l}/^\circ\text{C}$) [12]. Factors responsible for changes in the hold-up volume with temperature include expansion of the

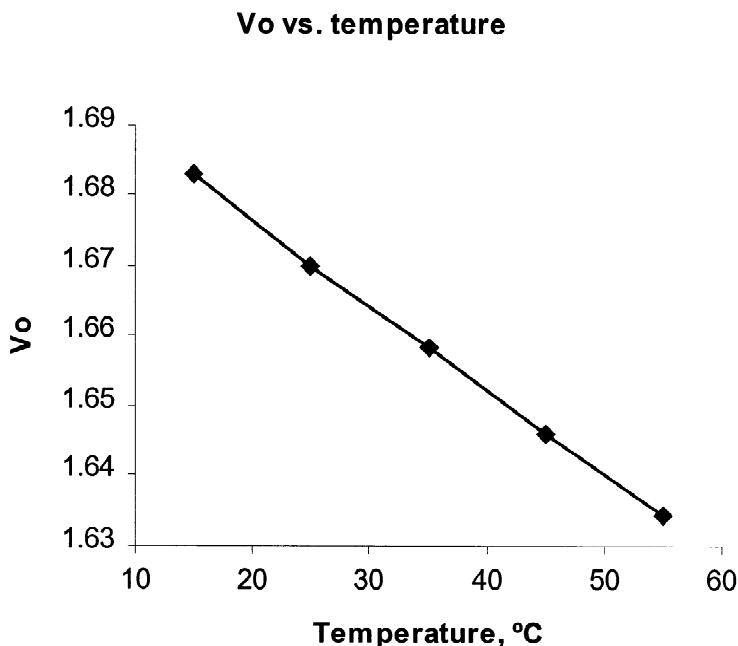


Fig. 3. Temperature dependence of the hold-up volume of the Prodigy-ODS2 column (150×4.6 mm).

column hardware, silica matrix, bonded ligands layer, and the eluent. This could not be calculated, practically because of the unknown temperature coefficient for porous amorphous silica.

3.2. Interaction energy

As mentioned before, positive retention (we are using this term to describe the retention relative to the hold-up volume of the system) indicates some kind of excess interaction of the analyte compared to the eluent. Using Eq. (7) and data from Table 3 we can calculate excess entropy and enthalpy values of *n*-alkanes eluted by *n*-hexane from reversed-phase columns.

Excess enthalpy values calculated using Eq. (7) are shown in Fig. 4. The scale of the ordinate in Fig. 4 was chosen for easy comparison to the data in Fig. 5. Enthalpy values are scattered around 0 and do not exceed ± 1 cal/mol (1 cal = 4.1868 J). On the other hand entropy values show a linear dependence with the number of analyte carbon atoms. Although entropy values do not exceed 0.12 cal/mol their influence is greater than ΔH . Enthalpy effects on retention are temperature dependent, and this term in Eq. (7) divided by the absolute temperature ($\Delta H/RT$) is about 50-times less than the $\Delta S/R$ effect.

From this we can conclude that for the given chromatographic system alkane retention is mainly

entropy driven. As can be seen from Fig. 5, excess entropy is close to zero for the component eluting with the hold-up volume. Entropy increases with decreasing retention.

Mockel in his article [17] gave his experimental results for a similar system. We have used his data to calculate entropy and enthalpy values according to the procedure described above. Figs. 6 and 7 represent the calculated ΔH and ΔS values for Mockel's data.

The dependence of the excess entropy is almost the same as in Fig. 5. Extrapolation of ΔS to *n*-pentane which has been used as an eluent gives $\Delta S = 0$, which is consistent with our proposed explanation. The absolute values of ΔS are very close despite the fact that different columns and eluents were used in these two experiments.

Excess entropy is also close to zero in Mockel's data, but the deviation is larger than in our experiments. This could indicate the presence of larger amount of accessible silanols in his column, which would introduce a specific interaction into the system.

