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ROLE OF THE CHAIN LENGTH OF CHEMICALLY BONDED PHASES AND THE RETENTION MECHANISM IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The influence of the chain length of *n*-alkyldimethylsilyl bonded phases (RP-1 to RP-22) on retention has been investigated using methanol–water mixtures as the mobile phase. It was found that the capacity factor increases exponentially up to a certain chain length, after which it remains constant. For a given solute the onset of retention, denoted as the "critical chain length", is independent of the mobile phase composition. It increases with the size of the solute molecule from a minimum chain length of 6 up to about 14 carbon atoms. Up to the critical chain length the selectivity increases slightly with increasing chain length. However, the influence of the stationary phase on retention and selectivity is far outweighed by the influence of the mobile phase. The role of the chemically bonded stationary phase in the separation process is proposed as a "compulsory absorption mechanism". Only a limited part of the bonded chains contributes to the retention mechanism.

INTRODUCTION

Chemically bonded stationary phases are still a fruitful subject for discussion, as demonstrated by the numerous publications and several reviews that have been published[1–10]. From these papers it appears that the most important issues are (i) the retention mechanism in reversed-phase liquid chromatography (RPLC), (ii) the role of the *n*-alkyl chain length in separation[1] and (iii) the optimal chain length in RPLC[11,12].

The retention mechanism has been the subject of several investigations. Partition[13–15], adsorption[16–23], dispersive interaction[24], solubility in the mobile phase[25] and mixed[6] and solvophobic adsorption mechanisms[26,27] have been proposed. It is clear that there is still a lack of understanding of the details of retention in RPLC. We agree with Karger and Giese[1] that a better understanding of the retention process should be developed in order to control the practice of separation better.

Most workers have observed that every mechanism proposed is related to the surface concentration of bonded chains. An important variable in surface modi-

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fication is the *n*-alkylsilyl bonded chain length. With longer chains the organic content per unit mass of packing increases. In general, increased carbon content or chain length results in greater retention under given mobile phase conditions[11,24,28–32]. However, these observations have not led to the formulation of an optimal carbon percentage or bonded chain length, apart from the fact that the loading capacity for solutes increases as the chain length increases.

Another debatable question is the dependence of selectivity on chain length. Majors and Hopper[28] observed an improvement in selectivity with increasing chain length up to C_{12}, after which it remained constant. Karch *et al.*[30] also observed an increase in selectivity with increasing chain length, but Hennion *et al.*[31] and, in the first instance, Hemetsberger and co-workers[20,22] and Unger and co-workers[11,21] found no selectivity differences. More recently, Hemetsberger *et al.*[32] found that different alkyl chains exhibit no selectivity differences towards solutes of comparable structure, whereas for solutes with different structures the selectivity increases with chain length. Unger[12] stated that no final conclusion is possible about preferential selectivity of bonded phases and that this must be checked in every instance.

Conflicting results have also been reported for the efficiency of different alkyl chains. Karch *et al.*[30] observed no influence. However, Karger and Giese[1] stated that a shorter chain length leads to better efficiences, whereas Grushka and Kikta[33] observed the opposite.

EXPERIMENTAL

Apparatus

A Waters liquid chromatograph was used, equipped with an M6000 pump, a U6K injector and a 401 refractive index detector. The injector, column and detector were thermostated at 27.5°C, whereas the connections between column and injector and column and detector were carefully isolated.

Chemicals

The solvents used as mobile phases were mixtures of water and methanol. The methanol was of pro analysi quality (J. T. Baker, Deventer, The Netherlands) and water was doubly distilled and deionized. All bonded phases were home-made on 10-μm SI 100 silica particles (Merck, Darmstadt, G.F.R.); the properties of these materials have been described elsewhere[34]. All phases were post-treated with trimethylchlorosilane to reduce the number of free silanol groups. However, as established previously[34], no influence of this post-silanization on chromatographic behaviour could be observed under reversed-phase conditions. The solutes used in the chromatographic experiments were of the highest purity commercially available.

Column packing

All columns (300 mm × 1/4 in. O.D. × 4.6 mm I.D.) were slurry packed using the balanced density technique. The procedure used has been described previously[35], but we made some modifications. After washing and drying (at 150°C under vacuum) of the packing materials we noticed that columns packed with these materials gave very poor results. The column content did not settle, but remained as a thick slurry that slowly flowed out of the column after disconnecting a reducing union. The

addition of a trace amount of water or a small amount of salt, *e.g.*, sodium acetate, to a slurry of carbon tetrachloride and methanol (90:10, v/v) improved the column efficiency considerably. This procedure is thought to remove electrostatic charges from the bonded materials[36].

Columns packed with the reversed-phase materials (RP-1 to RP-22) exhibited no variation in efficiency with RP chain length. Plate numbers of about 5000 (H = 60 μm) were obtained in methanol–water (60:40) for anisole as solute. The moderate efficiency is probably due to an unfavourable particle size distribution[35].

Hold-up time

The column hold-up time, t_0, was obtained from the linearization of the logarithmic capacity factor as a function of the number of carbon atoms of a homologous series of *n*-alcohols[37]. The capacity factors were calculated from the equation

$$k = \frac{t_R - t_0}{t_0 - t_{0r}} \quad (1)$$

where t_R is the absolute retention time and t_{or} the outer column residence time.

CHROMATOGRAPHIC PROPERTIES OF BONDED PACKINGS

Influence of bonded phase chain length on retention

The retention behaviour of alkylsilyl bonded packings with seven different chain lengths varying from RP-1 to RP-22 was investigated for mobile phases composed of methanol and water, ranging from pure methanol (Φ = 1) to pure water (Φ = 0) (Φ = volume fraction of methanol). The results are presented in Figs. 1–3.

