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## PREPARATION AND PROPERTIES OF REVERSED PHASES\*

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### SUMMARY

The preparation of monomeric reversed phases (RPs) with  $C_1$ - $C_{18}$  alkyl groups are described. Methods for checking the quality of RPs are discussed. The retentions of polar and non-polar samples were found to be influenced by the eluent, the length of the organic "bristle", the carbon content of the RP, the pore size distribution of the silica support and the total porosity of the stationary phase. The efficiency is a function of (among other factors) the composition of the eluent, the capacity ratio, the length of the bristle, the sieve fraction and the packing density. The maximum sample size is 10 times greater than that with silica and increases with increasing carbon content of the RP. The speed of analysis increases with decreasing length of the bristle. The mechanism of sorption procedures should be discussed with extreme caution after the quality of the RP has been defined.

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### INTRODUCTION

Recently, the properties of reversed phases and their mechanism in high-performance liquid chromatography (HPLC) have frequently been discussed. Unfortunately, however, there is a great variety of such phases, depending on: (1) the quality of the organic "bristle" chemically bonded on the surface of silica, *i.e.*,  $C_1$ - $C_{18}$  alkyl groups; (2) the specific surface area and pore size distribution of the silica; and (3) the method of preparation of these phases, resulting in different carbon contents per unit surface area of the silica, *i.e.*, in different concentrations of the more or less shielded polar silanol groups.

Stationary phases with organic groups permanently bonded to the surface of silica were introduced in chromatography in 1969<sup>1</sup>. The organic molecules can be bonded to the surface of silica in three different ways:

(1) Reaction of the silanol groups on the surface with alcohols, resulting in an ester bond (Si-O-C). The alcoholic reactant can contain different functional groups. These chemically bonded phases have excellent properties in gas chromatography<sup>2</sup>, but their use in liquid chromatography is restricted owing to their insufficient stability against hydrolysis.

(2) Reaction of the silanol groups, especially their chloride derivatives, with

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primary or secondary amines, yielding aminosilanes (Si-N-C) with reasonable stability even in aqueous systems<sup>3,4</sup>. The influence of functional groups in the organic molecules on selectivity has been demonstrated<sup>4,5</sup>. Owing to the monofunctionality of the silanols and of the organic reactants, only monomeric coverage of the surface is possible, resulting in bristle-type phases with a high speed of mass transfer.

(3) Reaction of the silanol groups with chlorosilane<sup>6</sup>, resulting in chemically bonded phases that are stable over the pH range 1–8.5 (at higher pH values the silica carrier starts to dissolve)<sup>7</sup>. In a first approach, bristle-type (*i.e.*, monomeric) phases can be prepared only by using monochlorosilanes. It is to be expected, however, that owing to the bulky alkyl groups bonded to the silicon atom, the coverage and the shielding of the silanols might be less than if di- or trichlorosilanes are used. On the other hand, with polyfunctional chlorosilanes, siloxane-type polymers fixed on the surface can be prepared<sup>8,9</sup> if water is added during the reaction or it is not strictly excluded. A polymeric structure, however, is not always desirable in HPLC because of the slow mass transfer in the polymer coating<sup>10,11</sup>. With porous layer beads (PLB) covered with a "polymeric" structure of chemically bonded phase, it was found that the efficiency of the columns was less than that with comparable liquid loadings with mechanically held organic phases<sup>6,12</sup>. With "monomeric" chemically bonded phases, higher efficiencies were obtained than with comparable liquid loadings on the same material<sup>13</sup>.

Chemically bonded phases have been reviewed in several papers<sup>14–17</sup>, including discussions on selectivity and sorption mechanisms<sup>18–21</sup>. These papers, as well as current research<sup>22–25</sup>, deal mainly with apolar stationary phases, *i.e.*, "reversed-phase" (RP) systems. These systems have been widely used, after their introduction<sup>26</sup>, in all kinds of column and flat-bed chromatography<sup>26–29</sup>.

In HPLC, chemically bonded phases offer many advantages. The selectivity of the stationary phase (silica) can be changed and modified. The absolute and relative retentions in columns packed with chemically bonded phases (including RP) are hardly affected by small changes in the water content of the eluents, while the opposite is true for silica. The equilibration of the stationary phase with a changed water content of the eluent (extremely important in gradient elution) is much more rapid with chemically bonded phases than with silica or alumina.

The stability of chemically bonded phases against hydrolysis increases from Si-O-C through Si-N-C to Si-C bonding. Unfortunately, the difficulties in the preparation increase in the same order. It is not a problem to prepare an RP, but it is extremely difficult to "cook" a good one.

#### PREPARATION OF REVERSED PHASES

Silicas with average pore diameters of 60, 100 and 500 Å were used (Lichrosorb SI-60, SI-100 and SI-500; E. Merck, Darmstadt, G.F.R.). According to the manufacturer, the specific surface areas are about 360 m<sup>2</sup>/g for SI-100 and about 35 m<sup>2</sup>/g for SI-500. The silica was purified before use by acid treatment and thorough washing to neutrality with distilled water. The silica was dried overnight at 180–200°.

The following chlorosilanes were used: I, dimethyldichlorosilane, Cl<sub>2</sub>Si(CH<sub>3</sub>)<sub>2</sub>; II, di-*n*-butyldichlorosilane, Cl<sub>2</sub>Si(C<sub>4</sub>H<sub>9</sub>)<sub>2</sub>; III, methyl-*n*-decyldichlorosilane, Cl<sub>2</sub>Si(CH<sub>3</sub>)C<sub>10</sub>H<sub>21</sub>; IV, methyl-*n*-octadecyldichlorosilane, Cl<sub>2</sub>Si(CH<sub>3</sub>)C<sub>18</sub>H<sub>37</sub>; and V,

*n*-octadecyltrichlorosilane,  $\text{Cl}_3\text{SiC}_{18}\text{H}_{37}$ . Chlorosilanes I and V are commercially available (Merck), II was prepared by a Grignard reaction<sup>30</sup> and III and IV by hydrosilylation<sup>31</sup>. The components were characterized by chemical analysis, boiling point measurements and nuclear magnetic resonance spectroscopy.

