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## INFLUENCE OF THE NUMBER AND LENGTH OF ALKYL CHAINS ON THE CHROMATOGRAPHIC PROPERTIES OF HYDROCARBONACEOUS BONDED PHASES

M. C. HENNION, C. PICARD and M. CAUDE

*Laboratoire de Chimie Analytique, Ecole Supérieure de Physique et de Chimie de Paris, 10 rue Vauquelin, 75231 Paris Cedex 05 (France)*

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### SUMMARY

Non-polar bonded stationary phases have been prepared by reaction of organochlorosilanes with silica. The influence of the surface coverage on chromatographic properties was studied. The capacity ratio and selectivity do not increase continuously with increasing surface coverage and reach maxima at a surface coverage of *ca.*  $2 \mu\text{mol}\cdot\text{m}^{-2}$  for polar and non-polar solutes. The influence of the length of the bonded alkyl chains at constant surface coverage was studied. The capacity ratio and selectivity increase continuously with increasing length of bonded bristles.

A compromise between surface coverage, alkyl chain length and mobile phase composition is discussed.

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### INTRODUCTION

In the last 5 years there has been a considerable increase in the use of chemically bonded stationary phases in high-performance liquid chromatography (HPLC). Most chromatographic separations (about 60% of HPLC work in 1976) have been carried out by reversed-phase chromatography. Much work dealing with bonded-phase chromatography has been published, and Pryde<sup>1</sup>, Rehak and Smolkova<sup>2</sup>, Bristow<sup>3</sup>, Cox<sup>4</sup> and Colin and Guiochon<sup>5</sup> have given comprehensive reviews of the work so far. Majors<sup>6,7</sup> reviewed the commercial packing materials currently available.

This paper describes the preparation of non-polar bonded materials with various carbon contents and different *n*-alkyl chain lengths. The effects of the surface coverage (or carbon content) and the length of the bonded chains on retention and selectivity properties have been studied for both polar and non-polar solutes. Work carried out in this area<sup>8-15</sup> has shown that the capacity ratio, *k'*, is related to the surface coverage and the length of the bonded chains. Kirkland<sup>8</sup>, Majors and Hopper<sup>9</sup> and Gilpin *et al.*<sup>10</sup> observed that an increase in chain length is accompanied by an increase in retention time. Hemetsberger *et al.*<sup>11</sup> found a linear relationship between *k'* and chain length for polar solutes. Karch *et al.*<sup>12</sup> concluded that while

a C<sub>4</sub> alkyl chain gave optimum coverage, it was better to have a long retention with a longer chain, such as C<sub>18</sub>. For many solutes tested, selectivity is independent of chain length. It was concluded that a long chain is preferred, but the different roles of surface coverage and chain length were unclear.

## EXPERIMENTAL

### *Chemicals*

The porous silica used to synthesize bonded packings was 5- $\mu$ m Partisil (Whatman, Clifton, N.J., U.S.A.) and experimental 5- $\mu$ m Spherosil (Rhône-Poulenc, Paris, France).

Butyl-, octyl- and dodecyltrichlorosilane were kindly provided by Rhône-Poulenc. Hexyltrichlorosilane was purchased from Pfaltz and Bauer (Stamford, Conn., U.S.A.) and octadecyltrichlorosilane from Merck (Darmstadt, G.F.R.). Toluene, xylene, methanol and tetrahydrofuran were of analytical reagent grade (Prolabo, Paris, France). Toluene was dried and kept on 4 Å molecular sieve.

### *Column packing techniques*

The columns were polished stainless-steel tubes (15 cm  $\times$  4.8 mm I.D.), fitted with a 7- $\mu$ m porous stainless-steel frit.

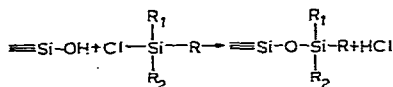
Columns were prepared by using a high-pressure slurry packing technique. Two different slurry liquids were tried. First the bonded silica was suspended in carbon tetrachloride at a concentration of *ca.* 10% (w/v) and a flow of ethanol was applied with a pressure of 500 bar. We achieved a better column efficiency by suspending the bonded silica in methanol-water (80:20) with a low concentration (10<sup>-2</sup> M) of sodium acetate and applying a flow of methanol-water (50:50) with a pressure of 300 bar<sup>16</sup>. The salt is thought to remove electrostatic charges from the bonded material and may produce better packed columns<sup>4</sup>.

