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# Property–structure relationship of solute–stationary phase complexes occurring in a molecular mechanism by penetration of eluite in bonded alkyl chains in reversed-phase liquid chromatography

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## Abstract

The analysis of the chromatographic behaviour of homologous series with a large range of chain lengths,  $1 \leq n_c \leq 32$ , allowed a second break in the curves of  $\log k'$  vs.  $n_c$  to be observed. This occurs independently of the nature of the series, the length of bonded chain ( $6 \leq n_{bp} \leq 22$ ), the analysis temperature (when it is higher than the temperature of phase transition); also, the qualitative mobile phase composition does not affect the phenomenon in partially aqueous reversed-phase liquid chromatography or in the non-aqueous reversed-phase mode (for mixtures  $\text{CHCl}_3$ -MeOH,  $\text{CHCl}_3$ -MeCN,  $\text{H}_2\text{O}$ -MeOH or  $\text{H}_2\text{O}$ -MeCN rich in MeOH or MeCN). The two breaks in the curves mark the limits of three zones which are characterized by two different values of  $n_c$ :  $n_{crit.}$  and  $n_{sat.}$ . The first describes the phenomenon for values of  $n_c \leq n_{crit.}$ . In this range  $n_{crit.}$  is independent of the homologous series but varies with the bonded chain length,  $n_{bp}$ . The methylene selectivity  $\alpha$  is different for each series and a correlation with the increment of molecular volume of a methylene group,  $\Delta V_{\text{CH}_2}$ , for each of them is established. The second break appears for values of  $n_{crit.} \leq n_c \leq n_{sat.}$ . In this case, the value of the methylene selectivity is the same regardless of the series. The same evolution can be demonstrated by examination of the increment of molecular volume of a methylene group. The third zone such as  $n_c \geq n_{sat.}$  shows a behaviour identical with that of the second zone but with lower values of  $\alpha$  and  $\Delta V_{\text{CH}_2}$ . The value of  $n_{sat.}$  is independent of the nature of the homologous series but is related to the bonding density of alkyl chains on silica. The results obtained are consistent with a molecular mechanism of interaction by insertion of the solute inside the bonded phase. The contacts are more or less tight depending on the space available between the chains of the stationary phase. This leads to three geometries of contact whose energetic phenomena are different.

## 1. Introduction

In an effort to verify the prediction of the solvophobic theory in reversed-phase liquid chromatography (RPLC) [1–4], many workers

have shown that the logarithms of the capacity factors vary linearly with the number of carbon atoms  $n_c$  in constituents of homologous series [5–10]. These results were obtained with different alkyl-bonded silicas and different aqueous–organic mobile phases. However, this linearity is not always rigorously observed: we can obtain

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sigmoidal plots of  $\log k'$  vs.  $n_c$ , according to the nature of the silane used for the graft, and/or the temperature of the analysis, or the nature of the solvent of the mobile phase [11]. A second case of non-linearity appears when one works with a large number of homologues: there is a discontinuity in the plots of the logarithm of capacity factor versus  $n_c$  which is observed in the PARP (partially aqueous reversed phase) as well as in the NARP (non-aqueous reversed phase) mode. It has been explained by a molecular mechanism of interaction in which the solute interacts with the bonded chains by vertical penetration [4,12–14]. Such a molecular view of the phenomenon was verified experimentally by measuring the variations of transfer enthalpies and entropies vs.  $n_c$  [15].

To validate these mechanisms further, we decided to perform some complementary experiments. A linear increase in the cavity size in the mobile phase with the number of carbon atoms is not thermodynamically established. Hence we have to understand how such long-chain solutes behave when they are 10–20 carbon atoms longer than the length of the alkyl chain bonded to silica. Lundanes and Greybrokk [16] have previously published a similar study limited to a single homologous series and only one type of mobile phase (tetrahydrofuran–water). However, this solvent leads to a conformational modification of the grafted alkyl chains and a different chromatographic behaviour than those observed in methanol or acetonitrile [17].

We also investigated the behaviour of solutes containing saturated carbon rings or straight chains with the same number of carbon atoms. Finally, the role of free silanols was investigated. In the case of a polar head of solute which could interact with residual silanols, is there penetration of the polar part of the solute into the carbon chains of the support, to create supplementary interaction with silanols?

The purpose of this study was to answer these questions. To achieve this goal, the influence of the nature of and variations in the molecular volume of homologous series was studied. The influence of bonding ratio and alkyl chain length was also addressed.

## 2. Experimental

The equipment used and precautions taken to ensure the precision of the measurements of capacity factors were reported elsewhere [5,15]. The phenomenon is independent of the value of the dead time in the range of column porosities tested, as demonstrated earlier [5]. To permit comparison of the results collected at different temperatures, all the reported values were obtained with  $k'$  calculated with a dead time corresponding to the maximum column porosity. This is valid considering that there is no significant modification of the void volume with temperature [18]. This value was calculated by using the weighing method [19]. For calculation we have taken into account the true dead volume in the column compared with the total dead volume of the equipment [15].

Homologues ( $C_nH_{2n+1}Z$ ) with  $Z = H, Cl, Br, OH, CPh, COOMe, Ph, CN, COOH, CH = CH_2, OCOMe, COMe, OCOPh, OCOPhCOO$  (*para*),  $COC_nH_{2n+1}$  ( $1 \leq n_c \leq 32$ ) and liquid crystals [ $OCO(C_6H_4)-p-N=N(C_6H_4)-p-OC_2H_5$ ] were used ( $3 \leq n_c \leq 6$ ). In addition, cycloalkanones ( $4 \leq n_c \leq 12$ ), 2,2,6,6-tetramethyl-4-piperidinyloxyl diesters of dicarboxylic acids (NO) ( $3 \leq n_c \leq 10$ ) and symmetrical dialkyl ketones ( $3 \leq n_c \leq 13$ ) were studied.

All the compounds were injected at least four times, at 25, 30.5, 36.2, 41.3, 45 and 50°C and sometimes at 60°C. For the analysis in NARP three experiments were carried out at 5, 10 and 25°C.

The analysis temperature on the monomeric  $C_{22}$  bonded phase was chosen in order to be above the phase transition temperature. Below this temperature the grafted chains are less mobile [12] and the retention mechanism is different [11].

The precolumn, column and injection valve were all thermostated using either a laboratory-made water jacket or a Crocosil oven (Cluzeau, Sainte-Foy-la-Grande, France). The bath temperature was controlled by a Huber HS 40 cryostat (Offenburg, Elgersweier, Germany) with a precision of  $\pm 0.1^\circ C$ .

