

Description of retention mechanism by solvophobic theory

Influence of organic modifiers on the retention behaviour of homologous series in reversed-phase liquid chromatography

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ABSTRACT

The retention behaviour of different homologous series ($2 \leq n_c \leq 22$) was studied on three different bonded chain lengths (C_1 , C_{14} and C_{18}) and in aqueous–organic solvent mixtures commonly used in reversed-phase liquid chromatography [methanol, acetonitrile and tetrahydrofuran (THF)]. For all solutes (ligands) without π electrons, and regardless of bonded chain length, the plots $\log k'$ vs. φ (organic modifier ratio) in methanol–water or acetonitrile–water are correlated with the evolution of surface tension of these mixtures. On the other hand, for ligand which possess π electrons, there is a modification in these curves which is due to specific π – π interactions added to the pure solvophobic effect. In this respect, pure methanol was found to be a better eluent than pure acetonitrile excepted for the ligands with π electrons, for which the opposite was observed. For the THF–water mixtures, the plots of $\log k'$ vs. φ were always below those for methanol–water or acetonitrile–water. Moreover, the plots of $\log k'$ vs. n_c did not increase linearly. In this pure solvent, retention is due to an exclusion mechanism. These results were in agreement with conformational infrared studies of alkyl chains in THF, methanol and acetonitrile. The overall results could rationalize the fact that retention prediction based on methylene selectivity was often erroneous when THF was used. Nevertheless, they show the possibility of optimizing separations of homologous series from the results obtained from a single gradient experiment with any commonly used organic modifier.

INTRODUCTION

Separation of the constituents of homologous series by reversed-phase liquid chromatography (RPLC) is not the central theme of most chromatographic research. Nevertheless, the study of these ligands is of value from a general point of view, because it leads to a better understanding of the parameters determining the chromatographic process.

The rationalization of experimental results is based on the solvophobic theory [1], which explains all phenomena from the standpoint of energies and has been adapted to chromatography by Melander and Horváth [2]. Its mathematical expression is

$$\ln k' = \ln\left(\frac{V}{V_m}\right) + \frac{1}{RT} \left[W_s + \Delta A (a_s + \gamma) + NA_s \gamma (\chi_e - 1) - \frac{\Delta Z}{\epsilon} \right] + \ln \left(\frac{RT}{P_0 V_s} \right) \quad (1)$$

This leads to the prediction of the behaviour of ligands as a function of their hydrophobic volume [3] and their polarity, and to that of the nature of the modifier on their retention. It involves two simultaneous phenomena:

(1) Retention of homologues increases with increasing carbon number, n_c , since the size of the cavity ΔA increases with increasing carbon number [3]. Numerous experiments have been reported on this effect [4-10] and allowed the evaluation of the elution strength of solvents based on the reasoning of Snyder [11], in both partial aqueous reversed-phase liquid chromatography (PARP) [12-15] and non-aqueous reversed-phase liquid chromatography (NARP) [9,12].

(2) The retention of the same solute in different aqueous-organic solvent mixtures must be correlated with their surface tension. This has been shown for three alkanes for which it was possible to correlate $\log k'$ vs. φ (volume ratio of organic modifier) with the curves of the variation of the surface tension of mixtures [16] (methanol-water and acetonitrile-water). The generality of the phenomenon independent of the nature of the ligand, has not been established however.

Moreover, the particular role of the aromatic ring as a polar head has been reported previously, suggesting a particular behaviour for systems with π electrons [8,17-19].

The aim of this work was to define further the role of the type of polar head of the ligand on retention and on methylene selectivity as a function of the nature of the modifier: methanol, acetonitrile or tetrahydrofuran (THF). This was accomplished by a systematic study of the retention of different homologous series as a function of the percentage of water in the mobile phase. We also attempted to correlate variations in the surface tension curves of aqueous-organic solvent mixtures.

This study was carried out on long-chain (C_{18} , C_{14}) and short-chain (C_1) monofunctional phases (identical with the previously mentioned monomeric phase [8]) in order to detect the possible role of residual silanols in the retention mechanism. The effects of the chemical nature of the bonded phase, *e.g.*, propylphenyl, will be reported subsequently.

EXPERIMENTAL

Reagents

Methanol, acetonitrile and tetrahydrofuran were of high-performance liquid chromatographic (HPLC) grade (Carlo Erba, Milan, Italy and Merck, Darmstadt, Germany). All solvents were filtered through a 0.5- μm Millipore filter. Water was of ultra-high quality (18 M Ω cm) from an Elgastat UHQ system (Elga, Bucks, UK).

Solutions were made up precisely by volume and degassed by sonication.

Homologous series ($C_nH_{2n+1}Z$, where $Z = \text{H, Ph, Cl, COOMe, OCOPh, OH}$) were obtained from various sources [7,8]. Studies on C_{18} and C_{14} bonded chains were

carried out with $C_nH_{2n+1}Z$ with $Z = H, OH, COOMe, Cl$ and Ph and studies on C_1 with $Z = H, OH, COOMe$ and $OCOPh$; $1 \leq n \leq 22$.

Equipment

The LC system consisted of a Model 110B pump (Beckman, San Ramon, CA, USA), a Model 7125 injection valve with a 20- μ l loop (Rheodyne, Cotati, CA, USA), a Model R401 refractive index detector (Waters Assoc., Milford, MA, USA), a UV detector (254 nm) (Beckman) and a Model DDL 21 light-scattering detector (Cunow, Cergy St. Christophe, France).

For all experiments, the precolumn, the loop and the column temperature was controlled using a Croco-cil oven (Cluzeau, Sainte-Foy-la-Grande, France) thermostated with water by means of a Model HS 40 cryostat (Huber, Offenburg-Elgersweier, Germany) with a precision of 0.1°C.

