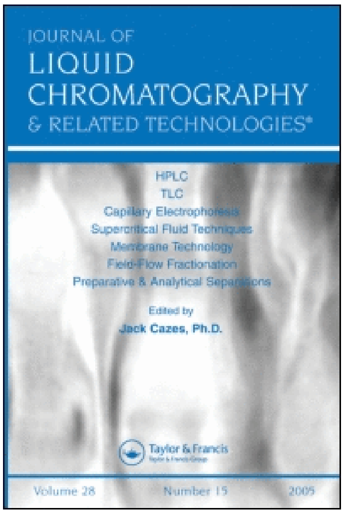

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Influence of the Amount of Bonded Non-Polar Phase and of the Length of Attached Alkyl Chains on Retention Characteristics of Silica-Based Sorbents for Reversed-Phase High Performance Liquid Chromatography

P. Špaček[a]; M. Kubín[a]; S. Vozka[ab]; B. Porsch[a]
[a] Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague [b] Laboratory Instruments Works, Prague, Czechoslovakia

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INFLUENCE OF THE AMOUNT OF BONDED NON-POLAR PHASE AND OF THE LENGTH OF ATTACHED ALKYL CHAINS ON RETENTION CHARACTERISTICS OF SILICA-BASED SORBENTS FOR REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

P. Špaček, M. Kubín, S. Vozka* and B. Porsch
Institute of Macromolecular Chemistry, Czechoslovak
Academy of Sciences, 162 06 Prague 6
and
*Laboratory Instruments Works, Prague 6, Czechoslovakia

ABSTRACT

The retention characteristics of two sets of chemically bonded non-polar silica packings with a high surface concentration of functional groups have been compared in the reversed-phase mode of liquid chromatography: (i) the conventional packings prepared by chemical modification with trimethylsilyl, heptyl, dodecyl and octadecyl groups, and (ii) mixed-phase materials where differences in the amount of organic bonded phase were achieved by bonding octadecyl and trimethylsilyl groups in different proportions. The retention data of two homologous series of solutes show that these two classes of packings are distinctly different; in particular, the capacity factors and selectivities are always higher on the mixed-phase bonded silica packings at the same percentage of carbon.

INTRODUCTION

In the last several years chemically bonded stationary phases, in particular non-polar phases for reversed-phase chromatography, have found widespread application in column and flat-bed chromatographic techniques, as evidenced by a number of papers (1 - 15) and review articles (16 - 18) devoted to the subject. However, despite their success in solving many difficult separation problems, the structure and properties as well as the retention mechanism of chemically bonded phases are still a matter of dispute (2,4,6 - 10, 12 - 14,19). It is known that the retention of a given solute at constant mobile phase composition increases with the length of the chemically attached alkyl chains; it is, however, less clear whether this increase in capacity factors is entirely due to the increased amount of the bonded organic phase (expressed e.g. by the carbon percentage) achieved with longer chains. In principle, this question could be settled by following the relative retention (selectivity) factors $\alpha_{ij} = k'_i/k'_j > 1$ for different bonded phases, but controversial results have been reported in the literature as to the dependence of selectivity on the number of carbon atoms in the bonded chains: Unger, Becker and Roumeliotis (4) maintain that the selectivity factors do not change on varying the chain length, whereas Hemetsberger, Maasfeld and Ricken (6) report an increase in α_{ij}'s with the number of carbon atoms in the bonded chains at essentially the same and high surface coverage (see also Karch, Sebastian and Halâsz (8)).

Hennion, Picard and Caude (10) also found a strong dependence of selectivity factors on the chain length of the bonded moiety, but their results appear to be less conclusive because of the low (less than optimal) surface coverage in this particular series of sorbents, so that a mixed retention mechanism can be suspected due to the presence of unreacted and accessible silanols on the silica surface. The danger that residual silanols will participate in retention is common to all attempts aimed at studying the dependence of capacity factors on the carbon content at constant chain length of the bonded phase (5,10,20).

