Journal of Chromatography A, 656 (1993) 81–112
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 268

Review

General view of molecular interaction mechanisms in reversed-phase liquid chromatography

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ABSTRACT

The compilation of the results obtained during the last 10 years on the retention behaviour of homologous series in reversed-phase liquid chromatography makes it possible to build a coherent model of molecular interactions for this type of sample. This model takes into account the effects of the chain length and the polarity of the sample molecule. It rationalizes the influence of the bonding density and the function nature (monomeric/polymeric) of the stationary phase and the role of temperature and the nature of the organic modifier in partially aqueous and non-aqueous reversed-phase liquid chromatography. The use of homologous series to investigate retention mechanisms gives the opportunity to distinguish between the solvophobic and the partition theories. Depending on the experimental conditions, both theories can explain the results observed and the changes in retention pattern when altering one of the chromatographic parameters previously mentioned. The description of the structure of the stationary phase as a brush, a picket fence or a stack is also integrated in the model. The experimental results accumulated with homologous series also suggest that the molecular interaction mechanisms governed by molecular recognition sites differ for molecules that are members of different homologous series, polycyclic aromatic hydrocarbons or carotenes.

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0021-9673/93/$24.00

1. INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is one of the most popular analytical techniques. It is usually based on the use of non-polar bonded silicas as the stationary phase. The vast differences that exist among these phases have been revealed by various approaches such as physico-chemical techniques [1–47] or the injection of test mixtures [1,48–62]. It is clear that the active parts of the stationary phases are the bonded species themselves. However, the underlying silica often plays a non-negligible role because of the unmodified silanol groups. There are two fundamental theories which account for the experimental results obtained in RPLC: the solvophobic theory [63] adapted to RPLC by Horváth *et al.* [64] and the partition theory developed by Dill and co-workers [65–68] following the work of Martire and Boehm [69] and derived from the statistical theory of crystalline lattices [70–72]. The main difference between these two approaches originate in the fact that the stationary phase plays a passive role in the first case whereas it is active in the second. Both theories, however, allow one to make similar predictions. For instance, the capacity factor is expected to increase with increase in the solute hydrocarbonaceous surface area and the surface tension of the mobile phase. Much work has been done on verifying the contribution of the first parameter and homologous series are a very powerful tool in this respect. All the studies made at different temperatures, with various types of bonded silicas, using aqueous–organic or non-aqueous mobile phases of different compositions (both volume composition and nature of the solvents) provide a better understanding of the molecular interaction mechanisms governing the retention of solutes possessing long hydrocarbon chains, compared with that envisioned for rigid molecules.

It is known that the retention time or, better, the capacity factor (k') of a substance is related to the change in partial molar free energy associated with the transfer of the solute (assumed to be in the standard state at infinite dilution) from the mobile phase to the stationary phase, ΔG_0, according to [73]

$$\log k' = -\frac{\Delta G_0}{2.3\,RT} + \log \phi \qquad (1)$$

where ϕ is the phase ratio. ΔG_0 depends on the stationary and mobile phases, the solute and temperature. In order to correlate ΔG_0 with the structure of the sample molecule, a simple approach consists of assuming that the molecule is made up of several groups, each of them being characterized by its own energy of transfer, $\Delta G_{0,i}$:

$$\Delta G_0 = \sum \Delta G_{0,i} \tag{2}$$

On the basis of eqn. 2, one would expect, in the particular case of homologous series, a linear relationship between ΔG_0 (and log k') and the number of carbon atoms (n_c) in the alkyl chain of the solute molecule:

$$\log k' = A' + n_c B' \tag{3}$$

This linear relationship between log k' and n_c has been used by numerous workers to calculate the column dead volume [74–76] or predict retention [77–88]. In a more general manner, the influence of the chain length (n_c), mobile phase composition (volume fraction, X) and temperature can be described by an equation such as [88]

$$\log k' = A + n_c B + XC + n_c XD + \frac{n_c E}{T} + \frac{XF}{T} + \frac{n_c XG}{T} \tag{4}$$

Eqn. 4 assumes the existence of a linear relationship between log k' and the mobile phase volume composition, X. If such a linearity does not exist over the whole range of mobile phase composition (from $X = 0$ to $X = 1$), log k' *vs.* X plots can be linearized in a limited range of eluent composition, however. If one can then assume the existence of a linear relationship between log k' and X, then the corresponding equation combined with eqns. 1 and 3 generates eqn. 4.

Much effort has been devoted to the study of the retention behaviour of homologous series, but unfortunately many workers have used only limited numbers of homologues. For most of these studies only two parameters have been investigated, the third being kept constant [62,82,89–92]. A limited number of thorough studies have been published, however, including the work of Guiochon and co-workers [61,74,77–82,91,93–97]. All the data available on homologous series lead to the observation that, for each series investigated, log k' *vs.* n_c plots at different solvent compositions and a given temperature or different temperatures and a given solvent composition are sets of straight lines with a convergence point. This point has well defined energetic significance and, in particular, the associated compensation temperature [98,99] is a useful tool for studying retention mechanisms and comparing chromatographic systems. The existence of the convergence point is the consequence of the phenomenon of enthalpy–entropy compensation: the enthalpy change is linearly related to the entropy change. According to Melander and Horváth [100], the enthalpy–entropy compensation is expected to occur only if uniformity of retention mechanism exists. The compensation temperature is characteristic of the type of mechanism. Compensation temperatures have been found to be identical for the retention of a variety of solutes on different types of reversed-phase columns [98]. Values of the compensation temperature in the range 500–900 K have been measured with reversed-phase systems, whereas the values associated with adsorption processes on naked silica are around 140 K. In the case of pure partition mechanisms, the compensation temperature has been found to be 450 K.

The first aim of this review is to compile information obtained with homologous series in order to obtain a global overview of the effects on retention properties of such parameters as temperature, nature of the solvent, chain length and structure of the chains bonded to the silica (monomeric and polymeric).

The second aim is to try to propose a uniform mechanism of molecular interaction. Sander and Wise [55] have published a comprehensive review on retention aspects in RPLC. They have also shown [53–56], using rigid molecules such as polycyclic aromatic hydrocarbons (PAHs), that the structure of the stationary phase controls the mechanisms of interaction responsible for retention. The separations of carotenes and other rigid molecules also seem to be very sensitive to the structure of the stationary phase [101–104]. In order to understand better retention mechanisms and particularly the effect of the structure of the stationary phase, it is of interest to compare the results obtained with PAHs and carotenes with those derived from studies on homologous series. The purpose of this contribution is to investigate whether the mechanisms of interaction are identical when the solute is a flexible or a rigid molecule. This problem is discussed in the second part of this review.

2. OVERVIEW OF THE RETENTION PROCESS

In the following, we shall try to present a molecular model accounting for the interactions that can take place between the solute molecules and the stationary phase in reversed-phase chromatography. This model, based on retention data for homologous series, must account for the effects of the solute nature, mobile phase composition and temperature. Several retention models have been proposed, based on either adsorption or partition. Originally, Horváth *et al.* [64] explained the retention mechanism using the solvophobic theory (see Figs. 1 and 2). Later, Dill [65] and Dorsey and Dill [66] explained it in terms of partitioning. This is illustrated in Fig. 3a, where S, A and L stand for the mobile phase, the solute and the stationary phase, respectively. In the partitioning process, the solute A is transferred from a bulk phase S into another bulk phase L. Dorsey and Dill explained the solvophobic theory of Horváth *et al.* [64] as schematized in Fig. 3c. The key point in this approach is that there is no fundamental difference between A and L. Using the same type of diagram, we have represented the adsorption of A at the surface of the stationary phase L in Fig. 3b. Four descriptions of the interactions between the solutes and the stationary phase have been proposed, based on different models of organization of the stationary phase:

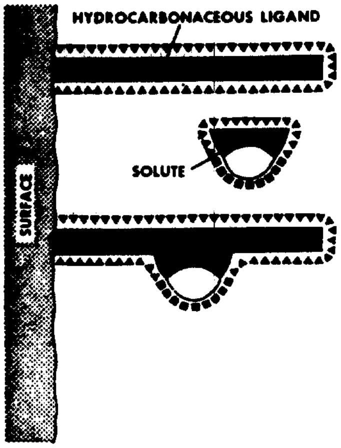

Fig. 1. Schematic illustration of the formation of the molecular association complex between the solute and the hydrocarbonaceous function on the surface of the stationary phase. From ref. 64.

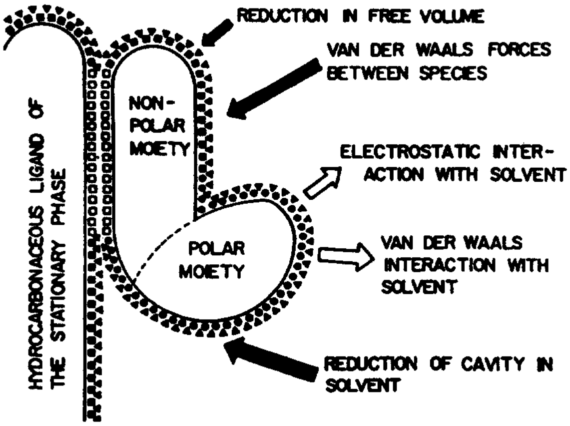

Fig. 2. Schematic illustration of the association between an amphiphilic solute and the hydrocarbonaceous ligand of the stationary phase in reversed-phase chromatography. Water represented by open squares covers the molecular surface area by which the total cavity area is reduced on contact of the two species. The arrows symbolize the forces involved in this process. [From Cs. Horváth and W.R. Melander, *Int. Lab.*, Nov./Dec. (1978) 11–35.]

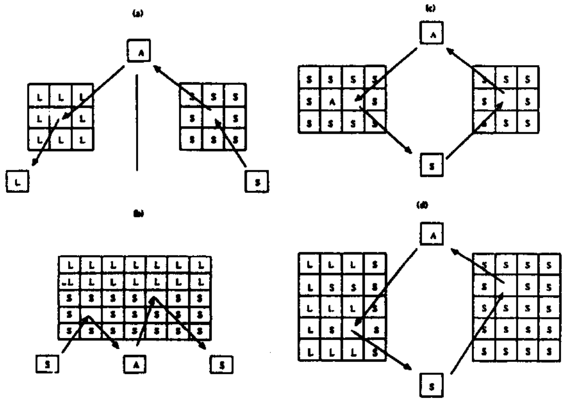

Fig. 3. Schematic representations of possible retention mechanisms in reversed-phase chromatography. (a) Partitioning process: the solute molecule A is transferred from the mobile phase S to the stationary phase L. (Copied and adapted from ref. 67.) (b) Adsorption process. (c) Pure solvophobic theory (copied and adapted from ref. 67). (d) Reversed-phase process on a monofunctional phase or a polyfunctional phase at a temperature above the phase transition.

(i) *Picket fence*. This suggests an interaction on the terminal parts of the bonded chains, similar in a way to an adsorption process (Fig. 4a).

(ii) *Brush or fur*. In this case, there is an insertion of the solute molecules inside the stationary phase (Fig. 4b). This is also somewhat similar to an adsorption process.

(iii) *Greasy droplet or haystack*. The solute molecules are immersed inside the stationary phase, similar to a partition process (Fig. 4c).

(iv) The last representation takes into account the various conformations of the stationary phase chains. The bonded chains are no longer considered like rigid sticks (Fig. 4, bottom left) and their interactions with the solute and the mobile phase must be considered (Fig. 4, bottom right).

It is not a simple task, however, to define the conformation of the bonded chains. Actually, four different conformational states exist (see Fig. 5): all-*trans* (tt), end-*gauche* (tggt), *gauche–gauche* (tgt) and *kink* (gtg′). The contribution of each of these states can be estimated from IR spectroscopy, with and without a solvent [14,95,105]. The energy differences between these conformations are small at ambient temperature and thus all conformations can exist simultaneously. With increasing temperature, the probability of *gauche* and *kink* conformations increases slowly. In this respect, it is worth comparing the bonded chains and the corresponding *n*-alkane molecules. According to Dorsey and Dill [66], the bonded chains cannot be treated as a bulk alkane phase because they do not have the freedom do take any possible conformation. It is clear that attaching the alkyl chains by one end prevents them from making certain motions. Also, lateral interactions between chains in the case of high bonding density phases favour certain conformations of the chains. According to Dorsey and Dill, the chains are more ordered, *i.e.*, more aligned in a bonded phase compared with the corresponding alkane molecules. This does not mean, however, that the chains are rigid and rod-like (see Fig. 4a–c). In order to understand how retention proceeds, a knowledge of the conformational state is required, as suggested by Stalcup *et al.* [106].

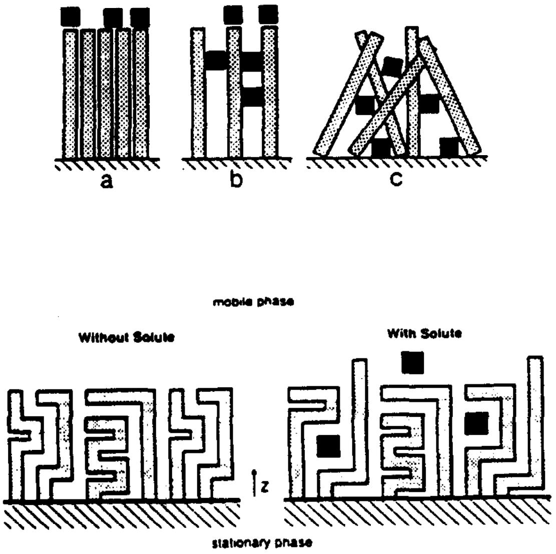

Fig. 4. Top: conventional models of molecular organization of stationary phase in RPLC [119]: (a) "picket fence"; (b) "fur"; (c) "haystack". Bottom: interphase model of molecular organization of stationary phases chains. From ref. 65.