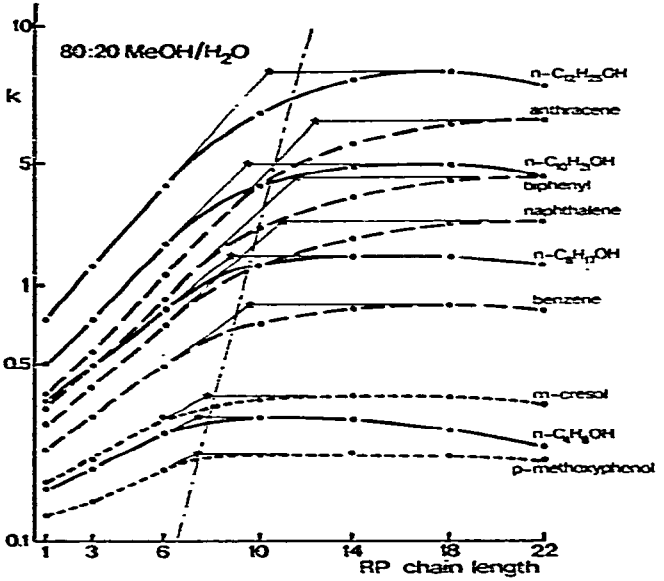

Fig. 1. Capacity factor as a function of RP chain length in methanol–water (80:20). Asterisks denote the critical chain length, determined as the intersection point of the extrapolated branches of the curves.

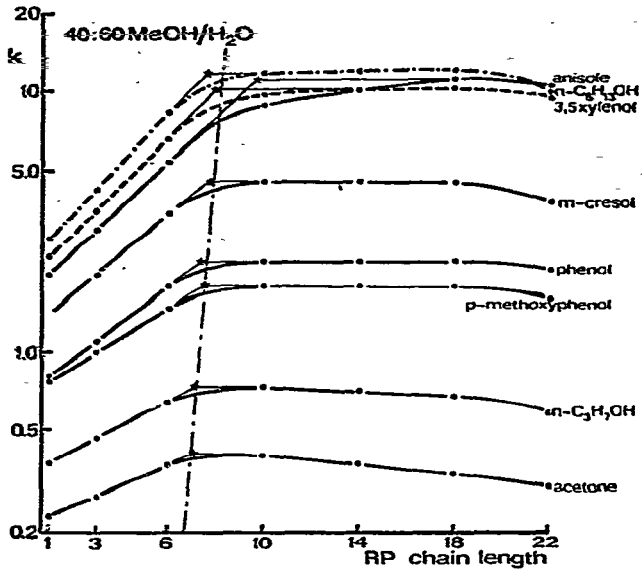

Fig. 2. Capacity factor as a function of RP chain length in methanol–water (40:60). Asterisks denote the critical chain length, determined as the intersection point of the extrapolated branches of the curves.

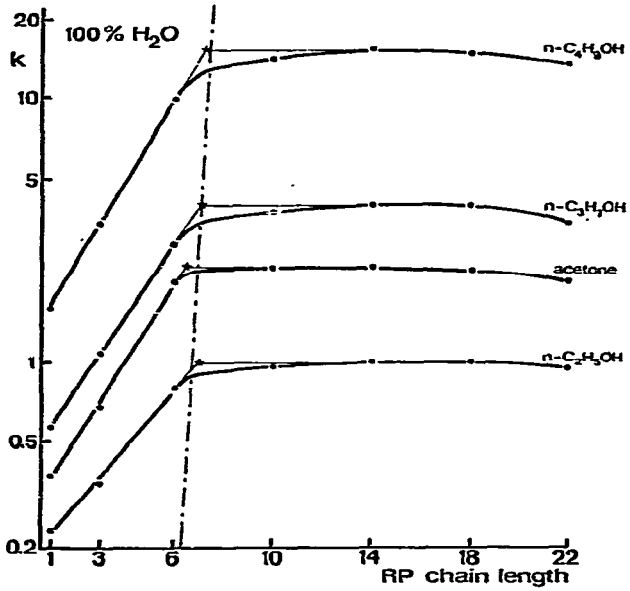

Fig. 3. Capacity factor as a function of RP chain length in pure water. Asterisks denote the critical chain length, determined as the intersection point of the extrapolated branches of the curves.

On the abscissa we prefer to plot the chain length (or the carbon number) of the alkyl bonded chain rather than the percentage of carbon. However, for all bonded phases maximal surface coverage was realized[34].

Contrary to other investigators[11,24,28–32], we did not observe a continuous increase in retention with increasing chemically bonded chain length. Instead, the retention increases more or less rapidly from the shortest chain length (RP-1) up to a certain chain length, after which the retention gradually levels off. From Figs. 1–3 it appears that the "critical chain length" where the retention starts to become constant lies between 6 and 10 carbon atoms in the alkyl bonded chain. Extension of this chain length above seven carbon atoms (RP-7) does not increase the retention significantly. This seems to indicate that for longer chains only the exterior part of the alkyl chains participates in the retention process. This conclusion contrasts with the opinion of Roumeliotis and Unger[11] that the complete chain length takes part. In fact, for very long chains the capacity factor appears to decrease slightly.

