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CORRELATION BETWEEN REVERSED-PHASE RETENTION AND SOLUTE MOLECULAR SURFACE TYPE AND AREA

IV. INFLUENCE OF ELUENT WATER CONTENT ON THE RETENTION PRODUCED BY DIFFERENT SURFACE TYPES

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SUMMARY

In reversed-phase systems, the retention of non-ionized solutes increases with increasing eluent water content. For all investigated solutes, the ln k' increase is linear from 0 to about 25% (v/v) of water in methanol. It parallels an eluent surface tension increase which, in turn, is related to a linear increase of cavity formation free energy. If normalized to equal solute molecules surface area, changes in cavity free energy are the same for all solutes larger than the eluent molecules.

However, quite remarkable differences in solute behaviour towards changes of eluent water content are observed. The solute retention index may drop, stay constant, or rise with increasing eluent water content. The strongest index decrease is observed with rigid symmetric solutes the surface of which does not carry protons. Both changes of the sorbed solute entropy and the existence of CH····CH interaction between reference alkanes and eluent methanol seem to be responsible for this effect. Solutes having a strong interaction with methanol, like alkynes, exhibit a marked index increase when water is added to the eluent since these interactions are suppressed when the methanol is claimed by the water.

Aromatic surface reacts less strongly on eluent composition changes than aliphatic surface, possibly due to an increasing polarization of π systems by water dipoles.

INTRODUCTION

It has been shown that, in general, retention in octadecylsilica (ODS)–methanol systems is related to the solute molecular surface area[1]. The logarithm of the capacity factor, ln k', depends linearly on the surface area (SA) as long as chain-like or flat solute molecules are considered. Different types of surface produce different amounts of retention[2]. Broadly speaking, to each type of surface has to be attributed a certain polarity[3]. With pure methanol as eluent, the proton-free surfaces of mole-

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cules having no dipole moment, like S_8, appear to have the lowest surface polarity[4]. The term "polarity" refers to various types of molecular interactions. Thus it is anticipated that the respective types of molecular surface area behave differently towards changes in eluent polarity and structure.

In methanol–water mixtures containing up to 30% (v/v) water, the surface tension, σ, increases almost linearly with increasing water content. Consequently, the free energy of cavity formation is expected to increase in the same way. On the other hand, zero or very low polarity surfaces like $-S_n-$ or $-(CH_2)_n-$ are expected to be subject to a strongly increasing solvophobic effect with increasing eluent water content, while clearly solvophilic surfaces like –OH, –O–, –C≡C are not or to a lesser extent.

We now describe results obtained on ODS columns using methanol–water eluents.

EXPERIMENTAL

The conditions were as described before[1]. The column was a Waters RadPAK A. Water was taken from a Millipore purification set. To make the methanol–water eluents, appropriate amounts of degassed water and methanol were mixed on a balance; the volume percentage was converted into a weight percentage using tables[5–6].

RESULTS AND DISCUSSION

Most of the solutes investigated were members of homologous series or groups of chemically similar compounds. In Fig. 1 the ln k' of n-alkanes as a function of the carbon atom number, n_C, is shown for various methanol–water mixtures from p = 0 to p = 25, where p denotes the volume percentage of water. The capacity ratio was evaluated from raw retention times using a constant raw dead time of 2.755 min, including an extra-column dead time of 0.175 min (precolumn tablet included):

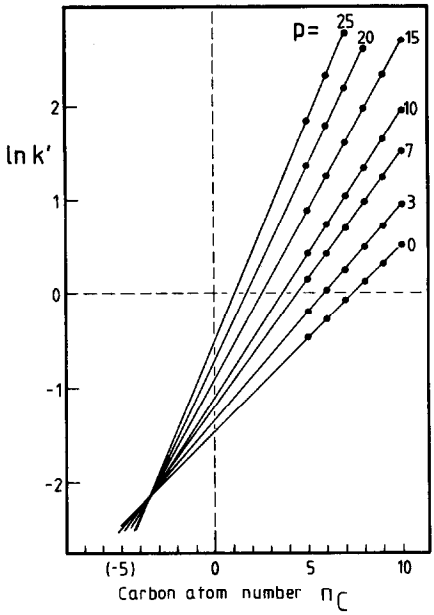
$$k' = \frac{t_{\mathrm{ms,\ raw}} - t_{\mathrm{m,\ raw}}}{t_{\mathrm{m}}} \tag{1}$$

For each p a straight line is obtained with correlation coefficient, $r \geqslant 0.9999$. All lines intersect at one point whose abscissa is denoted by $\tilde{n}_C$. This common intersection point (CIP) has frequently been cited[7–9], but its physical meaning is somewhat obscure. In the present case, $\tilde{n}_C = -3.32$, and the corresponding ln $k' = -2.1$.