### *Chromatographic equipment*

The liquid chromatograph was a Varian 8510 (Varian, Palo Alto, Calif., U.S.A.). Solute were detected with a fixed-wavelength UV detector (254 or 280 nm, Varian).

### *Synthesis of bonded-phase packings*

The synthesis was effected by the reaction of surface silanol groups with an organochlorosilane (mono-, di- or trichlorosilane):



This is a classical bonding method and the procedures have been previously described by Gilpin and Burke<sup>17</sup>, Kingston and Gerhart<sup>18</sup> and Wheals<sup>19</sup>.

Silica (10 g) is pre-treated in a mixture of concentrated sulphuric and nitric acids (50:50) in order to obtain the maximal number of reactive surface silanol groups. After washing silica to neutrality with distilled water, it is dried at 180°

under vacuum for 12 h in order to remove physisorbed water. Then the silica is suspended in a 10% (v/v) solution of silane in a suitable solvent (toluene or xylene) and refluxed for various periods. Subsequently the bonded silica is washed with 150 ml of toluene, 50 ml of tetrahydrofuran, water-tetrahydrofuran (90:10) and water.

The bonded silica obtained is characterized by its carbon content (result from microanalysis) or by the surface concentration of bonded bristles (calculated) and by its chromatographic properties.

In order to achieve reproducible results we thought it necessary to study the kinetics of the silanization reaction.

#### KINETICS OF SILANIZATION REACTION

The study was made with Partisil (specific area determined by the BET method,  $402 \text{ m}^2 \cdot \text{g}^{-1}$ ; pore volume,  $0.72 \text{ cm}^3 \cdot \text{g}^{-1}$ ; average particle size,  $6 \mu\text{m}$ ).

##### *Effect of reaction time*

As shown in Table I, the carbon content obtained by reaction of silica with a solution of 10% (v/v) octadecyltrichlorosilane ( $\text{C}_{18}$ ) in refluxing dry toluene depends on the reaction time. We found that a reaction time of about 18 h is necessary in order to obtain a maximal carbon content of about 10% (w/w) and that a longer reaction time does not produce an increase in carbon content.

TABLE I  
VARIATION OF SILICA SURFACE COVERAGE WITH SILANIZATION TIME

Parameter	Reaction time (h)					
	0.8	2.5	5	6.5	18	24
Carbon content (%, w/w)	3.0	3.6	4.2	6.5	10.5 11.3	11
Surface concentration ( $\mu\text{mol} \cdot \text{m}^{-2}$ )	0.36	0.45	0.52	0.84	1.46 1.60	1.54

##### *Effect of reaction temperature*

In the above study the silane solvent was toluene and the reaction temperature was that of refluxing toluene ( $111^\circ$ ). We carried out the same reaction with another solvent (xylene, boiling point  $138^\circ$ ) and also directly with the silane heated at  $170^\circ$ . The results (Table II) indicate that a higher temperature leads to a higher yield of silanization products and a shorter reaction time. These results agree with the observation that the reaction rate is controlled by the diffusion of silane molecules to the active sites through the silica pores<sup>15</sup>.

There is a general consensus that approximately five surface silanol groups<sup>20</sup> per square nanometre ( $8 \mu\text{mol} \cdot \text{m}^{-2}$ ) are available for bonding. The maximal carbon content obtained at  $170^\circ$  is 23% (w/w), which corresponds to a surface concentration of  $4.2 \mu\text{mol} \cdot \text{m}^{-2}$ . In this instance only 2.5 out of 5 hydroxyl groups are used during silanization.

TABLE II  
VARIATION OF MAXIMUM SURFACE COVERAGE WITH SILANIZATION TEMPERATURE

Parameter	Solvent for silane		
	Toluene	Xylene	Silane alone
Reaction temperature (°C)	111	138	170
Maximal carbon content obtained (% w/w)	10	15	23
Reaction time necessary to obtain maximal carbon content (h)	18	15	2

### CHROMATOGRAPHIC PROPERTIES OF BONDED PHASES

#### Choice of samples

We studied the separation of non-polar solutes (polycyclic aromatic hydrocarbons and alkylbenzenes) and polar solutes (phenols). These two types of solutes were chosen because their behaviours in reversed-phase chromatography are different and the results could be compared with those reported in the literature<sup>15,21-24</sup>.