Nine columns were used: an Ultrasphere ODS

(Altex, CA, USA), 5  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D. supplied by Beckman (Gagny, France); a LiChrospher RP-18 and a LiChrosorb RP Select B (Merck, Darmstadt, Germany), 5  $\mu\text{m}$ , 240  $\times$  4.0 mm I.D.; a C<sub>8</sub> high-density (Shandon SFCC, Eragny, France), 5  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D.; LiChrosorb RP-18 (5  $\mu\text{m}$ ), LiChrosorb RP-8 (5  $\mu\text{m}$ ) (Merck) and Spherisorb C<sub>6</sub> (5  $\mu\text{m}$ ) (Phase Separations, Queensferry, UK), 150  $\times$  4.6 mm I.D., laboratory packed. Experiments were also carried out with LiChrosorb RP-14, 10  $\mu\text{m}$ , 300  $\times$  4.6 mm I.D., and Spherosil XOB 075 C<sub>22</sub>, 5  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D., kindly supplied by L. de Galan (University of Delft, Netherlands) and J. Serpinet (LSA, University Claude Bernard Lyon I, France), respectively.

### 3. Theoretical

According to the thermodynamic approach [1] to solute interactions with non-polar alkyl chains of stationary phases, the capacity factor of a given elute at fixed eluent properties and temperature can be approximated by the relationship [4]

$$\log k' = a \Delta A + f(V_{\text{SP}}) + C \quad (1)$$

where  $\Delta A$  is the contact area between the elute molecule and the hydrocarbonaceous chains at the stationary phase surface,  $V_{\text{SP}}$  corresponds to the molecular size of the bonded chain and  $a$  and  $C$  depend on the solute and mobile phase properties and include the logarithm of the phase ratio for the  $C$  term.

Consequently, not only the size and the shape of the stationary phase but also its position at the surface in contact with a given eluent will have an effect on the chromatographic equilibrium constant [4]. In other words, the capacity factor of a substance is related to the change in partial molar free energy associated with the transfer of the solute (in the standard state at infinite dilution) from the mobile phase to the stationary phase,  $\Delta G^\circ$ , according to Martin [20]:

$$\log k' = -\Delta G^\circ / 2.3 RT + \log \phi \quad (2)$$

As reported in a recent review [21], the knowledge of the retention process consists in determining how  $\Delta G^\circ$  and  $\Delta A$  vary with the structure of the elutes. To answer to this problem, one approach is to use homologous series as probe compounds. There have been many studies that have demonstrated a linear relationship between  $\log k'$  and the number of carbon atoms of homologues  $n_c$  in these series [21]:

$$\log k' = pn_c + q \quad (3)$$

However, there are various ways to analyse retention data. As reported elsewhere, among them the use of methylene group selectivity for each pair of successive homologues,  $\alpha_n$ , allows one to quantify the magnitude of the interactions involved.

Consequently, with the aim of elucidating the intermolecular mechanism of retention, we used this last chromatographic parameter calculated in two different ways,  $\alpha_n$  and  $\tilde{\alpha}$  [21]. Moreover, for each of them we calculated their mean values ( $\bar{\alpha}_n$  and  $\tilde{\alpha}$ , respectively).

First, because of the levelling effect of the logarithm function, the linearity of the  $\log k'$  vs.  $n_c$  plots was investigated in terms of quadratic methylene selectivity  $\alpha_n$  values [5]. For a given homologue, it was calculated for each studied homologous series using the equation

$$\alpha_n = (k'_{n_c+1} / k'_{n_c-1})^{1/2} \quad (4)$$

As demonstrated previously, the  $\alpha_n$  vs.  $n_c$  plots permit subtle details to be revealed that cannot appear on a logarithmic scale.

Considering that the curves  $\alpha_n$  vs.  $n_c$  were similar independently of the nature of the tested series, we calculated the global methylene selectivity for a given chain length corresponding to  $n_c$  carbon atoms independently of the nature of the head of the homologue,  $\bar{\alpha}_n$ . This is the average value of  $\alpha_n$  for all the solutes investigated possessing the same chain length:

$$\bar{\alpha}_n = \sum_i^x \frac{\alpha_n}{x} \quad (5)$$

where  $x$  is the number of elutes tested. It has been reported that  $\bar{\alpha}_n$  could be used to investi-

gate the influence of the bonded chain length [21].

The curves of  $\bar{\alpha}_n$  vs.  $n_c$  gave us the opportunity to discriminate between four possibilities: a perfectly linear  $\log k'$  vs.  $n_c$  plot corresponds to horizontal  $\alpha_n$  vs.  $n_c$  plots (never observed experimentally), a curve with a sigmoidal shape corresponds to a curve with an apex [11], a smooth curve of  $\log k'$  vs.  $n_c$  corresponds in terms of  $\alpha_n$  vs.  $n_c$  to a straight line with a negative slope and a curve with break points corresponds to a curve with jumps or changes in slope [5]. Each of the last three have already been interpreted in terms of different mechanisms of interaction [11]. Among them, the last merits to be examined in another way, as follows.

In the range of  $n_c$  values where plots of  $\alpha_n$  vs.  $n_c$  are straight lines with a negative slope, the average methylene selectivity  $\bar{\alpha}$  can be determined to correlate the molecular properties of the homologues of different polarity with their retention.

The value of  $\bar{\alpha}$  was calculated for each homologous series from the equation

$$\bar{\alpha} = 10^p \quad (6)$$

where  $p$  is the slope of the experimental curves of Eq. (3). In terms of a thermodynamic approach, the combination of Eqs. 2 and 3 leads to

$$\log \bar{\alpha} = -\Delta\Delta G^\circ/2.3 RT = -\Delta G_{\text{CH}_2}^\circ/2.3 RT \quad (7)$$

which accounts for the increment of change in partial molar free energy associated with the transfer of a single methylene group. Now, considering the molecular signification of Eq. 1,  $\bar{\alpha}$  must permit a correlation of the retention data with the molecular volume increment  $\Delta V_{\text{CH}_2}$  of a  $\text{CH}_2$  group calculated for each investigated homologous series. This must be done to propose a molecular mechanism of interaction between homologous series and bonded alkyl chain.

Finally, it is possible to calculate the mean average methylene selectivity,  $\bar{\alpha}$ . This is the average value of  $\bar{\alpha}$  calculated for all the series investigated. It was calculated with the equation

$$\bar{\alpha} = \sum_1^N \frac{\bar{\alpha}}{N} \quad (8)$$

where  $N$  is the number of series tested.

Finally, the mean average methylene selectivity  $\bar{\alpha}$  permitted the retention between different grafted silicas to be compared and the eluent strength scale to be determined.