Similar assemblies of straight lines are found for other series like alkenes, alkynes, phenylalkanes, alkyl bromides and n-alcohols. Since the determination of CIP involves extrapolation from real data points over quite a long distance, there is a fairly large scatter in the $\tilde{n}_C$ value[9]. After calculating all possible intersections and eliminating mavericks, averaging yields data with moderate standard deviations.

For each solute series, at p = constant, the dependence of ln k' on the recurring unit number (carbon atom number in most cases) can be written as

$$\ln k' = a + bn_C \tag{2}$$



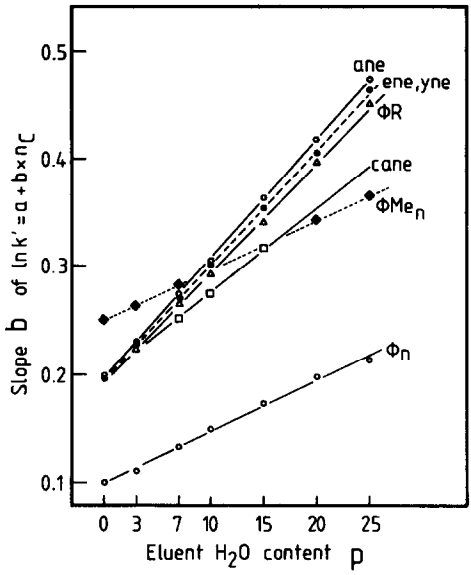


Fig. 1. Dependence of ln k' of n-alkanes on the carbon number, n_C. The lines for different eluent water contents, p, intersect at a common point.

Fig. 2. Slope "b" of ln k' = $a + bn_C$ as a function of the eluent water content, p. Solute classes: n-alkanes (ane); cycloalkanes (cane); 1-alkenes (ene); 1-alkynes (yne); phenylalkanes (ϕR); methylbenzenes (ϕMe$_n$); p-polyphenyls (ϕ_n).

where a and b depend on the eluent composition. As is seen from Fig. 2, the slope "b" is a linear function of the water percentage, p. The excellent linearity is independent of whether the dead time is assumed to be constant or is assumed to depend on p. This is not the case with intercept "a". It is a linear function of p if t_m = constant is assumed. If, however, t_m is a linear function of p

$$t_{m,\,p} = t_m^0 + \delta t \cdot p \tag{3}$$

where δt is the dead time change for 1% increase of eluent water content. The intercept "a" appears as an exponential

$$a_p = a^0 + \delta a \cdot p^z \tag{4}$$

with $z > 1$ depending on the solute class. A dead-time dependence like that in eqn. 3 has been postulated in the literature for the range p = 0–25[10]. On the other hand, from our fairly numerous data, we could not detect an highly significant dependence of t_m on p. The question of whether t_m is constant or not is closely related to the existence of a common intersection point (CIP).

If a' and b' are the parameters for one eluent composition, p', and a'' and b'' for the other, p'', then the intersection is defined by:

$$\ln k'(\tilde{n}_C) = a' + b'\tilde{n}_C = a'' + b''\tilde{n}_C \tag{5}$$

$$\tilde{n}_C = \frac{a' - a''}{b'' - b'} \tag{6}$$

Since "b" is always a linear function of p:

$$b' = b^0 + \delta b \cdot p' \text{ and } b'' = b^0 + \delta b \cdot p'' \tag{7}$$

If "a" is linear, then

$$a' = a^0 + \delta a \cdot p' \text{ and } a'' = a^0 + \delta a \cdot p'' \tag{8}$$

and if "a" is non-linear:

$$a' = a^0 + \delta a \cdot (p')^z \text{ and } a'' = a^0 + \delta a \cdot (p'')^z \tag{9}$$

From eqn. 7

$$\tilde{n}_C = -\delta a/\delta b = \text{constant} \tag{10}$$

and the CIP does exist. From eqn. 8, however

$$\tilde{n}_C = -\frac{\delta a}{\delta b} \cdot \frac{(p')^z - (p'')^z}{(p' - p'')} \tag{11}$$

and the CIP does not exist.