Silica and chlorosilanes were reacted as described previously<sup>6</sup>. Water and water vapour were excluded in order to prevent the polymerization of the chlorosilanes to polysiloxanes. After the reaction and the washing procedure, unreacted accessible silanol groups were determined by methyl red adsorption<sup>32</sup>. This reaction is a sensitive test for acidic protons on the silica surface. After the reaction, only a slight reddish colour was sometimes observed. Such phases were silanized further with hexamethyldisilazane or bis(trimethylsilyl)acetamide in toluene. Only phases with a negative methyl red test were used.

The phases were further characterized by their carbon content, determined by classical C, H analysis. In order to achieve good results, a relatively large amount (10–15 mg) was used for the analysis. The reproducibility of the analysis was better than  $\pm 10\%$  although the carbon content of the stationary phases was always below 22%. The reproducibility of the preparation of the stationary phases was as good or better than the C, H analysis, because no difference in the carbon content could be observed between different batches.

Table I lists the different stationary phases prepared with Lichrosorb SI-100. For the calculations, a specific surface area of 300–350  $\text{m}^2/\text{g}$  was assumed [the silanol concentration on the surface of silica is about 8  $\mu\text{mole}/\text{m}^2$  (ref. 33)]. With increasing chain-length of the bristle, the total carbon content increases from 3.1 to about 20% (w/w). The number of organic molecules per unit surface area (100  $\text{\AA}^2$ ) decreases from five molecules with the  $\text{C}_1$  bristle to about 2 molecules with the  $\text{C}_{18}$  bristle. Even with a place requirement of 50  $\text{\AA}^2$ , no accessible (unshielded) silanol groups were determined by methyl red adsorption. The unreacted hydroxyl groups are shielded by the alkyl chains.

In addition to the phases shown in Table I, other types of silica were also used. With Lichrosorb SI-500 (average pore diameter 500  $\text{\AA}$ ; surface area according to the manufacturer, 35  $\text{m}^2/\text{g}$ ) the carbon content of a  $\text{C}_{18}$  phase was 6.25%. With the given surface area, a surface concentration of 5.4 molecules per 100  $\text{\AA}^2$  and a corresponding

TABLE I  
STATIONARY PHASES MADE WITH SI-100

Bristle	Reagent	SI-100, $d_p$ ( $\mu\text{m}$ )	Carbon content (%, w/w)	Surface concentration ( $\mu\text{mole}/\text{m}^2$ )	Average place requirement of a bristle ( $\text{\AA}^2$ )
$\text{C}_1$	$\text{Cl}_2\text{Si}(\text{CH}_3)_2$	25–32	3.13	8.7	18.5
$\text{C}_4$	$\text{Cl}_2\text{Si}(\text{C}_4\text{H}_9)_2$	25–32	6.98	4.9	31.6
$\text{C}_4$		10	8.1	4.8	31.0
$\text{C}_{10}$	$\text{Cl}_2\text{Si}(\text{CH}_3)\text{C}_{10}\text{H}_{21}$	25–32	13.6	3.8	38.0
$\text{C}_{10}$		10	14.8	3.5	42.2
$\text{C}_{18}$	$\text{Cl}_3\text{SiC}_{18}\text{H}_{37}$	25–32	15.9	2.4	55.0
$\text{C}_{18}$		10	22.3	2.9	41.4
$\text{C}_{15}$		5	18.9	2.5	51.5
$\text{C}_{18}$	$\text{Cl}_2\text{Si}(\text{CH}_3)\text{C}_{18}\text{H}_{37}$	10	19.8	2.6	48.8

average place requirement of  $18.5 \text{ \AA}^2$  were calculated. These values seemed to be too "good" for a  $C_{18}$  phase. Polymerization or inexact specific surface area data used for the calculation could be the reason for this unusually low average place requirement. When the reaction was repeated under extremely dry conditions, the same values were obtained. Independent surface area measurements with different samples gave values between 78 and  $106 \text{ m}^2/\text{g}$ . The use of an average value of  $92 \text{ m}^2/\text{g}$  for the calculations resulted in a concentration of 2.05 organic molecules per  $100 \text{ \AA}^2$  (place requirement of  $48.6 \text{ \AA}^2$ ), which is in good agreement with the values of a  $C_{18}$  phase on SI-100.

With SI-60 (surface area *ca.*  $400 \text{ m}^2/\text{g}$ ), a carbon content of 22% (w/w) was found.

The stability of the phases was tested in the pH range 1–9. Within this range, no loss in the carbon content could be observed by C, H analysis. At pH above 9, the silica matrix dissolved. Temperature stability is not of major importance in HPLC; the temperature stability decreases with increasing chain-length of the bristle. For the  $C_{18}$  phase, noticeable decomposition starts at temperatures above  $220^\circ$  (ref. 30).

#### CHROMATOGRAPHIC MEASUREMENTS

A home-built equipment was used<sup>34</sup>, including a device for the control of the inlet pressure<sup>35</sup>. A differential refractometer (Model R401, Waters Assoc., Milford, Mass., U.S.A.) or a home-built UV detector operating at  $254 \pm 10 \text{ nm}$  was used. The samples were injected on the top of the stationary phase. The columns were drilled, and packed by a modified slurry technique, by either the balanced density or viscosity method<sup>36</sup> using 5- or  $10\text{-}\mu\text{m}$  particles. Packing of  $25\text{--}35\text{-}\mu\text{m}$  particles was performed by vibration and tapping. Only purified eluents were used.