Five columns were used: Spherisorb ODS-1 (5 μ m), 125 \times 4.6 mm I.D. (SFCC, Neuilly-Plaisance, France); LiChrosorb RP-2 (10 μ m), 250 \times 4.6 mm I.D. (Merck); RP-14 (10 μ m) 300 \times 4.6 mm I.D., previously used [7,8]; LiChrosorb RP-18 (5 μ m), 150 \times 4.6 mm I.D. (Merck), laboratory packed; and Hypersil ODS (5 μ m), 150 \times 4.6 mm I.D. (Shandon, Runcorn, UK), laboratory packed.

The eluent flow-rate was 1 ml/min.

Methods

All retention values are reported in terms of capacity factor, k' . The calculation of this parameter requires the determination of the void volume of the column used. The weighing method has been used for this purpose [8]. Each k' value reported is the mean from at least three reproducible injections. The accuracy of the measurements and the mathematical treatment of the results were as described in previous papers [7-9].

IR

Fourier transform IR spectra were obtained with a Perkin-Elmer 1730 FTIR spectrometer equipped with a DGTS detector and operated to give 2 cm^{-1} resolution. Conventional sodium chloride windows (38.7 μ m) or plates were used for pure samples. A calcium fluoride window (52 μ m) was used for mixed 2H_2O -organic modifier solutions. A total of 100 scans were collected for the neat reagents or ligands in solution, but bonded-phase samples required at least 500 scans.

The organic solvents (S.S.T., Champs sur Marne, France) were completely deuterated ($^2H_2O, C^2H_3O^2H, C^2H_3CN, THF-^2H_8, C^2HCl_3$) so the overlapping solvent peaks can be shifted to lower energies where they no longer interfere.

All spectra were recorded in transmission units between 1500 and 1300 cm^{-1} . Each time the solvent spectrum was subtracted from those of the ligands in solution. In order to compare different spectra, the data are normalized to the intensity of the methyl scissoring band at 1378 cm^{-1} . No modification of the wavenumber of the four absorption bands of interest appears when the organic solvent changes. The even-carbon alkanes were studied with $8 \leq n_C \leq 16$. Concentrations were calculated to be near the concentration corresponding to the phase ratio in the chromatographic column.

RESULTS AND DISCUSSION

Role of the modifier on the retention of the same solute: effect of surface tension

Behaviour of solutes of different polarity. In order to determine the possible influence of the physical nature of the ligate, we have drawn the plots of $\log k'$ vs. percentage of organic modifier (φ) compiled from data in ref. 12. This was done for each compound in five homologous series with both a monofunctional octadecyl linkage (Hypersil C₁₈) (Fig. 1) and a difunctional one (LiChrosorb RP-18) (Fig. 2). For purposes of clarity, only one compound per series is presented, but the same variations were observed, regardless of ligand chain length.

For all the series tested, (except phenylalkanes) and independently of the mono- or difunctional nature of the bonded phases, it was noted that the curves of variation of $\log k'$ obtained with methanol as organic modifier crossed those obtained with acetonitrile at about a 90% organic modifier content. This value was independent of the injected ligand length. For the phenylalkanes, we observed that the intersection of these curves was around a value of 95% of organic modifier instead of 90%. This difference may seem negligible, but this tendency to shift the crossover point of the two curves towards 100% organic modifier is supported by results published by Thevenon [16] and Thevenon-Emeric *et al.* [20] and the examination of those obtained by Chartier [17] showing that acetonitrile was a better eluent than pure methanol for components with π electrons.

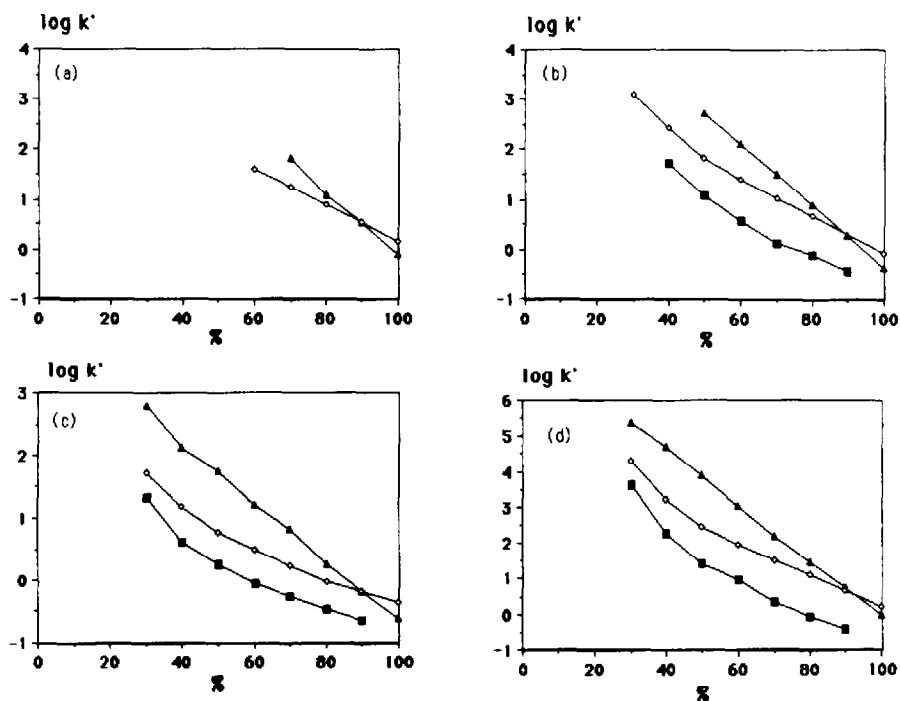


Fig. 1. $\log k'$ vs. volume fraction of organic solvent in water on the Hypersil C18 column. \blacktriangle = Methanol-water mixtures; \diamond = acetonitrile water mixtures; \blacksquare = THF-water mixtures. (a) *n*-Alkane with $n = 9$; (b) chloro-*n*-alkane with $n = 9$; (c) *n*-alkanol with $n = 9$; (d) phenyl-*n*-alkane with $n = 9$.