In this paper we report the results of a study undertaken with the aim of distinguishing between the effects of the amount of bonded phase and the length of attached alkyl chains by comparing the retention on two series of reversed-phase preparations: (i) a set of sorbents where the different amount of the bonded phase (carbon percentage) was achieved by bonding octadecyl and trimethylsilyl groups in different proportions to silica under conditions of virtually complete surface coverage, and (ii) a series of sorbents, prepared from the same batch of silica, that differed in the length of the bonded hydrocarbon moiety.

EXPERIMENTAL

Sorbents

The original silica was a commercial product marketed under the trade-name SEPARON SI VSK by Laboratory Instruments Works, Prague (surface area S = 451 m²/g, internal porosity $\varepsilon_i = 2.0$ ml/g). The four types of mixed-phase modified silicas (MX9, MX13, MX15 and MX20) were experimental batches prepared in the Chemical R & D Department, Laboratory Instruments Works, by bonding octadecyl and trimethylsilyl groups in different proportions to the original silica under conditions of virtually complete surface coverage (21). The conventional reversed-phase materials C1, C7, C12 and C18 were prepared in our laboratory from SEPARON SI VSK by chemical modification with alkyl chains of different length as described below.

Chemicals

Octadecyltrichlorosilane (ODTS, Aldrich Chemicals Co.), heptyltrichlorosilane (HTS), dodecyltrichlorosilane (DTS, both from PCR Research Chemicals, Inc.) and hexamethyldisilazane (HMDS, Lachema) were used as received. Toluene (Lachema, reagent grade) was dried by sodium and distilled from sodium with the exclusion of moisture. Pyridine (P.O.C.M., Poland, reagent grade) was dried with solid KOH and distilled from KOH under dry nitrogen. The other solvents were reagent grade and were used as received.

Apparatus

The chromatograph was assembled from a syringe-type positive displacement pump (VLD 30) and a stop-flow sample injector (products of Development Workshop, Czechoslovak Academy of Sciences), stainless steel columns (250 x 4 mm or 100 x 6 mm) marketed by Laboratory Instruments Works, Prague, and RI detector (Type 401, Waters Associates, Inc., Milford, Mass., USA). The detector output was fed to a potentiometric recorder (Servogor 2S, Goerz Electro, Austria).

The columns were slurry-packed at 40 MPa. The mobile phase employed throughout the study was methanol - water (70 : 30, v/v).

Preparation of Conventional Bonded Phases

Silica was first activated by boiling for 30 minutes in dilute hydrochloric acid (1 : 10, v/v), washed with distilled water until neutral, then boiled in a fresh portion of distilled water (30 minutes) and dried at 150°C in vacuo for at least 12 hours. All modifications were performed in refluxing toluene under strictly anhydrous conditions in a 3-neck flask equipped with a mercury-sealed stirrer, condenser with a U-tube packed with molecular sieve, and an inlet tube for dry nitrogen.

Conditions of the surface modification are summarized in Table 1. The work-up procedure was always the same: the bonded phase was first washed quickly with dry toluene, then with acetone, methanol and methanol/water (2 : 1, v/v), and left in this mixture overnight. Finally, the particles were washed by acetone and dried at 100°C.

TABLE 1

Preparation of Conventional Reversed-Phase Materials

Bonded phase	Reagent			Pyridine[c]	Time[d]
	Type	Amount[a]	Concentration[b]		
C 1	HMDS	4.7	10	-	7
C 7	HTS [e]	4.7	11	20	6
C12	DTS [e]	4.7	11	20	6
C18	ODTS[e]	6.0	9	12.1	6

[a] Amount in mmole reagent per gram of dry silica; [b] concentration in toluene, volume %; [c] mmole per gram of dry silica; [d] reaction time in hours; [e] residual silanols end-capped by HMDS (9 mmole per gram of modified silica, 10 volume % in toluene, refluxed for 900 minutes).

RESULTS AND DISCUSSION

The characteristics of bonded phases are summarized in Table 2. The surface coverage of the C1 conventional material (2.23 bonded groups per nm²) is very close to the maximum value (2.3) predicted theoretically by Berendsen and Galan (11) from the space requirements of the trimethylsilyl group. The surface density of the other conventional materials is in agreement with the highest values reported by other workers for analogous silylation reagents and, assuming that there are about 4.8 silanol groups per nm² (4,11), suggests a bifunctional reaction of trichlorosilanes with SiOH groups (6). Any remaining, non-reacted silanols are effectively shielded by the bonded aliphatic chains: all bonded phases in Table 2 gave negative results in the methyl red adsorption test and passed the criterion of "good" reversed-phase materials advocated by Karch, Sebastian and Halász (8) in tests with benzene and nitrobenzene as solutes and dry n-heptane as the mobile phase.