On closer examination, the critical chain length is found not to be equal for all solutes, but increases slightly with the capacity factor. In other words, more strongly retained solutes require longer RP chains to reach their constant capacity factor. This is indicated by the asterisks in Figs. 1–3, which in each instance represent the intersection point of the extrapolated straight lines drawn through the two branches of each curve. Despite an appreciable scatter, a systematic variation towards larger chain lengths is noticeable with increasing capacity factor.

It seems from Figs. 1–3 that the variation in critical chain length with capacity factor is smaller in mobile phases with a low modifier content. However, this is due only to the fact that in such mobile phases only small solutes can be eluted within a reasonable time. In fact, if the retention behaviours of one solute in different mobile phases is compared, both the overall shape of ln k *versus* RP chain length and the critical chain length remain virtually the same. The latter aspect is demonstrated for several solutes in Table I. As expected, the retention decreases sharply with increasing methanol content. However, the critical chain length, defined as the intersection point of the two linear branches of the ln k *versus* RP chain length curve, is independent of the methanol content. Again, the critical chain length increases with solute retention.

The picture that emerges from Figs. 1–3 and Table I is therefore that each solute requires its own minimum alkyl bonded chain length to maximize its retention. For the most weakly retained solutes an absolute minimum chain length of 6 carbon atoms is necessary. For more strongly retained solutes, eluted in increasingly stronger eluents, the minimum chain length increases gradually to 14 carbon atoms.

Selectivity

In chromatographic practice the selectivity is more important than the absolute retention. Returning to Figs. 1–3 we note that the rising branches of the capacity curves generally diverge, which means that initially the selectivity increases with increasing RP chain length. For long chains all capacity factors have reached their limiting value, so that the selectivities do not change further. This point is reached sooner with smaller molecules. Consequently, for large molecules the selectivity increases continuously up to RP-22, whereas for small molecules the selectivity generally remains constant for chains longer than, say, 14 carbon atoms.

A typical example is presented in Fig. 4, where the change in selectivity with chain length is shown for the solutes *n*-butanol and ethanol in mobile phases of various composition. Up to RP-14 the selectivity increases regularly. The increase is greater in pure water than in pure methanol. For longer RP chains the selectivity

TABLE I

ILLUSTRATION OF THE INDEPENDENCE OF THE CRITICAL CHAIN LENGTH ON ELUENT COMPOSITION

Component	*Limiting k values*					*Critical chain length*				
	$\Phi = 0.2$	$\Phi = 0.4$	$\Phi = 0.6$	$\Phi = 0.8$	$\Phi = 1.0$	$\Phi = 0.2$	$\Phi = 0.4$	$\Phi = 0.6$	$\Phi = 0.8$	$\Phi = 1.0$
Acetone	0.66	0.40	0.24	0.18	0.16	6.3	6.8	6.6	6.2	6.7
Phenol	6.10	2.22	0.72	0.27	0.15	7.2	7.4	7.3	7.3	7.3
m-Cresol	17.3	4.60	1.22	0.36	0.18	7.7	7.9	8.1	7.8	7.8
3,5-Xylenol	—	10.0	2.03	0.52	0.20	—	8.2	8.4	8.3	8.1
Naphthalene	—	—	9.80	1.72	0.47	—	—	10.4	10.9	10.2

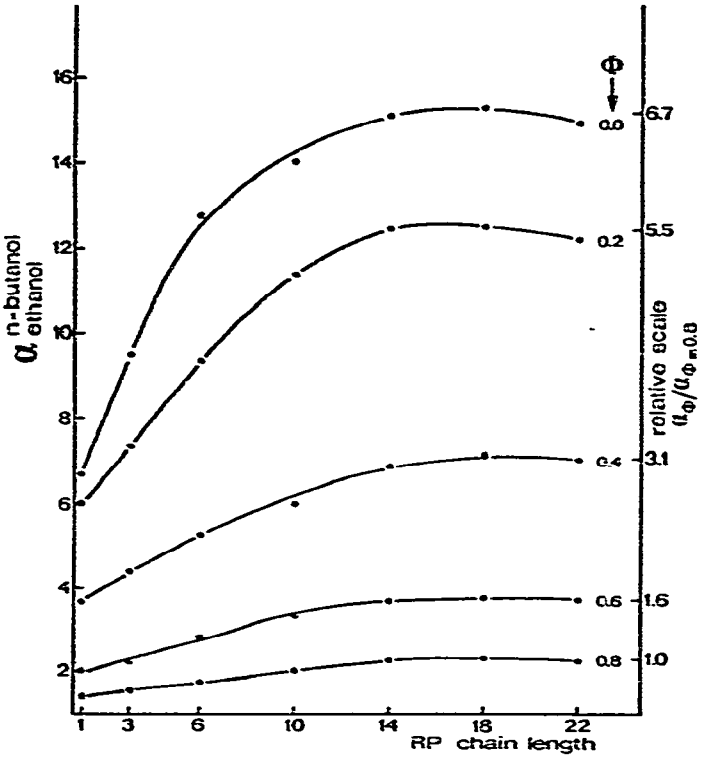

Fig. 4. Normal influence of RP chain length and mobile phase composition on the selectivity. The left ordinate scale indicates the capacity ratio ($\alpha = k_2/k_1$) of *n*-butanol and ethanol. The right ordinate scale indicates the selectivity relative to the volume for $\Phi = 0.8$ reached at long carbon chains.

remains constant. Also, the selectivity changes drastically with a change in mobile phase composition. In fact, the influence of the methanol content of the mobile phase generally exceeds that of the RP chain length. For example, at constant mobile phase composition, the selectivity in Fig. 4 changes by a factor of about 2 in going from RP-1 to RP-14. On the other hand, the maximum selectivity reached at long chain length increases by a factor of 7, when the mobile phase is changed from pure methanol to pure water.