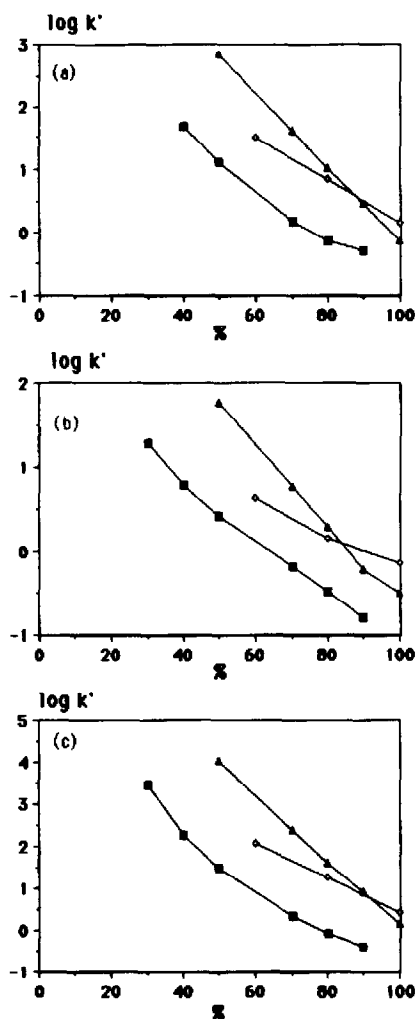


Fig. 2. Log k' vs. volume fraction of organic solvent in water on the RP-18 column. ▲ = methanol-water mixtures; ◇ = acetonitrile-water mixtures; ■ = THF-water mixtures. (a) Chloro-*n*-alkane with $n = 9$; (b) *n*-alkanol with $n = 9$; (c) phenyl-*n*-alkane with $n = 9$.

Similarly, the curves obtained with THF were systematically lower than those obtained with methanol and acetonitrile. The study of published data [21] for variously substituted aromatic compounds led to the same type of behaviour. This shows the general nature of the phenomenon, independently of the chemical nature of the ligand (aromatic or hydrocarbon chain substituted differently). Even though results with pure THF could not be obtained as a result of its particular behaviour [12], the variation of $\log k'$ vs. % THF was linear in the range studied. Extrapolating k' values for pure THF always furnished values much lower than those obtained with the other organic modifiers investigated.

Effect of bonded chain length. The same study was performed by using a methyl

phase. An example of the results is reported in Fig. 3. The general behaviour is similar to that observed with C_{18} phases.

Long-chain alkanols have an increased k'_{CH_3CN}/k'_{CH_3OH} selectivity in comparison with the other series tested. This is shown by a k' value in pure acetonitrile which is not aligned with those obtained with partially aqueous mixtures. This reflects the additional influence of residual silanols on retention and demonstrates a specific silanol–ligand interaction for this type of ligand via hydrogen bonds when this solvent is used. The covering of silica by methyl links permits easy access of residual silanols.

Relationship between retention characterized by k' and the surface tension of the mobile phase. Eqn. 1 according to Melander and Horváth [2] can be used to relate the change in the $\log k'$ value of a given ligand on a given column to the variation in surface tension, γ , of the mobile phase. We therefore plotted on the same curve the relative variation of this parameter for aqueous mixtures of three organic modifiers often used in PARP (Fig. 4).

If we consider only surface tension, it can be predicted that for organic modifier contents lower than 70%, the retention of the same ligand in aqueous–organic mixtures of the same volume composition would be in the order THF < CH₃CN < CH₃OH. For higher percentages, the order would be CH₃OH < THF \approx CH₃CN.

The curves recorded for methanol–water and acetonitrile–water mixtures follow the above predictions relatively closely (Figs. 1 and 2). The difference between the

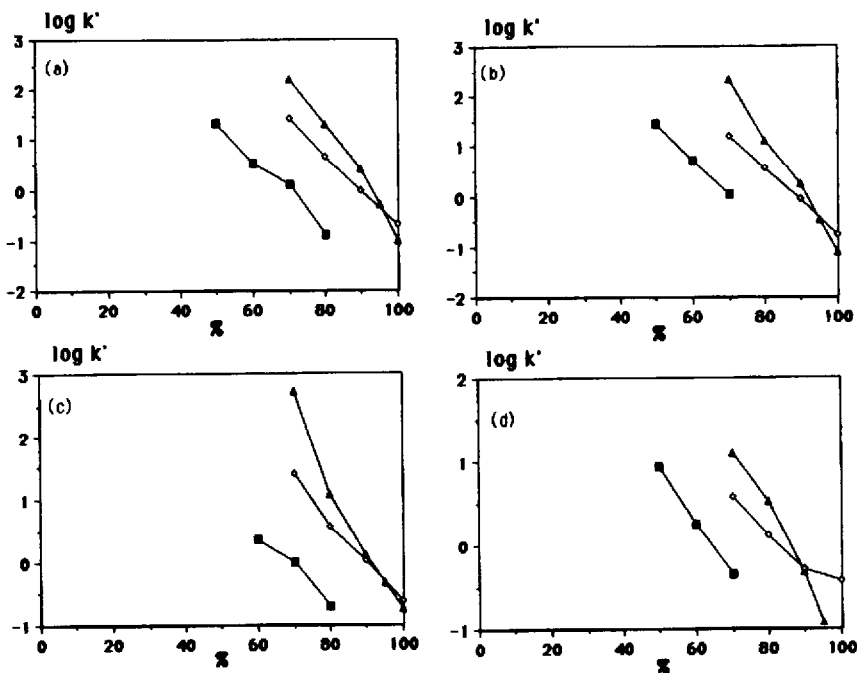


Fig. 3. $\log k'$ vs. volume fraction of organic solvent in water on the RP-2 column. \blacktriangle = methanol–water mixtures; \diamond = acetonitrile–water mixtures; \blacksquare = THF–water mixtures. (a) Phenyl-*n*-alkane with $n = 18$. (b) *n*-alkyl benzoate with $n = 18$; (c) *n*-alkane with $n = 18$; (d) *n*-alkanol with $n = 18$.

experimental value and the theoretical value of 70%, predictable from Fig. 4, can be explained by χ_e , a factor which adjusts the macroscopic surface tension to molecular dimensions, especially since the 90% crossover is constant, regardless of the chain length and polarity of the ligand.