TABLE 2

Characteristics of Reversed Phases Investigated

Designation	Carbon content (%C)[a]	Surface coverage µmole/m²	(groups/nm²)	Surface area (m²/g)
C 1	5.3	3.7	2.23	260
C 7	13.4	4.58	2.76	-
C12	19.0	4.07	2.45	-
C18	21.9	3.22	1.94	195
MX 9	9.25			-
MX13	13.4			310
MX15	15.2			-
MX20	19.7			255

[a] Carbon percentage P_c from elemental analysis

The surface area is considerably lowered by the chemical modification, particularly in the case of the octadecyl bonded phase, in agreement with the findings of Unger, Becker and Roumeliotis (4).

All materials were tested in the reversed-phase mode with methonol - water (70 : 30, v/v) as the mobile phase and two homologous series of compounds, viz., aliphatic alcohols and their corresponding acetates. The capacity factors, k', of some selected solutes are plotted in Fig.1 against the amount of bonded phase expressed as the carbon percentage, P_c. A fairly good linear correlation was found both for the conventional and mixed reversed phases with all solutes (including those not shown in the Figure), but the straight lines for the two classes of sorbents were distinctly different, with the capacity factors always significantly higher on the mixed phases. This indicates that the amount of the bonded phase, as measured by the percentage of carbon, is in itself not sufficient to characterize the retention on chemically

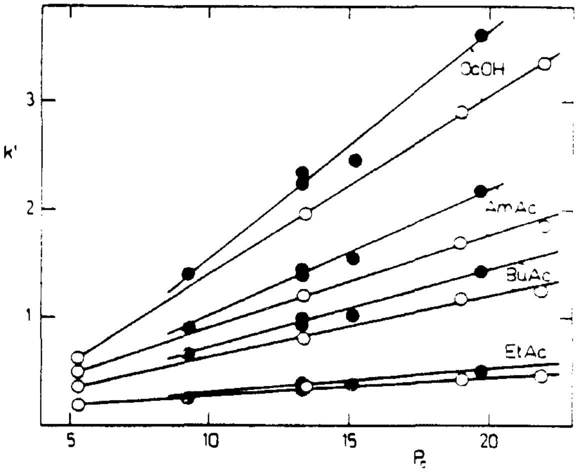

FIGURE 1. Capacity factors of some selected solutes as a function of carbon content of: ● mixed, ○ conventional phases

bonded hydrocarbon phases even when the effect of residual silanols is non-existent.

The mixed phases can be shown to differ from the conventional materials also according to other criteria. It is known that when log k' is plotted against the number of carbon atoms in solutes forming a homologous series, straight line dependences are obtained (5,8,21). Berendsen and Galan (15) observed that these straight lines for different reversed phase materials intersect at a common point. These plots for the two classes of bonded phases and for the two homologous series investigated are shown in Fig.2 for acetates and in Fig. 3 for alcohols. For each series of homologs, two different bundles of straight lines with two distinct intersection points are obtained, one for each class of sorbents (the lines for the C 1 bonded phase do not pass through the common intersection points - a similar behavior has been observed by others (8,15)).