The influence of the methanol content on retention and selectivity has been studied extensively by Schoenmakers *et al.*[38], who noted that (i) ln k varies linearly with methanol content down to a capacity factor of about unity and (ii) the negative slope of the linear part is strongly correlated with the solute retention. The examples of acetone, phenol, *m*-cresol and *p*-phenylphenol in Fig. 5 confirm this general behaviour.

Consequently, for the popular combination of an alkylsilyl bonded stationary phase and a methanol–water mobile phase, we can draw the general conclusion that *the selectivity increases with increasing RP chain length and with increasing water content*. In other words, the selectivity increases with increasing solute retention, which also means that a larger selectivity requires longer analysis times. The influence of the mobile phase on both retention and selectivity exceeds the influence of the stationary phase.

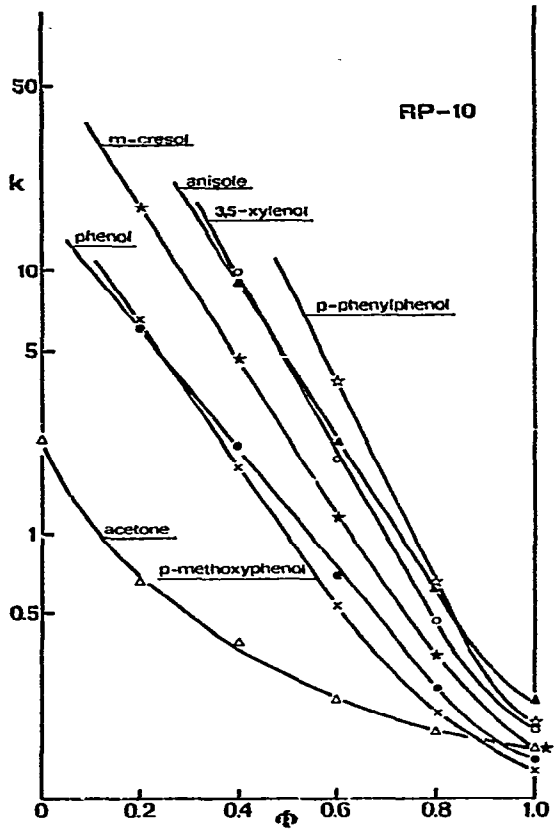

Fig. 5. Influence of the mobile phase composition (Φ = volume fraction of methanol) on the capacity factor.

Closer inspection of the data in Figs. 1–3 and 5 reveals exceptions to this general behaviour. On the one hand there are solutes for which the selectivity decreases with increasing chain length, *e.g.*, naphthalene and *n*-octanol in Fig. 1. Also, for some solute pairs the selectivity passes through a maximum. For example, in 80:20 methanol–water the solutes *n*-decanol and biphenyl show an optimum selectivity of $\alpha = 1.4$ on RP-10, but virtually coincide on RP-22 (Fig. 1). On the other hand, the generally observed increase in selectivity with increasing water content of the mobile phase also has exceptions. The crossing of some curves in Fig. 5 demonstrates that at some mobile phase composition two solutes show identical retention and, hence, reverse their elution order. In approaching this point the selectivity necessarily decreases with increasing water content.

Three examples of exceptional behaviour are presented in Fig. 6. For the solute pair phenol and *p*-methoxyphenol (Fig. 6a) the relative retention increases with RP chain length in the fashion expressed by the general rule formulated above, for $\Phi = 0.6$ and 0.4. Also, on decreasing the methanol content from 80% to 60% the relative retention and hence the selectivity increase, as expected. However, on further reduction of the methanol content the selectivity decreases again. In fact, as illustrated

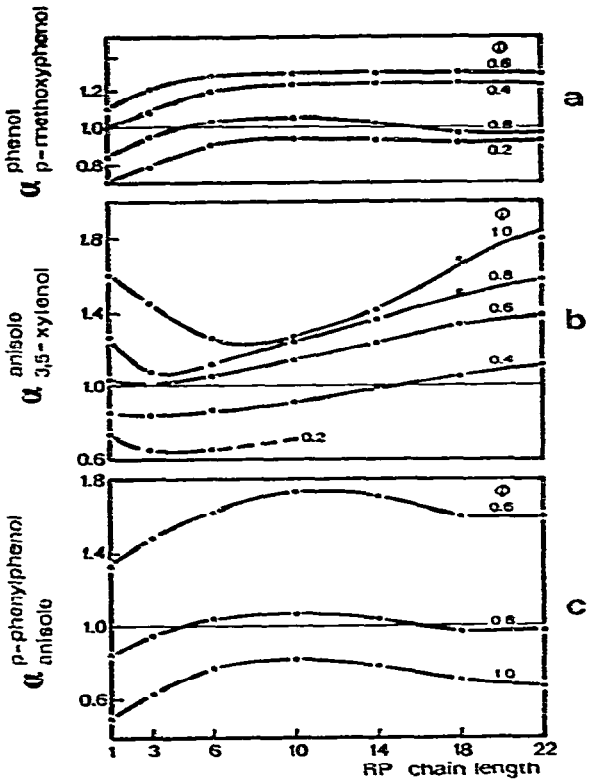

Fig. 6. Exceptional influence of RP chain length and mobile phase composition on the chromatographic selectivity.

in Fig. 5, the elution order reverses at very low methanol contents. Consequently, for $\Phi = 0.2$ in Fig. 6a the apparent regular behaviour of the curve actually represents a decrease in selectivity with increasing chain length, because $\alpha < 1$.