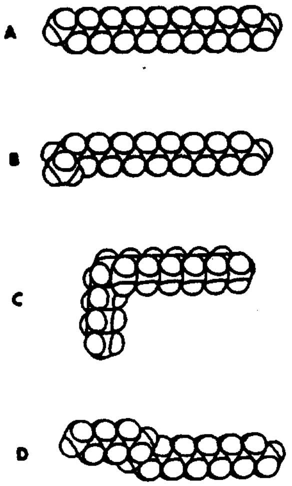

Fig. 5. Alkyl chain conformations. The non-planar conformations give rise to localized mode vibrations which are observed in the infrared spectrum. (A) All-*trans*; (B) end-*gauche*; (C) *gauche–gauche*; (D) *kink*. From ref. 14.

From the previous discussion, it is likely that, in contrast to what happens with a rigid phase such as silica or carbon, the molecular structure of an *n*-alkyl-bonded silica may change when the operating conditions are modified. Hence the differences between the chromatographic properties of a monofunctional and a polyfunctional phase should depend on temperature and solvent conditions.

Homologous series are particularly useful series of compounds to investigate retention processes over a wide range of experimental conditions (temperature, mobile phase volume composition and nature of the organic modifier). The results obtained with these solutes will be carefully examined and a synthesis of the approaches previously described will be attempted.

3. COMMENTS ON ANALYSIS OF RPLC DATA

Before discussing the results obtained with homologous series and how they can be used to investigate retention mechanisms, it is important to mention two points: the experimental conditions that must be verified in order to have reliable and meaningful data, and the mathematical treatments to be made with these data. The experimental conditions are as follows.

(1) Solutes belonging to a large number of homologous series differing widely in polarity must be used, so that the range of chain length (n_c) investigated is between 1 and 30–36. This is because measurements must be made with homologues with alkyl chains containing at least ten more carbons than the stationary bonded phase (n_{bp}).

(2) Many different stationary phases must be used: organic polymers, carbon-based materials, polymeric bonded silicas (resulting from the condensation of a polychloroalkylsilane with silica in the presence of water) and monomeric bonded silicas (resulting from the condensation of a monochlorosilane with silica). These last stationary phases can be homogenous (alkyl chain of varying length from 1 to 22 and propiophenyl), mixed (combination of such groups as cyanopropyl, *n*-octyl, *n*-octadecyl, cyanodecyl or *n*-undecyl) [76,107,108], polyfluorinated [109–111] or containing groups with localized charges built in the hydrophobic chains [112].

(3) A wide range of mobile phase and temperature conditions must be explored: mixtures of water with one or two organic modifiers (methanol, acetonitrile, tetrahydrofuran) and non-aqueous eluents made up methanol, acetone, methylene chloride, chloroform, etc., at different temperatures. Subcritical systems have also been used, based on carbon dioxide modified with methanol, acetonitrile, chloroform, etc. [76,113–115].

There are various ways to analyse retention data, as summarized below. Each of them can reveal a different aspect of the retention process.

(1) log k' *vs.* n_c plots are used to verify the expected linearity [96,116] resulting from eqns. 3 and 4. When this linearity is not observed throughout the range of n_c values investigated, the values at which changes in slope or even inflection points are observed should be determined. It is clear that the choice of the t_0 value is an important issue here, particularly when low k' are considered. It has been shown [96] that, although the choice of t_0 controls k' values, it does not change the overall appearance of the log k' *vs.* n_c plots and the fact that there is or is not a break point.

(2) The methylene group selectivity for each pair of successive homologues, α_n, allows one to quantify the average magnitude of the interactions involved:

$$\log \alpha_n = \log\left(\frac{k'_{n_c+1}}{k'_{n_c}}\right) = -\frac{\Delta(\Delta G_0)}{2.3\,RT} = -\frac{\Delta G_0^{\mathrm{CH2}}}{2.3\,RT} \quad (5)$$

Because of the levelling effect of the logarithmic function, the use of the methylene selectivity makes it possible to detect subtle phenomena that cannot be readily observed on the log k' *vs.* n_c plots.

(3) It is also recommended to calculate the quadratic selectivity, α_{nc} [91,94], defined as:

$$\alpha_{nc} = \left(\frac{k'_{n_c+1}}{k'_{n_c-1}}\right)^{1/2} \quad (6)$$

The quadratic selectivity allows one to compensate to some extent for experimental errors in the determination of k'. α_{nc} makes it possible to investigate more precisely how α_n changes with n_c.

(4) In the range of n_c values where plots α_{nc} *vs.* n_c are linear, it is possible to calculate the

average methylene selectivity, $\tilde{\alpha}$, for each homologous series from the value of the coefficient B' in eqn. 3: $\tilde{\alpha} = 10^{B'}$ [80,93]. $\tilde{\alpha}$ allows one to correlate the retention data with a molecular interaction through the molecular volume increment of a CH_2 group.

(5) Finally, it is possible to calculate the global methylene selectivity for a given n_c value, $\bar{\alpha}_n$: it is the average value of α_n for all the series investigated. The solvent strength can be defined from $\bar{\alpha}_n$ values.

(6) When the solute molecule possesses more than one alkyl chain (such as *n*-alkyl orthophthalates, di- and triglycerides), information on the number of chains interacting with the stationary phase can be obtained from the ratio R_α, defined as

$$R_\alpha = \frac{\log \tilde{\alpha}_Z}{\log \tilde{\alpha}_H} = \frac{-\Delta(\Delta H_{0_{RZ}}^{CH_2}) + T\Delta(\Delta S_{0_{RZ}}^{CH_2})}{-\Delta(\Delta H_{0_{RH}}^{CH_2}) + T\Delta(\Delta S_{0_{RH}}^{CH_2})} \qquad (7)$$

where $\tilde{\alpha}_Z$ represents the methylene selectivity for the solutes belonging to those homologous series possessing more than one alkyl chain and $\tilde{\alpha}_H$ represents that of the alkanes under the same conditions [91,94].

(7) Other information can also be derived from the R_α values for a given homologous series (the reference being the *n*-alkanes) measured using stationary phases of different structures. It allows one to recognize specific interactions [116] and determine whether the mechanisms of interaction with the stationary phase are homoenergetic, heteroenergetic or homeoenergetic [117–121].

(8) Plot the curves $\tilde{\alpha}$ *vs.* ΔV_{CH_2}, where ΔV_{CH_2} represents the increase in the molar volume resulting from the addition of a CH_2 group in the alkyl chain, for each series tested [122] in those areas where log k' *vs.* n_c can be considered as a straight line.

(9) Whenever possible, plots of log k' *vs.* $1/T$ for each of the homologues should be examined. When the plots are linear, the changes in partial free molar enthalpy, ΔH_0, and entropy, ΔS_0, associated with the transfer of the solute in its standard state at infinite dilution from the mobile phase to the stationary phase can be determined.

(10) When enthalpy–entropy compensation is operative, a linear relationship must be observed between log k' and the ΔH_0 values (eqn. 8), where T is the temperature at which k' was determined [97].

$$\log k' = \log k'_0 - \frac{\Delta H_0}{2.3\,R}\left(\frac{1}{T} - \frac{1}{\beta}\right) \qquad (8)$$

In this case, eqn. 8 can be used to determine the value of the compensation temperature β [98]. However, it is also possible to determine β values using plots of ΔH_0 *vs.* n_c and ΔS_0 *vs.* n_c according to the set of equations [91,98]

$$\begin{aligned} -\Delta H_0 &= -\Delta H_0^Z - n_c\,\Delta H_0^{CH_2} = n_c A'' + B'' \\ \Delta S_0 &= \Delta S_0^Z + n_c\,\Delta S_0^{CH_2} = n_c C'' + D'' \qquad (9) \\ \beta &= -\frac{A''}{C''} = \frac{\Delta H_0^{CH_2}}{\Delta S_0^{CH_2}} \end{aligned}$$

(11) Finally, one should plot log k' *vs.* X (X being the volume fraction of organic solvent in a binary mobile phase) for each homologue and compare these plots with the curve relating the change in mobile phase surface tension to X [95,97,100,123].

(12) The stationary phases can be classified into two main types, depending on their chemistry: monofunctional-like (or monomeric) and polyfunctional-like (or polymeric). In each type, the phase can be homogeneous or mixed; in the latter case there is more than one type of bonded moieties. In addition to these considerations, other important aspects are the chain length of the bonded moieties and the bonding density. Analysis of retention data clearly shows the differences between monofunctional-like and polyfunctional-like phases. It is possible to differentiate clearly between these two types of silica when working under typical RPLC conditions, *i.e.*, ambient temperature and with methanol or acetonitrile–water mobile phases. The following properties correspond to monofunctional phases: log k' *vs.* n_c curves potentially have two break points and are grouped in two sets of converging straight lines [96]; α *vs.* n_c, ΔH_0 *vs.* n_c and ΔS_0 *vs.* n_c plots have a discontinuity and a change in slope [91]; log k' *vs.* $1/T$ plots are grouped in two sets of converging straight lines [91]; $\alpha_{TBN/BaP}$ ("Sanders and Wise

test") must be ≥1.7 [48], where TNB is tetrabenzonaphthalene and BaP is benzo[*a*]pyrene (see Section 6.5); and *cis*- and *trans*-carotenes should not be separated in non-aqueous RPLC (NARP) [104].

The following properties correspond to polyfunctional phases: log *k'* *vs*. n_c curves are sigmoidal; α *vs*. n_c plots have a maximum; log *k'* *vs*. $1/T$ plots are curved (concave) with possibly a discontinuity; $\alpha_{TBN/BaP}$ must be ≤1; and *cis*- and *trans*-carotenes should be separated in NARP.

The situation is not always that simple, however. The stationary phase must be thought of as a dynamic rather than a static medium as suggested by Carr and Harris [19,124]. The supramolecular structure of the phase, taking into account the mutual interactions between bonded chains, must be taken into consideration.

The ensemble of the results acquired from the studies made on homologous series can be summarized as follows.

4. ROLE OF TEMPERATURE

4.1. *Difference between polyfunctional-like and monofunctional-like phases*

It has been shown that, with polyfunctional bonded silicas and polymeric supports, the variation of log *k'* *vs*. $1/T$ measured with homologues is not linear between 20 and 60°C, whatever the length of the solute molecule (Fig. 6). Such behaviour should also be observed with other types of solutes undergoing the same retention mechanism as homologues, but it has not been reported in the literature, to the best of our knowledge. This non-linearity has been observed with pure methanol and 90:10 and 80:20 methanol–water mixtures [125]. There is a temperature range where a change in slope (large n_c values) or even an inversion of slope (*i.e.*, *k'* increasing with increasing temperature) can be observed (small n_c values) [125]. This temperature range defines a phase transition zone such as described by Serpinet and co-workers [126–129]. A similar phase transition zone can sometimes be observed on long-chain ($n_{bp} \geq 18$) monofunctional bonded phases. It has not been reported for phases with $n_{bp} \leq 12$ [129–132].

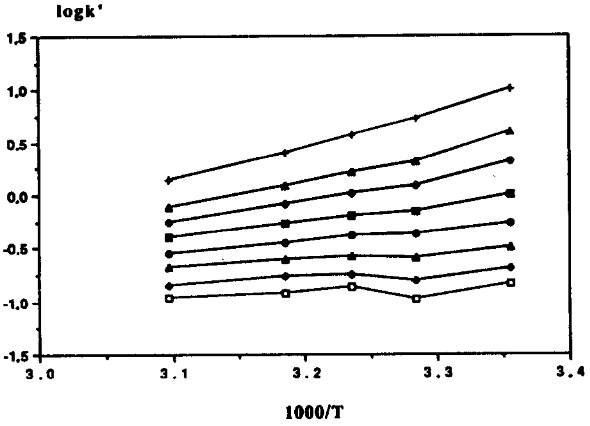

Fig. 6. Log *k'* *vs*. $1000/T$ plots for *n*-phenylalkanes ($2 \leq n_c \leq 18$) in pure methanol on a polyfunctional C_{18}-bonded silica (Supelcosil LC PAH). □: $n_c = 2$; ◆: $n_c = 4$; △: $n_c = 6$; ●: $n_c = 8$; ■: $n_c = 10$; ◇: $n_c = 12$; ▲: $n_c = 14$; +: $n_c = 18$.

The phase transition temperature is a function of the bonding density and the mobile phase composition [126–129]. For monofunctional C_{18} and C_{22} *n*-alkyl phases, the maximum values of the phase transition temperature are 30 and 54°C, respectively. This means that under typical room temperature conditions, a C_{18} phase (probably the most popular RP material) can very well be used either above or below its transition temperature. Thus, recently, non-linear Van 't Hoff plots have been reported for octadecyl monomeric bonded silicas with the shortest chain homologues of two series (*n*-alkylbenzenes with $0 \leq n_c \leq 3$ and nitroalkanes with $n_c = 3$, 4 and 6) [130]. In this study it was demonstrated that the non-linearity was observed for stationary phases with a bonding density larger than 2.48 μmol/m^2. Curves with a change of slope showing an obvious deviation from linearity at about 10°C were reported [130]. As the retention mechanism appears to be different whether the working temperature is above or below the transition point, it is important to determine the phase transition zone for the bonded phase used.