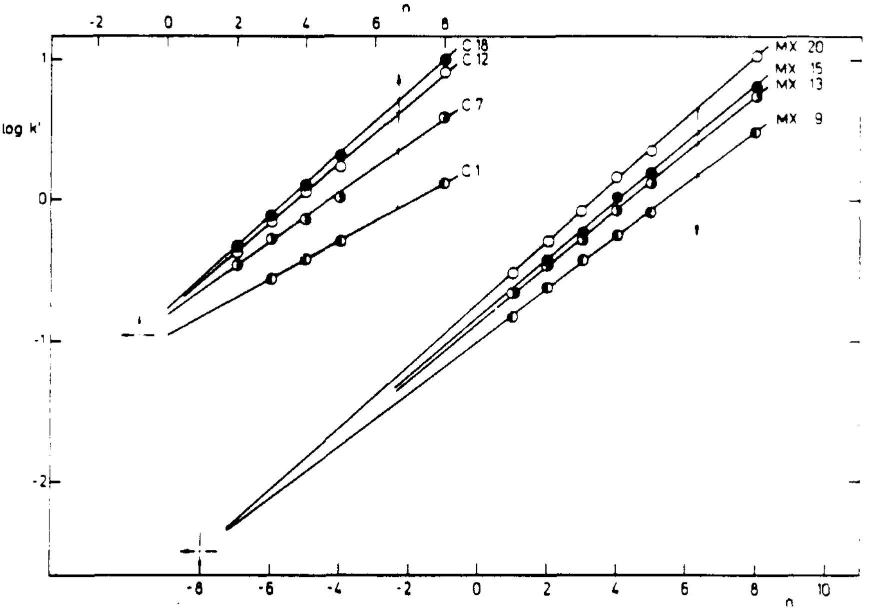

FIGURE 2. Dependence of log k' on the serial number n in the homologous series of acetates (n = 1 for methyl acetate etc.)

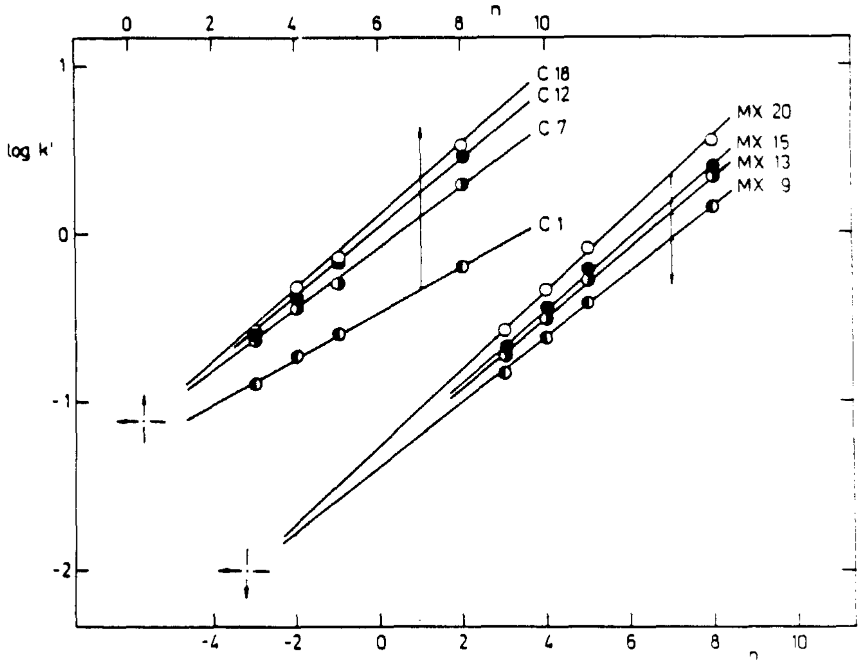

FIGURE 3. Dependence of log k' on the serial number in the homologous series of alcohols (n = 3 for propanol etc.)

This difference is made more graphic in Fig. 4, where the slopes of the straight lines in Figs 2 and 3 are plotted against the carbon percentage. The results show that the mixed phases have a higher selectivity with respect to members of homologous series than the conventional materials at the same percentage of carbon. The slopes for alcohols and acetates are the same within the limits of experimental error.

Another interesting difference between the two classes of bonded phases is revealed in Fig. 5, which shows the dependence of the fractional dead volume, ε_m, as measured on the different tested columns, on the carbon percentage ($\varepsilon_m = V_m/V_{tot}$, where V_m is the dead volume obtained from the unretained peak of methanol and V_{tot} is the volume of the empty column).