The example of anisole and 3,5-xylenol in Fig. 6b is even more complicated. On chains longer than RP-14 the selectivity decreases rather than increases with increasing water content. For smaller RP chains a reversal of elution order is observed at a mobile phase composition depending on the RP chain length. Finally, at higher methanol contents the selectivity passes through a minimum at an RP chain length between 3 and 8 carbon atoms.

The final example of *p*-phenylphenol and anisole in Fig. 6c shows a maximum selectivity in 60:40 methanol–water on RP-10, no selectivity for $\Phi = 0.8$ at nearly every RP chain length, and a reverse selectivity at still higher methanol contents in the mobile phase with a maximum on RP-1.

These exceptions make it impossible to recommend unequivocally a certain RP chain length as optimal for all separation problems. The interplay between the influence of RP chain length and methanol content on retention (*i.e.*, analysis time) and selectivity presents a challenging optimization problem. As an example, we consider the separation of a mixture in Fig. 7. At a constant mobile phase composition

of 60:40 methanol–water the retentions increase regularly with increasing RP chain length. The separation is incomplete on RP-1 (Fig. 7a), marginal on RP-6 (Fig. 7b), but excellent on RP-14 and RP-22 (Fig. 7c and d, respectively). The analysis time is slightly shorter on RP-22 than on RP-14. This is the combined result of smaller capacity factors and a shorter hold-up time[37].

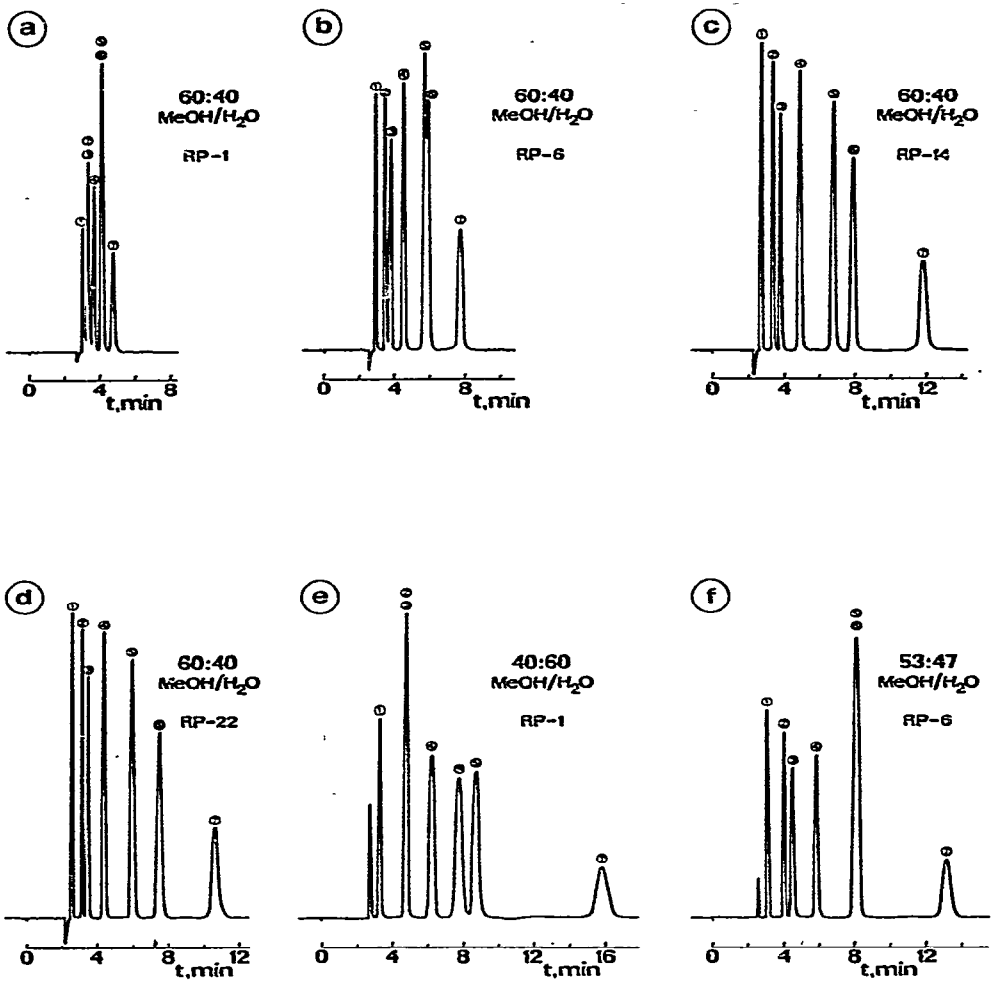

Fig. 7. Chromatograms of a mixture of seven solutes run under various conditions. 1 = Acetone; 2 = *p*-methoxyphenol; 3 = phenol; 4 = *m*-cresol; 5 = 3,5-xylenol; 6 = anisole; 7 = *p*-phenylphenol.

Trying to improve the selectivity of this mixture on the RP-1 and RP-6 phases in roughly the same analysis time, we decreased the methanol content in the mobile phase. Fig. 7e demonstrates that on reducing Φ from 0.60 to 0.40, the solute pair 3,5-xylenol and anisole is separated on the RP-1 packing, whereas *p*-methoxyphenol and phenol remain unseparated. Decreasing the methanol content in the mobile

phase from $\Phi = 0.60$ to 0.53 even deteriorates the separation of anisole and 3,5-xylenol on the RP-6 phase, as shown in Fig. 7f. In other words, both phases are incapable of separating this mixture in the same analysis time as on RP-22 (Fig. 7d). However, complete separation on both phases is possible in 20% methanol on RP-1 and in 40% methanol on RP-6, but requires 60 and 45 min, respectively.