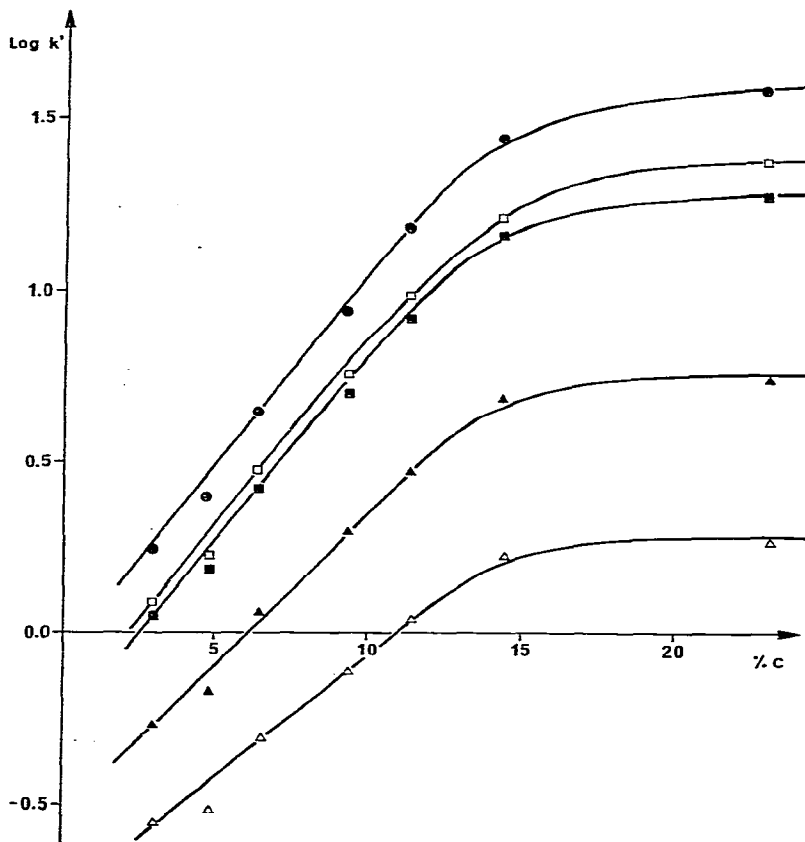


Fig. 1. Variation of the logarithm of the capacity factor of aromatic hydrocarbons with the carbon content of the stationary phase ( $C_{18}$  bonded Partisil). Mobile phase: methanol-water (70:30).  $\Delta$ , Benzene;  $\blacktriangle$ , naphthalene;  $\blacksquare$ , phenanthrene;  $\square$ , anthracene;  $\bullet$ , pyrene.

*Effects of surface coverage for a linear-chain C<sub>18</sub> bonded to 5- $\mu$ m Partisil*

*Effect on capacity ratio.* We prepared C<sub>18</sub> bonded phases with carbon contents varying from 3 to 23% (w/w), and tested these supports for each solute with water-methanol (30:70) as eluent. Capacity ratios were determined relative to the phenol eluted with pure methanol as the unretained solute. Figs. 1 and 2 demonstrate an exponential increase in capacity ratio with carbon content of the bonded phase for each solute up to about 15%. This value corresponds to a space requirement for a bristle of 0.70 nm<sup>2</sup>. Subsequently the capacity ratios are almost constant. We tried to relate this result to the mechanisms that have been proposed.