In a study by Tchapla *et al.* [91] in a smaller temperature range (20–40°C), it was observed that when monofunctional bonded silicas are used above their phase transition temperature, log *k'* *vs*. $1/T$ plots for homologues of increasing chain length are grouped in two sets of convergent straight lines. This has been verified with different homologous series and bonded chains length, n_{bp} 8–18 (Fig. 7). The first set corresponds to homologues with $n_c < n_{crit}$ and the second set to homologues with $n_c > n_{crit}$ [91]. Calculations of the changes in free enthalpy and entropy of transfer have been made for each homologue. The curves of ΔH_0 *vs.* n_c and ΔS_0 *vs.* n_c have two plateaux with a step occurring at $n_c = n_{crit}$. Just as for the curves of α *vs.* n_c, this corresponds to an increase in the entropy of transfer and a decrease in the enthalphy of transfer when n_c becomes larger than n_{crit}. This suggests a smaller solute–stationary phase contact energy and a larger disorder of the system when n_c is larger than n_{crit}. Similarly, the existence of two different compensation temperatures (one for $n_c < n_{crit}$ and the other for $n_c > n_{crit}$) strengthens the idea that two different mechanisms of interaction exist, depending on the value of n_c relative to n_{crit}. On the other hand, similar compensation temperatures in each range (below and above n_{crit}) have been observed with tetradecyl ($n_{bp} = 14$) and octadecyl ($n_{bp} = 18$) phases using pure methanol, a water-poor methanol–water mixture [91], methanol–chloroform and acetonitrile–chloroform mixtures [94] as eluents. This suggests the uniformity of molecular retention mechanisms on monofunctional bonded silica, independent of the nature of the mobile phase and the size of the bonded chain [97].

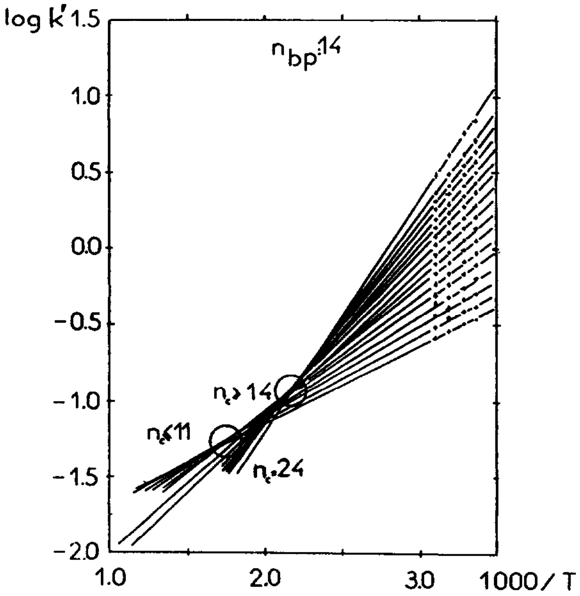

Fig. 7. Log *k'* *vs.* 1000/*T* plots *n*-alkanes ($6 \leq n_c \leq 22$) in pure methanol on a monofunctional C_{14}-bonded silica. From ref. 91.

It must be noted, however, that with a monofunctional C_{18} phase of low bonding density, for which the variation of α with n_c is perfectly linear, linear Van 't Hoff plots grouped in a single convergent set of lines have been measured [125].

With rigid stationary phases such as carbon-based materials, the log *k'* *vs.* 1/*T* plots are also linear. The changes in free enthalpy and entropy of transfer with n_c are perfectly linear [131] and the compensation phenomenon evidenced with bonded silicas also exists [131]. The value of the compensation temperature is independent of the mobile phase composition and close to that measured with traditional reversed-phase systems. This value is very different from that of normal-phase systems based on silica (140 K) and suggests that the retention mechanism on carbon materials is similar to that on traditional reversed-phase systems.

4.2. Effect of temperature on the conformation of bonded chains

The calculations of Staroverov and Fadeev [132], along with the work of Sander *et al.* [14], allow the difference between the two types of behaviour to be explained. It was shown that the alkyl chains of a silica bonded with C_{18} moieties or coated with *n*-octadecane are more ordered than the corresponding silane or alkane molecules. For a bonded silica there would be no change in the relative amounts of the four conformations shown in Fig. 5 from 10°C upwards. Actually, this minimum temperature depends on the bonding ratio [126]. Conversely, at −30°C, the chains are more ordered, although there is still a significant amount of chains with a left or kink conformation, as opposed to what is observed with pure hydrocarbons. Thus, even at low temperature, the bonded chains are not immobilized and are not all in a *trans* conformation. This must be correlated with the previously reported observation of a discontinuity of the log k' *vs.* $1/T$ plots for polymeric phases. The discontinuity corresponds to a very small temperature range where k' increases with increasing temperature. The temperature range corresponds to a change in the local order of the chains [133]. The consequences of a change in local order can also be observed with monomeric phases, provided that the experiments are conducted at low temperature. This is also suggested by the shape of the log k' *vs.* n_c [126,134–137] and α *vs.* n_c plots [125,138]: there is a maximum α value related to a maximum cohesion energy. This must be correlated with the observations made by Sander and Wise [56] on the retention of PAHs. The same phenomenon has also been observed for the separation of *cis*- and *trans*-carotenes [104].

5. ROLE OF THE STATIONARY PHASE

5.1. *Influence of the bonded chain length*

It has been reported that with medium- and long-chain ($6 \leqslant n_{bp} \leqslant 18$) monofunctional bonded silicas having a high bonding density, the log k' *vs.* n_c plots are often not simple straight lines, particularly when organic-rich mobile phases are used [96]. In many instances, they can be considered to consist of (at least) two segments of straight lines. The connection point of these segments, the "break point", defines a critical carbon number, n_{crit}. This number is independent of the homologous series studied and is a characteristic of the bonded phase chain length [96]. A break point has also been observed with a mixed phase such as cyanopropyl/octadecyl. The corresponding n_{crit} value is identical with that obtained with monofunctional C_{18} bonded silica [138]. When solutes with a long chain length are used (this is typically the field of NARP chromatography), a second break point can often be observed (Fig. 8). The corresponding carbon number value, n_{sat}, is a function of the bonding density only. The larger this density, the lower is the value of n_{sat}, n_{sat} always being larger than n_{crit} anyway. Further, it must be noted that with a long-chain monofunctional silica having a low bonding density, neither of these two break points has been observed [122]. The situation is identical with short chain-length (*e.g.*, methyl) phases whatever the bonding density [96] and with carbon-based adsorbents, at least up to a value of n_c from 1 to 14 [131] and from 13 to 26 [138]. These two types of curves (with and without break points) have been reported with many different mobile phase conditions: methanol and acetonitrile, either pure or mixed with small amounts of water or with chloroform [125].

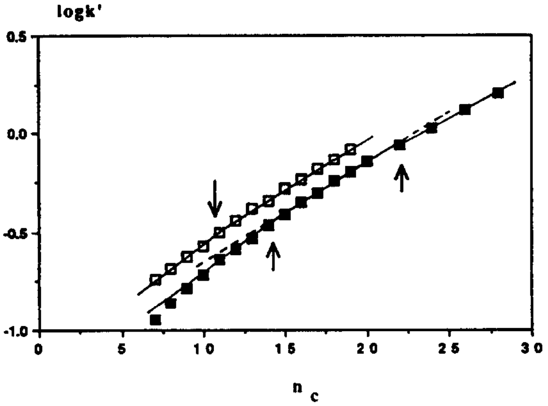

Fig. 8. Log k' *vs.* n_c plots on a mixed monofunctional bonded silica (Spherisorb column: octadecyl/cyano-1-propyl) in pure methanol at 21°C for (■) *n*-alkanes and (□) phenyl-*n*-alkanes. The arrows correspond to slope changes.

Close examination of the results recently obtained in supercritical fluid chromatography (SFC) with carbon dioxide modified with various amounts of methanol and monofunctional C_{18} stationary phase revealed a break point at about $n_{crit} = 14–16$ [139]. Also in SFC, Hagege [76] has shown that a break point appears at $n_{crit} = 10$ with a monofunctional C_{11} phase, under certain pressure conditions. These results indicate a similarity between NARP and SFC. This similarity is also confirmed by other data obtained in studies of the role of mobile phase additives on retention in SFC and NARP [113–115,140].

All this suggests that the molecular mechanisms of interaction are similar, if not identical, in RPLC, NARP and SFC.

5.2. Influence of the polyfunctional-like nature of the stationary phase

The situation is very different from that discussed previously for polyfunctional phases and polymeric materials such as polyvinylbenzene–polystyrene (either non-bonded or bonded by *n*-octadecyl moieties). The plots of log k' *vs.* n_c are sigmoidal (Fig. 9) rather than consisting of two straight segments [125]. The sigmoidal shape has also been observed with monofunctional silicas at low temperature, as can be seen in Figs. 10 [126] and 11 [138,141]. These results suggest that the retention mechanism is not the same with monofunctional and polyfunctional phases (or monofunctional at low temperature).

5.3. Reversibility between polyfunctional-like and monofunctional-like behaviour

The shape of the α_n *vs.* n_c curves gives interesting information on the retention mechanism. Three types of curves have been reported, suggesting different retention mechanisms in each case:

a-Type: this consists of two plateaux connected by a step occurring at n_{crit}. The second plateau may sometimes present a break point appearing at n_{sat}. This behaviour is characteristic of monofunctional bonded silicas, as can be seen in Fig. 12 [122,138]. It depends on temperature, as reported above.

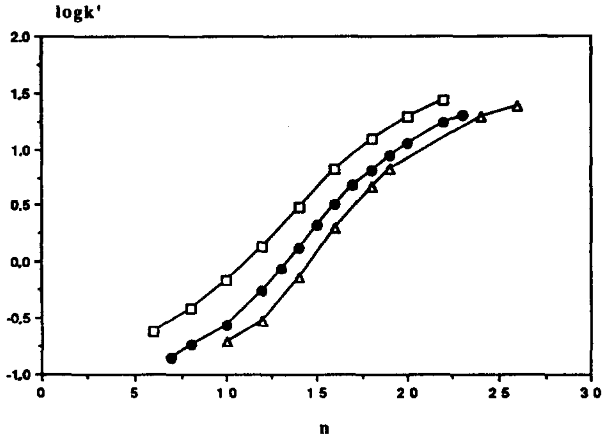

Fig. 9. Log k' *vs.* n_c plots on a polyfunctional C_{18}-bonded silica (Supelcosil LC PAH) in pure methanol at 25°C for (□) *n*-alkanes, (●) methyl-*n*-alkanoates and (△) *n*-alkanols.

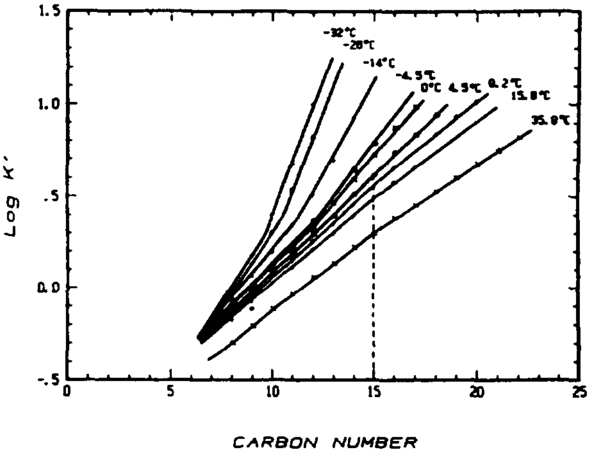

Fig. 10. Variation of log k' *vs.* n_c for *n*-alkanes at different temperatures on a LiChrosorb RP-18 column and pure methanol as mobile phase. From top to bottom curves: −32; −28; −14; −4.5; 0; 4.5; 9.2; 15.8; 35.9°C. From ref. 126.

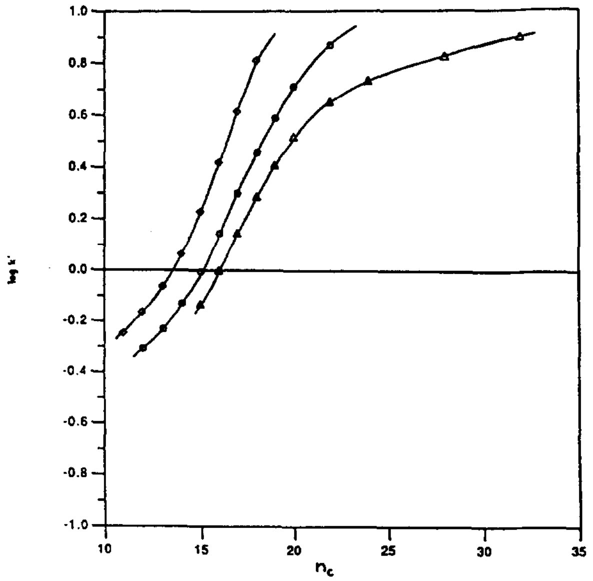

Fig. 11. Variation of log k' *vs.* n_c for *n*-alkanes at 20°C (below the phase transition temperature) on a Spherosil C_{22} column and mixtures of methanol–chloroform of different compositions. (◇) Methanol–chloroform (60:40); (○) methanol–chloroform (50:50); (△) methanol–chloroform (45:55). From ref. 141.