Consequently, in the present example, the combination of RP-22 with $\Phi = 0.60$ offers the optimal separation conditions with baseline separation of all solutes within 11 min.

Obviously, such an optimization process depends on the composition of the mixture, and if this mixture contains only solutes that behave in accordance with the general selectivity rules (*i.e.*, influence of both RP chain length and mobile phase), shorter chain lengths and a higher water content in the mobile phase can also lead to an acceptable separation in a reasonable analysis time.

In summary, it can be stated that especially for larger solutes, which can only be chromatographed in mobile phases with a high methanol content, longer RP chains provide longer retentions and generally better selectivity. For this reason we recommend RP-18 bonded packings. Final optimization can then be performed by varying the mobile phase composition, which generally has a much greater influence than the stationary phase material.

RETENTION MECHANISM

If we now set out to develop a consistent picture of the retention mechanism in RPLC on chemically bonded stationary phases, we can start by summarizing the main results of the previous discussion.

Firstly, we recall the predominant influence of the mobile phase over the stationary phase on retention. Whereas in going from RP-1 to RP-22 the capacity factor increases by a factor of 2–10, depending on the size of the solute (Figs. 1–3), the corresponding variation with mobile phase composition amounts to several decades (Fig. 5). Any proposed mechanism must therefore emphasize the influence of the mobile phase and impart to the stationary phase a role of secondary importance.

Secondly, we observed the existence of a critical RP chain length, indicating that a solute interacts only with a certain part of the bonded chains. Although this critical chain length is independent of the mobile phase composition, it does not mean that the stationary phase is unaffected by the mobile phase. In a previous study of the hold-up time in bonded packings we found strong indications of the presence of a solvation layer that increased with increasing methanol content of the mobile phase[37]. In pure water there is virtually no solvation, whereas with an increasing amount of methanol the solvation layer increases gradually, but not abruptly, to a thickness of about 0.6 nm. We consider it unlikely that the solvated molecules are adsorbed as a uniform layer on top of the closely packed bonded chains. Rather, the decreasing polarity of the mobile phase activates the bonded phase to swell and, therefore, the chains to stretch, thereby providing space for the mobile phase molecules (methanol and water) to penetrate between the bonded chains[34]. This phenomenon is confirmed by the shapes of the chromatographic peaks. In pure water the peaks are strongly fronting, suggesting a minimal contact area with the shrunken RP chains. In mobile phases containing methanol the chromatographic peaks become symmetrical.

Thirdly, although the critical chain length is independent of the mobile phase, it does depend on the size and the nature of the solute. This is illustrated by the data in Table II. With increasing solute size its retention and its critical chain length increase. From the series of *n*-alcohols and also from a comparison of phenol, *m*-cresol and 3,5-xylenol we can derive an increment of the critical chain length, with about 0.5 RP unit per added methylene or methyl group. Similarly, the addition of a phenyl ring (benzene, naphthalene, anthracene) increases the critical chain length by 1.3 RP units. Remarkably, however, the addition of a strongly polar hydroxyl group (benzene/phenol, biphenyl/*p*-phenylphenol, anisole/*p*-methoxyphenol) decreases the critical chain length by 2.2 RP units. The critical chain length is therefore a function of the size of the non-polar part of the solute molecule, which can be exposed to the bonded phase.

TABLE II

LIMITING CAPACITY FACTORS AND CRITICAL CHAIN LENGTHS WITH METHANOL–WATER (80:20)

Solute	*Limiting capacity factor, k*	*Critical chain length, RP*
p-Methoxyphenol	0.22	7.5
Phenol	0.27	7.3
n-Butanol	0.27	7.3
m-Cresol	0.37	7.8
3,5-Xylenol	0.53	8.3
p-Phenylphenol	0.76	9.3
Anisole	0.78	9.6
Benzene	0.85	9.6
n-Octanol	1.32	8.7
Naphthalene	1.81	10.9
Biphenyl	2.60	11.6
n-Decanol	3.02	9.4
Anthracene	4.29	12.2
n-Dodecanol	6.73	10.3

Let us now consider the two main mechanisms advanced for the role of the stationary phase in RPLC: partition and adsorption. For several reasons a partition mechanism is very unlikely. We agree with Horváth and Melander[39] that "the bonded alkyl molecule is not expected to behave as a bulk liquid, since it is only a monolayer thick and the anchored molecules have fewer translational and rotational degrees of freedom than those comprising an unbonded liquid phase". Even in pure water, where the alkyl chains shrink together and resemble a liquid most closely, the observed peak shape indicates anything but a partition process. Finally, the phenomenon of a critical chain length cannot be reconciled with a partition process in which the entire bonded molecule participates.

However, an adsorption mechanism also is not completely satisfactory. Firstly it suggests an active participation of the stationary phase, which negates the predominant role of the mobile phase and is difficult to visualize for the weak dispersive forces between non-polar molecules. Also, active adsorption must compete with a solvation layer of mobile phase molecules. We could thus expect the adsorptive process to be less effective in mobile phases with a high modifier content. As remarked earlier,

however, the critical chain length is independent of the mobile phase composition. In our opinion the stationary phase is more a passive receptor than an active attractor of solutes. Retention mechanisms that suggest active participation of the stationary phase, such as dispersive interaction[24], are therefore less appropriate.