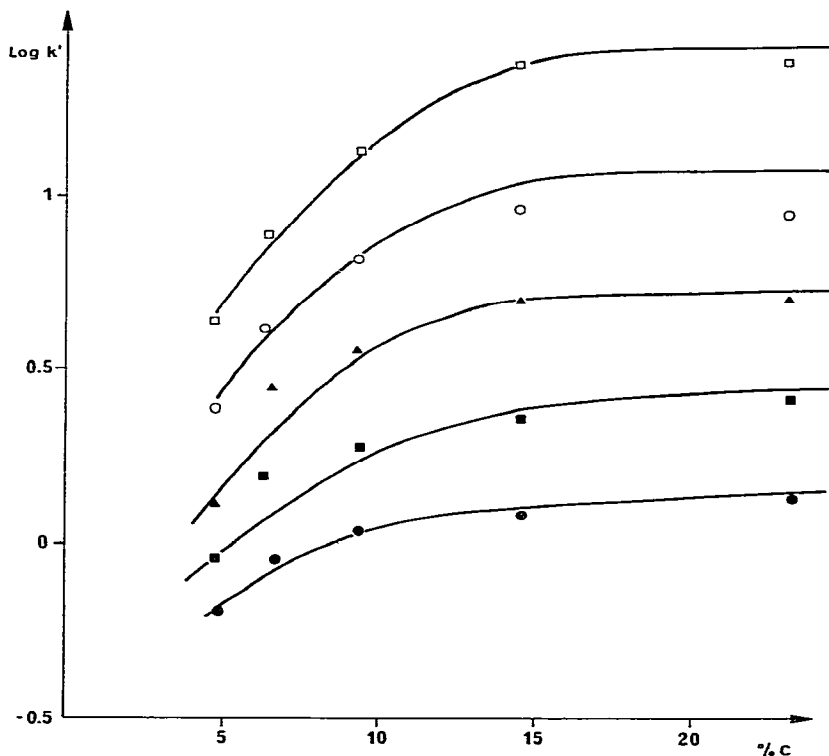


Fig. 2. Variation of the logarithm of the capacity factor of phenols with the carbon content of the stationary phase (C<sub>18</sub> bonded Partisil). Mobile phase: methanol-water (40:60). ●, Hydroquinone; ■, resorcinol; ▲, methylresorcinol; ○, *p*-methoxyphenol; □, phenol.

Some workers<sup>13,25</sup> propose a partition mechanism: the organic solvent of the mobile phase, consisting of a mixture of water and an organic water-miscible solvent, is adsorbed on the non-polar bonded chain surface and partition can occur between this modified stationary phase and the mobile phase.

On the other hand, Telepchak<sup>26</sup> introduced arguments in favour of an adsorption mechanism but did not describe it precisely. Karch *et al.*<sup>12</sup> consider that retention is due to the dispersive interactions between solute molecules and non-polar stationary phase. Locke<sup>27</sup> considers these interactions to be weak and non-

selective and that the retention is a function of the solubility of the solute in the mobile phase. As an example, there is a linear relationship between the logarithm of the capacity ratios of various monoaromatic hydrocarbons and their molar volume, which is related to their solubility in water (Fig. 3)<sup>28</sup>. Horváth *et al.*<sup>29</sup> describes the retention as an adsorption mechanism due to the formation of complexes between the solute molecules and the bonded chains resulting from solvophobic interactions.

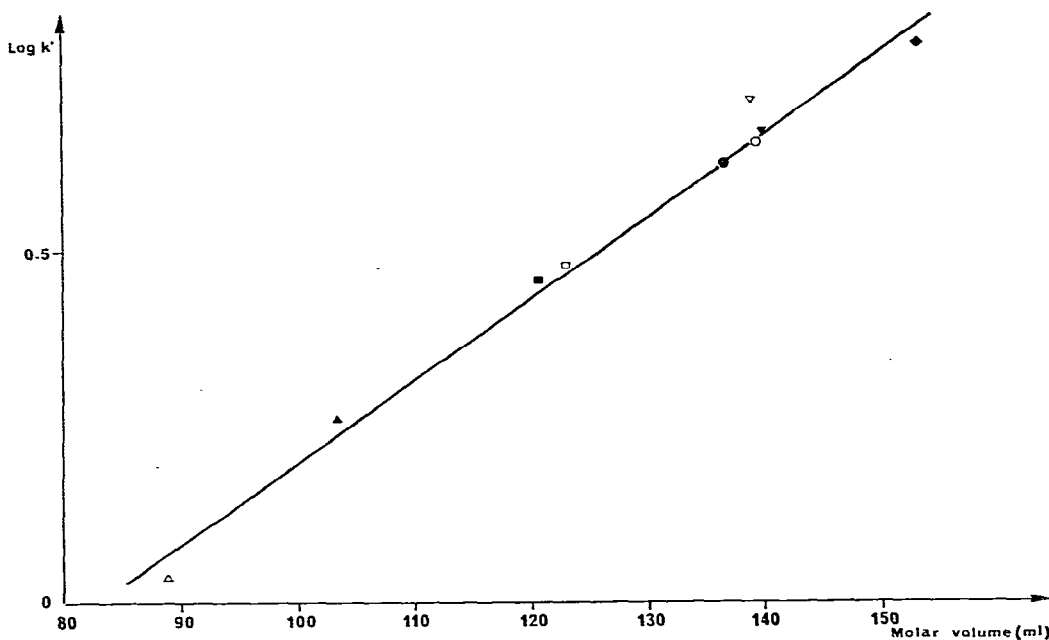


Fig. 3. Variation of the logarithm of the capacity ratio of alkylbenzenes with their molar volume ( $C_{18}$  bonded Partisil, 11.5% C). Mobile phase: methanol-water (70:30).  $\triangle$ , Benzene;  $\blacktriangle$ , toluene;  $\blacksquare$ , *o*-xylene;  $\square$ , *m*-xylene;  $\bullet$ , 2-ethyltoluene;  $\circ$ , 3-ethyltoluene;  $\blacktriangledown$ , 4-ethyltoluene;  $\nabla$ , 1,3,5-trimethylbenzene;  $\blacklozenge$ , 1,2-diethylbenzene.