In the case of ligands with π electrons, there is a shift of the crossover point. This is the consequence of a smaller retention in acetonitrile than in methanol with regard to the pure effect from pure solvophobic theory. This can be interpreted as the result of the development of specific interactions between a ligand and the solvent, leading to better solvation of the complex and its faster elution.

With THF, the $\log k'$ vs. % organic modifier curves do not cross those obtained with methanol, and apparently call into question an interpretation based solely on surface tension. This led us to compare the homologous series in THF using both chromatography and infrared spectrometry in order to elucidate the particular role of this modifier.

Role of THF in the retention of homologous series

As reported in the Introduction, the retention of homologues must increase with increasing size of the cavity ΔA . A linear increase is not verified thermodynamically but is justified by the results of numerous experiments in methanol. We therefore tested whether same phenomenon can be observed with THF.

The results are reported Fig. 5. Regardless of the series, the variation of $\log k'$ vs. n_C is not represented by broken lines, but by curves. This was confirmed by plotting α vs. n_C curves (Fig. 6), which are much more sensitive to slight changes in selectivity, and which exhibit a regular decrease. The mean value of α was identical for all the series tested for the same limiting values of n_C , confirming the convex shape of the first type of plot ($\log k'$ vs. n_C). The effect observed is due to the solvent and not to the nature of the link. Indeed, with the same column the plots of α vs. n_C with methanol-water mixtures (or pure methanol) furnished a curve with a jump n_{crit} equal to 14, as already reported with other monofunctional C_{18} -bonded phases.

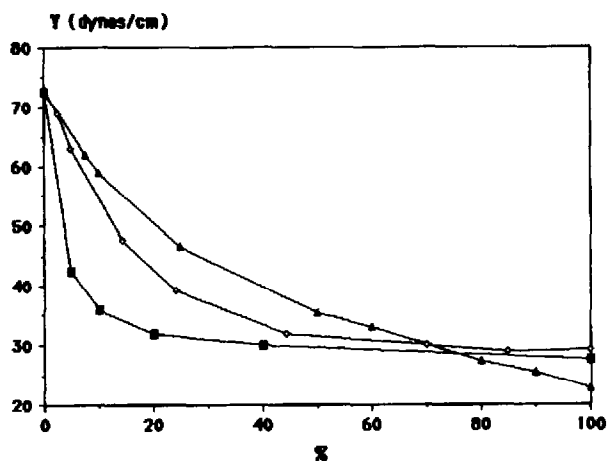


Fig. 4. Surface tension (γ) vs. volume fraction of organic solvent in water. \blacktriangle = Methanol-water mixtures; \diamond = acetonitrile-water mixtures; \blacksquare = THF-water mixtures. From ref. 2.

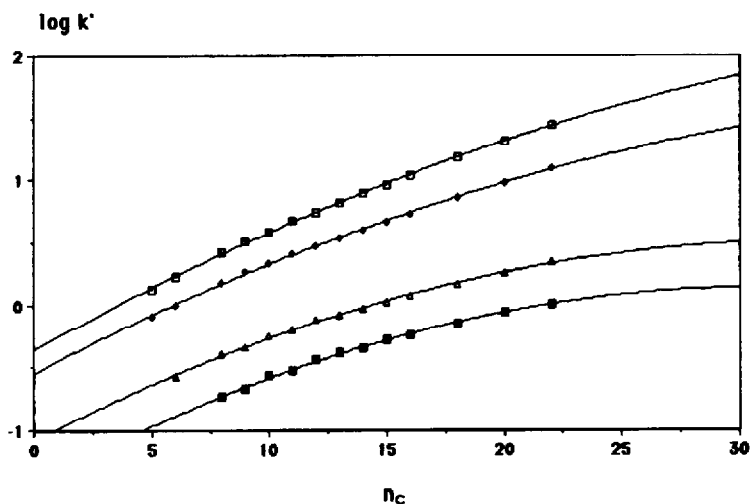


Fig. 5. Log k' vs. n_c plots on a Spherisorb ODS-I column for n -alkyl benzoates in THF-water mixtures. THF: water ratio: \square = 55:45; \blacklozenge = 60:40; \triangle = 70:30; \blacksquare = 75:25.

In another way, we were able to show that ligands are excluded by using pure tetrahydrofuran or tetrahydrofuran-chloroform mixtures, as had also been by others [11,22]. The results (Fig. 7) show that at water contents above 5% alkanes are well