To be sure of the interpretation of our results, two strategies were adopted: the use of many homologous series to demonstrate the generality of the phenomenon reported below, and a mathematical treatment of our results. This strategy was previously used successfully to demonstrate independently of the homologues tested the existence of a break at a value of  $n_c$  that is characteristic of the length of the bonded chain [15]. This value is called  $n_{\text{crit.}}$  in the following. It was also revealed at the value of  $n_c$  at which a jump occurs on the  $\alpha_n$  vs.  $n_c$  plots.

In this work we used the same method to establish the veracity of a second break point which occurs on the curves of  $\log k'$  vs.  $n_c$ . It is observed for homologues whose chain length is longer than  $n_{\text{crit.}}$ . It appears at a value of the chain length called  $n_{\text{sat.}}$  in the following. It was determined considering the value of  $n_c$  at which the experimental value of  $\log k'$  of the corresponding solute does not fall on a straight line on the curve of either  $\log k'$  vs.  $n_c$  or  $\alpha_n$  vs.  $n_c$  drawn from the highest values of  $n_c$ . Thus, in the range of  $n$  higher than  $n_{\text{crit.}}$ , we compared two different representations of  $\alpha_n$  vs.  $n_c$  plots for each homologous series. One of them was a single straight line whose linear correlation was always higher than 0.99. The other was two convergent lines with a break point. In this case, for each of the linear parts the linear correlation was also above 0.99. We verified that there is a significant difference (95% confidence interval) between the slopes and the origins of these two representations (single line or broken line) by using the standard deviations comparison test with the same degrees of freedom [22]. This fact, and the values at which the second break points occur ( $n_{\text{sat.}}$ ), observed independently of the series investigated, involve the choice that the best representation of the phenomenon is given by a

linear segment, an abrupt shift and a broken linear segment.

The molar volumes were calculated from relative molecular masses and densities of the different solutes studied. The values of these parameters were obtained from a reference book [23].

#### 4. Results and discussion

##### 4.1. Preliminary results: characterization of the bonded silica used

As reported by Unger [24], “from a purely geometric point of view, bonded phases can be divided into two types: monolayer and polymer layer”. A monolayer can be obtained by using a monofunctional silane as reactant on silica or di- and trifunctional silanes, provided that water is excluded. The surface reactions between silica and trifunctional silanes in the presence of water yield polymer layers through condensation. These two kinds of bonded silicas lead to two different chromatographic behaviours, as follows.

Previous results have shown that the alkyl-bonded silicas that were synthesized from monofunctional or difunctional silanes could lead to a

mechanism of insertion [5,15]. Hence they will be considered to have a “monomeric-like” behaviour. The silica bonded with a polymeric layer could lead to a partition mechanism [11]. It was described elsewhere by a molecular mechanism such that there is total insertion of the solute in the stationary phase. To be distinguished from the mechanism of insertion, it was defined as “polymeric-like” behaviour. In fact, this difference between “monomeric-like” and “polymeric-like” behaviour refers to a change in the conformation of the bonded chain and how the elute interacts with the stationary phase. This was described in detail in a recent review [21]. It was also demonstrated that there is a reversible solvent and temperature-induced “monomeric-like–polymeric-like” transition in alkyl-bonded silicas [11,21].

We first checked that the commercial stationary phases used under the experimental conditions described here were “monomeric-like” or “polymeric-like”. We used the results in the literature or the test of Sander and Wise [25,26] which allow this differentiation. Unambiguously, the C<sub>18</sub>-bonded silicas giving  $\alpha_{\text{TBN/BaP}} > 1.7$  reflect “monomeric-like” phases. The terminology “polymeric-like” is used for all phases obtained with polyfunctional silanes on silica, giving  $\alpha_{\text{TBN/BaP}} < 1$ . The results are given in Table 1.

Table 1  
Characteristics of bonded phases used: values at which the breaks occur in log  $k'$  vs.  $n_c$  (or  $\alpha_n$  vs.  $n_c$ ) plots

Column	Nature of the silane reagent used	Stationary phase density ( $\mu\text{mol}/\text{m}^2$ )	$n_{\text{bp}}$	$n_{\text{crit}}$	$n_{\text{sat}}$	Test of Sander and Wise [35]
Spherisorb C-6			6	6	12–13	1.57 [37]
LiChrosorb RP-8	Difunctional [30,31]	3.0	8	8	15	1.36 [37]
LiChrosorb RP-14	Monofunctional [27]	3.4	14	12	22	1.64 [37]
LiChrosorb RP-18	Difunctional [30,31]	3.5	18	14	21	–
Spherosil XOB C <sub>22</sub>	Monofunctional [12,32]	4.0	22	14	24	1.76 [37]
C <sub>8</sub> HD	Monofunctional [33]	4.7–5.2	8	8	10	1.71 [37]
LiChrosorb RP Select B	Difunctional [30,31]	2.4	8	8	14	1.36 [36]
LiChrospher RP-18	Difunctional [30]	3.3	18	14	22–23	Intermediate [35]
Ultrasphere ODS	Monofunctional [34]	3.6	18	14	20	1.11 [36], 1.44 [37] Monomeric [35], 1.98 [36], 1.89 [37]

The stationary phase densities were obtained either from L. de Galan (LiChrosorb RP-14), J. Serpinct (Spherosil XOB 075 C<sub>22</sub>) or from the manufacturers. Only the LiChrospher RP-18 support is not classified clearly using this test. Its response is characteristic of either dense monomeric or not dense polymeric materials. Our studies on this column showed that with both methanol and acetonitrile, with 100:0, 90:10 or 95:5 organic solvent–water mixtures, the  $\alpha_n$  vs.  $n_c$  plots showed a break similar to that observed with well known monomeric phases such as Zorbax ODS and Ultrasphere ODS. As a consequence, we assumed that this spherical silica

behaved similarly to “monomeric-like” phases with regard to homologous series [21].

#### 4.2. Studies of long carbon chain solutes on bonded silica allowing insertion

##### Effect of bonded phase length. Examination of $\alpha_n$ vs. $n_c$

This study was carried out with bonded silicas with different chain lengths ( $n_{bp} = 6, 8, 14, 18, 22$ ). The results are shown in Fig. 1. Whatever the chain length of the bonded phase, the homologous series or the organic solvent used (methanol, acetonitrile pure or mixed with water