Karger *et al.*<sup>24</sup> studied the formation of cavities when solubilizing a hydrocarbon in water and introduced a topological index based on the hydrocarbon skeleton of the solute. Other workers<sup>12</sup> think that it is a mixed mechanism.

We observe that every mechanism proposed is related to the number of bonded chains. More work is therefore needed in order to explain why the capacity ratio does not increase continuously with increasing surface coverage.

*Effect on selectivity.* The relative retention factor,  $\alpha$ , increases with increasing bonded-phase carbon content up to about 15% for each solute pair. Subsequently the value stays approximately constant, as is shown in Figs. 4 and 5.

*Effect on efficiency.* Fig. 6 shows that efficiency is maximal for a carbon content of about 14.5% (*i.e.*, a space requirement of 0.70 nm<sup>2</sup>). The corresponding height equivalent to a theoretical plate,  $H$ , according to the elution peaks of a polycyclic aromatic hydrocarbon with a capacity ratio of 4, is approximately 0.02 mm for a

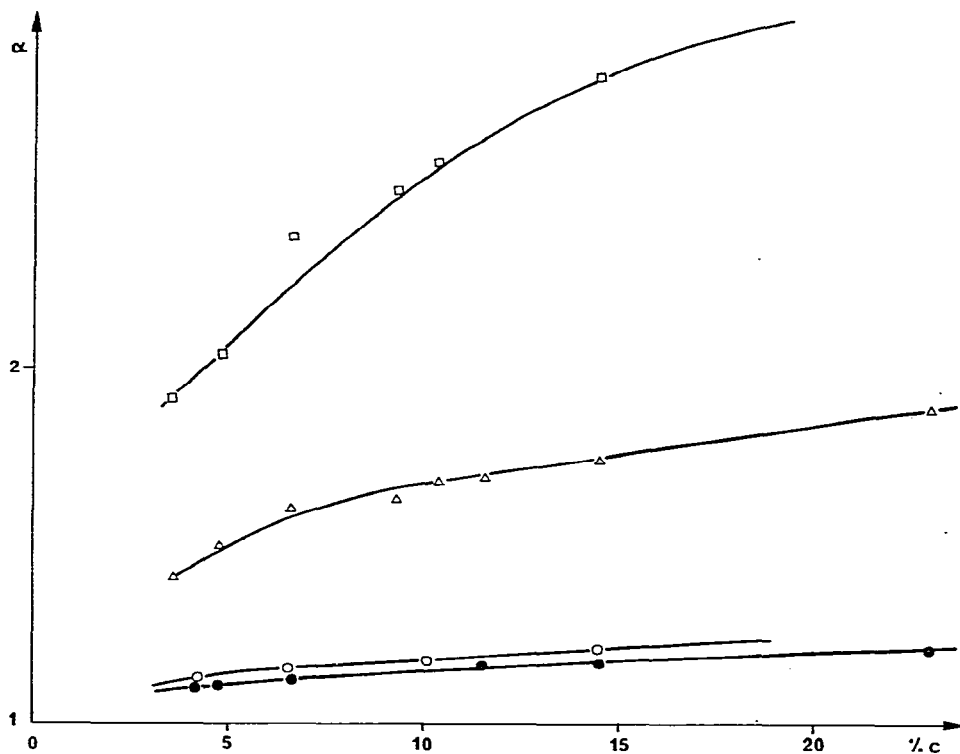


Fig. 4. Variation of the selectivity factor,  $\alpha$ , for various couples of hydrocarbon compounds with the carbon content of the stationary phase ( $C_{18}$  bonded Partisil). Mobile phase: methanol-water (70:30). ●, Anthracene-phenanthrene; ○, chrysene-triphenylene; △, toluene-benzene; □, *o*-xylene-benzene.

linear flow velocity of eluent of  $0.1 \text{ cm} \cdot \text{sec}^{-1}$ . This corresponds to a reduced plate height ( $h = H/d_p$ ) of about 4.