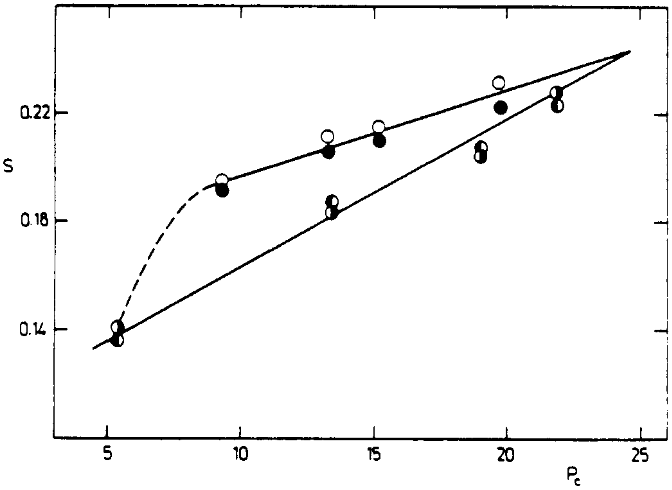

FIGURE 4. Slope s of straight lines in Figs 2 and 3 as a function of carbon content
○ alcohols and ● acetates on the mixed phases,
◑ alcohols and ◐ acetates on conventional phases

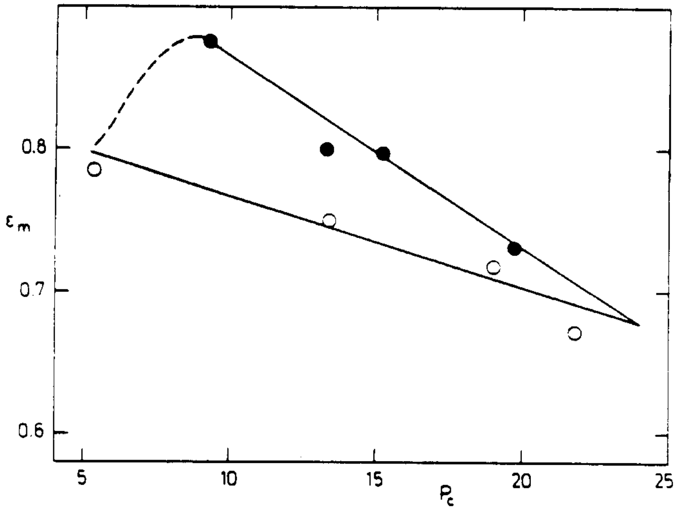

FIGURE 5. Fractional dead volume of columns plotted against the carbon content of ● mixed and ○ conventional phases

There is no reason why the dependences in Figs 4 and 5 should be linear (on the contrary, if the fraction of octadecyl groups in the mixed phase were further decreased, the two dependences must go through a common point corresponding to the phase C 1 at $P_c = 5.3\%$, as shown by dotted curves in both Figures), but it is interesting to notice that straight lines through the points in both Figures intersect at $P_c = 24$. This seems to indicate that 24 percent carbon (i.e., surface coverage 3.68 μmole/m²) would be the limiting value obtainable with octadecyltrichlorosilane under conditions of exhaustive silylation. (Indeed, when another sample of silica was refluxed in toluene with ODTS for 26 hours under otherwise identical conditions as in Table 1, surface coverage of 3.7 μmole/m² was achieved in excellent agreement with the previous figure.)

It has been observed (15) that if log k' is plotted for a series of n-alkanes as solutes against the effective number of carbon atoms in the conventional reversed phases prepared by chemically bonding alkyl chains of different length to the silica surface, a set of straight lines results which possess a common intersection point whose ordinate is virtually equal to the ordinate of the intersection point of lines obtained by plotting, for different reversed phase materials, log k' against the actual number of carbon atoms in the homologous series of solutes (see Figs 2 and 3). The effective carbon number of the reversed phase, $n_{c,ef}$, is defined as the nominal number of carbon atoms in the bonded alkyl chain, n_c, corrected for the surface density of the bonded molecules, and is proportional (15) to the expression

$$n_{c,ef} \propto P_c \left[1 + \frac{P_c(M-1)}{1200\, n_c - P_c(M-1)} \right] \qquad (1)$$

where P_c is the percentage of carbon and M is the molecular weight of the bonded group.

Our data obtained with two different homologous series of aliphatic alcohols and their acetates on conventional bonded phases C7, C12 and C18 also conform to this picture, as shown in Fig. 6.