#### CHARACTERIZATION OF THE STATIONARY PHASES

##### *Non-polar eluents*

With non-polar eluents (such as *n*-heptane) and silica, the retention order is due to the interaction between the silanol groups and the sample<sup>37</sup>. The absolute retentions (or the capacity ratios,  $k'$ ) are a function of the amount of silanol groups in the column. As this amount decreases, the value of  $k'$  also decreases. In RP columns, where no silanol groups are accessible, the non-polar samples will be eluted with the inert peak if the eluent is non-polar. The more unreacted silanol groups are present, the higher will be the  $k'$  value of the polar samples.

Table II shows the  $k'$  values of the non-polar to medium polar samples on different RP stationary phases with a non-polar eluent (*n*-heptane). With *n*-heptane, the  $k'$  values show a minimum with the  $C_4$  bristles, although the concentration of the alkyl groups on the surface is highest with the  $C_1$  bristle. With  $C_{18}$ , the  $k'$  values are almost as high as with  $C_1$ . The shielding of the unreacted silanol groups seems to be optimal with the butyl group. It would be expected that  $C_{18}$  groups would have better shielding properties, but it is overcompensated for by their high average place requirement, which is about 1.5 times greater than that for a butyl group. Similar results were obtained with more polar samples and dichloromethane as the eluent.

TABLE II  
CAPACITY RATIOS ON RP WITH *n*-HEPTANE AS ELUENT

Sample	Stationary phase				
	$C_1$	$C_4$	$C_{18}$ (22% C)	$C_{18\times}$ (11% C)	Silica SI-109
<i>n</i> -Hexane	0.05	0.04	0.05	—	—
Benzene	0.07	0.07	—	0.05	0.48
Biphenyl	0.08	0.05	—	0.08	1.69
Methoxybenzene	0.17	0.07	0.20	0.35	—
Nitrobenzene	0.34	0.11	0.47	0.65	13.8
Methyl benzoate	0.53	0.12	0.36	2.65	—
Ethyl formate	0.63	0.12	0.46	2.93	—
Benzyl phenylacetate	0.62	0.07	0.22	3.11	—
<i>o</i> -Dinitrobenzene	1.86	0.07	1.15	9.89	—

With this eluent, the elution order increased from butanol to methanol, *i.e.*, the retention order was a function of the unreacted and unshielded silanol groups on the surface of the RP.

The influence of unreacted and unshielded silanol groups is demonstrated by the much higher  $k'$  values obtained with the  $C_{18\times}$  having an 11% (w/w) carbon content compared with the  $C_{18}$  having a 22% (w/w) carbon content. With silica and *n*-heptane, the  $k'$  values are about 0.5 for benzene and 14 for nitrobenzene. The more polar esters and the dinitrobenzene are not eluted under the usual conditions.

As a result of these experiences, only RPs with the following properties were used for further work: (1) no methyl red adsorption; (2) capacity ratios of benzene and nitrobenzene less than 0.1 and 0.5, respectively, with *n*-heptane as eluent. These phases, when tested by gas chromatography, gave symmetrical ether peaks, which is a standard requirement for good chemically bonded phases<sup>2</sup>.

From the point of view of covering and shielding the surface silanol groups, the use of butylsilanes would be optimal. However, RP systems are hardly used with non-polar eluents, and the behaviour of the phases with polar eluents will determine the optimal bristle length.

#### Polar eluents

Typical eluents used in RP chromatography are methanol, water and their mixtures. In the following studies, we restricted ourselves to these eluents.

Fig. 1 shows the influence of the bristle length bonded on to the surface on the retentions of *n*-alkanes, the logarithm of the capacity ratios of  $C_6$ – $C_{17}$  *n*-alkanes being plotted against carbon number. For each bristle type between  $C_1$  and  $C_{18}$ , a linear relationship is obtained with methanol as eluent. This is not a typical separation problem in HPLC, but for systematic studies it might be valuable. The slope of the lines (*i.e.*, the relative retentions of the *n*-alkanes) increases with increasing length of the bristles. The difference between the behaviour of the  $C_{18}$  (22%, w/w, carbon) and the  $C_{18\times}$  (11%, w/w, carbon) stationary phases is notable. Comparing the carbon content of the different RPs in Table I, it seems that not only the chain length but also the amount of organic material bonded on to the silica determines the absolute and relative retentions (compare the data for  $C_{10}$  and  $C_{18\times}$  in Table I and Fig. 1).

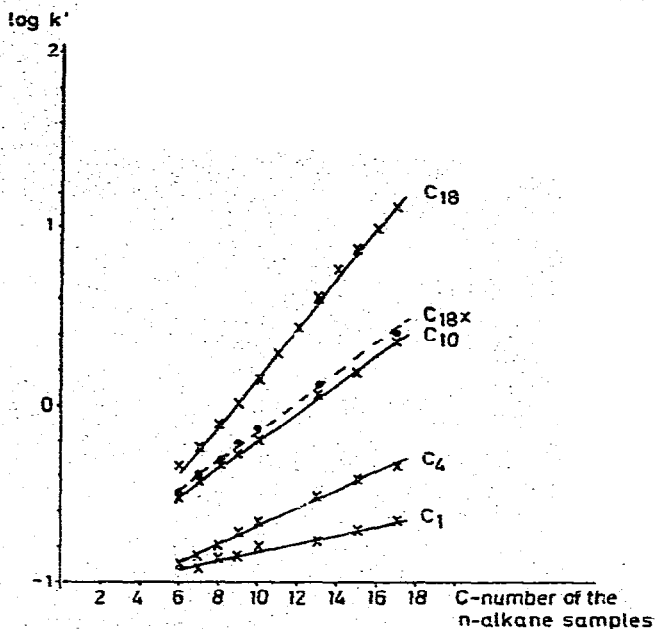


Fig. 1. Influence of bristle length on the retentions of *n*-alkanes. Samples:  $C_6$ - $C_{17}$  *n*-alkanes. Eluent: methanol. Stationary phases: silica SI-100; subjected to reaction with methyl- $(C_1)$ , butyl- $(C_4)$ , decyl- $(C_{10})$  and octadecyl- $(C_{18})$  silanes (see Table I).  $C_{18}$ : bristle  $C_{18}$ , 22% carbon bonded;  $C_{18x}$ : bristle  $C_{18}$ , 11% carbon bonded.