For carbon contents less than 10%, there is a decrease in column efficiency as the peaks are less symmetrical. This could be explained by possible interactions of the solute molecules with unreacted hydroxyl groups of the stationary phase<sup>14</sup>, but these interactions seem improbable. Indeed, the capacity ratios of nitrobenzene and benzene eluted by hexane do not exceed 0.5 and 0.1, respectively, for any of the bonded phases studied. This criterion is generally accepted as indicating that there are no available unreacted silanol groups that can interact with solute molecules<sup>3,12,14</sup>. For carbon contents above 15%, we also observed a decrease in column efficiency, which was accompanied by a higher pressure drop.

All of these results show that the reversed-phase material obtained by bonding octadecyltrichlorosilane to Partisil silica and with a carbon content of 15% has the most suitable chromatographic properties. This stationary phase is reproducibly synthesized by reaction of silica with a solution of 10% octadecyltrichlorosilane in refluxed xylene for 15 h.

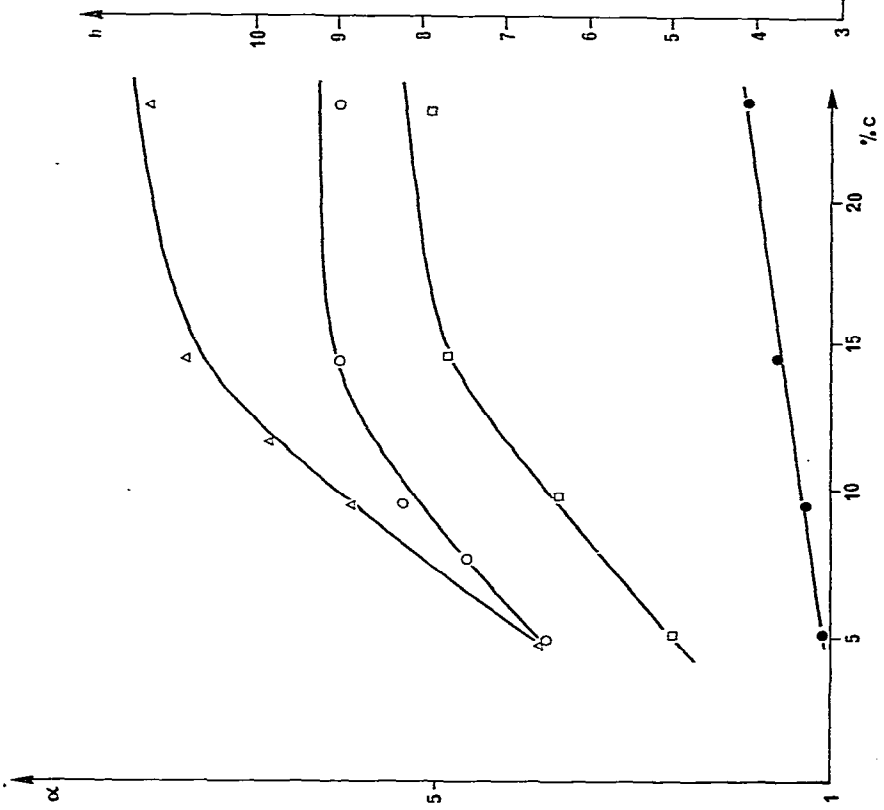


Fig. 5. Variation of the selectivity factor,  $\alpha$ , for various pairs of phenol compounds with the carbon content of the stationary phase ( $C_{18}$  bonded Partisil). Mobile phase: methanol-water (40:60).  $\bullet$ , Phenol-*p*-methoxyphenol;  $\circ$ , *p*-cresol-phenol;  $\Delta$ , *p*-cresol-methoxyphenol.