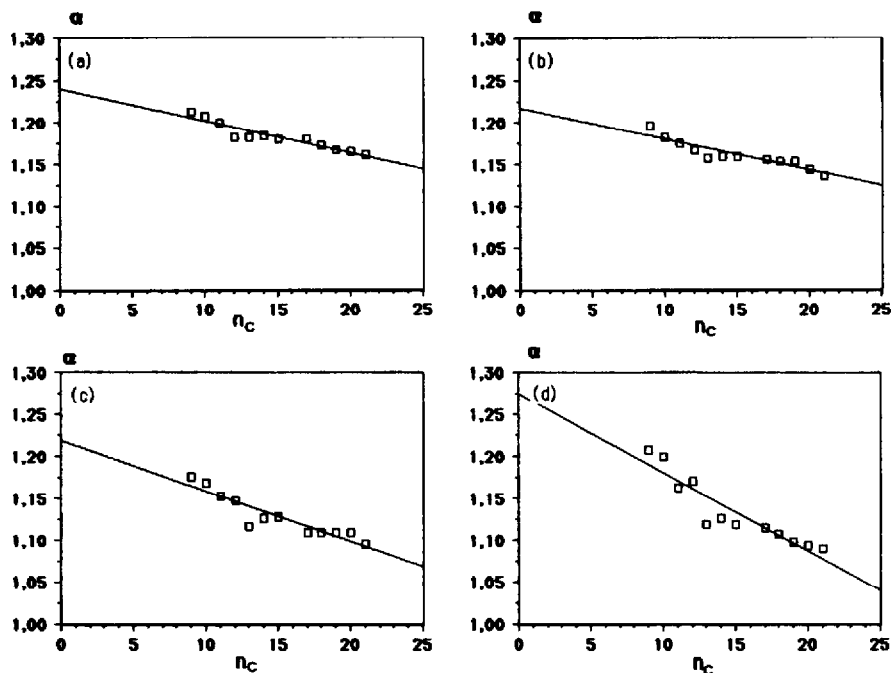


Fig. 6. Quadratic methylene selectivity [8] (α_n) vs. n_c plots on a Spherisorb ODS-I column for n -alkyl benzoates in THF-water mixtures. THF: water ratio: (a) 55:45; (b) 60:40; (c) 70:30; (d) 75:25.

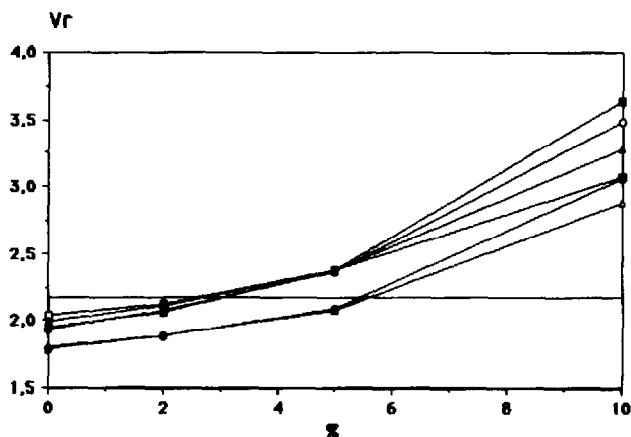


Fig. 7. Absolute retention volume (V_r) of ligands vs. volume fraction of water in THF on a C_{18} column. ■ = *n*-Alkane with $n = 36$; ○ = *n*-alkane with $n = 32$; ▲ = *n*-alkane with $n = 26$; □ = *n*-alkane with $n = 20$; △ = triglyceride with $n = 16$; ● = triglyceride with $n = 18$. Horizontal line, void volume of the column.

retained and a common point of intersection can be seen for a value of V_r close to but slightly higher than the column dead volume obtained with the method of two solvents. This phenomenon is the same for alkanes and triglycerides. The same study undertaken with tetrahydrofuran–chloroform mixtures (up to 25% chloroform) showed that the retention volume of each of the ligands used in this study remained the same as in pure tetrahydrofuran. The points on the $\log M$ vs. V_r plot are aligned for the ligands studied, contributing evidence for an exclusion phenomenon.

A conformation change of the chains in this modifier could explain all the above results. This will be described below.

Conformational infrared study

Calculations have shown [23] that four bands in the region 1480 – 1320 cm^{-1} were characteristic of the conformation of alkyl chains: $\nu = 1378$ cm^{-1} , all-*trans* conformation of the chain; $\nu = 1341$ cm^{-1} , end-*gauche* conformation; $\nu = 1354$ cm^{-1} , *gauche-gauche* conformation (two consecutive non-planar left bonds); $\nu = 1367$ cm^{-1} , kink model conformation (*gauche-trans-gauche* sequences).

Based on these attributes, we studied the conformation of different bonded alkyl phases and of the corresponding alkanes in different deuterated solvents. We observed that regardless of the solvent, THF- $^2\text{H}_8$, $\text{C}^2\text{H}_3\text{O}^2\text{H}$, C^2HCl_3 or $\text{C}^2\text{H}_3\text{CN}$, the four expected spectral bands were present in variable proportions. Solubilization in partially aqueous mixtures did not change the phenomenon qualitatively.

The results (Fig. 8) call for several comments. The proportion end-*gauche*, *gauche-gauche* and kink conformations increases consistently with the carbon number of the alkane in all four solvents tested. We confirmed the results of Sander *et al.* [24] and showed that the same changes occurred in the other three solvents. For an alkane of given chain length, the proportion of kink conformations in methanol and acetonitrile is similar to but lower than that in tetrahydrofuran or chloroform, whereas the inverse occurs for the weights of the *gauche-gauche* and end-*gauche* conformations.