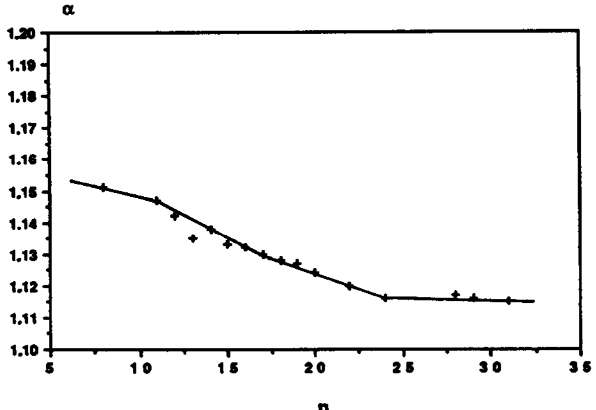

Fig. 12. Quadratic methylene selectivity α *vs.* n_c plots on a monofunctional C_{18}-bonded silica (Ultraspher ODS) for *n*-alkanes in acetonitrile–chloroform (60:40, v/v) at 20°C. From ref. 141.

b-Type: this is a straight line with a negative slope. It has been observed in aqueous [23,96] and non-aqueous [94] eluents, with either monofunctional short-chain silicas or monofunctional long-chain silicas having a low bonding density [122].

c-Type: this is a curve with a maximum. This is characteristic of polyfunctional bonded silicas or polymeric phases (see Fig. 13).

The type of curve observed depends on the experimental conditions (mobile and stationary phases) and temperature. For example, phases exhibiting an *a*-type behaviour with methanol and acetonitrile have a *c*-type behaviour in THF. This has been observed with monofunctional phases used below their phase transition temperature. The effect of temperature is particularly interesting. When increasing temperature from below to above the phase transition temperature, with both a monofunctional and a polyfunctional silica, the α_n *vs.* n_c curves change from *c*-type to *b*-type and then *a*-type [125,141,126]. It must be noted, however, that the addition of a strong solvent (dielectric constant $\varepsilon < 20$) in the mobile phase restores the transition from *c*-type to *a*- or *b*-type [109].

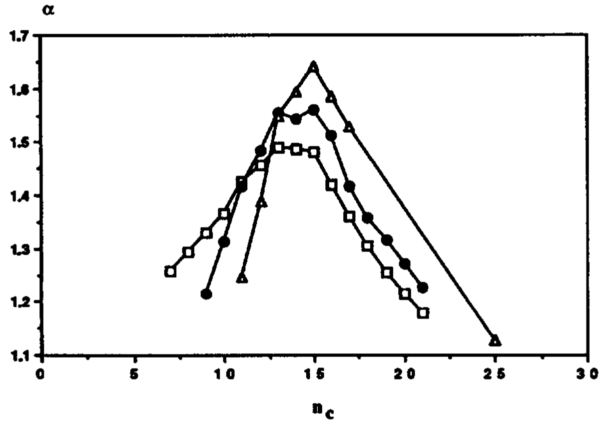

Fig. 13. Quadratic methylene selectivity α *vs.* n_c plots on a polyfunctional C_{18}-bonded silica (Supelcosil LC PAH) in pure methanol at 25°C for (□) *n*-alkanes, (●) methyl *n*-alkanoates and (△) *n*-alkanols.

Interesting results have also been reported in SFC using pure carbon dioxide. When simultaneously increasing the temperature and decreasing the eluent density, there is a change from a situation where the selectivity before n_{crit} is larger than that after, to the opposite situation. This has been observed with monofunctional homogeneous silica (*e.g.*, *n*-undecyl and cyano-1-decyl) and mixed phases (*n*-undecyl/cyano-1-decyl and *n*-undecyl/cyano-1-butyl) [76].

The previous results suggest that, depending on the operating conditions, it is possible to change reversibly from a behaviour characteristic of a monofunctional phase to that of a polyfunctional phase. The retention mechanism is probably related to the type of curve (*a*, *b* or *c*), as suggested by several experimental results. With a C_{18} silica (either mono- or polyfunctional), at 25°C (*i.e.*, above the phase transition temperature for a monofunctional phase but not necessarily for all polyfunctional phases), the ratio of the R_α values (see eqn. 7) at $n_c = 14$ (this is where the maximum selectivity is observed for a C_{18} phase with *c*-type behaviour) is equal to 1.9 ± 0.2, whatever the homologous series investigated. This indicates that the retention mechanism is not the same for both phases. At a temperature sufficiently high to be above the phase transition temperature for both types of phases, *i.e.* 50°C, one observes an *a*-type curve whatever the nature of the stationary phase. In this case, the value of R_α is 1.00 ± 0.05 for all the homologous series studied, suggesting the same retention mechanism. These results indicate that two different retention mechanisms exist, depending on whether the phase is operated above or below its transition temperature. The mechanisms are characterized by the type of curves: *a*- and *c*-type. Above the transition temperature, *i.e.* at 50°C for both a monofunctional and a polyfunctional C_{18} phase, the curve is of *a*-type and the retention mechanism is homoenergetic: the ratio of the methylene selectivity for two systems is constant and equal to unity. In contrast, below the phase transition temperature, the curve is of *c*-type and the mechanism is homeoenergetic: the ratio of the selectivity is constant, whatever the n_c value, but it is different from unity. When the selectivity is not constant with n_c, the mechanism is heteroenergetic.

5.4. Molecular interaction mechanism

5.4.1. Monofunctional phases used below their phase transition temperature

In the case of monofunctional phases, the conformational mobility of the chains makes it possible for the solute molecules to insert themselves between the bonded chains, with tight contact between both species. Solute molecules take the place previously occupied by organic solvent molecules: methanol or acetonitrile or, more generally, a solvent with a high dielectric constant. As mentioned by Rosset [142], the confinement of the methanol molecules in these phases is due to the dynamics of the chains. Because of their mobility and their multiple conformations, the bonded chains occupy a larger space than that corresponding to a single, well defined and rigid conformation. This "extra space" allows organic solvent or solute molecules to insert themselves between the bonded chains. This insertion model is similar to that of Serpinet and co-workers [128,143]. They investigated the chromatographic properties of long-chain (n_{bp} = 18 and 22) bonded silicas with a high bonding density (4 μmol/m^2) impregnated with *n*-alkanes of similar chain length. This produces a surface comparable to that of a silica with a very high bonding density (8 μmol/m^2), the chromatographic properties of which have been thoroughly studied by Serpinet and co-workers, who proposed such an insertion model to explain their experimental results.

5.4.2. Effect of the bonded chain length

The phenomenon of interchain (solute–stationary phase) interactions builds up as long as the carbon number of the solute is smaller than that of the bonded chains. The value of n_{crit} is equal to 12 when $n_{bp} = 14$ and close to 14 for $n_{bp} = 18$ and 22. This must be related to the IR spectroscopic data of Sander and co-workers [14,56], which show that C_{18} and C_{22} phases are conformationally identical. The stationary phase structure is well represented by the brush-type model of Karch *et al.* [144]. The polar head of the solute, if any, sticks outside of the bonded layer, immersed in the mobile phase. The conformation of the chains prevents the polar head from interacting with the stationary phase and, in particular, with the residual silanols. This justifies the fact that for a given stationary phase chain length, the jump in the curves of α *vs.* n_c always happens at $n_c = n_{crit}$ [96], whatever the homologous series investigated, including alkyl acetates. It also explains the results obtained with a C_{18}/C_3CN mixed phase, which has been found to behave similarly to homogeneous *n*-alkylsilica: there is no contribution of the cyano groups, at least as far as insertion of solute molecules is concerned. Actually, the presence of these cyano groups is very important as it allows in some instances compounds containing π electrons to develop π–π interactions, thus resulting in increased retention.

For homologues such as those with $n_c < n_{crit}$, a regular decrease in α with increasing n_c has been observed with all monofunctional phases investigated: n_{bp} = 6, 8, 14 and 18 [96]. Each additional methylene group contributes less than the previous one, possibly because of some compaction of the solute alkyl chain. Further, it has been shown [122] that the methylene selectivity depends slightly on the chemical nature of the polar head. This phenomenon can be observed when closely examining Berendsen *et al.*'s data [75,145]. This effect of the polar head seems to contradict the previous statement that the polar head of the solute does not interact with the stationary phase. However, the volume of a methylene group depends on the nature of the polar head and, when $n_c < n_{crit}$, a correlation exists between the value of α and the molecular volume of a CH_2 group [122]. In other words, the fact that the methylene selectivity is weakly related to the nature of the polar head is not a consequence of the interaction of the head with the stationary phase but rather an effect of the head on the structure of the solute alkyl chain itself. This complies with a retention mechanism involving a penetration phenomenon and tight contact between the bonded chains and the solute chain (Fig. 14C, top).

This description of the retention process is in agreement with wettability data of Korosi [146] on low-energy surfaces. He measured contact angles of high bonding density silicas (3.5–4 μmol/m^2) modified with C_{14}, C_{18} and C_{22} moieties and concluded that linear and branched alkanes and also solutes possessing long alkyl chains penetrate inside the bonded chain network. A similar "penetration and close contact" mechanism has also been suggested for a mixed phase (phenyl–decyl) [146]. The solute–stationary phase contact energy is maximum when $n_c = n_{bp} - 5$. When $n_c > n_{bp} - 5$, solute insertion resulting from the hydrophobic effect is still possible, however. In this case, the *gauche* conformations of the chains become predominant, associated with a looser contact and a weaker solute–stationary phase interaction. The solute molecules curl themselves between the bonded chains and the methylene selectivity decreases. For $n_c > n_{crit}$, $\tilde{\alpha}$ is constant and does not depend on the series tested, which is not the case when $n_c < n_{crit}$. The phenomenon just described corresponds to a less ordered stationary phase and to solute–stationary phase interactions involving *gauche* conformations of the solute molecules. The molecules penetrate the stationary phase because of the solvophobic effect but their contacts with the bonded chains are looser than when $n_c < n_{crit}$. This justifies the entropy of transfer increase and the simultaneous enthalpy decrease previously reported for solutes with chain length longer than n_{crit}, as can be seen in Fig. 15 [91]. This phenomenon has been observed for all homologous series tested, two different mobile phase compositions and two

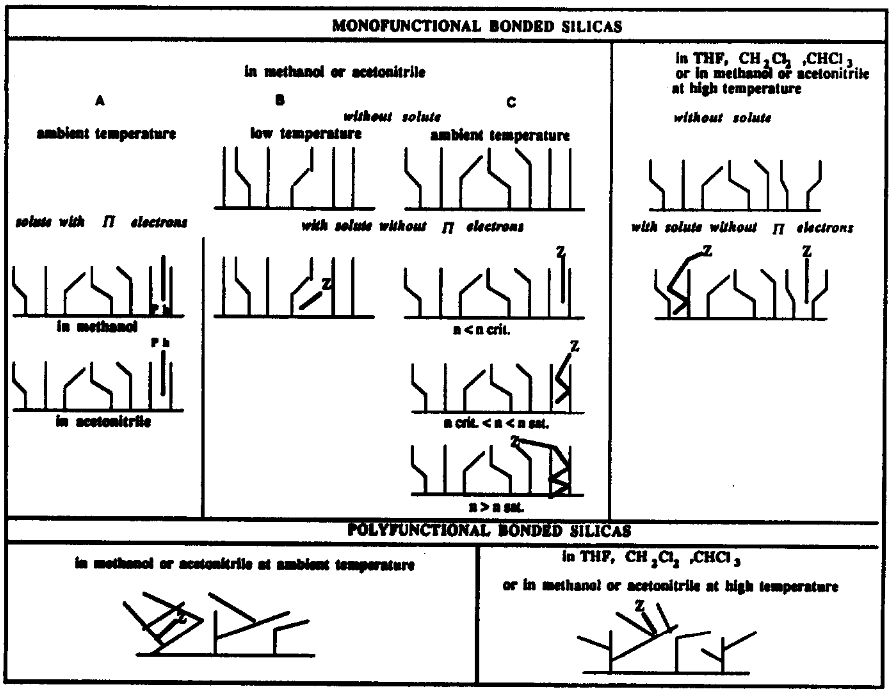

Fig. 14. Schematic representation of the molecular mechanism of interaction for the retention of homologous on monofunctional and polyfunctional bonded silicas. First column: particular case of aromatic homologues. Second and third columns: role of the temperature and chain length of the homologue. Fourth column: role of solvent with low dielectric constant.