The existence of the CIP implies that t_m does not depend on the eluent water content which, in turn, means that the phase volume ratio, φ, has a constant value, too. According to our data, this refers only to a range between 0 and 25% (v/v) of water in methanol. In the following we will assume t_m = constant. From eqn. 10 we can easily determine $\tilde{n}_C$ for a series by expressing the intercept "a" as a linear function of the corresponding slope "b" in eqn. 2 for all eluent compositions: $a = \tilde{n}_C b + C$. The results obtained with some series of solutes are given in Table I.

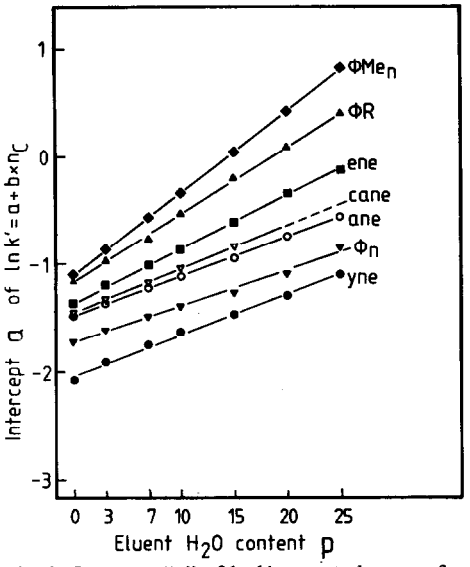
Fig. 3 shows the intercepts "a" for various solute classes. There is no systematic deviation from linearity. For $p = 0$ all the values are negative, and increase with increasing p.

The eluent dependence of the slope "b" (Fig. 2) has to be interpreted by changes in both the cavity and interaction free energies of the recurring structural

TABLE I

PARAMETERS OF $a = \tilde{n}_C b + C$ FOR SOME SOLUTE SERIES

Series	*C*	$\tilde{n}_C$	*Correlation coeff., r*
Alkanes	−2.12	3.32	0.999
Alkenes	−2.23	4.60	0.999
Alkynes	−2.69	3.48	0.999
Phenylalkanes	−2.31	6.09	0.998
Cycloalkanes	−2.41	4.95	0.998
Polyphenyls	−2.30	6.10	0.995
Methylbenzenes	−5.24	16.59	0.999



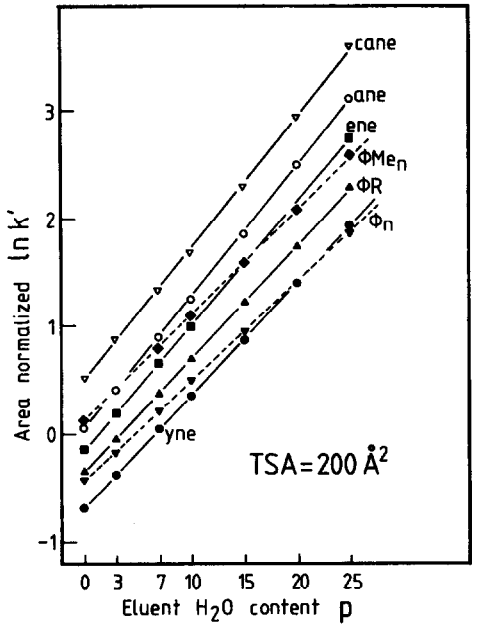


Fig. 3. Intercept "a" of ln k' = a + bn_C as a function of the eluent water content, p. A constant dead time is assumed. Solutes as in Fig. 2.

Fig. 4. ln k' as a function of the eluent water content, p. Solutes as in Fig. 2, but normalized to a common total molecular surface area of 200 Å.

unit. For methylene chains in different series, these changes are very similar. The methylene rings of cycloalkanes are slightly less sensitive towards eluent polarity changes, which is mainly due to the smaller surface area contribution of CH_2 in rings (18.34 Å^2 *versus* 22.67 Å^2 in chains). In Fig. 4 the solute surface areas are normalized to 200 Å^2 which is very close to the size of cyclodecane. If area-normalized, the changes in ln k' of cycloalkanes and n-alkenes, respectively, with eluent water content, p, are identical. The absolute values of ln k' for cycloalkanes are higher, however. The area normalization implies that the cavity free energies of both solutes are practically the same. An increased dispersion force, due to the presence of more matter covered by the same surface area (9.915 carbon atoms in the ring, 7.703 in the chain), would influence interaction with both the stationary and mobile phases. So we propose that the entropy decrease occurring upon formation of the sorbed state is higher for the chain-like n-alkanes.