For the mixed phases the effective number of carbon atoms cannot be calculated in a similar manner, as only the total percentage of carbon and not the proportion of bonded octadecyl and trimethylsilyl groups was known. However, on the basis of previous results in Figs 4 and 5 it seems reasonable to assume that the overall surface coverage was the same for the mixed phases and approximately equal to 3.7μmole/m^2. We can write for the percentage of carbon of the mixed phase the relation

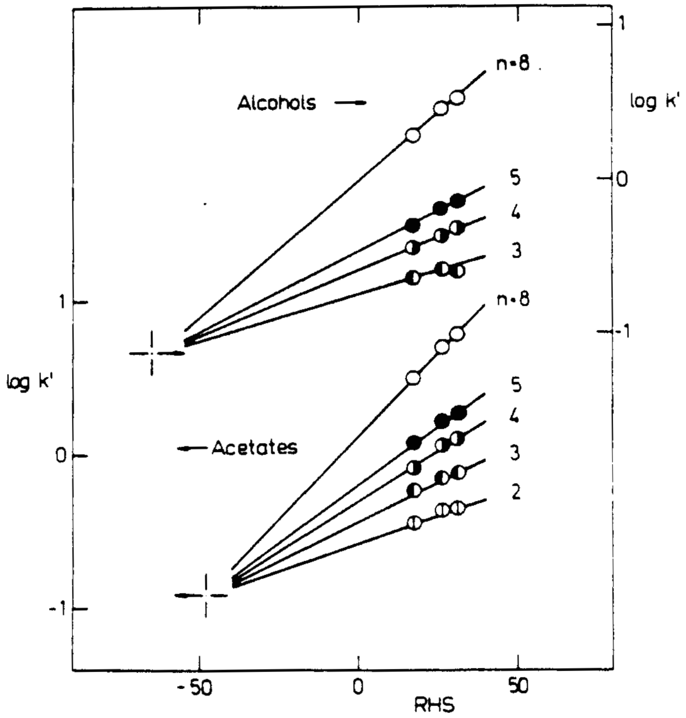

FIGURE 6. Dependence of log k' for individual members of homologous series upon the right-hand side (RHS) of Eq.(1); conventional bonded materials

$$P_c = 100 \frac{N_0 . 12\, n_0 + N_t . 12\, n_t}{1 + \left[N_0 (M_0 - 1) + N_t (M_t - 1)\right]} \qquad (2)$$

where the subscripts refer to the octadecyl and trimethylsilyl groups, N's designate the amounts of the corresponding bonded moieties (in mole per gram of original silica), M's are the respective molecular weights and $n_0 = 18$ and $n_t = 3$ are the nominal numbers of carbon atoms. (The term in square brackets in the denominator corrects for the increase in weight of silica due to the chemical modification.) Assuming that

$$N_0 + N_t = 3.7(\mu\text{mole/m}^2).451(\text{m}^2/\text{g}).10^{-6} = 1.669.10^{-3}(\text{mole/g original silica}) \qquad (3)$$

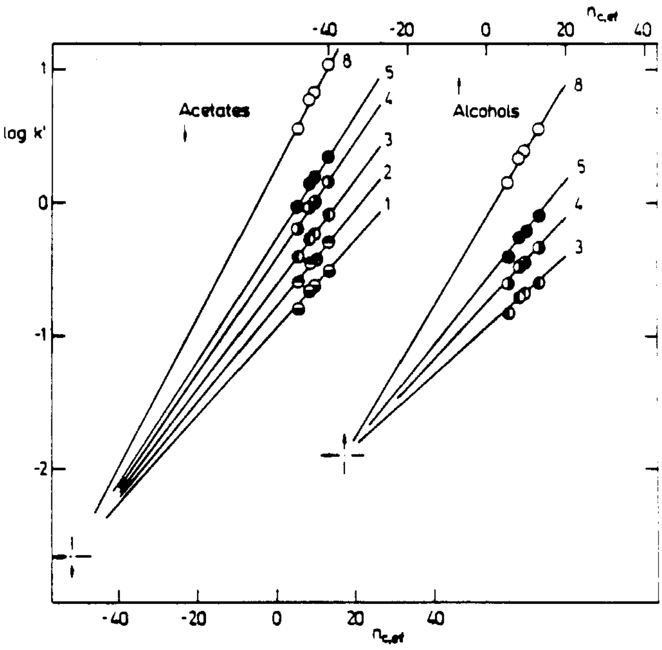

FIGURE 7. Dependence of log k' for individual members of homologous series plotted against the effective number of carbon atoms in the mixed phases