However, the more general solvophobic theory expresses correctly that retention is determined primarily by the competition between the interaction of the solute with the mobile phase molecules and the mutual interaction of the mobile phase molecules[26,39]. In this picture the solute molecules are squeezed out of the mobile phase and forced to enter the stationary phase. In order to do so the solute must break through or replace the solvated layer on the bonded phase. Hence, with increasing modifier content of the mobile phase two cooperating processes probably tend to keep the solute in the mobile phase. Firstly, the enhanced non-polar character reduces the repulsive force of the mobile phase, and secondly, the increased solvation layer, which is seen as a part of the stationary phase, makes it more difficult for the solute to enter the stationary phase.

This penetration is perhaps best described as a compulsory absorption, rather than an adsorption process. The solute is squeezed into spaces created between the bonded chains, as illustrated schematically in Fig. 8.

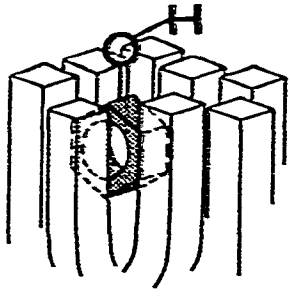

Fig. 8. Schematic representation of absorption of phenol by chemically bonded alkyl chains.

Here the phenyl ring of the phenol molecule resides between the upright chains, but its hydroxyl group still points towards the mobile phase. Obviously, there is a certain chain area required for the alkyl bonded phase to enclose the solute fully. Once this has been accomplished, further elongation of the alkyl chains is no longer effective. It is also clear that the minimum or critical chain length required increases with increasing size of the solute molecule. A relatively non-polar molecule such as benzene will be forced more deeply into the stationary phase than a polar molecule such as phenol. The phenomenon of a critical chain length thus follows logically from the enforced nature of the absorption process.

We now turn to the influence of the RP chain length on the selectivity of the separation. Let us consider two solute molecules of different non-polar surface area. On short RP chains both solutes can penetrate the alkyl chains to only a limited extent; in fact, they may well reside on top of the chains. The larger solute possesses the larger absolute contact area, so that it will be more retained. This is illustrated very schematically in Fig. 9a. As a result of the closely packed chemically bonded chains the larger molecule will meet resistance to its penetration of the bonded phase.

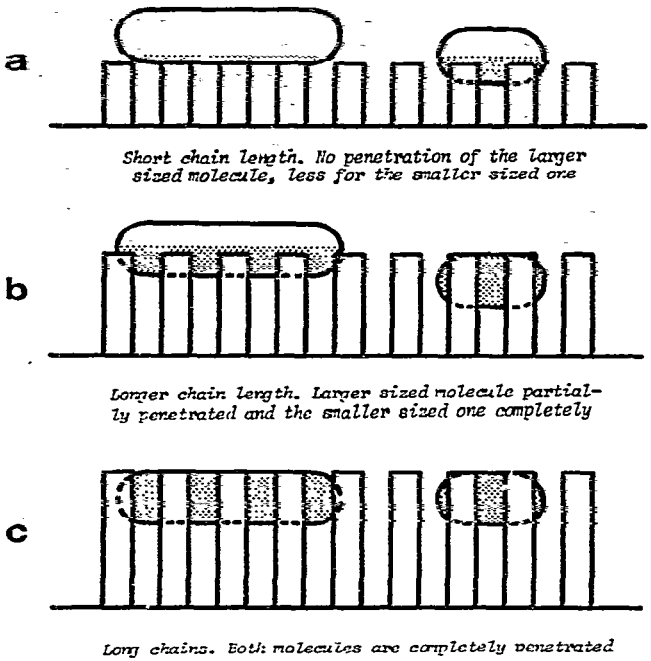

Fig. 9. Schematic illustration of partial penetration of solutes of different sizes in chemically bonded phases with (a) short chains, (b) longer chains and (c) long chains.

Consequently, the smaller molecule penetrates more deeply into the stationary phase and with increasing RP chain length it will be fully enclosed sooner (Fig. 9b). Hence, the relative increase in retention will be limited for a smaller solute and it will soon reach its critical RP chain length. The larger solute needs longer RP chains to become fully enclosed (hence its larger critical chain length), but ultimately its contact area is much larger than for the smaller solute (Fig. 9c). Its relative increase in retention will, therefore, also be larger. Consequently, the selectivity will generally increase with increasing RP chain length.

Obviously, retention can be obtained only when the solute resides for some period in the stationary phase, and the retention increases the longer is the residence time in the bonded phase. The latter is achieved by decreasing the modifier content in the mobile phase. In other words, the above-described enforced penetration process (Fig. 9) will take place more rapidly the lower is the modifier content. This results in a reduced desorption and, consequently, in longer residence times. With respect to the observed reversals in retention (Fig. 5), the enforced penetration depends largely on the chemical nature of both the mobile phase and the solute molecule. This should be further investigated in relation to Horváth and co-workers' solvophobic theory[26,39], or the solubility parameter theory[40].

Finally, in Figs. 1–3 we observe a decrease in capacity factor for many solutes in going from RP-18 to RP-22. This may be due to partial screening of the silica pores. If, in the extreme situation of very long RP chains, the silica pores are completely filled with bonded phase molecules, we would expect a solute to be excluded from such pores, and its k value would be zero. Consequently, there must be a transition

point where the capacity factor, after its initial rise and levelling off with increasing RP chain length, starts to decrease again. For the present silica support with an average pore radius of 8 nm, this might be the 2.95 nm long[34] RP-22 chain.