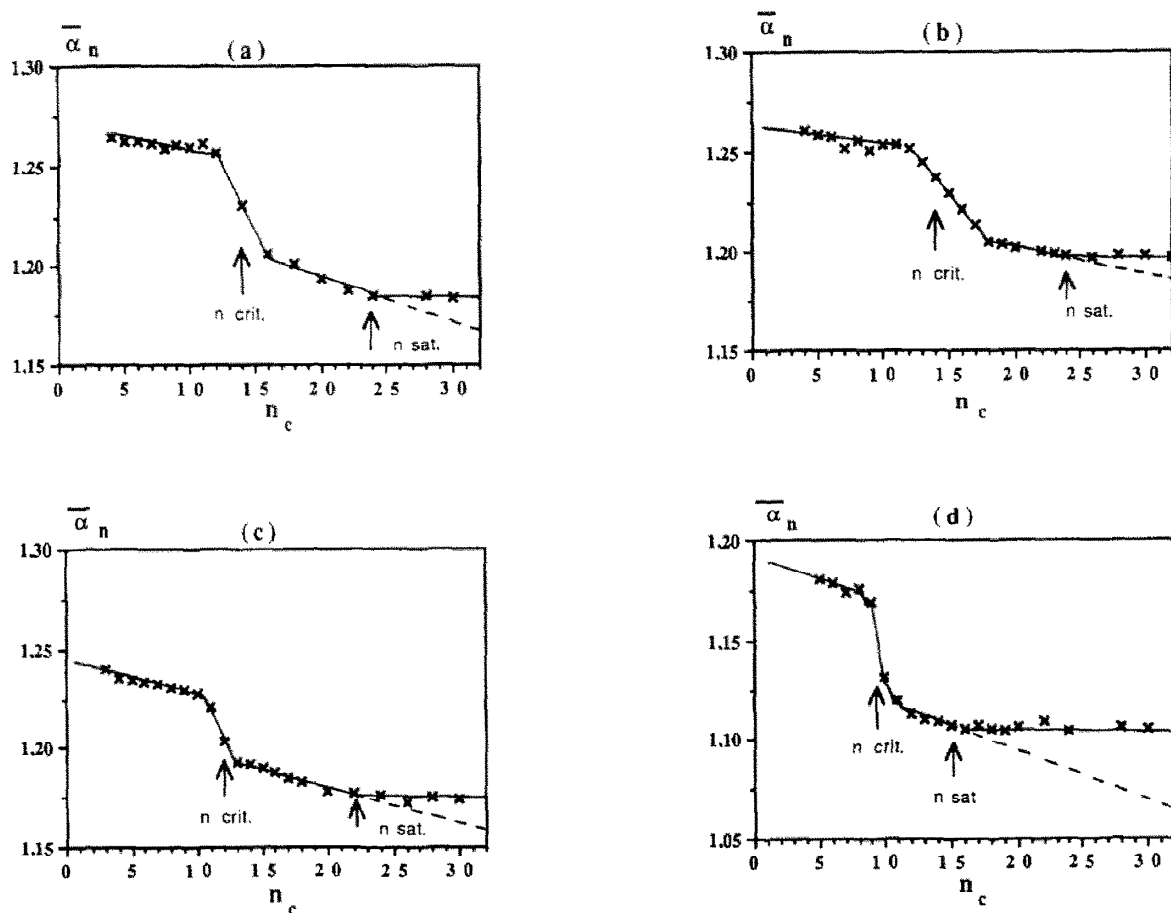


Fig. 1. Global methylene selectivity  $\bar{\alpha}_n$  vs.  $n_c$  plots in pure methanol on various bonded phases: (a) monomeric C<sub>22</sub>-bonded phase, 55°C; (b) monomeric C<sub>18</sub>-bonded phase, 25°C; (c) monomeric C<sub>14</sub>-bonded phase, 25°C; (d) monomeric C<sub>8</sub>-bonded phase, 25°C.

but rich in organic modifier), the same plots were obtained.

The left part of the curves has already been described [5]. Surprisingly, the critical value ( $n_{crit.}$ ) for docosyl-bonded silica is approximately the same as that for all the tested octadecyl phases. For long-chain solutes ( $n_c > n_{crit.}$ ) the right-hand part of the curves display, for a given  $n$  value (called  $n_{sat.}$ ), a change in the slope of methylene selectivity. After this  $n_{sat.}$  value,  $\bar{\alpha}_n$  remains relatively constant, or decreases slightly. This second break in  $\alpha_n$  vs.  $n_c$  curves is independent of the series studied, but is dependent on the length of the bonded chains (Table 1). The shorter the bonded chains (with comparable ligand density), the lower the observed  $n_{sat.}$  value. To specify the extent and limitations of this phenomenon, we studied columns with the same bonded alkyl chain length, but with different bonding ratios. In this case,  $n_{crit.}$  remains constant but  $n_{sat.}$  decreases with increase in the bonding ratio on silica. For example, for an  $n$ -octyl graft,  $n_{sat.} = 10$  for  $\Gamma = 5.0 \mu\text{mol}/\text{m}^2$ ,  $n_{sat.} = 14$ – $15$  for  $2.4 < \Gamma < 3.0 \mu\text{mol}/\text{m}^2$ .

The same plots have been obtained in various acetonitrile–chloroform mixtures [13]. Thus,

these results seem to prove the generality of the phenomenon regardless of the monofunctional nature of the bonded phase and the NARP or PARP liquid chromatography modes.

#### Examination of average methylene selectivity $\bar{\alpha}$

The value of the quadratic methylene selectivity  $\alpha_n$  (Eq. 4) is not exactly constant in the range of  $n_c$  values for which the plateaux were observed [5]. Consequently, the calculation of the average methylene selectivity  $\bar{\alpha}$  was made for each investigated homologous series from Eq. 6. The selectivity is directly related to the variation of standard free energy at infinite dilution for the transfer from the mobile phase to the stationary phase, corresponding to a single methylene group of the solute.

In this way,  $\bar{\alpha}_{in}$  is the reflection of this energy change for a solute with  $n_c < n_{crit.}$ ;  $\bar{\alpha}_{crit}$  characterizes the same phenomenon for  $n_{crit.} \leq n_c \leq n_{sat.}$  and  $\bar{\alpha}_{sat}$  for  $n_c > n_{sat.}$ . For the sake of example, we give in Table 2 the values of  $\bar{\alpha}_{in}$  and  $\bar{\alpha}_{crit}$  obtained with two different mobile phase compositions on a  $C_{14}$ -bonded silica. We observed that  $\bar{\alpha}_{crit}$  is approximately constant independently of the nature of the homologous

Table 2

Average methylene selectivity for  $n < n_{crit.}$  ( $\bar{\alpha}_{in}$ ) and for  $n_{crit.} \leq n \leq n_{sat.}$  ( $\bar{\alpha}_{crit}$ ) versus the polar head of the homologous series, with a monomeric  $C_{14}$ -bonded phase