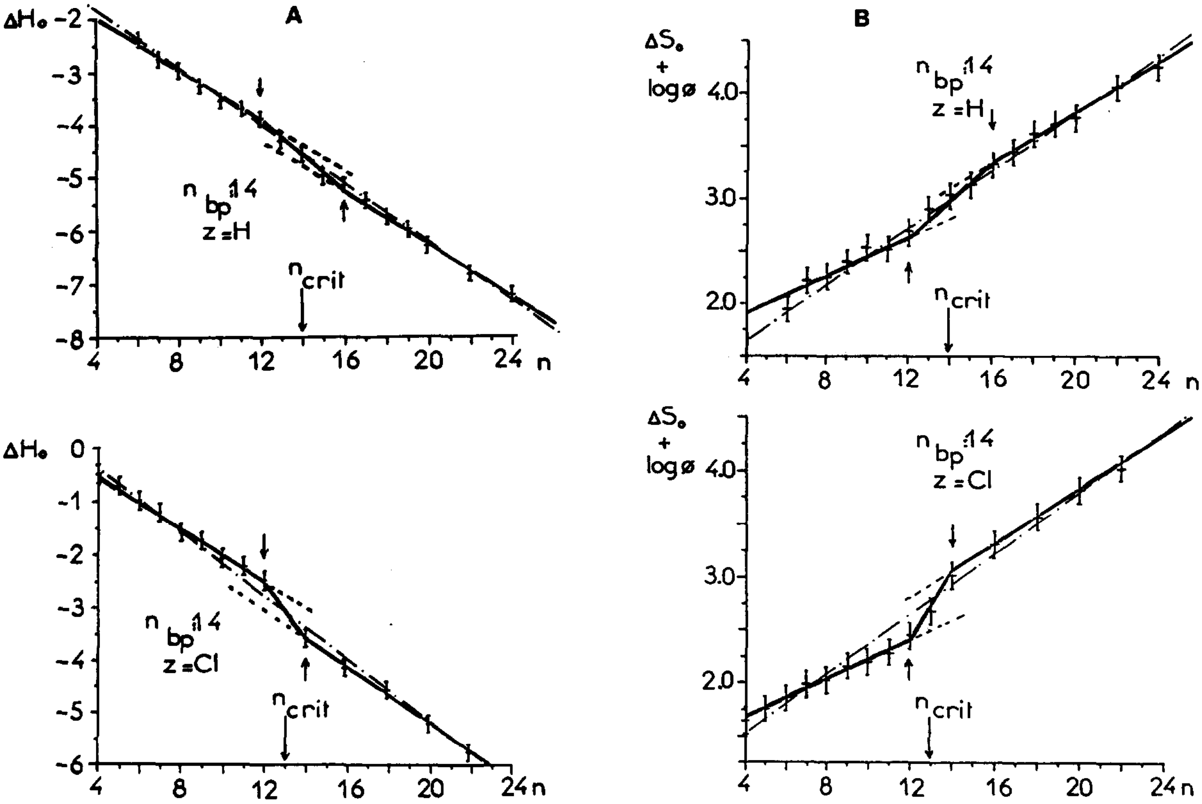

Fig. 15. (A) Transfer enthalpy $\Delta H°$ *vs.* n_c plots on a C_{14}-bonded phase in pure methanol. Top, *n*-alkanes; bottom, chloro-*n*-alkanes. (B) Associated entropy $\Delta S°$ *vs.* n_c plots. Same conditions as in (A). From ref. 91.

stationary phases ($n_{bp} = 14$ and 18). The concept of "penetration and loose contact" is in agreement with the existence of two compensation temperatures, the values of which do not depend on the experimental conditions: one for $n_c < n_{crit}$ and the other for $n_c > n_{crit}$ [95].

For very long chains, the complete insertion of the solute molecules between the bonded chains, even in a *gauche* conformation, is no longer possible. On part of the solute chain penetrates inside the stationary phase ("internal" interaction) while the rest sits on top of the bonded chains where it can only interact with the CH_3 terminal groups of these chains ("external" interactions; see Fig. 14C, top). A critical chain length is associated with this phenomenon: n_{sat}. When $n_c > n_{sat}$, the methylene selectivity should not change with increasing n_c [122,141]. The limit value n_{sat} above which the interactions with the bonded chains involve their CH_3 terminal groups is larger than n_{crit} which characterizes the transition from a close to a loose contact inside the stationary phase (thus always "internal" interaction). This explains why a lower compensation temperature has been observed for solutes with $n_c \geq 22$ compared with solutes with $14 \leq n_c \leq 20$ on a monofunctional $n_{bp} = 14$ phase [91].

The "penetration and close contact" approach explains several observations, such as the following.

(i) When $n_c < n_{crit}$, $\tilde{\alpha}$ is not the same for different homologous series. This is because, as previously mentioned, the molecular volume of a CH_2 group is not constant for different homologous series and thus the free energy of transfer and accordingly $\tilde{\alpha}$ depend on the series considered.

(ii) When $n_c < n_{crit}$, $\tilde{\alpha}$ and retention depend on the length of the bonded chains [50,145,148–

151]. This behaviour, which cannot be explained by the solvophobic effect, is due to the increase in the n-alkane surface tension and thus to the energy of contact with increasing carbon number [152,153]. This suggests that the stationary phase plays an active role in the retention process, as also suggested by the partitioning model [65,66]. In the case of a methyl phase, there is obviously no possible penetration and the retention mechanism should be very close to adsorption as described by Dill [65] and Dorsey and Dill [66] and suggested by Colin and Guiochon [154].

(iii) When $n_{bp} \leq 8$, $n_{crit} = n_{bp}$, and when $n_{bp} > 8$, $n_{crit} < n_{bp}$. This has to be connected with the decrease in structural order in bilayer membranes when the carbon number of the constituting chains becomes larger than 8 [155]. Such a decrease at $n_{bp} = 8$ is likely to produce a weaker contact between the host and the guest molecules.

(iv) The value of $\tilde{\alpha}$ increases slightly with increasing bonding density [156–158] at constant (bonded) chain length. This is because, with increasing bonding density, there is less space between the bonded chains and then the solute molecules are likely to interact with more than one chain. This also justifies the fact that, for a given carbon number, α is larger with linear bonded chains phases than cyclic or branched chains [159,160].

(v) The concept of critical chain length observed by Berendsen [145] with monofunctional phases of different lengths. Above a given stationary phase chain length, the value of which depends on the solute, k' stays fairly constant for a given solute. Berendsen suggested a retention mechanism for which only the free end (non-bonded) of the bonded chains is involved in the retention mechanism.

(vi) The selectivity decreases above the phase transition temperature with monofunctional phases. In this case interchain interactions become weak. The solute–stationary phase contact is not as favourable as before and then α decreases. The retention mechanism is then probably a mixture of hydrophobic effect and partitioning, although, on a structural basis, the stationary phase cannot be considered as a liquid (Fig. 3d).

5.4.3. Polyfunctional phases or monofunctional phases below their phase transition temperature

In the case of polyfunctional phases or monofunctional phases used below their phase transition temperature, the chains (or the sections of the chains) directly facing the mobile phase are more mobile in organic-rich than in water-rich solvent mixtures. Although the molecular structure of the phase is not well defined [161], it can be described as globular bushes or greasy patches somehow randomly distributed over the surface of the silica, as suggested by Lochmuller and Wilder [162]. Even if the carbon content of the phase is not high enough to envision the existence of a continuous thick liquid film covering all the silica surface, these droplets can nevertheless produce a solubilization of the solute molecules which are then totally immersed in the stationary phase. The mobility of the bonded chains is low and restrained, thus favouring solute–stationary phase contacts (see Fig. 14, bottom left, and Fig. 14B, top). This leads to a methylene selectivity being maximum when these contacts are the tightest, just as observed when immersing n-alkane molecules of various length in crystals of n-alkane [163].

It has been seen previously that with a polyfunctional phase or a monofunctional phase used below its phase transition temperature, the curve of α *vs.* n_c has a maximum [138]. For a C_{18} phase, the maximum value of α corresponds to $n_c = 14$–15 and for a C_{22} phase to $n_c = 17$. It has also been observed that the temperature at the end of phase transition decreases when the difference between the chain length of the solute and the stationary phase increases [136,164]. The end of the phase transition can be explained in terms of melting of a mixed and dense film made of bonded chains and immersed solutes molecules. The stability of this film (and hence the value of its melting temperature) is maximum when both molecules are of similar size. The theory explaining these facts corresponds to a pure partition mechanism. The higher the temperature, the weaker is the effect. When temperature increases, chain motions become more rapid. The solute molecules do not make their way down to the surface of the silica. There is only a superficial penetration of these molecules in the phase and the contact takes place with the external chains. Accordingly, there is a transition from a mechanism of total immersion (Fig. 14, bottom left) to a mechanism of insertion between the outer bonded chains (Fig. 14, bottom right).

6. ROLE OF THE NATURE OF THE SOLUTE

Rather than discussing in general terms the contribution of the solute, we shall examine some specific situations.

6.1. *Aromatic homologous series*

When using aqueous–organic eluents, the value of n_{crit} for phenylalkanes and *n*-alkyl orthophthalates is, respectively, three and five carbon atoms less than it is with other homologous series, whatever the nature of their polar functional group, including *n*-alkyl benzoates. It is very likely that the aromatic homologues penetrate the stationary phase by their polar head. On the other hand, in NARP, n_{crit} is the same for all series tested when using mobile phases containing acetonitrile [91,141]. In this case the aromatic part of the solute molecule sticks out of the stationary phase (Fig. 14A, top).

This behaviour has been interpreted as the consequence of specific π–π solute–solvent interactions (phenyl ring ↔ acetonitrile). Another consequence of these interactions will be described later (Section 7.3).

6.2. *Homologues with several hydrocarbon chains*

When the homologue molecules contain several alkyl chains, there is the possibility for more than one chain to interact with the stationary phase. In this case, the ratio R_α (the reference series being the *n*-alkanes) must be larger than unity. Its value is actually an indication of the number of chains interacting. For the *n*-orthophthalates (two chains), for instance, R_α is close to 2 [91], for the triglycerides (three chains) it is close to 2.4 and for the diglycerides (two chains) it is about 1.8 [94]. These results indicate that the molecular interactions between the *n*-orthophthalates and the stationary phase involve the two hydrocarbon chains of the solute molecule. For di- and triglycerides, the fact that the R_α values are not integers corresponding to the number of alkyl chains suggests that there are several conformers in dynamic equilibrium, each of them interacting with the bonded chains [94] by a different number of alkyl chains.

6.3. *Homologous series with a polar head*

Before discussing the behaviour of polar homologous series, it is worth mentioning observations made on the effect of the chain length (n_{c}) and the molecular volume increment of a $\mathrm{CH_2}$ group. It has been found that, below n_{crit}, the volume increment differs from series to series, being larger with polar series. Above n_{crit}, it is the same. Actually, the range $n_{\mathrm{c}} > n_{\mathrm{crit}}$ should be divided into two zones, $n_{\mathrm{crit}} < n_{\mathrm{c}} < n_{\mathrm{sat}}$ and $n_{\mathrm{c}} > n_{\mathrm{sat}}$. In each range the volume increment is the same for all homologous series, but the increments corresponding to the two zones are slightly different.

These results provide an explanation for observations made about average methylene selectivity $\tilde{\alpha}$ [118], since it is clear that a correlation exists between the molecular volume increment of a $\mathrm{CH_2}$ group and the selectivity; $\tilde{\alpha}$ is slightly larger for polar than non-polar series when $n_{\mathrm{c}} < n_{\mathrm{crit}}$, whereas it is approximately constant, but smaller, in the range $n_{\mathrm{crit}} \leq n_{\mathrm{c}} \leq n_{\mathrm{sat}}$, independent of the bonded phase chain length (at least when $6 \leq n_{\mathrm{bp}} \leq 22$); $\tilde{\alpha}$ is also constant when $n_{\mathrm{c}} > n_{\mathrm{sat}}$ and its value is smaller than in the range $n_{\mathrm{crit}} \leq n_{\mathrm{c}} \leq n_{\mathrm{sat}}$. This suggests two conclusions. First, the interaction between the solute alkyl chain and the stationary phase is larger when $n_{\mathrm{c}} < n_{\mathrm{crit}}$ than when $n_{\mathrm{c}} > n_{\mathrm{crit}}$, probably because in the latter instance the solute chain is in a *gauche* conformation. Second, when $n_{\mathrm{c}} < n_{\mathrm{crit}}$, homologous series with a polar head interact more with the stationary phase than series with no polar head (incidentally, it must be noted that the corresponding compensation temperatures differ by more than 100°C). This is in agreement with a larger molecular volume increment of a $\mathrm{CH_2}$ group for polar series in this range of n_{c}

values. The presence along the alkyl chain of a small polar group barely influences retention phenomena. In fact, symmetrical ketones behave in exactly the same way as alkyl methyl ketones of the same total chain length: the plots of log k' *vs.* n_c and α *vs.* n_c are identical.

It must be noted that the situation with cycloalkanones is a special case. With these solutes, the methylene selectivity for $n_c < n_{crit}$ is similar to that with non-planar homologues having chain lengths in the range $n_{crit} \leq n_c \leq n_{sat}$. This suggests that, because of their cyclic structure, cycloalkanones interact weakly with the stationary phase, as do linear homologues.

6.4. *Bifunctional homologues*

Bifunctional compounds can exhibit strange retention behaviour. For instance, when n_c is smaller than n_{crit}, the log k' *vs.* n_c plots for aliphatic dinitroxides are linear with a value of $\tilde{\alpha}$ corresponding to that of monofunctional homologous series having chain lengths in the range $n_{crit} \leq n_c \leq n_{sat}$. Also, for aromatic compounds with $1 \leq n_c \leq 6$, for cyanoalkylbenzenes, hydroxyalkylbenzenes [80] and difunctional phthalimides [165], log k' *vs.* n_c plots with a minimum have been reported.

One last exception must be noted. It concerns primary, secondary and tertiary α,ω-diaminoalkanes for which log k' *vs.* n_c plots with a break point have also been observed. The value of n_{crit} for $n_{bp} = 14$ is 10, which is different from that observed with monofunctional series ($n_{crit} = 14$). The slope change at the break point is also much larger. These observations can be explained in terms of a conformational effect specific to these compounds. This effect has also been suggested by potentiometric and NMR relaxation time studies [166].

6.5. *Particular case of solutes with a rigid structure*

Before proposing a general interpretation of the preceding results, which were obtained using solutes having a flexible cyclic or flexible linear structure, it is worth comparing them with those obtained under the same conditions, but with rigid solutes such as PAHs or carotenes. This allows one to examine the similarities and/or differences indicating whether or not the mechanisms of molecular interaction are identical for solutes of different structures.