RETENTION BEHAVIOUR OF HOMOLOGOUS SERIES: THE COMMON INTERSECTION POINT

In a previous paper[41] we reported the retention behaviour of a homologous series of *n*-alcohols on home-made short-chain bonded phases. From these data it appeared that when the linear relationships between ln k and the number of carbon atoms in the series, n_C (from $n_C = 5$ upwards), are extrapolated for these bonded phases, the lines appear to intersect at a common intersection point.

We continued this investigation with the synthesized *n*-alkylsilyl bonded phases RP-1 to RP-22 (ref. 34). We examined different homologous series in methanol–water mobile phase with compositions between $\Phi = 1$ and 0.7. Some results are shown in Fig. 10.

For a given short-chain RP phase, the results for different homologous series are linear and approximately parallel, in agreement with earlier observations[37]. Also, for a given homologous series the straight lines extrapolated for different RP phases intersect at a common intersection point. This is true for RP phases up to 10 carbon

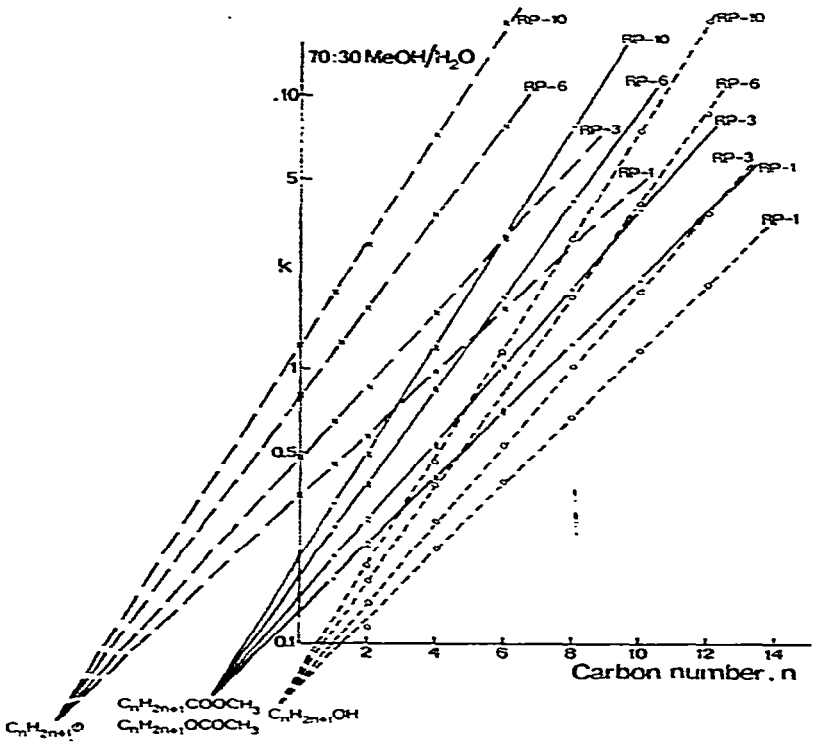

Fig. 10. Log k *versus* carbon number of homologous series ($C_nH_{2n+1}X$, where X = Ph, $COOCH_3$, $OCOCH_3$ and OH for different RP phases (C_1, C_3, C_6 and C_{10}), showing a common intersection point.

atoms. However, for even larger RP phases no further increase in retention can be observed. This could already be concluded from the levelling-off of the capacity factors in Figs. 1–3. Thus, the straight lines observed from RP-10 upwards virtually coincide.

This also means that the effects of the carbon number in the homologous series and in the RP chain are no longer interchangeable[41]. Indeed, the nearly linearly rising branches in Figs. 1–3 may also be extrapolated to show a more or less common intersection point for homologues. However, the straight line runs only from RP-1 to RP-6. It seems, therefore, that the above conclusion is valid only for short-chain RP phases. This is in contrast with Karch's data for RP-1 to RP-18 chains[42]. More recently, Hennion and co-workers[31,43] published retention data for polyaromatic hydrocarbons on undercovered (2.1 μmol/m^2) alkyl phases from RP-4 to RP-18 that also exhibit a common intersection point. However, both Hennion and co-workers' and Karch's phases were prepared from dichloro- and trichloroalkylsilanes, which might indicate the cause of the differences between their results and our present data.

CONCLUSIONS

In this study some chromatographic aspects of the chain length of *n*-alkyldimethylsilyl bonded phases have been investigated. With the packing procedure used no differences in efficiency could be observed between different RP phases. The influence of the alkyl chain on retention is secondary to the much greater influence of the mobile phase composition. At least for methanol–water the same conclusion could be drawn for the selectivity.

In general, both the retention and the selectivity initially increase with increasing RP chain length up to a limiting value reached for an RP chain length between 6 and 14 carbon atoms. The so-called critical RP chain length is independent of the mobile phase composition, but increases with increasing size of the solute molecule. In the same way, retention and selectivity generally increase, and much more strongly, with decreasing methanol content of the mobile phase.

Despite exceptions to this general behaviour, it appears safe to recommend an RP chain length of not less than six carbon atoms. However, larger molecules can be chromatographed only in mobile phases with a relatively high content of organic modifier, and therefore require longer RP chains in order to obtain sufficient retention and selectivity. For this reason we recommend an RP-18 bonded packing for general separation problems.

The observed constancy in retention after the critical RP chain length has been reached suggests that not the complete alkyl chain but only a limited moiety of the bonded chains participates in the separation process. It appears that the critical chain length of different solutes changes with the available non-polar surface area of the solutes. From these observations a "compulsory absorption mechanism" is proposed for the role of the chemically bonded stationary phase in the separation process.

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