Z	MeCN–H <sub>2</sub> O (95:05) at 30°C		Pure MeOH at 30°C	
	$\bar{\alpha}_{in}$	$\bar{\alpha}_{crit}$	$\bar{\alpha}_{in}$	$\bar{\alpha}_{crit}$
Cl	1.318	1.271		
COOMe	1.332	1.265		
COMe	1.362	1.283	1.333	1.181
OH	1.358	1.274		
OCOPh	1.302	1.265	1.222	1.170
COPh	1.351	–	1.323	–
OCOMe	1.329	1.268	1.239	1.175
Ph	1.319	1.272		
H	1.316	1.276		
Liquid crystals		1.261		1.160
OCOPhCOO	1.300	1.259	1.254	1.171
CH=CH <sub>2</sub>	1.306	1.273		
NO		1.281		
Br	1.324	1.274		

series for a given solvent and stationary phase. The values of  $\bar{\alpha}_{in}$  are lower for non-polar homologous series ( $Z = H, CH=CH_2, Ph$  and surprisingly  $Cl$  and groups possessing a phenyl ring) than for polar series. We observed this effect for grafts of different lengths ( $n_{bp} = 8, 14, 18$  and  $22$ ) percolated by mobile phases such as pure methanol, methanol–water (90:10, v/v), pure acetonitrile and acetonitrile–water (95:5, v/v). It must also be noted that a close re-examination of the results of Berendsen [27] and Berendsen and de Galan [28] leads to the same conclusion. This difference between polar and non-polar homologous series for  $n_c < n_{crit.}$  can be related to the determination of two different values of compensation temperatures for these two classes of solutes [15]. Moreover, independently of the polar or non-polar nature of the solute, the value of the temperature of compensation becomes smaller for solutes such as  $n_{crit.} < n_c < n_{sat.}$  than those such as  $n_c < n_{crit.}$ . It also appears that solutes with a chain length higher than 24 do not have exactly the same behaviour as the smaller ones ( $15 < n_c < 22$ ) on a  $C_{14}$ -bonded silica [21]. They give a linear dependence of  $\log k'$  vs.  $1/T$  like the others, but the corresponding straight lines do not converge to a single point which is related to the temperature of compensation [15]. This last conclusion agrees with the fact that two breaks could be observed on the curves of  $\log k'$  vs.  $n_c$  and that the corresponding values of the mean average

methylene selectivity  $\bar{\alpha}_{sat.}$  were smaller than  $\bar{\alpha}_{crit.}$  independently of the nature of the series investigated (Table 3). The difference between  $\bar{\alpha}_{crit.}$  and  $\bar{\alpha}_{sat.}$  is approximately 0.02 methylene selectivity unit, independently of the chain density of the bonded phase, but their respective values are related to the chain length of the stationary phase.

#### Particular behaviour of $\mu$ Bondapak $C_{18}$ and LiChrosorb RP-2 silicas

These two types of phases were not included in the results discussed previously because the  $\alpha_n$  vs.  $n_c$  plots exhibit a regular decrease. This corroborates the fact that  $\mu$ Bondapak  $C_{18}$  silicas are different from the others investigated here. Contrary to our first assertion, this stationary phase is not “polymeric-like” silica [15] but “monomeric-like” as reported by Sander and Wise [26]. For the sake of comparison, the reported value of the methylene selectivity in Table 3 was calculated as the global methylene selectivity  $\bar{\alpha}_{14}$  at a value of  $n_c$  of 14. Surprisingly, the corresponding value for this particular  $C_{18}$ -bonded silica was close to the value of the mean average selectivity  $\bar{\alpha}_{sat.}$  obtained for the other bonded silicas ( $C_{14}, C_{18}$ ) (see Table 3). These experimental results were also observed in NARP liquid chromatography with various mobile phase compositions (MeCN–CHCl<sub>3</sub> or MeOH–CHCl<sub>3</sub>) [13]. These results are not con-

Table 3

Mean average methylene selectivity for  $n < n_{crit.}$  ( $\bar{\alpha}_{in}$ ),  $n_{crit.} < n < n_{sat.}$  ( $\bar{\alpha}_{crit.}$ ) and  $n > n_{sat.}$  ( $\bar{\alpha}_{sat.}$ ) versus the nature of the grafted silica

Mobile phase	Column	$n_{bp}$	$\bar{\alpha}_{in}$	$\bar{\alpha}_{crit.}$	$\bar{\alpha}_{sat.}$	$\bar{\alpha}_{nc=14}$
Pure MeOH (25°C)	LiChrosorb RP-8	8	1.18	1.12	1.106	–
	LiChrosorb RP Select B	8	–	1.123	1.106	–
	LiChrosorb RP-14	14	1.23	1.185	1.172	–
	LiChrosorb RP-18	18	1.24	1.21	1.198	–
	$\mu$ Bondapak $C_{18}$	18	–	–	–	1.169
	LiChrosorb RP-2	1	–	–	–	1.15
MeOH–H <sub>2</sub> O (90:10) (25°C)	LiChrosorb RP-14	14	1.34	1.315	1.298	–
	LiChrosorb RP-18	18	1.39	1.36	–	–
	$\mu$ Bondapak $C_{18}$	18	–	–	–	1.27
	LiChrosorb RP-2	1	–	–	–	1.17



tradiictory to those reported above. They suggest a loose contact between the bonded phase and the solute which is coherent with a monomeric stationary phase with a short chain length for which lying on the phase is the only possibility [21]. This is also valid for the octadecyl phase with a low bonding density for which a close insertion of solute in the phase cannot occur [21].

*Correlation between average methylene selectivity  $\tilde{\alpha}$  and molecular mechanism of retention*

We have shown that  $\tilde{\alpha}_{in}$  is not identical for all the homologues tested (cf., Table 2). The first hypothesis is to consider that the retention of the polar compounds ( $Z = \text{COOMe}$ ,  $\text{COMe}$ ,  $\text{OH}$ ,  $\text{COPh}$ ,  $\text{OCOMe}$ ,  $\text{Br}$ ) can be due to a mixed mechanism of retention as previously described by Nahum and Horváth [29]. The hydrocarbonaceous part of the solute interacts with the hydrocarbonaceous part of the bonded phase by dispersion interactions and the polar part of solute  $Z$  interacts with silanols located at the bottom of the chains of the stationary phase. In such a case, the polar head  $Z$  of the solute penetrates deeply inside the chains of the bonded phase. The influence of this contribution to the retention should decrease with increasing chain length of the solute owing to the increase in its size. This could explain why smaller alcohols ( $n_c < 4$ ) are more retained than predicted by extrapolation from higher homologues of the straight lines of the  $\log k'$  vs.  $n_c$  curves [5].

However, in this case the depth of penetration of a polar solute inside the bonded phase depends on the steric hindrance of the polar head. In order to verify this hypothesis, we tested the behaviour of alkyl acetates ( $Z = \text{OCOMe}$ ). In this particular case, the polar head corresponds approximately to three methylene groups and should lead to a value of  $n_{crit}$  less than three carbon atoms than the other homologues tested. We have never observed this effect. The behaviour of alkyl acetates was identical with that of the other polar series. Such a mechanism of insertion of the head group of the homologues

into the stationary phase was only observed for phenylalkanes and dialkyl *o*-phthalates [5,15].