Sander and Wise [53–56] established a coherent retention model which takes into account solute–stationary phase molecular interactions in the case of polycyclic aromatic solutes (Fig. 16). Wise and co-workers [101,102] also investigated the separation of carotenes, simultaneously with the work of Lesellier and co-workers [103,104]. It results from the work of Sander and Wise that the elution order and the selectivity factors for benzo[*a*]pyrene (BaP), phenanthrylphenanthrene (PhPh) and tetrabenzonaphthalene (TBN) can be used to differentiate between polyfunctional and monofunctional packings. The most useful criterion is the selectivity factor $\alpha_{TBN/BaP}$: it is smaller than 1 for polyfunctional phases with a low carbon content and larger than 1.7 for monofunctional phases with a high carbon content. For an intermediate value, the authors concluded that it is not possible to clearly characterized the type of packing material.

Working only with the eluent mixture acetonitrile–water (85:15) in combination with monofunctional packings and with acetonitrile–water mixtures of different compositions in combination with polyfunctional phases [48,53–55], Sander and co-workers studied the effects of many parameters such as temperature [56,167] (Fig. 17), bonding density [14,53], bonded chain length [54] (Fig. 18), pore diameter [168] and pretreatment of the silica before bonding [168]. The conclusions of their work can be summarized as follows. For monofunctional phases and

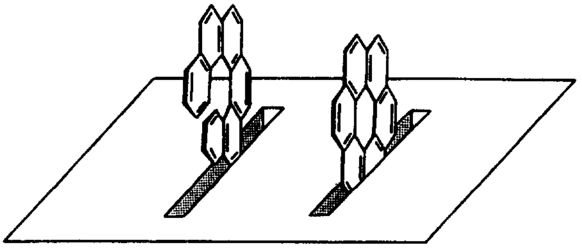

Fig. 16. Schematic representation of the "slot model" for the retention of PAHs on polyfunctional C_{18}-bonded silicas as stationary phase. From ref. 48.

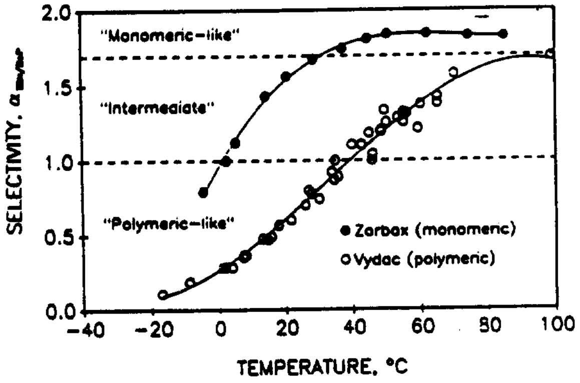

Fig. 17. Change in shape selectivity as a function of temperature for (●) a monofunctional (Zorbax ODS) and (○) a polyfunctional (Vydac 201TP) C_{18} column. Mobile phase, water–acetonitrile (15:85, v/v) for the monofunctional column, various compositions for the polyfunctional column. From ref. 48.

at a constant bonding density, there is a continuous transition from a monofunctional to a polyfunctional type when n_{bp} increases. The reverse is observed for polyfunctional phases. At a given bonded chain length, there is an continuous transition from a monofunctional to a polyfunctional type with decreasing temperature. The reverse is true for a polyfunctional phase with increasing temperature. Similar results have been observed with carotenes [103,169] under the same conditions. Some data from the work of Sander and Wise are reported in Table 1. The observed trend is in agreement with what can be deduced from the log k' *vs.* n_c and α *vs.* n_c plots obtained with homologous series. Sander and Wise did not investigate the effect of the volume composition of the mobile phase with monofunctional phases. It must be noted, however, that Stalcup *et al.* [106] have shown that when temperature is changed, the retention of PAHs is different whether the mobile phase is a water–methanol mixture or an acetonitrile–water mixture [106].

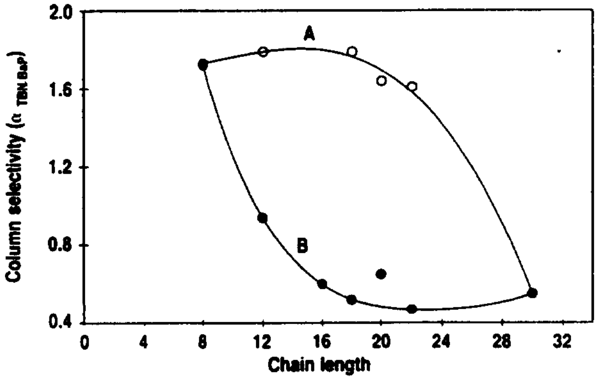

Fig. 18. Plots of shape selectivity ($\alpha_{TBN/BaP}$) *vs.* alkyl chain length for (A) monofunctional and (B) polyfunctional alkyl phases. From ref. 48.

In order to understand retention mechanisms better, we have studied the behaviour of the three PAHs selected by Sander and Wise in the same mobile phases as those used during the work on homologous series. The results reported in Fig. 19 show the effect of the water content on the selectivity $\alpha_{TBN/BaP}$ for a monofunctional and a polyfunctional bonded silica. As can be seen, the polyfunctional character of a polyfunctional phase increased when the water content decreases. For monofunctional phases, the α value always corresponds to a monofunctional character, whatever the water content, except in pure acetonitrile where the situation is not clear. The same trend has been observed with methanol. Similar studies made in NARP and in SFC have shown identical effects of the solvent composition on retention [113–115,140].

It is interesting and surprising that when a strong solvent (low ε) is added to acetonitrile or methanol, the value of $\alpha_{TBN/BaP}$ decreases, which would suggest an increase in the polyfunctional character of the packing. These observations on the effects of the solvent composition on retention of PAHs do not agree with those made with homologous series (whereas the observations about the role of temperature are in agreement). The smaller the dielectric constant of the solvent, the larger is the effect: $\alpha_{TBN/BaP}$ values are smaller in THF, tetrachloroethylene and chloroform than in acetone. In turn, $\alpha_{TBN/BaP}$ in acetone is smaller than in methanol or acetonitrile, the last two values being very close.

Conversely, a temperature decrease in NARP (whatever the mobile phase composition, both in terms of volume fraction and nature of the solvents) and in SFC (using CO_2) results in a decrease in $\alpha_{TBN/BaP}$. This reinforces the polyfunctional character of the phase, which agrees with results obtained with homologous series.

TABLE 1

APPARENT EVOLUTION OF THE MONOFUNCTIONAL OR POLYFUNCTIONAL CHARACTER OF ALKYL-BONDED SILICAS AS A RESULT OF THE TEST OF SANDER AND WISE [48]: THRESHOLD AT WHICH THE TRANSITION OCCURS

Parameter	Monofunctional	Intermediate	Polyfunctional
n_{bp} (monofunctional bonding)	$n_c < 20$	$20 < n_c < 28$	$n_c > 28$
n_{bp} (polyfunctional bonding)	$n_c < 8$	$8 < n_c < 12$	$n_c > 12$
Temperature (°C) (C_{18} monofunctional silica)	$T > 25$	$0 < T < 25$	$T < 0$
Temperature (°C) (C_{18} polyfunctional silica)	$T > 90–100$	$40 < T < 90–100$	$T < 40$
Bonding density ($\mu mol/m^2$) (C_{18} monofunctional silica)	–	<1.26	–
Pore diameter (Å) (C_{18} monofunctional silica)	–	<60	–
Bonding density ($\mu mol/m^2$) (C_{18} polyfunctional silica)	–	<4	>4
Pore diameter (Å) (C_{18} polyfunctional silica)		between 60 and 100	>150

6.6. Comparison between the retention mechanism of flexible and rigid solutes

In their unified theory of retention and selectivity in liquid chromatography, Martire and Boehm [69] concluded that the bonded phases present a shape selectivity that increases when the solute alkyl chains are predominantly in the stretched conformation. This is the reason why rigid and linear solutes are more retained than globular solutes. It is in agreement with the retention model that we propose: penetration and close contact with monofunctional phases and total immersion with polyfunctional phases. The methylene selectivity is larger for polyfunctional than for monofunctional phases. Sander and Wise [55] came to the same conclusion in their study on PAHs.

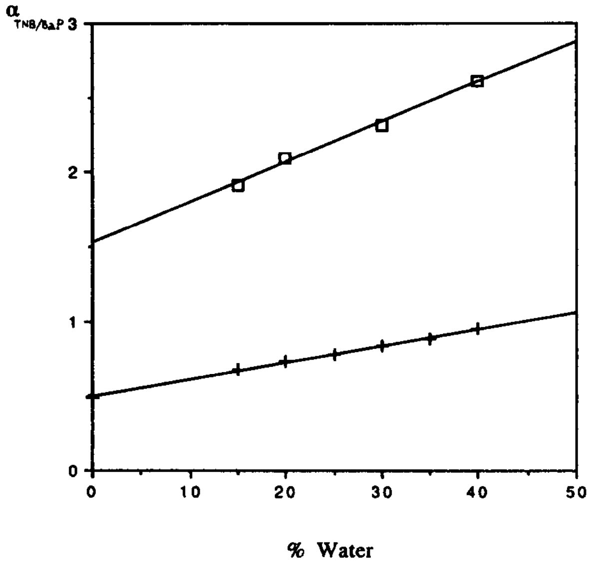

Fig. 19. Plots of shape selectivity ($\alpha_{TBN/BaP}$) *vs.* mobile phase composition (acetonitrile–water) for (□) monofunctional (Hypersil C_{18}) and (+) polyfunctional (Supelcosil LC PAH) bonded silicas at 20°C.

The addition of an organic modifier to water or a strong solvent to methanol, acetonitrile or subcritical CO_2 leads to separation properties characterizing either a monofunctional conformation of the stationary phase with homologous series or carotenes, or a polyfunctional conformation with PAHs. This suggests that the sites of interactions are not the same for these families of solutes. The thickness of the bonded layer should be a primary factor controlling the selectivity. For monofunctional silicas, this thickness is directly related to the length of the bonded chain (n_{bp}). For polyfunctional silicas, the thickness depends not only on n_{bp}, but also on the degree of polymerization. The slot model of Sander and Wise[a] could be applied if one

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[a] According to this model, planar solutes penetrate deeper into the slots than non-planar solutes of similar molecular weight and length-to-breadth ratio. The effective contact is greater for planar solutes than for non-planar analogues because of a partial exclusion of the latter compounds from the stationary phase. Sander and Wise suggested that monofunctional and polyfunctional phases are differently organized and explained the change in selectivity $\alpha_{TBN/BaP}$ by their slot model.

considered that the spaces created in the stationary phase between zones of high bonding density and zones of low bonding density correspond to the cluster representation of Lochmuller and Wilder [162].

The model we propose implies more localized interactions inside each bonded zone. The slot model and the penetration model are not contradictory but rather complementary. It must be pointed out that the shape selectivity observed for carotene isomers is the reverse of that observed for the PAHs: angled rigid isomers (*cis*) are more retained than linear rigid isomers (*trans*) under conditions where the stationary phase is in a polyfunctional state. It is likely that carotene molecules penetrate in the slots by their cyclic (cyclohexenic) portion (similar in a way to PAH penetration), but because of their stereochemistry the interaction of the rest of the molecule with the top section of the bonded chains is weaker for the *trans* than for the *cis* isomers.

7. ROLE OF THE MOBILE PHASE COMPOSITION

7.1. *General case*

The two theoretical models (solvophobic and partition theories) accounting for the retention mechanism involve the formation of a cavity in the solvent. The energy associated with this process is directly related to the value of the mobile phase surface tension. Hence one should expect methanol to be stronger (that is more eluting) than THF, THF in turn being stronger than acetonitrile. It is remarkable that the plots relating the surface tension to the volume composition for the binary mixtures water–methanol, water–acetonitrile and water–THF cross at the same point corresponding to about 70% organic component (Fig. 20). At this composition, which can be called "isoeluotrope", the three mixtures have the same surface tension and should give identical retention times for all solutes, independent of their chemical natures.

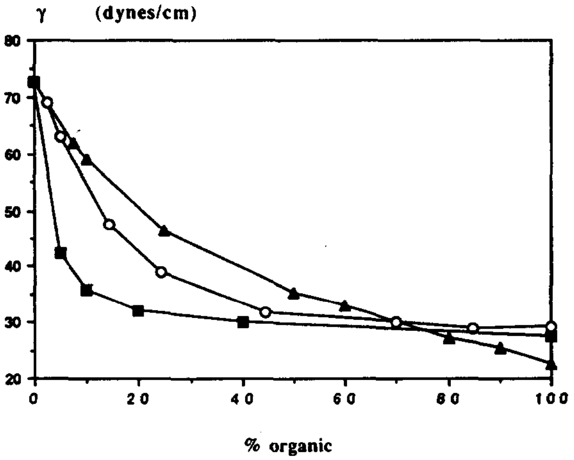

Fig. 20. Surface tension (γ) *vs.* volume fraction of organic solvent in water. ▲ = Methanol–water mixtures; ○ = acetonitrile–water mixtures; ■ = THF–water mixtures. From ref. 95.