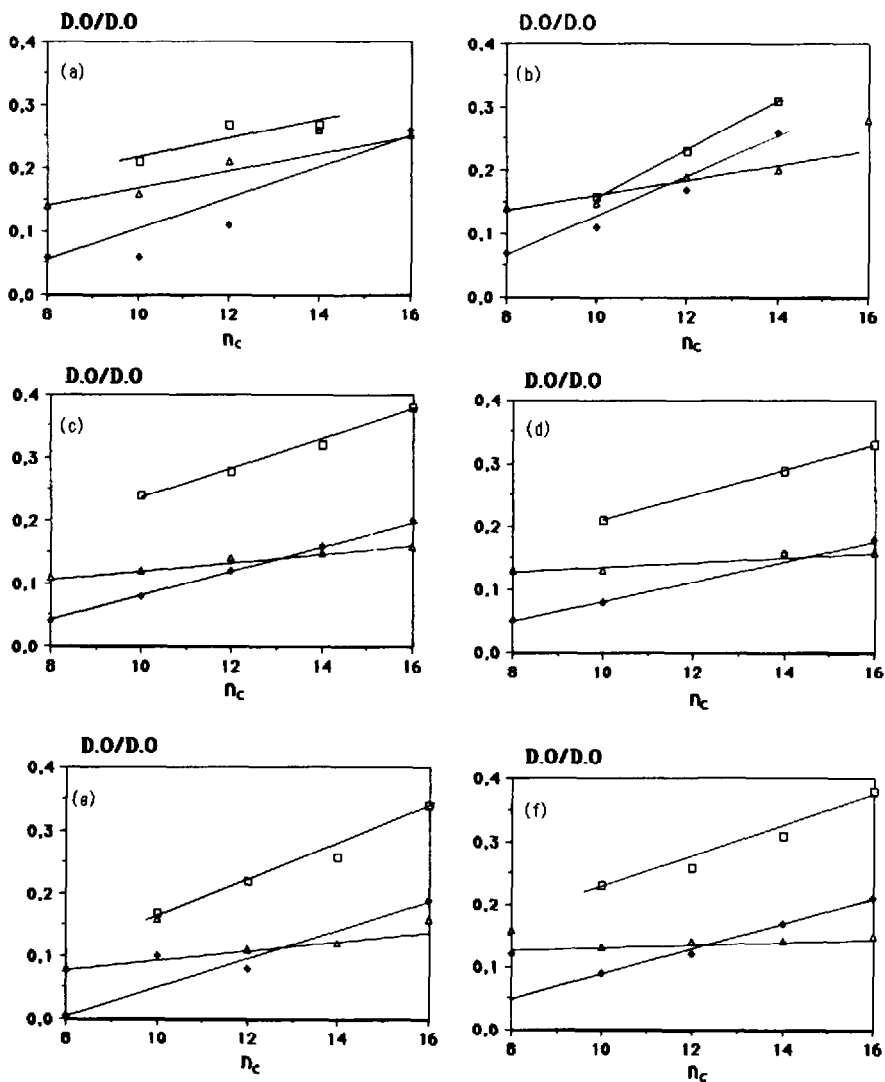


Fig. 8. Ratio of vibration band absorbances related to different alkyl chain conformations vs. alkane chain length. \square = Ratio D.O. kink/D.O. all-*trans*; \triangle = ratio D.O. *gauche-gauche*/D.O. all-*trans*; \blacklozenge = ratio D.O. end-*gauche*/D.O. all-*trans*. (a) in $C^2H_5O^2H$; (b) in 2H_3CN ; (c) in $THF-{}^2H_6$; (d) pure ligand; (e) in C^2HCl_3 ; (f) in $THF-{}^2H_6-{}^2H_2O$ (90:10). D.O. = optical density.

Hence the fact that the values obtained in THF are different from those in methanol and acetonitrile confirm, as we have seen in chromatography, that THF has a particular behaviour towards alkyl chains.

The proportions of the conformations in both acetonitrile and methanol being similar, we studied the role of the first solvent with regard to the chromatographic process. We studied particularly ligands with π electrons. For all these ligands, the results are entirely comparable to those obtained with methanol [8] (Fig. 9). There is a

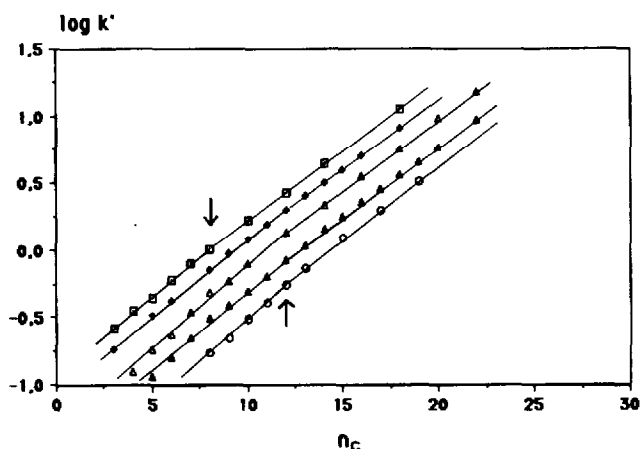


Fig. 9. Log k' vs. n_c plots on an RP-14 column [solvent: acetonitrile–water (95:5)]. \square = Phenyl- n -alkanes; \blacklozenge = n -alkyl benzoates; \triangle = chloro- n -alkanes; \blacktriangle = methyl esters of n -alkanecarboxylic acids; \circ = 2- n -alkanones.

break in the curve for a critical chain length, independent of the series tested, characteristic of the bonded chain length and independent of the percentage of water and temperature. A shift in this critical value, n_{crit} in the case of phenylalkanes and *o*-phthalates in comparison with the other homologous series tested confirms that these ligands penetrate the interior of the linkage by their aromatic part in acetonitrile–water mixtures as in methanol water mixtures.

On the other hand, it should be noted that in NARP in pure acetonitrile the aromatic ring does not penetrate the linkage but rather remains at the exterior [16]. We have recently confirmed these results and showed that at least 5% acetonitrile in methanol is necessary for the development of a specific interaction between acetonitrile and the aromatic ring to cause non-insertion of this ring in the ligate. This phenomenon of non-insertion had been reported for alkyl benzoates [8] for all the solvent studied.

Acetonitrile is thus a particular modifier, introducing specific interactions with ligands having π electrons.

Interpretation of the results in relation to the solvophobic theory

Regardless of the modifier used, the behaviour of homologous series is directly related to the increase in the hydrophobic volume, ΔA , of the ligand with increasing n_c (eqn. 1).

IR results in methanol and acetonitrile show that regardless of the length of the chain, the all-*trans* conformation is the predominant one. However, when n_c increases, the proportion of kink and *gauche gauche* conformations causes a deviation from linearity and flatness of the chains. This leads to a less intimate contact of solutes with the linkage, especially as the number of carbon atoms in the solute increases. If we consider the energy of contact of each methylene, the addition of an additional methylene does not cause a constant variation of the energy of interaction when n_c increases. Hence the methylene selectivity is not constant but declines slightly

with increasing carbon number. In THF, the solutes behave differently than in methanol and acetonitrile. The fact that in THF the proportion of the kink conformation is higher than that in the other two does not prevent penetration between the ligates. The contact of solutes is distributed according to the position of the kink in the linkage chain. This contact is less intimate and increases less regularly than with a ligate which is stretched. Hence methylene selectivity decreases slightly with increasing chain length and the plots of $\log k'$ vs. n_C are curved.