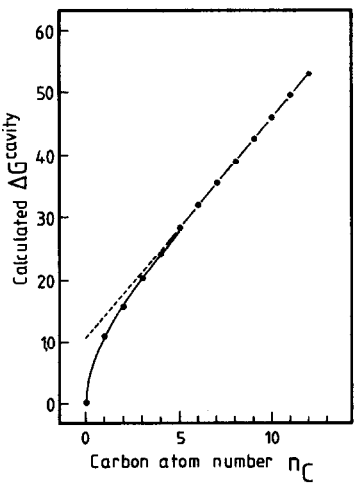
The methylated benzene rings and p-polyphenyls are least affected by increasing p. Apparently, the interaction of the π systems with the eluent cannot easily be suppressed by increasing solvophobic expulsion forces.

The interpretation of the "a" terms in Fig. 3 is more complex. Since n_C in eqn. 2 is defined such that in chains it includes only the paraffinic carbon atoms, the intercept "a" has been looked upon as the retention contribution of the functional group, plus ln φ (phase ratio)[11]. This interpretation, however, is incomplete. A quite substantial fraction of "a" originates from the way eqn. 2 was established. Since ln k' increases linearly with n_C, starting from $n_C \approx 5$, above $n_c = 5$ the slope term,

"b", has a physical meaning, namely $\Delta\Delta G^{\text{Sorption}}/RT$. The application of a continuous function to a discontinuous system consisting of discrete structural units, however, is incorrect in principle. It leads to the extrapolation of real data from $n_C \geqslant 5$ to $n_C = 0$ where the function does not exist. Furthermore, non-linearities at $n_C < 5$ are overlooked.

As an example, ΔG^{cavity} for n-alkanes in pure methanol was calculated according to Sinanoglu[12] and Horváth *et al.*[13]. As is seen from Fig. 5, the increase in ΔG^{cavity} with increasing alkyl chain length becomes linear at $n_C \geqslant 5$. It has a value of 3.5 kJ/mol per methylene group. Extrapolation to $n_C = 0$ yields an intercept of 10.7 kJ/mol which is in fact meaningless since a non-existant cavity does not require any formation free energy. Furthermore, the cavity free energy for $n_C = 1$ (methane), 11.1 kJ/mol, is much higher than for recurring CH_2 groups. So there is a strong increase in the cavity free energy before the region is reached in which experimental data are obtained. Similar non-linearities may occur in the free energy of interaction of the solute with the mobile and stationary phases. The sum of these effects is projected into the value of intercept "a" when the straight line for the recurring groups is extrapolated to $n_C = 0$.

So we have to conclude that intercept "a" is comprised of three contributions: one from extrapolation, another from the functional group and, finally, ln φ. The latter is assumed to be constant in the range $p = 0$–25, for reasons stated above.



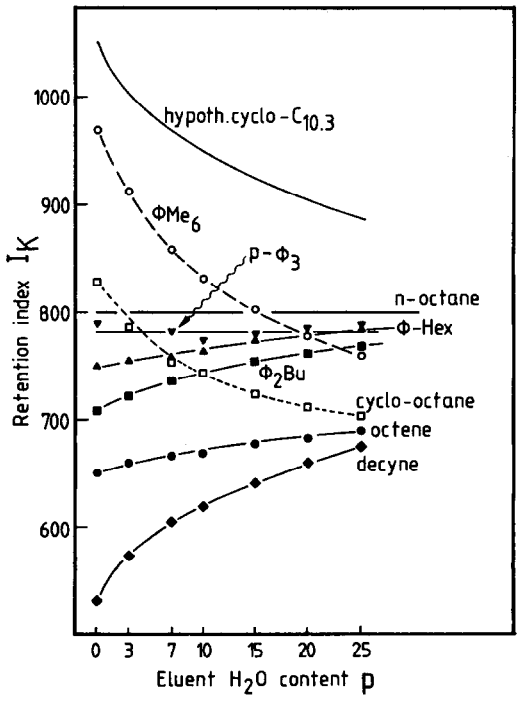


Fig. 5. Cavity free energies for n-alkanes in pure methanol, calculated according to refs. 12, 13. Units are kJ/mol.