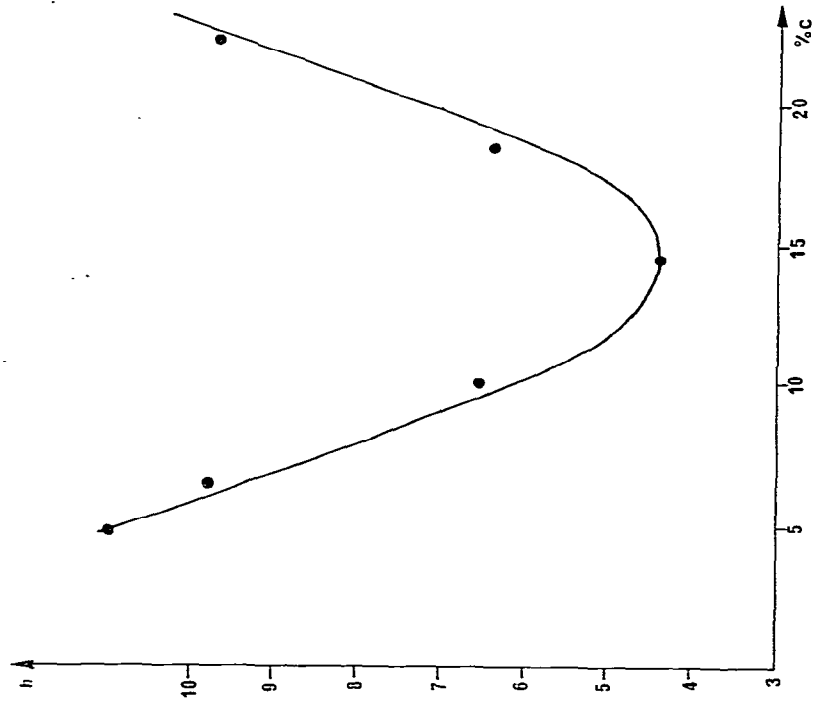


Fig. 6. Variation of the reduced plate height,  $h$ , with the carbon content of the stationary phase ( $C_{18}$  bonded Partisil) measured for a capacity ratio  $k' = 4$ .



*Effects of alkyl chain length at constant surface coverage ( $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ )*

This study was carried out with experimental Spherosil (specific surface area,  $520 \text{ m}^2\cdot\text{g}^{-1}$ ; pore volume,  $0.96 \text{ cm}^3\cdot\text{g}^{-1}$ ; average particle diameter,  $5.4 \mu\text{m}$ ).

The number of carbon atoms in the bonded alkyl chain varies from 4 to 18. Although the specific surface area of the silica used in this study was larger ( $520 \text{ m}^2\cdot\text{g}^{-1}$ ) than that of the silica used in the former study ( $402 \text{ m}^2\cdot\text{g}^{-1}$ ), the most suitable conditions found for the  $\text{C}_{18}$  Partisol bonded phase were used. The surface coverage is approximately  $2.1 \mu\text{mol}\cdot\text{m}^{-2}$  for every bonded phase synthesized in this study.

*Effect on capacity ratio.* Fig. 7 shows the variation of the logarithm of the capacity ratio of non-polar solutes with the length of the bonded chains. Hence, for non-polar solutes,  $k'$  increases exponentially with increase in the number of