The overall results are in favour of a mechanism of retention in which the polar head of solutes stays out of the chains of the stationary phase to maximize the polar interactions with the solvent [15]. For polar solutes the potential polar head–residual silanols interaction plays a role in the retention but it occurs just in the place of low-density of coverage of silica.

The second point to elucidate is the relationship between the London dispersive interactions and contacts between chains with increasing number of carbon atoms.

In the case of the penetration model, there is a close contact between the hydrocarbonaceous chain as described above. The regular increase in the values of  $k'$  is a consequence of the increase in molecular volume with  $n_c$  [1–4,11]. Thus,  $\tilde{\alpha}_{in}$  must reflect the variation of the transfer energy related to the average molecular volume increment due to a single methylene group of the homologues. This aspect is discussed in the following section.

*Correlation between average methylene selectivity  $\tilde{\alpha}$  and molecular structure of the solute*

For all the homologues tested, curves of variation of molecular volume  $V_M$  vs.  $n_c$  were plotted. Over a range of  $n_c$  values, the curves were not perfect straight lines. The values of the slope between two nearest homologues vs.  $n_c$  are not parallel to the  $n_c$  axis but decrease slowly. However, in the ranges  $1 < n_c < 14$  and  $14 < n_c < 24$  the linear regressions give a correlation coefficient always higher than 0.998, which shows that the curves can be treated as straight lines. Consequently, we considered for the following discussion that the average molecular volume of a methylene group,  $\Delta V_{\text{CH}_2}$ , is given by the slope of the curves of  $V_M$  vs.  $n_c$ .

In general, we have observed that  $\Delta V_{\text{CH}_2}$  is different for each homologous series tested. It is higher for the homologues with a polar head. The order is  $\text{OCOPh} > \text{COMe} > \text{OCOPhCOO} > \text{COOMe} > \text{OCOMe} > \text{CHO} > \text{COOH} > \text{CN} = \text{OH} > \text{I} > \text{Br} > \text{Ph} > \text{CH}=\text{CH}_2 = \text{Cl} > \text{H} > \text{F}$ .

For each series, and on different phases of various alkyl chain lengths, three values of the average molecular volume of a methylene group were calculated: the first, called  $\Delta V_{in}$ , with  $n_c < n_{crit.}$ ; the second,  $\Delta V_{crit.}$ , with  $n_{crit.} < n_c < n_{sat.}$ ; and the third,  $\Delta V_{sat.}$ , with  $n_c > n_{sat.}$  (Table 4).

The values of  $\Delta V_{crit.}$  are close together but those of  $\Delta V_{in}$  are different for a given homologous series. Consequently, we tried to correlate the values of  $\Delta V_{in}$  corresponding to each homologous series with the values of the average methylene selectivity  $\tilde{\alpha}$  observed in RPLC with the graft of different investigated chain lengths. The results are shown in Fig. 2, where the linear regression coefficients are also reported. Their examination reveals that there is possibly a relationship between these two parameters in a range of  $n_c$  such that we think that the retention is described by a close contact between the hydrocarbonaceous parts of the solute and the bonded phase. This conclusion reported here with different bonded phases was also observed independently of the nature of the mobile phase (PARP and NARP modes).

The phenylalkanes are the only homologous series for which this correlation was not observed, but the fact that the phenyl ring penetrates into the bonded phase instead of the alkyl chain [5,15] leads to rationalization of this observation. The chain–chain contact must be

weaker than for the other series owing to a more important steric hindrance of the aromatic ring.

Further, independently of the nature of the head Z of the homologue, it could be seen that the values of the average molecular volume of a methylene group,  $\Delta V_{crit.}$  and  $\Delta V_{sat.}$ , are approximately constant in the ranges  $n_{crit.} < n_c < n_{sat.}$  and  $n_c > n_{sat.}$ , respectively. These values decrease in the order  $\Delta V_{in} > \Delta V_{crit.} > \Delta V_{sat.}$ . This is consistent with the experimental observation that  $\tilde{\alpha}_{in}$  values are higher than  $\tilde{\alpha}_{crit.}$  values (which are constant for all the homologues). The same conclusion could be drawn for the  $\tilde{\alpha}_{sat.}$  values as for  $\tilde{\alpha}_{crit.}$  values.

#### Particular behaviour of solutes with saturated cyclic structure

Independently of the nature of the mobile phase composition, the value of the average methylene selectivity  $\tilde{\alpha}$  found for cycloalkanones with  $n_c < n_{crit.}$  was equal to that obtained for linear chains in the range such that  $n_{crit.} < n_c < n_{sat.}$ . In the same way, we obtained for symmetrical aliphatic ketones ( $3 < n_c < 11$ ) a value of this variable close to  $\tilde{\alpha}_{in}$ . This difference between alicyclic and linear ketones is not due to the carbonyl group. It could be attributed to the saturated cyclic configuration. In a penetration process it does not give a chain–cycle contact as close as two linear hydrocarbon chains.

Table 4

Values of the average methylene volume of different homologous series for  $n_c < n_{crit.}$  ( $\Delta V_{in}$ ) and  $n_{crit.} < n_c < n_{sat.}$  ( $\Delta V_{crit.}$ )

Z	$\Delta V_{in}$			$\Delta V_{crit.}$	
	$n_{bp} = 8$	$n_{bp} = 14$	$n_{bp} = 18$	$n_{bp} = 8$	$n_{bp} = 14$ and 18
Cl	16.39	16.4	16.41	16.44	16.42
COOMe	16.91	16.76	16.70		
COMe	16.7	16.73	16.77		
OH	16.7	16.63	16.63	16.65	16.45
OCOMe	16.74				
Ph	16.58	16.49	16.53	16.50	16.30
HC = CH <sub>2</sub>	15.28	15.80	15.89	16.32	16.42
Br	16.53	16.52	–	16.30	16.47
CN	16.87	–	–	16.48	16.51
H	15.57	15.95	15.85	16.45	16.34