Some studies have been carried out to attempt to establish a correlation between the mobile phase composition and the retention properties of homologous series. Except in three cases which are discussed below, the log k' *vs.* composition plots are in agreement with what can be predicted from the surface tension curves for each homologue studied (independent of the homologous series to which it belongs). This has been verified with monofunctional (Fig. 21) and polyfunctional (Fig. 22) stationary phases of the same chain length [125]. It has also been shown that the retention of *n*-alkanes follows the same pattern of variation as the surface tension with the solvent composition for organic mixtures such as methanol–acetonitrile, methanol–chloroform and methanol–acetonitrile–chloroform [138], as can be seen in Fig. 23. As was predictable, pure methanol appears to be stronger than pure acetonitrile. The log k' *vs.* X plots cross at about $X = 0.8$, also in agreement with Fig. 20.

The three exceptions are as follows. Each time they have been observed, specific solvation effects existed between two of the three species involved in the chromatographic process (solute–eluent, solute–stationary phase and eluent–stationary phase).

7.2. *Solvents with low dielectric constant ($\varepsilon < 20$)*

The first exception is observed when using water–THF mixtures: for a given water content,

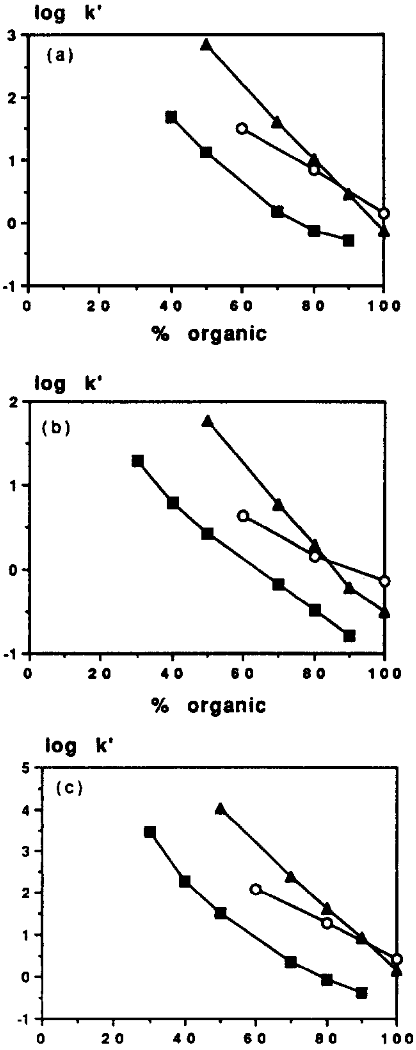

Fig. 21. Log *k'* *vs*. percentage of organic solvent in water on C_{18} column (LiChrosorb RP-18). ▲ = Methanol–water mixtures; ○ = acetonitrile–water mixtures; ■ = THF–water mixtures. (a) Chloro-*n*-alkane with $n_c = 9$; (b) *n*-alkanol with $n_c = 9$; (c) phenyl-*n*-alkane with $n_c = 9$. From ref. 95.

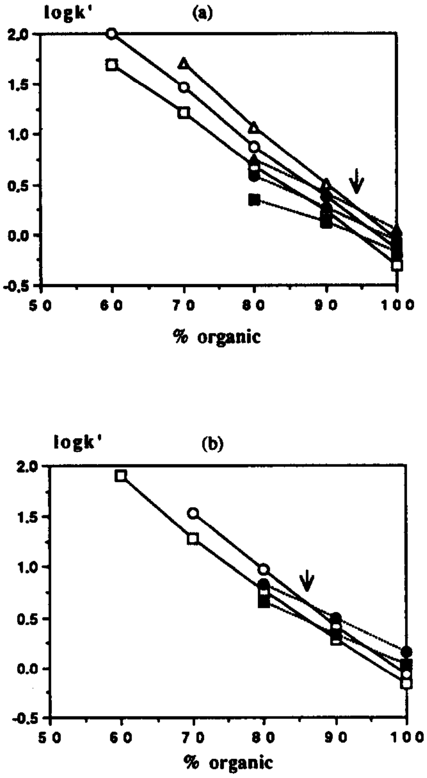

Fig. 22. Log *k'* *vs*. percentage of organic solvent in water on a polyfunctional C_{18}-bonded silica (Brownlee ODS) at 21°C. △, ○, □ = Methanol–water mixtures; ▲, ●, ■ = acetonitrile–water mixtures. (a) Phenyl-*n*-alkanes with n_c = (□, ■) 4, (○, ●) 5 and (△, ▲) 6. (b) *n*-Alkanes with n_c = (□, ■) 6 and (○, ●) 7. The arrows correspond to experimental "isoeluotrope" mobile phase composition.

these mobile phases are always the best eluents. It has even been shown that with pure THF, solutes are actually not retained and sometimes they are partially excluded. The chromatographic process is thus similar to a size-exclusion process, even for short homologues [95]. This phenomenon, which should not be expected on the basis of surface tension curves (see Fig. 20), is independent of the nature of the solute and the type of stationary phase. This situation is not specific of THF. It is related to a conformational effect induced by solvents of low dielectric constants. This effect has been revealed by a comparative FT-IR study on the role of solvents on the conformation of hydrocarbon chains [88,95,105,170]. It concerns a modification of the spatial structure of the stationary phase which induces a variation of the area of the contact surface between the solute and the bonded chains, as well as the size of the cavity to accommodate the solute molecule. This phenomenon is particularly important in NARP chromatography and in SFC.

It must be pointed out that if the phenomenon

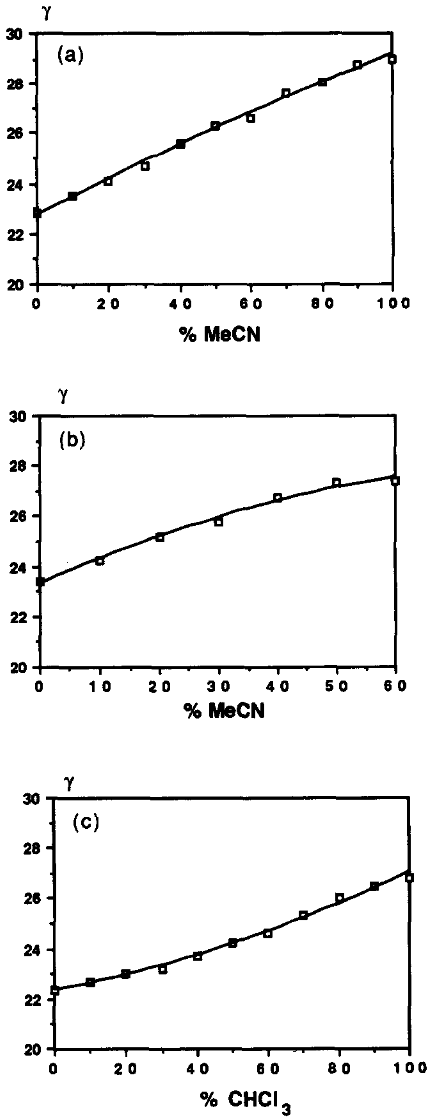

Fig. 23. Surface tension (γ) *vs.* volume fraction of organic solvent. (a) Methanol–acetonitrile mixtures; (b) methanol–acetonitrile–chloroform mixtures ($60-x$:x:40; the percentage of chloroform is maintained constant at 40%); (c) methanol–chloroform mixtures. Obtained from V. Husse and A.M. Siouffi specially for this study.

previously described is, in some respects, an indirect role of low dielectric constant solvents which happens with any type of solute. Obviously, in the case of electrically charged solutes, there is a direct effect of the solvent dielectric constant on retention [100].

7.3. π–π *Interactions*

When the solute and the solvent molecules contain π electrons there is a translation towards low k' values of the log k' *vs.* X plots obtained with acetonitrile–water mixtures relative to those obtained with methanol–water mixtures (Fig. 22). In other words, acetonitrile is stronger than expected. Thus, with *n*-alkyl stationary phases the isoeluotrope methanol–acetonitrile composition is richer in organic solvent for phenyl-*n*-alkanes than the *n*-alkanes [95]. This effect is emphasized when the stationary phase also contains π electrons, such as the phenyl-1-propyl (Fig. 24), *n*-octyl/cyano-1-propyl or *n*-octadecyl/cyano-1-propyl phases, for instance [116]. The specific π–π interactions can be sufficiently strong in this case so that acetonitrile is always a stronger solvent than methanol, whatever the mobile phase volume composition. In other words, the log k' *vs.* X plots obtained with the acetonitrile–water system are always below those obtained with the methanol–water system. This has been observed with solutes possessing a large number of unsaturations (triglycerides, carotenes and phenylhydantoins of amino acids, for instance) using totally non-polar monofunctional phases (such as octadecyl or undecyl), slightly polar phases (such as cyano-1-decyl) and mixed phases (such as undecyl/cyano 1-decyl) [76,107,108].

7.4. *Hydroxyl and silanol interactions*

When hydroxyl and silanol interactions take place, the situation is the reverse of that described in the previous section: the log k' *vs.* X plots are shifted to higher k' values with acetonitrile–water mixtures compared with methanol–water mixtures [95,108]. In order for this phenomenon to be observed, it is necessary that the stationary phase exhibits accessible OH groups, originating either from the silica itself (unreacted silanol groups) or from the bonded moieties. This is the case with such materials as short-chain (e.g., methyl) bonded phases, long-chain phases with a low bonding density and hydroxyl-1-undecyl or hydroxyl-1-butyl phases, for instance. With methyl-bonded silicas, the isoeluo-

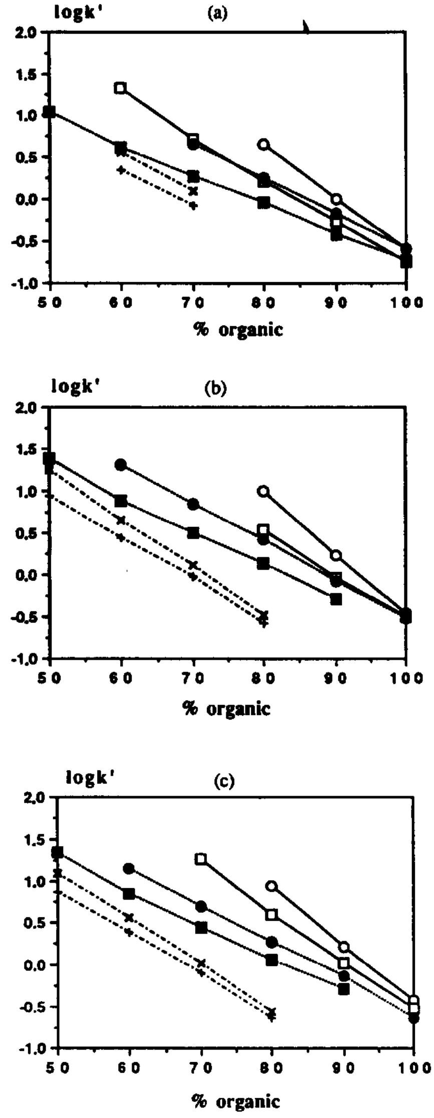

Fig. 24. Log k' *vs.* percentage of organic solvent in water on a monofunctional phenyl-1-propyl-bonded silica (Zorbax Phenyl) at 21°C. □, ○ = methanol–water mixtures; ■, ● = acetonitrile–water mixtures; +, × = THF–water mixtures. (a) *n*-Alkanes with n_c = (□, ■, +) 8 and (○, ●, ×) 12; (b) phenyl-*n*-alkanes with n_c = (□, ■, +) 8 and (○, ●, ×) 12; (c) *n*-alkyl benzoates with n_c = (□, ■, +) 9 and (○, ●, ×) 12.

trope composition for water–methanol and water–acetonitrile mixtures is poorer in organic solvent for the *n*-alkanols than for the *n*-alkanes. This is due to the presence of specific interactions with the residual silanols which are accessible to the solutes. These interactions are stronger with acetonitrile than methanol, which tends to block the silanol and/or hydroxyl groups. Also, specific solvation effects in methanol–water eluents will tend to accelerate solutes containing OH groups compared with those having no such groups.

An indirect proof of these interactions has been provided by a study of the asymmetry factor of *n*-alkanol and *n*-alkane peaks in pure acetonitrile. With a low bonding density C_{18} phase (μBondapak C_{18}), the elution peaks of *n*-alkanols are so distorted (asymmetry factor >6) that it is almost impossible to measure meaningful chromatographic data [141]. Under the same conditions, the elution peaks of *n*-alkanes are almost perfectly symmetrical. Adding only 0.5% of methanol to the mobile phase reduces the asymmetry factors for *n*-alkanols to 1.5, whereas it has no effect on the *n*-alkanes peaks.

7.5. *Effect of solvent nature*

If temperature clearly has a strong effect on the properties of the stationary phase, as previously described, the presence of solvent and/or solute molecules between the bonded chains must be an important factor. These molecules modify the conformation of the bonded chains (see Fig. 4, bottom right). The magnitude of the effect increases with increasing chain mobility, that is, with increasing distance from the anchoring point [66]. The longer this distance, the more the conformation of the bonded chains resembles that of a bulk phase. Accordingly, the role of inserted solute–solvent molecules depends on the length of the bonded chains. It also depends on the bonding density. This chain organization can be expressed in terms of "a lattice of sites arranged in layers parallel to the interface" [66]. The situation is therefore fairly complex and depends on the nature of the organic modifier. Thus, the selectivity is smaller in THF compared with methanol or acetonitrile [81]. The presence of THF or any solvent with a low dielectric constant ($\varepsilon < 20$) favours kink and *gauche* conformations and thus produces a less ordered stationary phase. As the selectivity is a measure of the solute hydrocarbon chain–bonded chains interactions, at a given capacity factor for one homologue a lower selectivity should be expected in THF. Similarly, at a given average capacity factor, the selectivity differences between acetonitrile and methanol [81,151] can be correlated with a conformational modification of the stationary phase due to the different composition of the adsorbed solvent layer [171,172]. With methanol–water mixtures, the adsorbed layer and the bulk mobile phase have almost similar compositions, which is not the case with acetonitrile (and also THF): the adsorbed layer is richer in organic solvent, favouring again *kink* and *gauche* conformations. This conforms with the prediction that the chains should stretch with increasing bonding density and compatibility with the mobile phase [69]. The experimental observations can be summarized as follows.