Similar results were obtained with *n*-alcohols under identical conditions (Fig. 2). The introduction of one hydroxyl group into the hydrocarbon chain of the sample molecule has a significant influence on the retention. The  $k'$  value decreases from 4.6 for heptadecane to 1.1 for hexadecanol, neglecting the carbon atom that carries the hydroxyl group. On the other hand, the longer the bristle (and the higher the carbon content of the RP), the higher are the  $k'$  values and the relative retentions.

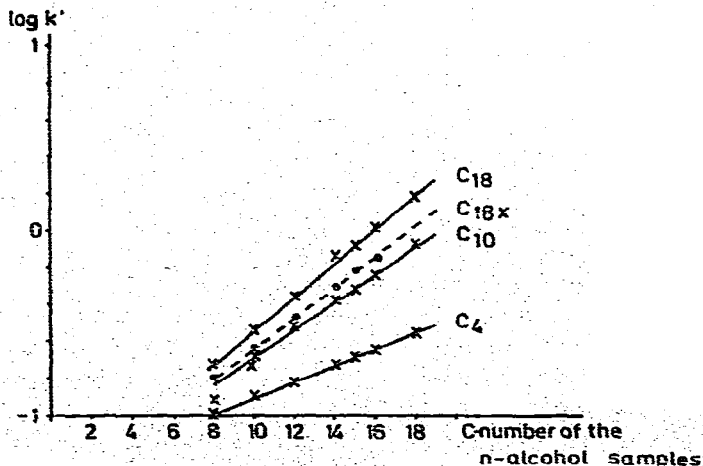


Fig. 2. Influence of bristle length on the retentions of *n*-alcohols. Samples:  $C_6$ - $C_{18}$  *n*-alcohols. Other conditions as in Fig. 1.

Increasing the polarity of the eluent from methanol to water, the differences in the absolute and relative retentions as a function of the quality of the RP decrease, as demonstrated by comparison of Figs. 2 and 3. However, the relative retentions of neighbouring samples still increase with increasing bristle length. The relative retention of *n*-propanol-ethanol increases from 2.8 on the C<sub>4</sub> bristle to 3.6 on the C<sub>18</sub> bristle.

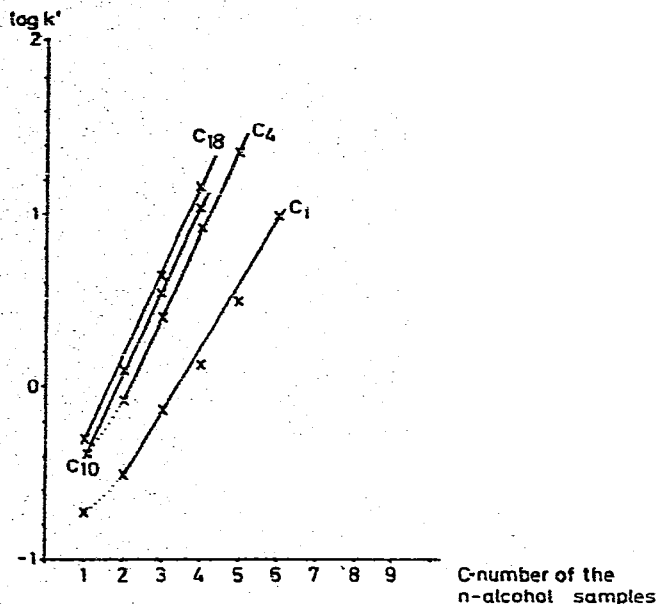


Fig. 3. Influence of bristle length on the retentions of *n*-alcohols. Samples: C<sub>1</sub>–C<sub>6</sub> *n*-alcohols. Eluent: water. Other conditions as in Fig. 1.

Similar observations were made with other organic samples, *e.g.*, phenols, with water as eluent, as shown in Fig. 4. The relative retentions are similar, the absolute retention of a given sample increases with the chain-length of the bristle. The anomalous behaviour of the C<sub>18</sub> phase bonded exceptionally on the surface of a silica with an average pore diameter of 60 Å (SI-60; broken line in Fig. 4) will be discussed later. Other samples, such as aromatic hydrocarbons, acids and esters, gave similar results.

Although the shielding properties are better with the C<sub>4</sub> bristle, higher *k'* values and better relative retentions are obtained with the longer bristles. Therefore, the use of C<sub>18</sub> bristles in RP separations seems to be optimal.

#### *Influence of the pore size distribution of the silica*

Three silicas with different average pore sizes were reacted with octadecylsilanes. The carbon content of the RP was similar with the SI-60 and SI-100 (22%, w/w), whereas SI-500 had only 6.5% carbon bonded on to the surface as a consequence of its much smaller specific surface area.

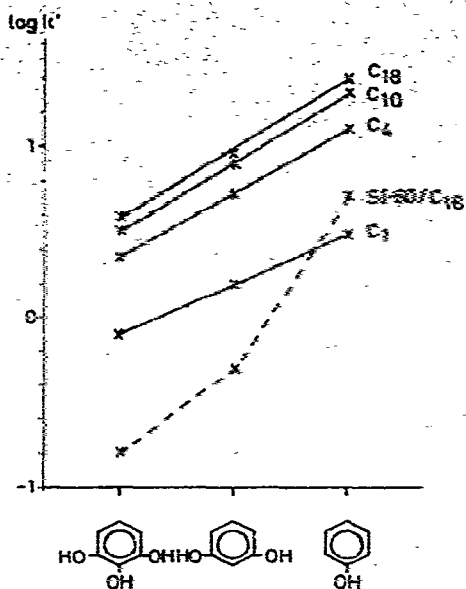


Fig. 4. Influence of bristle length on the retentions of phenols. Samples: phenol, resorcinol, pyrogallol. Eluent: water. Other conditions as in Fig. 1, except for the broken line (SI-60 used instead of SI-100).