Fig. 6. Dependence of the retention indices, I_K, of some typical solutes on the eluent water content, p.

Since the intercept, "a", varies more strongly than the slope, "b", as a function of p, even for alkanes having no functional group, it appears that the free energies involved in sorption of the small members at the beginning of a series are more subject to changes in eluent polarity and structure than are those pertinent to the molecular size region where the slope, "b", has been defined.

It should be noted that the total sorption free energy of a CH_2 group in n-alkanes is -490 J/mol for $p = 0$. The cavity free energy for the same group is 3.54 kJ/mol, about seven times as much.

From the ΔG balance for dissolution

$$\Delta G^{\text{dissol}} = \Delta G^{\text{cavity}} + \Delta G^{\text{inter}}_{\text{mob}} + \Delta G^{\text{evapor}} + RT \cdot \ln(RT/p^0\bar{V}) \tag{12}$$

and data on alkane solubility[14], $\Delta G^{\text{inter}}_{\text{mob}}$ for interaction of n-decane with methanol was calculated to be -70 kJ/mol. Taking into account the different volumes of CH_3 and CH_2, $\Delta G^{\text{inter}}_{\text{stat}}$ for the interaction of a CH_2 group with the stationary phase turns out to be only -3.27 kJ/mol, indicating a strong entropy loss in the formation of the sorbed solute state. The free energy of sorption which is finally responsible for retention appears to be the fairly small difference between much larger energy and entropy quantities associated with the underlying physical processes.

Eluent-induced changes in retention of a particular type of solute are best visualized when compared to the corresponding changes in n-alkanes. For this purpose we express retention in terms of the retention index, I_K, using the retention of n-alkanes as a reference system. The index of n-alkanes is defined as

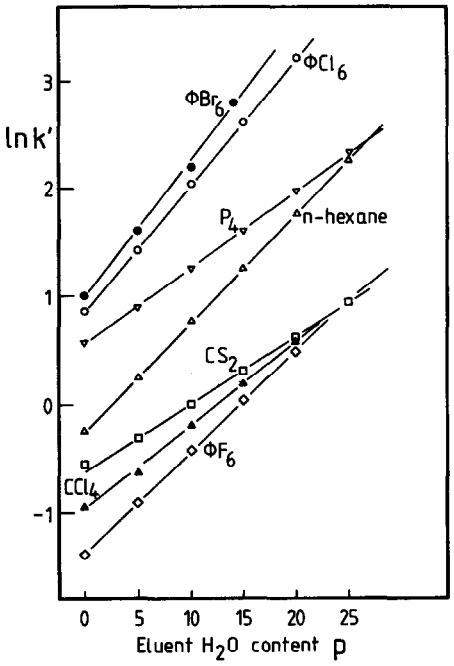
$$\bar{I}_K = 100 \cdot n_C \tag{13}$$

under all chromatographic conditions. The bar over a symbol denotes a quantity pertinent to n-alkanes.

Then the index for a solute Y is:

$$I_K(\text{Y}) = \frac{100}{\bar{b}}[\ln k'(\text{Y}) - \bar{a}] \tag{14}$$

Fig. 6 shows the I_K of several hydrocarbon solutes, each representing a solute class. Three types of solute behaviour can be distinguished: practically no change in I_K, and an increase or decrease in I_K with increasing p. p-Terphenyl shows no change. Its retention increase with increasing eluent water content parallels that of n-alkanes. An increase in I_K with increasing p is exhibited by hexylbenzene, diphenylbutane, octene and decyne. These compounds have a relatively strong interaction with the pure methanol eluent. When water is added, two main effects seem to occur. First, the water claims more and more methanol molecules for its own hydrogen bonding, so the methanol remaining for interaction with solutes is effectively diluted. Secondly, the more water is present the stronger are the non-specific hydrophobic forces, the more specific interactions being of only minor importance. Both effects are interrelated, of course. A decrease in I_K with increasing p is observed for hexamethylbenzene (ϕMe_6), cyclooctane. These solutes lack carbon chains. The highly symmetric ϕMe_6 shows the most pronounced decrease in I_K. At $p = 0$ it is eluted slightly more rapidly