The most interesting experimental results are the effect of the eluent molecular size. As can be seen from Fig. 1, an increase of the number of CH_2 groups in the analyte molecule leads to a decrease in retention volume. At the same time an increase of the number of CH_2 groups in the eluent leads to an

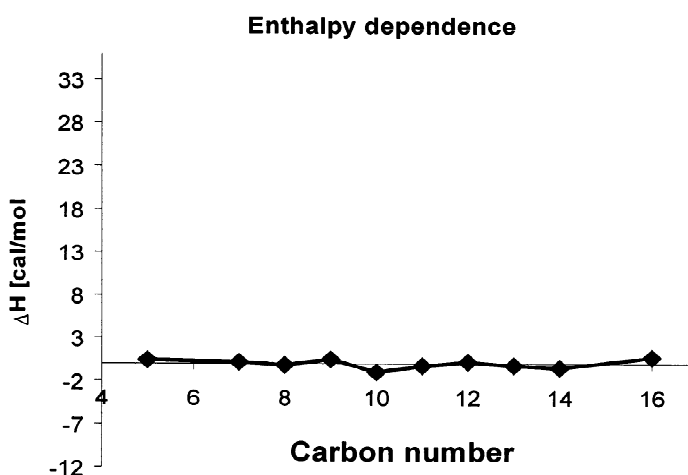


Fig. 4. Excess enthalpy for *n*-alkanes eluted from *n*-hexane on the Prodigy-ODS2 column. This graph is plotted with the temperature-corrected scale to make it comparable with Fig. 5.

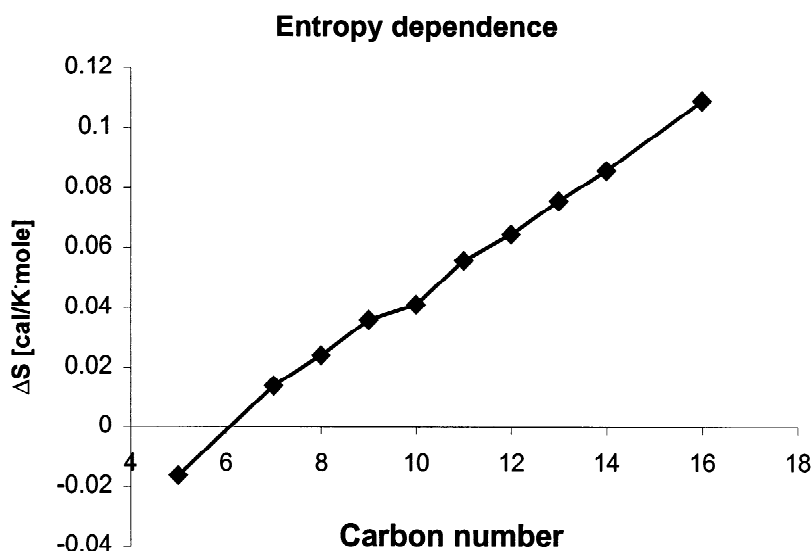


Fig. 5. Excess entropy for *n*-alkanes eluted from *n*-hexane on the Prodigy-ODS2 column.

increase of retention volume for the same analyte (Fig. 1). Most important is the fact that the incremental increase of retention volume per eluent CH₂ group has the same value but different sign for eluent and analyte. Fig. 8 represents the same data shown in Fig. 1, but now plotted against the number of the eluent carbon atoms.

The average slope for the curves in Fig. 1 is $-9.2 \mu\text{l}$ per analyte CH₂ unit. The average slope for the curves in Fig. 8 is $+8.5 \mu\text{l}$ per eluent CH₂ unit. Deviations in the slopes for different analytes in Fig. 8 are less than 3%.