Fig. 5 shows the influence of the average pore size on the  $k'$  values of  $n$ -alcohols with water-methanol (1:1, v/v) and water alone as eluents. The  $k'$  values on the SI-500 RP are much smaller than on the other phases owing to the lower carbon content. With water-methanol as eluent, the  $k'$  values on the SI-60 RP are slightly

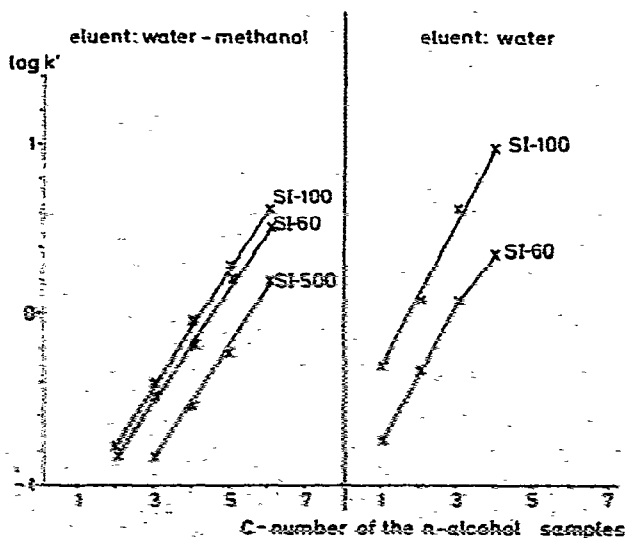


Fig. 5. Influence of the average pore size of the silica with  $C_{18}$  bristles. Samples:  $C_1$ - $C_7$   $n$ -alcohols. Eluents: methanol-water (1:1, v/v) (left); water (right). Silicas: SI-60 (average pore size 60 Å); SI-100 (average pore size 100 Å); SI-500 (average pore size 450 Å).



smaller than on the SI-100 RP. With water as eluent, this effect is much more significant. This result can also be observed with other samples (as in Fig. 4, broken curve, *i.e.*, for the  $k'$  values of phenols with this phase).

Without discussing the mechanism of the processes described above, it should be pointed out that the total porosity of the RP with SI-100 and SI-500 supports was always about 0.8 ( $\pm 0.02$ ), as is usual with porous stationary phases. The total porosity of the SI-60- $C_{18}$  phase decreased from 0.49 to 0.43 on changing from methanol-water to water as eluent. These values are similar to the total porosity of porous layer beads. It seems that owing to the  $C_{18}$  bristles only a small part (if any) of the pore volume is penetrable by the eluent. If the maximum sample size does not decrease with this effect, silica supports with an average pore size of 60 Å or a little smaller should be optimal for the reversed phases, because with decreasing total porosity the pressure drop over the column decreases at constant linear velocity of the eluent if the geometry of the column is kept constant. The risk with such phases is, however, that their total porosity may be a function of the eluent or that of the sample. Further research is required in this field.

#### Column efficiency

In the following, the support for the RP always had an average pore diameter of about 100 Å, *i.e.*, SI-100 silica was always used.

In Fig. 6,  $h$  versus  $u$  curves are plotted for samples with capacity ratios between 0.15 and 1.8. The eluent was methanol-water (1:1, v/v). The viscosities and the

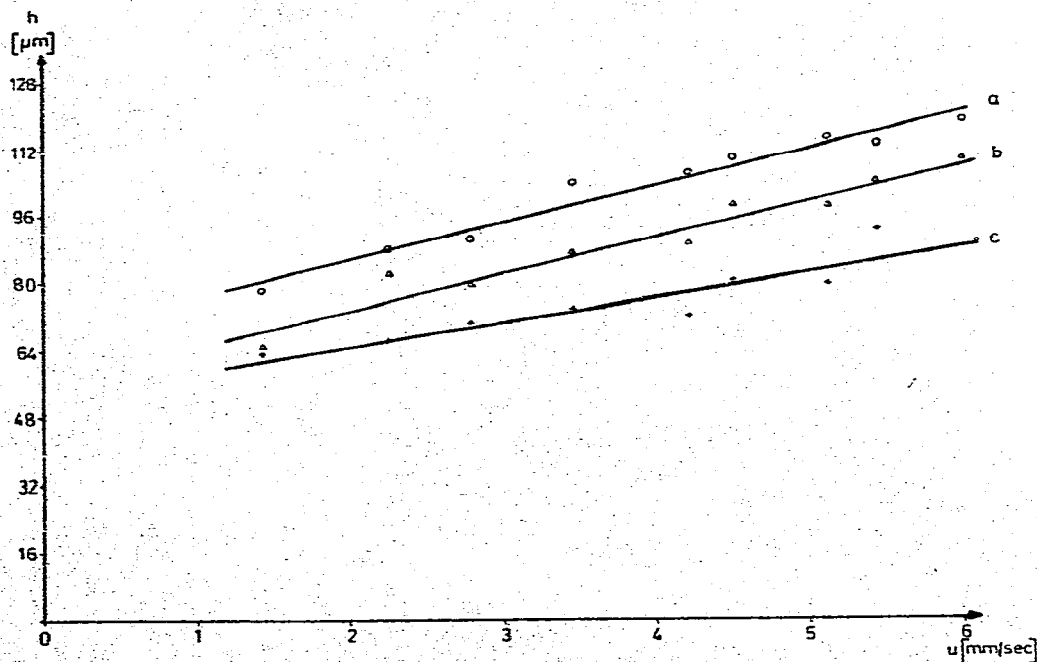


Fig. 6. Peak broadening as function of mobile phase velocity. Eluent: water-methanol (1:1, v/v). Column: length, 30 cm; I.D., 4.2 mm; drilled. Stationary phase: SI-100- $C_{18}$ ,  $d_p = 10 \mu\text{m}$ . Permeability:  $K = 1.6 \cdot 10^{-9} \text{ cm}^2$ . Samples: (a) *n*-pentanol ( $k' = 1.8$ ); (b) phenol (1.3); (c) ethanol (0.15).