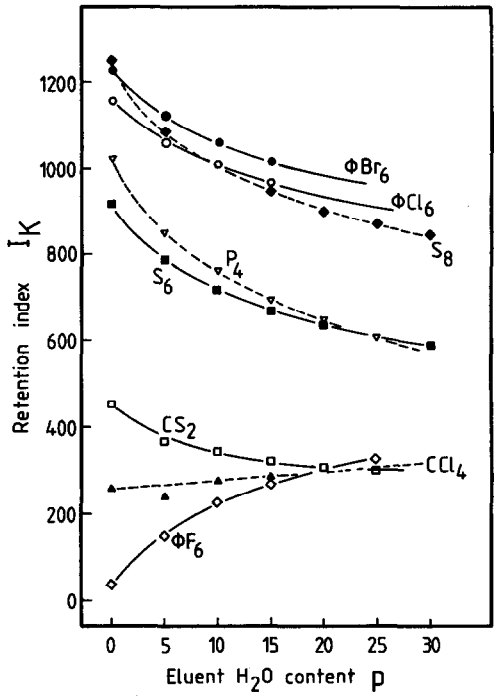


Fig. 7. ln k' of some low polarity proton-free solutes as a function of the eluent water content, p.

Fig. 8. Retention index, I_K, of highly solvophobic solutes as a function of the eluent water content, p.

than n-decane, at $p = 15$ it is coeluted with octane, and shifts towards heptane upon increasing p. The surface area of ϕMe_n corresponds to an hypothetical $C_{8.5}$ alkane. The experimental elevation of I_K is 120 units for $p = 0$, which is equivalent to 0.6 kJ/mol in this system. This difference in sorption free energy appears to result from two effects. First, the interaction of the aromatic system with the surrounding methanol reduces retention. Secondly, during the formation of the sorbed state, less degrees of freedom of intramolecular motion are lost with the hexamethylbenzene. Consequently, its entropy, ΔS_{stat}^{inter}, is less negative.

An hypothetical cycloalkane with 10.3 carbon atoms would have the same surface area, 206.7 Å^2, as n-octane. Its calculated I_K is indicated in Fig. 6, being 1050 at $p = 0$. Compared to n-octane, the difference in sorption free energy is 1.23 kJ/mol. If one assumes that the increased dispersion interaction of the cycloalkane produces about equal effects on the mobile and stationary phases, the difference in ΔS_{stat}^{inter} should be of the order of 4 kJ/K · mol.

The eluent composition dependence of the I_K of proton-free solutes is even more pronounced. Fig. 7 shows the ln k' of hexafluoro-, hexachloro- and hexabromobenzene, P_4, carbon tetrachloride and carbon disulphide, plus, for comparison, n-hexane. All values increase linearly with increasing eluent water content, p. It should be noted thast the ln k' increase for n-hexane is markedly steeper than that of P_4. The lines intersect at $p = 27$. With lower eluent water contents, n-hexane is eluted before P_4, with higher water contents this order is reversed. This effect has

also been shown experimentally for *n*-hexane and cyclohexasulphur[4]. There are two possible reasons for this behaviour, one originating in the stationary phase, the other in the mobile phase. First, the more negative entropy, ΔS_{stat}^{inter}, of *n*-alkanes in the sorbed state could be more strongly influenced by the presence of water than that of compounds having an high rotational symmetry. This effect would account for the decrease in I_K with increasing p for cycloalkanes and hexamethylbenzene as well as for P_4, sulphur homocycles and carbon disulphide.

A comparison of Figs. 6 and 8, in which the dependence of I_K on p for proton-free solutes is displayed, indicates that the decrease in I_K is more pronounced with P_4, S_6, S_8 which do not have dipole moments or hydrogen atoms. If alkane C–H bonds participate in an interaction with eluent C–H bonds in addition to the purely dispersive forces exerted by, *e.g.*, S_6, then the replacement of methanol in the eluent by water would decrease this interaction. This, in turn, would cause a retention increase in addition to an increase in cavity energy. Most probably, a combination of the two effects is responsible for the observed decrease in I_K.

The higher retention of *n*-hexane as compared to that of S_6 and P_4 at eluent water percentages above 26 is explained by the larger surface area, since with increasing p, solvophobicity increasingly dominates the solute–solvent interactions of low polarity solute molecules.

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