Moreover, the very low retention observed with this solvent cannot be explained only by surface tension. It reflects its high solvation power for hydrocarbon chains. Restricted movements in methanol [25,26] or acetonitrile [27] favour longer contact times between the alkyl chains and thus a more intimate contact than in chloroform or dioxane [25,26] (and by analogy THF), where movements are much freer. In eqn. 1, this may be related to the dielectric constant ϵ of THF, which is very low in comparison with those of acetonitrile and methanol. This is the third factor responsible for retention when surface tensions are similar.

Verification of the linear approximation in binary mixtures

The detailed study of $\log k'$ vs. % organic modifier shows apparent differences between the three solvent systems used.

The separation factors between two ligands L_1 and L_2 are represented by the vertical difference between the $\log k'$ vs. % organic modifier curves for a given composition of mobile phase:

$$\log \alpha_{L_1L_2} = \log k'_{L_1} - \log k'_{L_2} \quad (2)$$

In the case of homologous series, $\log \alpha_{L_1L_2}$ represents the methylene selectivity [15]. For mixtures with a high percentage of organic modifier, the separation factor increases in the order THF < acetonitrile < methanol. The reverse order is observed for mixtures poor in organic modifier (< 50%). Hence, in the case of solutes requiring analysis at a low percentage of organic modifier, more homologues can be separated in methanol than in tetrahydrofuran.

The curves of the different ligands are convergent, regardless of the modifier used. This had been noted with another series of ligands with different chemical structure, *i.e.*, differently substituted aromatic compounds [21]. The originality of the present results is that for homologous series this is also verified in acetonitrile, which is not the case for the other ligands tested. A practical application of this property permitting the separation of homologous series to be optimized is suggested below.

Application to the determination of parameters permitting the prediction of retention of homologous series

A scale of elution strength of different solvents used in HPLC is based on the value of the methylene selectivity [11,28]. This elution strength scale is defined by analogy with Snyder's processing [29] of adsorption on polar surfaces. It characterizes only the purely solvophobic effect and thus has a real basis provided that this effect is manifested by an increase in retention by a constant increment of the compounds in a homologous series. This property is not verified for THF-water mixtures.

We therefore attempted to predict the retention of ligands based on Snyder *et al.*'s processing [13] with

$$\log k' = \log k'_0 - S\phi \quad (3)$$

This equation takes all interactions into account.

We determined the values of $\log k'_0$ and S by rendering the curves in Figs. 1 and 2 linear for $\log k'$ values between 0 and 2. Regardless of the organic modifier, the correlation coefficients were systematically higher than 0.998. The linear correlation of these values of S and $\log k'_0$ was made with eqn. 4:

$$S = p + q \log k'_0 \quad (4)$$

Such a treatment had already been applied to methanol, with 32 aromatic ligands and with different columns. The values of p and q deduced permitted the successful prediction of the retentions of different ligands, using an isocratic mobile phase and a single gradient [30]. Application of the same treatment to the homologous series tested in this work resulted in S vs. $\log k'_0$ curves shown in Fig. 10. As can be seen, and in contrast to previous results, the points are aligned regardless of the solvent (Table I), suggesting the possibility of optimizing the separation of homologous compounds or long-chain saturated compounds with different polarity by the use of a single elution gradient [31], without being required to start by using a mobile phase containing methanol.

CONCLUSIONS

This study has confirmed the application of the solvophobic theory to explain reversed-phase chromatography. Capacity factors increase with increasing hydrocarbon surface of ligand-ligand contacts. This is true regardless of whether the mechanism of molecular interaction occurs via an intimate contact of the ligand (in acetonitrile or methanol) or by a more or less loose contact (in THF or on methyl-bonded columns for all solvents).

The variation of the retention of solutes as a function of the percentage of organic modifier in the mobile phase is calculated based on the variation in the surface tension of the eluting mixture. In the most general case, this is realized with methanol or acetonitrile, whether or not the ligand penetrates the stationary phase and regardless of its polarity. Pure methanol is generally a better eluent than pure acetonitrile.

The development of specific ligand modifier or ligand-modifier interactions causes a change in selectivity leading to a crossover of $\log k'$ vs. % organic modifier curves when the latter is higher than 90%. In some instance, acetonitrile is an even better eluent than methanol. The linearity of the $\log k'$ vs. n_c curves is unaffected, but the nature of the penetration mechanism may be modified depending on the nature of the polar head of the ligand. Thus, acetonitrile develops specific π - π interactions, in particular with aromatic or multi-double-bond systems. They may be profitably used in NARP to optimize the separation of these compounds.

As a result of its low dielectric constant, THF solvates hydrocarbon chains

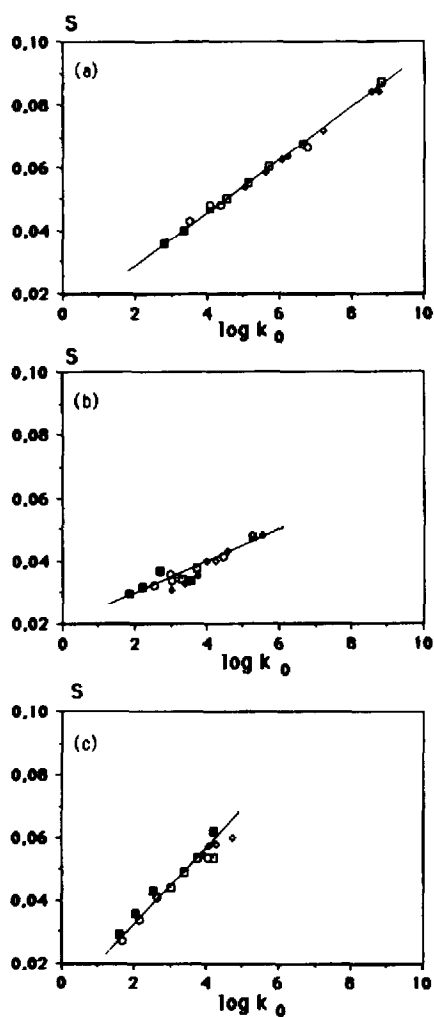


Fig. 10. S (slope of $\log k'$ vs. % organic modifier) vs. $\log k_0$ on a Hypersil C_{18} column. (a) Methanol-water mixtures; (b) acetonitrile-water mixtures; (c) THF-water mixtures. \square = Chloro- n -alkanes; \blacklozenge = n -alkanes; \blacksquare = n -alkanols; \diamond = phenyl- n -alkanes; \circ = methyl esters of n -alkanecarboxylic acids.