interdiffusion coefficients of these eluents as a function of concentrations are anomalous. The 1:1 mixture has about the maximum viscosity (the minimum diffusion coefficients of the samples), the consequence of which should be a lower efficiency if it is not also a function of the  $k'$  values which, of course, change with the composition of the eluent also. Because the sieve fraction is around  $10\ \mu\text{m}$ , the curves may be approximated to straight lines. The slopes are surprisingly small (5.8–8.7 msec) and the intercepts relatively high (ca.  $60\ \mu\text{m}$ ). In our experience, the slope is hardly affected if the composition of the eluent is changed. In contrast, the intercept of the line is a sensitive function of the composition of the eluent and, of course, of that of the packing method.

The efficiencies are not influenced by the length of the organic bristle. With  $10\text{-}\mu\text{m}$  particles and methanol as eluent, a  $C$ -term of 7–10 msec was measured (with water the value was 10–12 msec), independent of the bristle length between  $C_4$  and  $C_{18}$ . On returning to the initial eluent, the column efficiency was always reproducible.

The influence of particle size on efficiency is the same as observed with plain silica<sup>38,39</sup>. On reducing the particle diameter from 25–32  $\mu\text{m}$  to about  $10\ \mu\text{m}$ , the  $h$  values decrease by a factor of about 8; on decreasing the average particle diameter from 10 to 5  $\mu\text{m}$ , the  $h$  values decrease only by a factor of about 2. With an average particle diameter of 5  $\mu\text{m}$ , the  $h$  versus  $u$  curves, of course, show a minimum (see Fig. 7). In this column with a length of 30 cm, about 8500 theoretical plates are generated

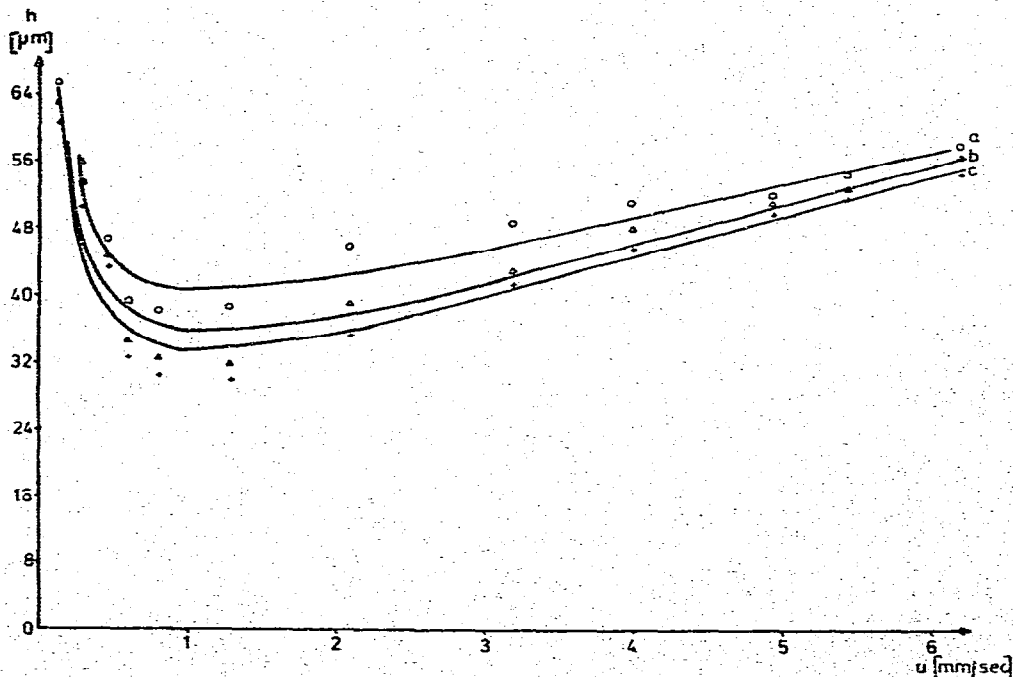


Fig. 7. Peak broadening as function of mobile phase velocity. Column as in Fig. 6. Eluent: methanol. Stationary phase: SI-100- $C_{18}$ ,  $d_p = 5\ \mu\text{m}$ . Permeability:  $K = 3 \cdot 10^{-10}\ \text{cm}^2$ . Samples: (a)  $n$ -decanol ( $k' = 0.31$ ); (b)  $n$ -decane (1.24); (c) ethanol (inert).

at the minimum velocity of 1.5 mm/sec, *i.e.*,  $h \approx 35 \mu\text{m}$ . The C-term is about 5 msec, and consequently the speed of analysis increases significantly with increasing velocity.

The average pore diameter of the silica had no influence on column efficiency if it was about or larger than 100 Å. The  $h$  values on columns packed with SI-100 and SI-500 RP were similar with water or methanol as eluent.

In the reaction of silica with trichlorooctadecylsilane, polymerization is possible if traces of water are not excluded. For steric reasons, a maximum of two of the three chlorine atoms can react with the silanol groups of the silica. If a polymeric stationary phase is formed, the efficiency with such a phase will be lower than that with a monomeric (bristle-type) phase.

In order to study this effect, dichloromethyloctadecylsilane was prepared and reacted with silica. Under same conditions, the same type of silica was reacted with trichlorooctadecylsilane. During these reactions, traces of water were excluded as usual. The product obtained with the trichlorosilane was washed, dried and reacted with hexamethyldisilazane in order to block the newly formed silanol groups. On comparing the two  $C_{18}$  phases, the average plate requirement of a bristle, the absolute and relative retentions and the efficiencies were very similar. Consequently, if water is strictly excluded, the chromatographic properties of the dichloro- and trichlorosilane-treated silica are virtually identical if the later products are subsequently silanized.