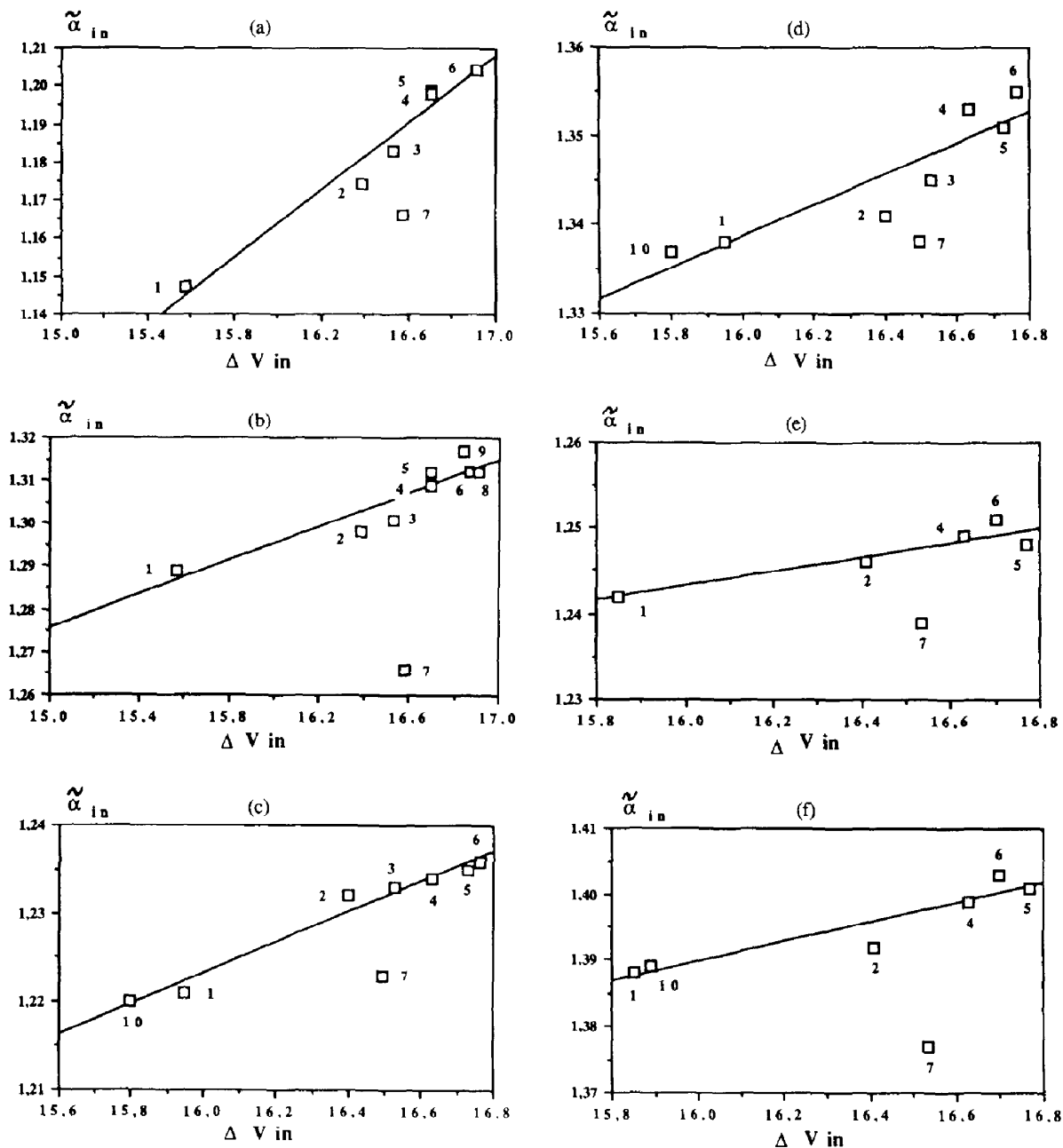


Fig. 2. Average methylene selectivity ( $n_c \leq n_{crit}$ )  $\tilde{\alpha}_{in}$  vs. average molecular volume of a methylene group  $\Delta V_{in}$  ( $n_c \leq n_{crit}$ ) (a)  $C_8$ -bonded phase, pure methanol,  $r = 0.978$ ; (b)  $C_8$ -bonded phase, methanol-water (90:10),  $r = 0.925$ ; (c)  $C_{14}$ -bonded phase, pure methanol,  $r = 0.987$ ; (d)  $C_{14}$ -bonded phase, methanol-water (90:10),  $r = 0.914$ ; (e)  $C_{18}$ -bonded phase, pure methanol,  $r = 0.92$ ; (f)  $C_{18}$ -bonded phase, methanol-water (90:10),  $r = 0.941$ . 1 = alkanes; 2 = alkyl chlorides; 3 = alkyl bromides; 4 = alkanols; 5 = 2-alkanones; 6 = methyl alkanoates; 7 = phenylalkanes; 8 = cyanoalkanes; 9 = carboxylic acids; 10 = alkenes.

Such a phenomenon was also observed with the 2,2,6,6-tetramethyl-4-piperidinoxyl diesters of dicarboxylic acids (reported as NO in Table 2) with  $2 < n_c < 10$ , which interact with the stationary phase even by an external chain–chain contact or by penetration with the very hindered ring. For these types of compounds we observed a value of methylene selectivity intermediate between  $\tilde{\alpha}_{in}$  and  $\tilde{\alpha}_{crit}$  but close to the latter and not equal to  $\tilde{\alpha}_{sat}$ , characteristic of an external contact [21]. Hence it is reasonable to think that this cyclic structure superficially penetrates the chains of bonded phase and their hydrocarbon chains interact more freely with the chains of the bonded phase.

We also report the results obtained with liquid crystals. Although the cyclic structures are aromatic, we observed a value of mean methylene selectivity close to  $\tilde{\alpha}_{crit}$  and not to  $\tilde{\alpha}_{in}$ . An examination of their structures leads to the conclusion that the aromatic part (EtOPhN = NPh) is equivalent to thirteen methylene groups. Consequently, the two aromatic rings penetrate the chain of the stationary phase. As their equivalent length is equal to the depth of penetration ( $n_{crit} = 14$ ) no break could be observed for these solutes on the bonded silicas investigated. The contact between saturated chains is less tight than in the case of direct penetration in the stationary phase. According to this last hypothesis, the average methylene selectivity would be equal to  $\tilde{\alpha}_{in}$ . Finally, this phenomenon is the same as that reported previously for phenylalkanes and dialkyl *o*-phthalates for which the penetration of the aromatic ring in the stationary phase was established without ambiguity [15].

## 5. Conclusions

Several conclusions can be drawn from the results presented above.

(1) The overall results permit us to propose a more complete model of molecular mechanism of interaction than that established previously for monomeric bonded silicas. Various criteria could be used to describe this mechanism: the plots of

quadratic methylene selectivity  $\alpha_n$  vs.  $n_c$  present two plateaux, the second of which is divided into two parts separated by a break. The drop between the two plateaux appears at a critical value of  $n_c$ ,  $n_{crit}$ , and the break at a value named the saturation value  $n_{sat}$ . Three different values of mean average methylene selectivity can be calculated:  $\tilde{\alpha}_{in}$ , which corresponds to the homologues such that  $n_c < n_{crit}$ ,  $\tilde{\alpha}_{crit}$  for  $n_{crit} < n_c < n_{sat}$ , and  $\tilde{\alpha}_{sat}$  for  $n_c > n_{sat}$ .