Increase in analyte retention with increasing number of eluent CH₂ groups can not be explained by a

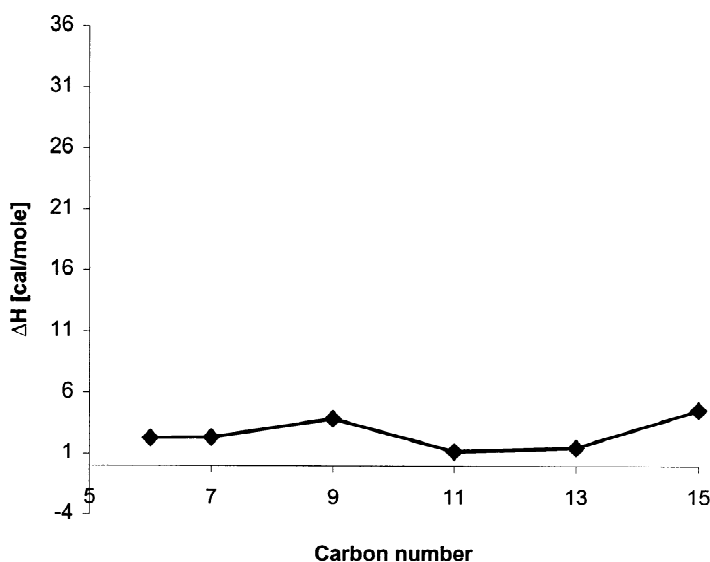


Fig. 6. Excess enthalpy of the *n*-alkane retention from *n*-pentane on the Eurosphere-ODS column. Calculated from data published in Ref. [15].

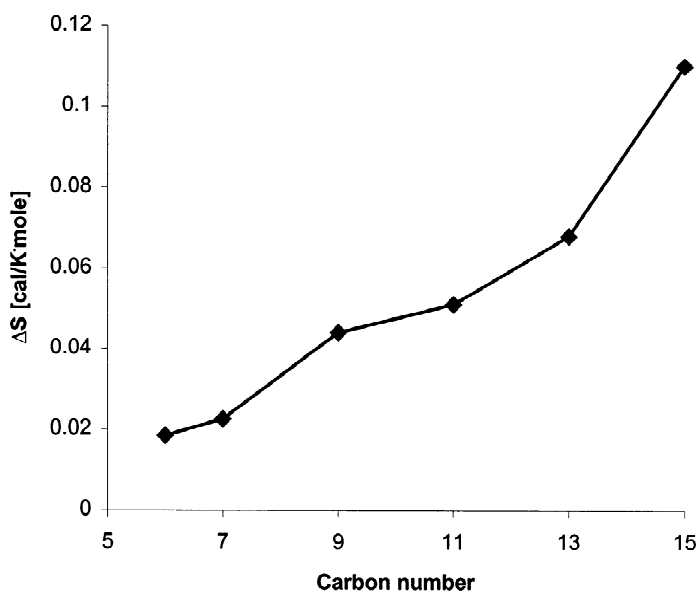


Fig. 7. Excess entropy of the *n*-alkane retention from *n*-pentane on the Eurosphere-ODS column. Calculated from data published in Ref. [15].