#### Loadability

The loadability was determined by measuring the increase in  $h$  values and the decrease in  $k'$  values with increasing sample size at constant velocity. Overloading starts, by definition, if the deviation exceeded 10% of the values measured with extremely low sample sizes.

Table III lists the measured loadabilities for  $C_4$  and  $C_{18}$  stationary phases. The maximum sample size is given in units of  $10^{-4}$  g of sample per gram of stationary phase. Increasing the bristle length from  $C_4$  to  $C_{18}$  doubles the loadability of the columns. The maximum sample size increases with increasing carbon content of the stationary phase, but the relationship is not linear. Moreover the loadability increases less than the amount of bonded organic material. With the  $C_{18}$  RP, the maximum sample size is about  $2 \cdot 10^{-3}$  g of sample per gram of stationary phase. The loadability is an order of magnitude greater than with bare silica.

TABLE III

#### LOADABILITY OF RP SYSTEMS

RP systems  $C_4$  and  $C_{18}$ . Silica SI-100,  $d_p = 10 \mu\text{m}$ . Column length, 30 cm; I.D. 4.2 mm. Eluent: water-methanol (1:4, v/v).

Sample	$k'$ value		Maximum sample size ( $10^{-4}$ g/g)	
	$C_4$	$C_{18}$	$C_4$	$C_{18}$
Hexanol	0.3	0.5	7.1	10.5
<i>m</i> -Xylenol	0.2	0.5	9.8	20
Gammexane	0.7	2.1	11.5	18.5
Lanatosid A	0.3	0.6	9.5	21

## CONCLUSIONS

Chemically bonded non-polar stationary phases of the reversed-phase type have been prepared. The aim was to minimize the influence of the silanol groups of the silica on solute retentions. A non-polar stationary phase must not have accessible silanol groups, as indicated by a negative methyl red adsorption. Further, the retention of samples in non-polar eluents should be extremely small. By increasing the bristle length from methyl to octadecyl, it was found that the average place requirement increases from about  $20 \text{ \AA}^2$  for the methyl group to about  $50 \text{ \AA}^2$  for the octadecyl group. The coverage and/or the shielding of the surface silanol group is optimal with butyl groups. On  $C_4$  RP, the retention of polar components in non-polar eluents such as *n*-heptane or dichloromethane is minimal.

With typical eluents for RP separations such as methanol and water, the  $k'$  values and the relative retentions increase with increasing length of the bristles. The efficiencies of columns packed with these materials are not influenced by the length of the bristles. Therefore, for difficult separations (small relative retentions),  $C_{18}$  RP systems with a high carbon content should be used. However, faster separations are obtained with shorter bristles, *i.e.*, with butyl groups ( $C_4$ ). The higher the carbon content of the RP, the greater the amount of a sample that can be separated without loss of resolution. Such systems offer good possibilities for use in preparative liquid chromatography.

RP systems offer great potentialities for the separation of polar samples as well as for the separation of hydrocarbons. Fig. 8 shows the separation of different phenols with water as eluent with a  $C_4$  RP. The time of analysis is doubled if a  $C_{18}$

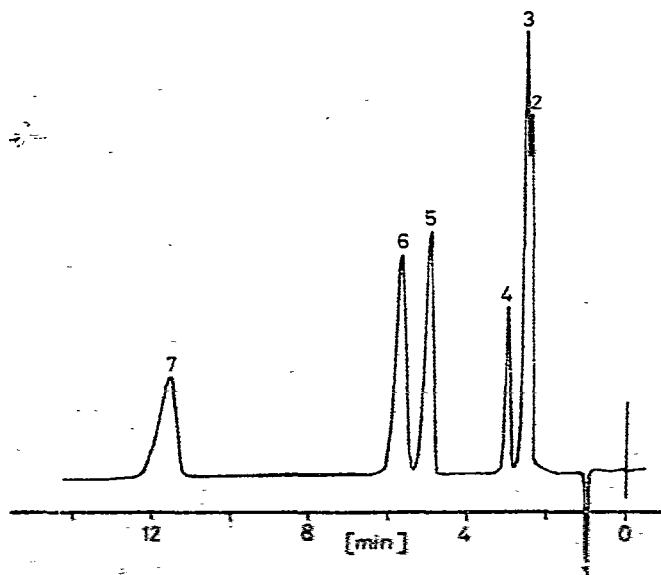


Fig. 8. Separation of phenols. Column as in Fig. 6. Stationary phase: SI-100- $C_4$ ,  $d_p = 10 \mu\text{m}$ . Eluent: water;  $u = 0.5 \text{ cm/sec}$ ;  $\Delta p = 125 \text{ atm}$ . Samples: 1 =  $D_2O$  (inert); 2 = phloroglucinol ( $k' = 2.5$ ); 3 = pyrogallol (2.6); 4 = hydroquinone (2.95); 5 = resorcinol (4.95); 6 = catechol (5.65); 7 = phenol (11.5).

RP is used. Fig. 9 shows the separation of aromatic and cycloalkane hydrocarbons on a  $C_{18}$  RP system. Such separations should not be solved by RP chromatography in a first approach, but the advantages of RP in the separation of polynuclear aromatic hydrocarbons has already been demonstrated<sup>30</sup>.

In our experience, it is more sophisticated to pack columns with a reversed phase than with silica. The packing density (*i.e.*, the weight of the stationary phase per unit empty column volume) can be varied by the packing method. In an extremely approximate approach, it could be argued that with decreasing packing density the efficiency of the column increases up to a given limit. With these columns, the efficiency often decreases during the lifetime of the column. On the contrary, high packing densities result in excellent reproducibility and longer lifetimes, but sometimes poorer efficiency.