(2) The values of  $n_{crit}$  are independent of the nature of the homologous series and the mobile phase composition (PARP rich in organic modifier and NARP rich in methanol or acetonitrile);  $n_{crit}$  is directly connected to the length of the alkyl-bonded chain. Surprisingly, it is the same for octadecyl and docosyl chains. The value of  $n_{sat}$  is related to the density of the grafts. It decreases when the density increases. Conversely, for alkylsilicas with a low value of the carbon load the second break in the experimental curves cannot be observed. Finally, for a silica and a very low density no break appears.

(3) The average methylene selectivities  $\tilde{\alpha}_{in}$  vary from one homologous series to another. The values of  $\tilde{\alpha}_{crit}$  and  $\tilde{\alpha}_{sat}$  are independent of the polar head.

(4) A correlation between the values of average methylene selectivity  $\tilde{\alpha}$  and the average molecular volume of a methylene group  $\Delta V_{CH_2}$  can be established. Thus, for values such that  $n_c < n_{crit}$ ,  $\Delta V_{in}$  varies with the homologues. A linear relationship could be drawn between  $\tilde{\alpha}_{in}$  and  $V_{in}$  for each tested series, independently of the nature of the length of monomeric bonded silica. For the homologues such that  $n_{crit} < n_c < n_{sat}$  and  $n_c > n_{sat}$ , the corresponding values of  $\tilde{\alpha}_{crit}$ ,  $\Delta V_{crit}$ , and  $\tilde{\alpha}_{sat}$  and  $\Delta V_{sat}$  are different but constant and independent of the nature of the homologous series.

(5) The precise determination of the value of the average methylene selectivity  $\tilde{\alpha}$  of a particular series in a range of  $n_c$  such that  $n_c < n_{crit}$  permits the molecular mechanism of interaction with the stationary phase to be determined. Thus the liquid crystals interact with the alkyl-bonded chains by insertion of their aromatic rings. For the cycloalkanes their structures do not permit a

deep insertion with close contact. The interaction is more superficial exactly like aliphatic chains in a range of  $n_c$  such that  $n_{crit.} < n_c < n_{sat.}$ .

(6) These results suggest a molecular mechanism of retention for monofunctional bonded silicas with penetration and tight contact between the aliphatic chains of the bonded phase and the solute for  $n_c < n_{crit.}$ . This contact is responsible for the close correlation between  $\Delta V_{in}$  and  $\tilde{\alpha}_{in}$  and rationalizes the fact that  $\tilde{\alpha}_{in}$  is different with regard to the nature of homologues. When there are supplementary methylene groups in the structure of the solute there is always penetration of the solute inside the alkyl chains of the bonded phase but the contact is less tight. It occurs with solute molecule in folded conformations such that all the available space between the chains of the bonded phase is engaged. There is no contact between each methylene group of the bonded phase and of the solute. The role of the polar head is levelled.  $\Delta V_{CH_2}$  is independent of the polar or non-polar nature of the solute and also  $\alpha_{crit.}$ . When there is no additional space between the chains of the bonded phase their contact with the chain of a longer solute could be established by lying on the external methyl groups of the bonded phase. In this case no differentiation can be observed between the different homologous series. The corresponding  $\Delta V_{sat.}$  and  $\tilde{\alpha}_{sat.}$  are constant but smaller than  $\Delta V_{crit.}$  and  $\tilde{\alpha}_{crit.}$ .

## 6. Symbols

- $a$  = Characteristic constant which depends on the solute and the mobile phase.  
 $C$  = Characteristic constant which depends on the solute and the mobile phase.  
 $k'$  = Capacity factor of solute.  
 $n_{crit.}$  = carbon number of homologue for which the first break is observed for  $\log k'$  vs.  $n_c$  plots.  
 $n_{sat.}$  = carbon number of homologue for

which the second break is observed for  $\log k'$  vs.  $n_c$  plots.

$p$  = Slope of the curve  $\log k'$  vs.  $n_c$ .

$q$  = Intercept with the y-axis of the curve  $\log k'$  vs.  $n_c$ .

$R$  = Gas constant.

$T$  = Absolute temperature.

$V_M$  = Molecular volume of solute.

$V_{SP}$  = Molecular size of the bonded chain.

$Z$  = Functional group of homologous series.

$\alpha_n = (k'_{n_c+1}/k'_{n_c-1})^{1/2}$ : quadratic methylene selectivity.

$\bar{\alpha}_n = \sum_1^x \alpha_n/x$ : global quadratic methylene

selectivity where  $x$  is the number of tested eluities with a chain length of  $n$  carbon atoms.

$\tilde{\alpha}$  = average methylene selectivity calculated from the slope of  $\log k'$  vs.  $n_c$ .

$\bar{\alpha} = \sum \alpha/N$ : mean average methylene selectivity where  $N$  is the number of tested homologous series.

$\alpha_{TBN/BAp}$  = selectivity between tetrabenzonaphthalene and benzo[*a*]pyrene obtained with MeCN–H<sub>2</sub>O (85:15) as mobile phase.

$\tilde{\alpha}_{in}$  = average methylene selectivity for homologues such that  $n_c < n_{crit.}$

$\tilde{\alpha}_{sat.}$  = average methylene selectivity for homologues such that  $n_c > n_{sat.}$

$\tilde{\alpha}_{crit.}$  = average methylene selectivity for homologues such that  $n_{crit.} < n_c < n_{sat.}$

$\Gamma$  = Surface concentration of bonded organosilyl group.

$\phi$  = Phase ratio.

$\Delta A$  = Contact area between the solute and the stationary phase.

$\Delta G^\circ$  = Partial molar free energy associated with the transfer of the solute and the stationary phase (assumed to be in the standard state at infinite dilution) from the mobile phase to the stationary phase.

$\Delta G_{CH_2}^\circ$  = Partial molar free energy associated with the transfer of single methylene

group of solute from the mobile phase to the stationary phase.

$\Delta V_{\text{CH}_2}$  = average molecular volume of a methylene group.

$\Delta V_{\text{in}}$  = average molecular volume of a methylene group for  $n_c < n_{\text{crit}}$ .

$\Delta V_{\text{sat}}$  = average molecular volume of a methylene group for  $n_c > n_{\text{sat}}$ .

$\Delta V_{\text{crit}}$  = average molecular volume of a methylene group for  $n_{\text{crit}} < n_c < n_{\text{sat}}$ .

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