7.5.1. Conditions of low temperature and/or water-rich mobile phase

When the mobile phase is very water-rich, the average methylene selectivity increases with n_c. This is true for both methanol–water and acetonitrile–water. It seems that a change in the molecular interaction mechanism occurs when the eluent contains more than 40% of water. Unfortunately, solutes are usually too strongly retained under these conditions (very intense solvophobic effect). Accordingly, it is only possible to make measurements with the first members of the series (in general n_c does not exceed 4 or 5) and it is difficult to draw clear conclusions. In fact, considering the n_c range investigated, it has not been possible to detect with water-rich eluents whether or not the curves of α *vs.* n_c have a maximum (experimental data have only shown that α increases with n_c). This would have indicated a retention mechanism identical with that observed, using organic-rich mobile phases, with a polyfunctional phase or a monofunctional phase at low temperature. This must be the case, however. Indeed, increasing the water content of the eluent produces a more intense hydrophobic effect, which should result in expulsion of the organic solvent molecules inserted between the bonded chains [173]. As a consequence, these chains should fold on top of each other. It has indeed been shown that the mobility of the alkyl chains is reduced in mobile phases with a high water concentration [20,173]. The structure of the stationary phase is more rigid than in neat organic solvents or water-poor solvent mixtures. The structure is close to that existing at low temperature [14], corresponding to chains with a reduced number of conformational states but statistically entangled.

Accordingly, the use of water-rich mobile phases and/or a low temperature has similar consequences on the structure of the conformational state of the stationary phase. The chains are folded on to themselves with slow intermolecular motions and the structure can no longer be represented as a brush (Fig. 4b) as described by Karch *et al.* [144]. The retention mechanism is likely to be of the partition type as described by Dorsey and Dill [66], whatever the state of the stationary phase at ambient temperature. The results of Lochmuller and co-workers [162,174] and Gilpin and Squires [175] confirm this point of view, which has also been reported by Scott and Simpson [176].

7.5.2. Conditions of organic-rich mobile phase with a large dielectric constant ($\varepsilon > 30$)

In methanol- or acetonitrile-rich mobile phases, the chain mobility is larger than in water. Methanol [177] and acetonitrile molecules are inserted in the network of alkyl chains (near the surface) in the case of polymeric phases or between the bonded chains in the case of monomeric phases [132,171]. This produces a stationary phase which is fairly ordered, but not static. The chains are not completely stretched and parallel to each other, as is the case with biological membranes, for instance [33]. The stationary phase statistically (on a time basis) looks like a brush, allowing the alkyl chain(s) of the solute to penetrate deeply the bonded layer and establish close contact with the bonded chains. This last statement must be related to the observation that the speed of interconversion between the different conformations of the bonded chains is slow in methanol [22]. At high bonding ratios, the chains are folded on to each other, as shown by NMR relaxation studies by Gilpin and Gangoda [29].

Even if one accepts, as did Gilpin [171] and Sentell [156], that the chains are better solvated (*i.e.*, the solvent molecules penetrate more easily between them) in acetonitrile than in methanol, thus leading to a more ordered structure of the stationary phase, it has been shown by IR spectroscopy that the chain conformations are very similar with both solvents. For homologous series, these two organic solvents should therefore give similar retention patterns. However, a study of the behaviour of several phenylalkanes at different temperatures has shown a large difference between data obtained in methanol and acetonitrile [178,179]. We did not observe such a difference when comparing the log k' *vs.* n_c and α *vs.* n_c plots. It must also be noted that the retention order of PAHs changes when methanol is replaced with acetonitrile [106]. According to Martire and co-workers [106,180], the stationary phase chains are more stretched in acetonitrile than in methanol. This stretching is more pronounced as the percentage of organic solvent in the mobile phase is increased. A "picket-fence" model (Fig. 4b) seems to describe properly the state of the stationary phase in acetonitrile. This seems contradictory with the IR spectroscopic results mentioned previously, which do not indicate a conformational difference of the bonded chains between neat methanol and neat acetonitrile. This disagreement is possibly due to the modification of the state of the stationary phase when it interacts with phenylalkane molecules, to such an extent that specific acetonitrile ↔ phenylalkane π–π interactions take place and modify the conformation of the bonded chains (see Fig. 14, bottom right). Obviously, such interactions do not appear with methanol.

7.5.3. Conditions of organic-rich mobile phase with $\varepsilon < 30$ and/or high temperature

In solvents of low dielectric constant having a strong solvating power for alkyl chains, the conformational mobility of the chains (solute and stationary phase) is responsible for looser and more transient contacts.

For monofunctional phases, neither a slope change nor a jump has been observed in the α *vs.* n_c plots. The retention mechanism is globally identical with that observed with low bonding density monofunctional phases and solvents with a low solvating power for the alkyl chains ($\varepsilon >$ 30). It can be described by a stationary phase–solute contact where the length of the stationary phase is no longer a critical parameter. The mobility of the bonded chains prevents the possibility of insertion of the solute molecules in a rigid network and the contacts with the solute molecules become looser (Fig. 14, top right). In these low dielectric constant solvents, the chain mobility may become such that pore access is difficult and sometimes impossible.

With polyfunctional phases, the use of such solvents should also produce an increase in the motions of the chains but the (deep) diffusion of solute molecules inside the stationary phase may be effectively restricted by the chain entanglement. Accordingly, there is only an insertion of the solute molecules between the external bonded chains which then acts somehow like a (short) monofunctional phase when it is used with solvents with a high dielectric constant. A similar effect is observed in this case when the temperature is increased (Fig. 14, bottom right).

It is possible to change from a polyfunctional to a monomeric type of structure either by increasing the temperature or by adding a solvent of very small dielectric constant ($\varepsilon < 20$) to the mobile phase. The inverse is also true. The results of Northrop *et al.* [181] confirm this point. This must be correlated with ^{13}C NMR data obtained with bonded alkylsilicas. Measurements of the shapes and widths of spectral bands have shown that the chain motions are restrained in methanol, whereas in chloroform or dioxane they are much easier [30,31]. Also, it has been shown that there is a significant change in the conformation of alkyl chains in THF in comparison with methanol or acetonitrile, probably because of the presence of a larger number of THF molecules between the bonded chains [95]. This is in agreement with the results of Martire and Boehm [69], who showed that the quantity of THF adsorbed on a bonded phase is very large. Accordingly, the conformation of the chains can vary between collapsed (methanol- and acetonitrile–water mixtures) and completely stretched (neat THF). Incidentally, the complete stretching of the chains in neat THF produces a "dynamic sealing" of the pores, thus generating an exclusion-type retention mechanism.

In conclusion, the use of solvents with a low dielectric constant induces a conformation change of the chains and consequently a modification of the solute–stationary phase molecular mechanism of interaction. More generally, this conformation change explains why, at a given temperature and mobile phase composition, monofunctional and polyfunctional phases do not give identical retention properties [31,182]: the freedom of motion of the chains is less for polyfunctional than for monofunctional phases. It is worth noting in passing that monofunctional phases with a large bonding density tend to resemble polyfunctional phases with a low bonding density. In fact, these two types of phases behave very similarly from a chromatographic standpoint [48,53,156].

All the previous considerations can be summarized as follows: Figs. 3d and 4b describe a monofunctional phase at ambient temperature or a polyfunctional phase at high temperature; Figs. 3a and 4c describe a monofunctional phase at low temperature or a polyfunctional phase at ambient temperature; the larger selectivity of a polyfunctional compared with a monofunctional phase (*e.g.*, $R_\alpha = 1.9$ for a C_{18} material) results from a larger number of chain–chain contacts with the polyfunctional phase. $n_{crit} = n_{bp}$ for $n_{bp} \leq 8$, the chains being well organized and the solute penetration deep, whereas $n_{crit} < n_{bp}$ for $n_{bp} = 14$, 18 and 22, the mobility of the end section of the bonded chains preventing deep penetration of the solute molecules all the way to the base of these chains.

It seems clear that the most appropriate model to describe solute–stationary phase interactions depends on the experimental conditions (mobile phase composition and temperature). At low temperature and/or with a mobile phase of high dielectric constant, the stationary phase has a polyfunctional character as shown in Fig. 14. At high temperature and/or with a mobile phase of low dielectric constant, the stationary phase has a monofunctional character. This is also the conclusion of recent work by Staroverov and Fadeev [132]. The work of Sander and Wise on PAHs [53,54,56,147] indicates a similar effect of temperature but a different effect of the solvent nature. All these results are summarized in Fig. 14.

8. GENERAL CONCLUSIONS

The following conclusions can be drawn from studies conducted on homologous series, PAHs and carotenes in reversed-phase chromatography (either aqueous or non-aqueous) and supercritical fluid chromatography.

(1) The distinction between monofunctional and polyfunctional stationary phase is not as clear as might be thought from the structure of these phases. It is possible to switch from the properties of one type to those of the other type by lowering or increasing the temperature. The addition of a solvent of low dielectric constant in water, methanol, acetonitrile or supercritical CO_2 changes the separation properties of a polyfunctional phase to those of a monofunctional phase, except for PAHs, which behave the opposite way.

(2) The appropriate model to describe the retention process depends on the operating conditions. Thus, partition, adsorption and solvophobic theories have their own privileged area of validity.

(3) The dielectric constant is the major parameter permitting the rationalization of the contribution of the solvent because the use of a mobile phase of low dielectric constant modifies the conformation of the stationary phase. It can even produce dynamic blocking of the pores with the associated exclusion behaviour observed in pure THF.

(4) The use of either a stationary phase containing π electrons (bonded silica or organic polymer) or acetonitrile-based eluents produces major selectivity changes for PAHs, carotenes, and homologues processing π electrons. However, this does not alter the basic solute–stationary phase interaction mechanism.

(5) The molecular mechanism of interaction is not the same for PAHs, carotenes, and homologous series. Because of the structural differences existing between these solutes, the interaction sites are not the same. In the case of monofunc-

tional phases and solutes such as homologous series and triglycerides, the retention mechanism involves the local structure of the stationary phase. Above the phase transition temperature, there is penetration of the hydrocarbon chain of the solute into the stationary phase. The contact is tight when organic-rich aqueous–organic eluent mixtures are used. The polar head of the solute, if any, stays outside the bonded layer, except if it is a phenyl or an *o*-phthalyl group. A "brush" representation of the stationary phase in combination with the solvophobic theory account well for this retention model. The same penetration phenomenon can be observed on the outer chains of a polyfunctional phase when a low dielectric constant mobile phase is used. For polyfunctional phases, there is total immersion of the solute in the bulk of the stationary phase. A "greasy-droplets" or "haystack" representation of the stationary phase in combination with the partition theory account well for the observed results. For phases with a very high bonding density, a "picket-fence" representation is appropriate. The situation is the same with short-chain bonded silicas and materials based on carbon. The adsorption model accounts well for the retention properties. In the case of PAHs (cyclic and rigid solutes), the retention mechanism is well described by the "slot" model of Sander and Wise, characterizing interaction sites in regions of the stationary phase located between strongly and weakly bonded areas.

(6) Solutes with polyfunctional hydrocarbon chains and cyclanes penetrate the stationary phase only superficially. In contrast, solutes carrying several alkyl chains, such as di- and triglycerides and alkyl *o*-phthalates penetrate deeply the stationary phase by their hydrocarbon chains. The solute–bonded chain equilibrium is dynamic. All the alkyl chains of the solute molecule do not necessarily interact in the same way with the bonded chains. Nevertheless, the selectivity is much larger than that observed with solutes having the same polar head but carrying only one alkyl chain.

(7) The role of the mobile phase composition cannot be described just by its conformational effects on the stationary phase. In general terms, the retention of a solute for a given organic modifier mixed with water is directly related to the surface tension of the mixture, as long as the dielectric constant is larger than 20–30. Accordingly, it follows that the variation of the capacity factor with the eluent composition when mixing solvents of similar dielectric constants follows the surface tension changes of the mixture, for solvents of similar surface tensions the mechanisms of interaction are different if the values of ε are different and mixing such solvents can produce very large selectivity changes, and a specific effect must be noted with solute molecules containing π electrons when using acetonitrile-containing mobile phases. It must first be recalled that solutes with a polar head penetrate the stationary phase by the head when aqueous mobile phases are used (methanol–water and acetonitrile–water mixtures), whereas they penetrate by their alkyl chain in pure acetonitrile or acetonitrile–methanol mixtures containing more than 5% of acetonitrile, or acetonitrile mixed with a medium-polarity organic solvent (NARP). This gives the possibility of solvation effects taking place, the polar head being accessible. Solute molecules containing π electrons are less retained in acetonitrile–water than in methanol–water mixtures, which is not the case with non-aromatic solutes when the organic solvent fraction is larger than 0.80–0.85. This is due to specific solvation effects in acetonitrile-based eluents [122,125]. It must also be noted that the selectivity for phenylalkane–alkane of similar chain length decreases when methanol is replaced with acetonitrile.

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