It is extremely dangerous to discuss the mechanism of chromatographic separations with reversed phases without defining with great accuracy the quality of the stationary phase used.

Further applications of RP chromatography and the influence of sample structure and eluent composition on solute retention are discussed in a following paper<sup>41</sup>.

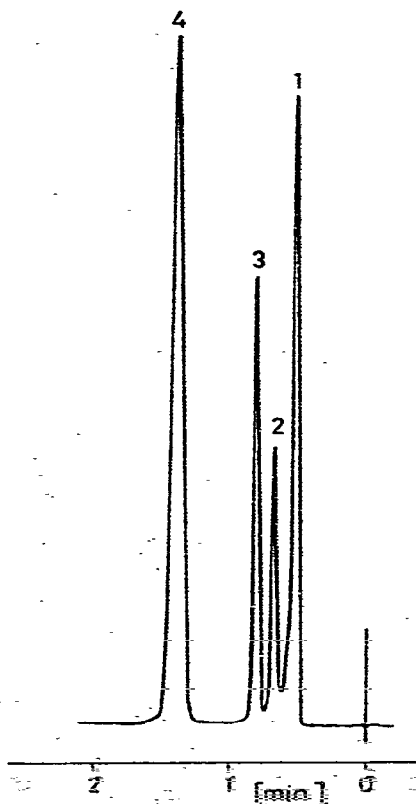


Fig. 9. Separation of hydrocarbons. Column as in Fig. 6. Stationary phase: SI-100- $C_{18}$ ,  $d_p = 10 \mu m$ . Eluent: methanol;  $u = 1.1 \text{ cm/sec}$ ;  $\Delta P = 175 \text{ atm}$ . Samples: 1 = ethanol (inert); 2 = biphenyl ( $k' = 0.3$ ); 3 = phenylcyclohexane (0.6); 4 = bicyclohexyl (1.86).

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## REFERENCES

- 1 I. Halász and I. Sebastian, *Angew. Chem.*, 81 (1969) 464.
- 2 I. Sebastian, *Dissertation*, University Frankfurt/Main, 1969; I. Halász and I. Sebastian, *J. Chromatogr. Sci.*, 12 (1974) 161.
- 3 I. Sebastian, O.-E. Brust and I. Halász, in S. G. Perry (Editor), *Gas Chromatography 1972*, Applied Science Publ., Barking, 1973, p. 281.
- 4 O.-E. Brust, I. Sebastian and I. Halász, *J. Chromatogr.*, 83 (1973) 15.
- 5 U. Neue, *Thesis*, University of Saarbrücken, Saarbrücken, 1973.
- 6 J. J. Kirkland and J.-J. de Stefano, *J. Chromatogr. Sci.*, 8 (1970) 309.
- 7 I. Sebastian and I. Halász, *Chromatographia*, 7 (1974) 371.
- 8 W. A. Aue and C. R. Hastings, *J. Chromatogr.*, 42 (1969) 319.
- 9 W. A. Aue and S. Kapila, in E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1974, p. 13.
- 10 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
- 11 R. A. Henry, J. A. Schmit and R. C. Williams, *J. Chromatogr. Sci.*, 11 (1973) 358.
- 12 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 206.
- 13 D. F. Horgan, Jr. and J. N. Little, *J. Chromatogr. Sci.*, 10 (1972) 76.
- 14 R. E. Leitch and J. J. de Stefano, *J. Chromatogr. Sci.*, 11 (1973) 105.
- 15 D. C. Locke, *J. Chromatogr. Sci.*, 11 (1973) 120.
- 16 A. Pryde, *J. Chromatogr. Sci.*, 12 (1974) 486.
- 17 E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1974.
- 18 B. B. Wheals, C. G. Vaughan and M. J. Whitehouse, *J. Chromatogr.*, 106 (1975) 109.
- 19 J. N. Seiber, *J. Chromatogr.*, 94 (1974) 151.
- 20 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
- 21 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
- 22 R. E. Majors and M. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.
- 23 E. Grushka and E. J. Kitka, Jr., *Anal. Chem.*, 46 (1974) 1370.
- 24 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 25 R. V. Gilpin, J. A. Korpi and C. A. Janicki, *Anal. Chem.*, 46 (1974) 1314.
- 26 A. J. P. Martin and G. A. Howard, *Biochem. J.*, 56 (1950) 539.
- 27 E. Lederer (Editor), *Chromatographie en Chimie Organique et Biologique*, Vols. I and II, Masson, Paris, 1959, 1960.
- 28 K. Macek and I. Hais, *Handbuch der Papierchromatographie*, Band I, Gustav Fischer Verlag, Jena, 1958.
- 29 E. Stahl (Editor), *Dünnschichtchromatographie*, Springer, Berlin, Heidelberg, New York, 2. Aufl., 1967.
- 30 K. Karch, *Thesis*, University of Saarbrücken, Saarbrücken, 1972.
- 31 A. J. Barry, L. de Free, J. W. Girkey and D. E. Hook, *J. Amer. Chem. Soc.*, 69 (1947) 2916.
- 32 J. Shapiro and J. M. Kolthoff, *J. Amer. Chem. Soc.*, 72 (1950) 776.
- 33 K. Unger, *Angew. Chem.*, 84 (1972) 331.
- 34 G. Deininger and I. Halász, *J. Chromatogr. Sci.*, 9 (1971) 83.
- 35 G. Deininger and I. Halász, *J. Chromatogr.*, 60 (1971) 65.
- 36 J. Asshauer and I. Halász, *J. Chromatogr. Sci.*, 12 (1974) 139.
- 37 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 38 I. Halász, R. Ende and J. Asshauer, *J. Chromatogr.*, 112 (1975) 37.
- 39 I. Halász, *Z. Anal. Chem.*, 277 (1975) 257.
- 40 H. Engelhardt and W. Böhme, *Erdöl Kohle, Compendium 74/75* (1975) 741.
- 41 K. Karch, I. Sebastian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.