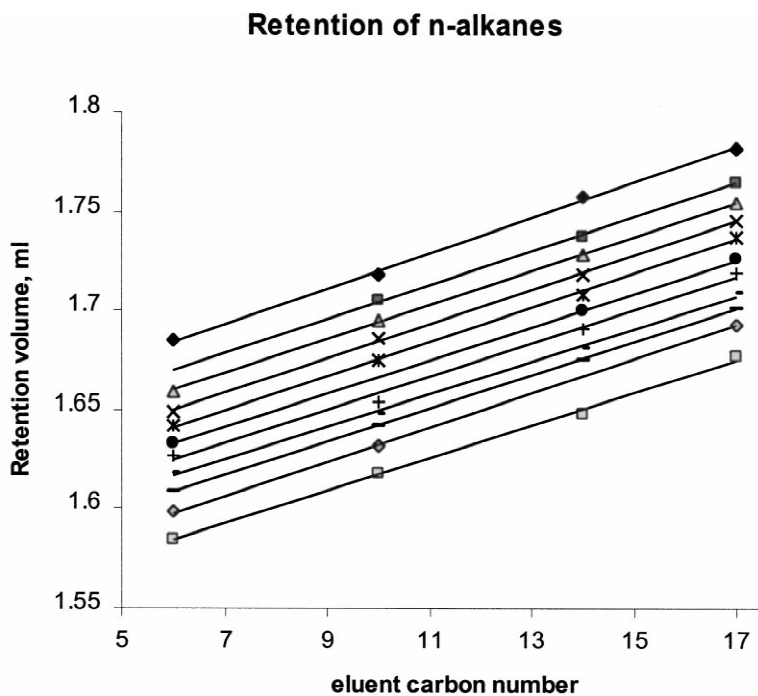


Fig. 8. Retention of *n*-alkanes homologous series on the Prodigy-ODS2 column plotted against the number of carbon atoms in the eluent molecules.

size-exclusion mechanism, because the eluent plays no role in size exclusion except to transport the analyte through the column. If there would be any excess interactions of the eluent molecules with the surface, for example due to larger number of CH₂ groups, then this would only decrease the analyte retention, not increase it. The chromatographic system used here does not show any preferable surface interactions (Figs. 4 and 6 shows the enthalpy values of essentially zero).

The most logical explanation must be an entropy effect. An increase of the eluent molecular size will restrict the freedom of movement of the analyte molecules, which in turn will lead to an increase of excess entropy of the analyte. We can rewrite Eq. (7) in the form:

$$V_R = V_0 \exp\left(\frac{\Delta(\Delta H)}{RT} - \frac{\Delta(\Delta S)}{R}\right) \quad (9)$$

As we have seen the enthalpy term is essentially zero and the decrease of the excess entropy will obviously lead to an increase of the exponential value and the analyte retention volume.

Fig. 9 represents the dependencies of the excess

entropy values on the number of analyte carbon atoms for four different eluents. As can be seen, an increase of the eluent molecular mass leads to a decrease of the analyte excess entropy.

We should emphasize that all four lines (Fig. 9) cross the zero at that point where the eluent and the analyte molecules have the same size. For this hypothetical chromatographic system when the eluent is also used as an analyte, we obviously have all excess thermodynamic parameters equal to zero. Thus, the value of the exponent in Eq. (9) will be 1, and the analyte retention volume will exactly correspond to the hold-up volume.

4. Conclusions

Retention of *n*-alkanes on reversed-phase (C₁₈) columns with *n*-alkanes as eluent shows zero excess analyte–adsorbent interactions. The entropy of the system governs retention dependencies on both the number of analyte as well as eluent carbon atoms. Analytes with their molecular mass (size) smaller than the eluent show an increase of retention relative