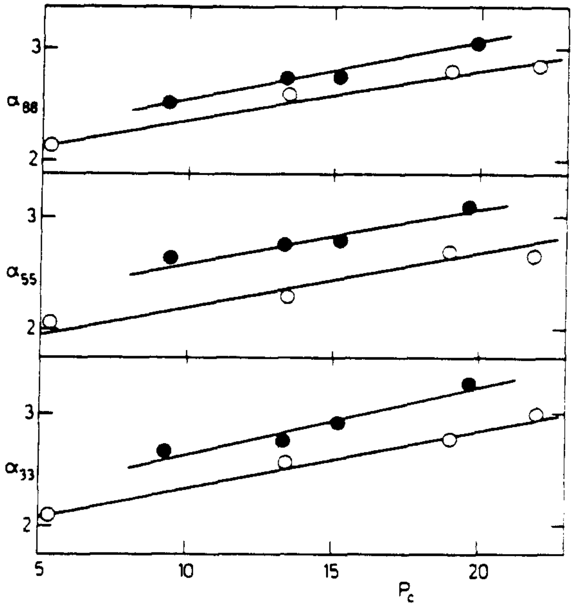

FIGURE 8. Selectivity of mixed (●) and conventional (○) bonded phases

α_i is the ratio of capacity factors of i-th acetate and the corresponding alcohol (e.g. $\alpha_3 = k'_{PrAc}/k'_{PrOH}$)

we can solve Eqs (2) and (3) for N_0 and N_t and define the effective number of carbon atoms of the mixed phase, $n_{c,ef}$, by the equation

$$(N_0 + N_t).n_{c,ef} = N_0 . n_0 + N_t . n_t \qquad (4)$$

Fig.7 shows log k' as obtained on the mixed phases with alcohols and acetates plotted against $n_{c,ef}$ calculated from Eq.(4). The dependences for the individual homologous series are again linear; moreover, not only do they intersect at a common point, but the ordinates of these points are again similar to those of the corresponding intersection points in Figs 2 and 3, respectively. Although no physical interpretation of this interesting behavior can be given at present, it is clear that the rule first observed by Berendsen and Galan (15) for n-alkanes holds also for other homologous series on other types of bonded phases in reversed phase chromatography.

The mixed phases show higher selectivity also with respect to corresponding members of the two homologous series investigated. This is documented in Fig.8: the selectivity factors α_i are plotted for three pairs acetate/alcohol against the carbon content P_c (e.g. $\alpha_3 = k'_{PrAc}/k'_{PrOH}$). The points for the mixed phases lie invariably above those for the conventional sorbents.

The mixed phases are thus seen to represent a very interesting class of materials for reversed-phase chromatography with higher capacity factors and higher selectivity than the conventional phases at the same content of carbon. As an example of their practical utilization, Fig.9 shows a reversed phase separation of some

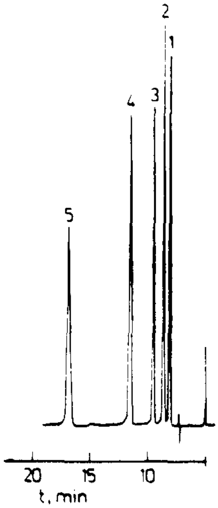

FIGURE 9. Separation of phenols on the mixed phase MX 20 Column 250 mm long, 4 mm I.D., methanol - water (70 : 30, v/v) at 0.5 ml/min, UV detection, 254 nm

1 phenol, 2 m-cresol, 3 2,6-xylenol, 4 4-tert-butylphenol, 5 4-cyclohexylphenol

substituted phenols on the mixed phase MX20 (this material is now marketed by Laboratory Instruments Works, Prague, under the trade -mark SEPARON Si C18).

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