TABLE I

GRADIENT SHAPE PARAMETERS

Stationary phase	Modifier	p (eqn. 4)	q (eqn. 4)	r (correlation coefficient)
Hypersil C_{18}	Methanol	3.06	0.8	0.998
	Acetonitrile	4.26	0.53	0.936
	THF	2.85	1.07	0.980
Lichrosorb RP-18	Methanol	3.27	0.8	0.999
	Acetonitrile	1.61	0.87	0.992
Spherisorb ODS-1	THF	3.88	1.0	0.985

much better and even though the solvophobic effect is generally respected with this modifier when mixed with water, the retention of ligands is always lower than in other solvents. The mobility of chains and the wide range of conformations in this solvent (shown by IR and NMR) even lead to a phenomenon of exclusion when it is used pure. On the other hand, this property can be used to solubilize ligands with a large hydrocarbon volume by adding several percent of this modifier to methanol or acetonitrile, which will lead to a mechanism of interaction by insertion.

The non-linear behaviour of ligands in THF as a function of their chain length explains the erroneous predictions obtained when optimizing long-chain separations based on the idea of elution strength deduced from methylene selectivity. An approach to this problem on the basis of the p and q values deduced from studies of the linear variation of retention in binary mixtures is more appropriate.

SYMBOLS

a_s	Characteristic constant of solvent
$\Delta A =$	$A_L + A_1 - A_{L_1}$
A_L	Surface area of ligand (solute)
A_1	Surface area of ligate (stationary phase)
A_{L_1}	Surface area of complex
A_s	Surface area of solvent
k'	Capacity factor of ligand
k'_0	Extrapolation of the k' value in pure water
N	Avogadro's number
p_0	Atmospheric pressure
R	Gas constant
S	Slope of $\log k'$ vs. φ plot.
T	Absolute temperature
V_s	Molar volume of solvent
V_r	Absolute retention volume of ligand
V	Volume of stationary phase
V_m	Volume of mobile phase
W_s	Characteristic constant of solvent
$\Delta Z =$	$Z_{L_1} - Z_1 - Z_L$
Z_{L_1}	Partial electrostatic charge of complex
Z_1	Partial electrostatic charge of ligate
Z_L	Partial electrostatic charge of ligand
α	Methylene selectivity = $\log(k'_{n+1}/k'_n)$ ratio of capacity factors for two consecutive homologous ligands
α_n	Quadratic methylene selectivity = $(k'_{n+1}/k'_{n-1})^{1/2}$
$\alpha_{L_1L_2}$	Separation factor: ratio of capacity ratios between two ligands L_1 and L_2 for a given mobile phase composition
γ	Surface tension of bulk solvent
ϵ	Dielectric constant of solvent
χ_c	Factor adjusting the macroscopic surface tension to molecular dimension
φ	Volume ratio of organic modifier

58.7 cm/s and $H_{\min} = 0.170$ mm, and a plate number of 146 910. By considering also the Du^2 term, these values become 57.0 cm/s, 0.172 mm and 145 027, respectively (substituting $D_G = 0.25$ cm²/s in the Golay equation, corresponding to *n*-octane at room temperature with hydrogen as carrier gas). The differences are negligible. However, with a specially designed system for ultra-fast analyses (85 cm × 0.065 mm I.D. column and total extra column variance only 0.0000685 s², calculated from Fig. 1) extra-column effects become dramatic: the optimum velocity falls to 110 cm/s instead of 226 cm/s, the minimum HETP is 0.072 mm instead of 0.044 mm and we have only 11 834 instead of 19 211 plates; the Du^2 term will amount to 21.7% of the total HETP value calculated at $u = 110$ cm/s.

It would be possible to characterize simultaneously the resolution and the rapidity by a new, not yet defined, chromatographic term, which we can call "performance". We shall use it in the following manner: (a) for two chromatographic systems, that with the higher performance gives the same resolution in a shorter analysis time; or (b) the performance is higher if the system gives a higher resolution in the same time. In both instances we assume that the same mixture is injected and the same type of stationary phase is used.

Gaspar *et al.* [7] introduced the notion of the time necessary to generate a theoretical plate (*TH*), defined as

$$TH = \frac{\sigma^2}{t_R} \quad (9)$$

which gives the zone broadening as the time-based variance increase per unit time spent in the chromatographic system and which can characterize the system performance. Considering eqn. 10 also, *TH* is the inverse of the magnitude already used, *i.e.*, the number of plates generated per unit time. *TH* is an analogous quantity to HETP; while the classical theory of GC is based on the efficiency, we shall use the performance measured by *TH*. The best performing system is the one which results in the smallest increase in band time variance in a given time. By transformations, we obtain from eqn. 9

$$TH = \frac{\sigma^2}{t_R^2} \cdot t_R = \frac{t_R}{N} = (k' + 1) \frac{L}{u} \cdot \frac{H}{L} = (k' + 1) \frac{H}{u} \quad (10)$$

and from eqn. 7

$$TH = (k' + 1) \left(\frac{B}{u^2} + C + Du \right) \quad (11)$$

By derivation, we can show that *TH* has a minimum at an optimum velocity given by

$$u' = (2B/D)^{1/3} \quad (12)$$

It is easy to show that this is the velocity at which the tangent, through the origin, touches the $H-u$ curve (*cf.*, Fig. 1). We have to understand the meaning of u' in the following way: starting from the optimum velocity (which gives the minimum HETP value) and increasing the carrier gas velocity up to u' , the increase in analysis speed is