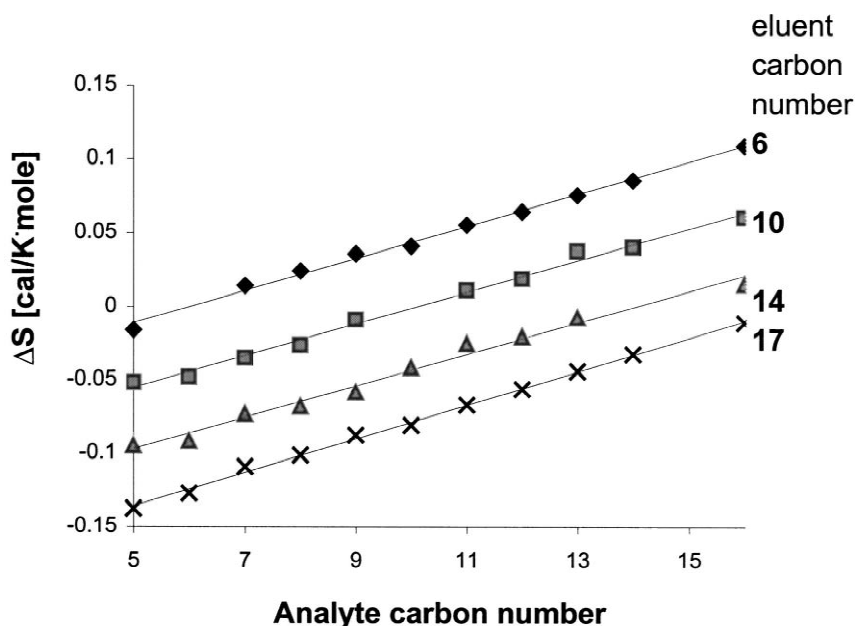


Fig. 9. Dependencies of the excess entropy of *n*-alkane retention on the Prodigy-ODS2 column eluted with *n*-alkanes. Number of carbon atoms of the eluent molecules are shown on the right.

to the system hold-up volume. This is associated with the decrease of their freedom in the solution (decrease of the entropy) and their exclusion from the solution onto the adsorbent surface.

All analytes have shown linear (not logarithmic) dependencies of their retention volume on the number of carbon atoms. This could be explained on the basis of adsorption theory. When the thermodynamic equilibrium constant is close to unity, its logarithm could be effectively approximated by a linear function. This confirms the necessity to use $\ln(k+1)$ instead of $\ln(k)$ as the proper energetic parameter for chromatographic systems.

References

- [1] S.I. Andersen, K.S. Birdi, *Prog. Colloid Polym. Sci.* 82 (1990) 52–61.
- [2] J.G. Dorsey, K. Dill, *Chem. Rev.* 89 (1989) 331–346.
- [3] Y.V. Kazakevich, O.G. Larionov, Y.A. Eltekov, *Pure Appl. Chem.* 61 (1989) 2037–2040.
- [4] J.H. Knox, G.J. Kaliszan, F. Kennedy, *Faraday Symp. Chem. Soc.* 15 (1980) 113.
- [5] H. Poppe, *J. Chromatogr. A* 656 (1993) 19–36.
- [6] L.C. Tan, P.W. Carr, *J. Chromatogr. A* 775 (1997) 1–12.
- [7] A. Vailaya, Cs. Horvath, *J. Chromatogr. A* 829 (1998) 1.
- [8] F. Riedo, E. Kovats, *J. Chromatogr.* 239 (1982) 1.
- [9] C.S. Koch, F. Koster, G.H. Findenegg, *J. Chromatogr.* 406 (1987) 257.
- [10] Y.A. Eltekov, Y.V. Kazakevich, A.V. Kiselev, A.A. Zhuchkov, *Chromatographia* 20 (1985) 525–528.
- [11] H.L. Wang, J.L. Duda, C.J. Radke, *J. Coll. Interface Sci.* 66 (1978) 153.
- [12] E.H. Slaats, W. Markovski, J. Fekete, H. Poppe, *J. Chromatogr.* 207 (1981) 299–323.
- [13] J. Knox, R. Kaliszan, *J. Chromatogr.* 349 (1985) 211–234.
- [14] Y.V. Kazakevich, H.M. McNair, *J. Chromatogr. Sci.* 33 (1995) 321–327.
- [15] Y.V. Kazakevich, H.M. McNair, *J. Chromatogr. Sci.* 31 (1993) 317–321.
- [16] H.J. Mockel, U. Dreyer, *Chromatographia* 37 (1993) 179–184.
- [17] H.J. Mockel, *J. Chromatogr. A* 675 (1994) 13–28.
- [18] G. Golckner, in: *Polymer Characterization by Liquid Chromatography*, *Journal of Chromatography Library*, Vol. 34, Elsevier, Amsterdam, 1987, p. 117.
- [19] D.H. Everett, *J. Chem. Soc., Faraday Trans.* 60 (1964) 1803.
- [20] J.N. Wilson, *J. Am. Chem. Soc.* 62 (1940) 1583.
- [21] D. DeVault, *J. Am. Chem. Soc.* 65 (1943) 532.
- [22] G. Schay, *Ber. Bunsenges Phys. Chem.* 77 (1973) 184.