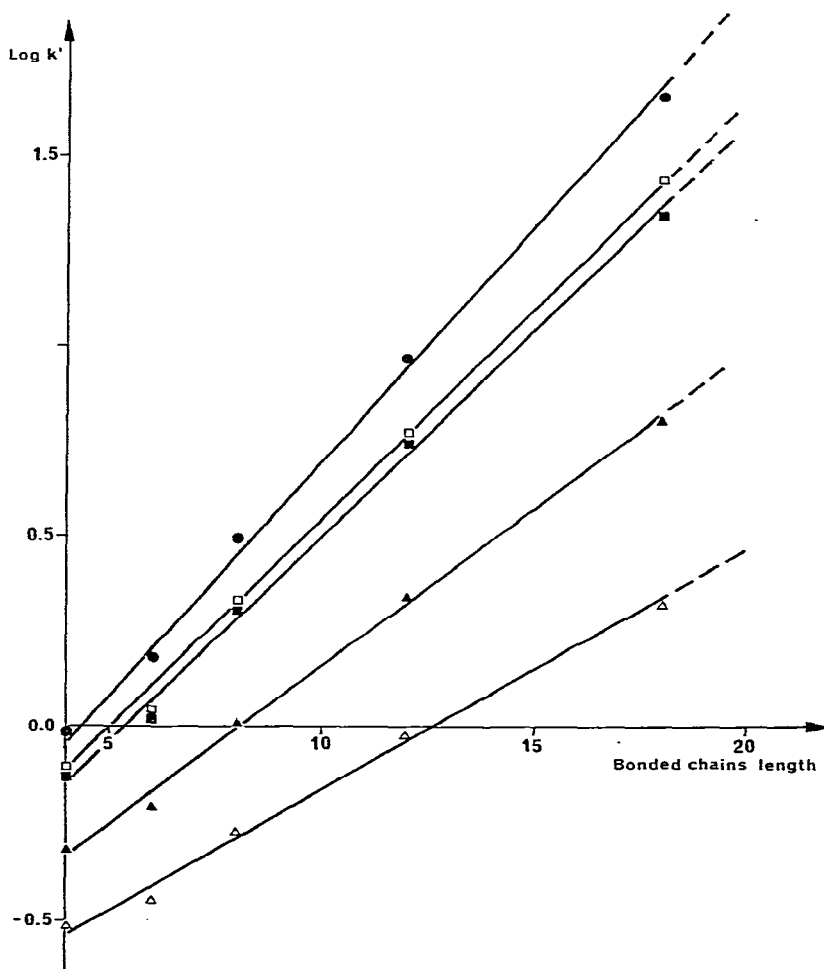


Fig. 7. Variation of the logarithm of the capacity ratio of aromatic hydrocarbons with the length of the alkyl bonded chain for a surface coverage of  $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ . Mobile phase: methanol-water (70:30).  $\triangle$ , Benzene;  $\blacktriangle$ , naphthalene;  $\blacksquare$ , phenanthrene;  $\square$ , anthracene;  $\bullet$ , pyrene.

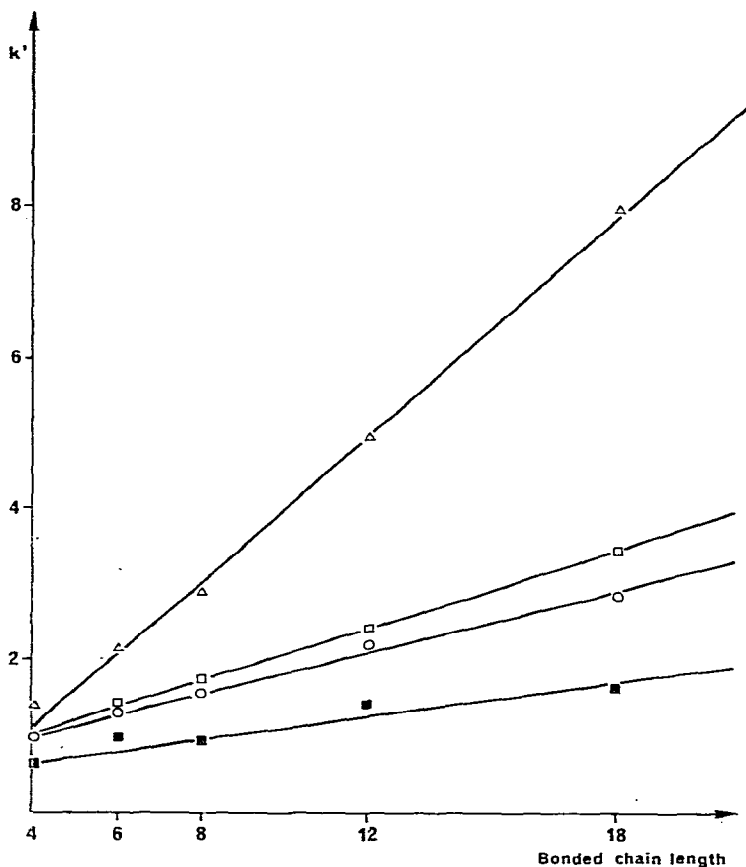


Fig. 8. Variation of the capacity ratio of phenol compounds with the length of the alkyl bonded chain for a surface coverage of  $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ . Mobile phase: methanol-water (40:60). ■, Resorcinol; ○, *p*-methoxyphenol; □, phenol; △, *p*-cresol.

carbon atoms in the bonded chain. Polar solutes show a different behaviour, as can be seen in Fig. 8: the relationship between  $k'$  and the bonded chain length is linear, as was reported by Hemetsberger *et al.*<sup>11</sup>.

These results indicate that the total length of the bonded chain interacts with the solute.

**Effect on selectivity.** As can be seen in Figs. 9 and 10, the selectivity factors increase with increasing length of the bonded chain for non-polar and polar solutes. This is not a general phenomenon in reversed-phase chromatography. Previous observations have shown a different selectivity behaviour with respect to the length of the bonded chains<sup>9,11,15</sup>.

**Effect on efficiency.** As the surface coverage is constant, the column efficiency remains approximately constant whatever the bonded chain length. However, for alkyl bonded chains with fewer than four carbon atoms, the column efficiency decreases because the unreacted silanol groups can take part in the separation mechanism more easily<sup>9</sup>.

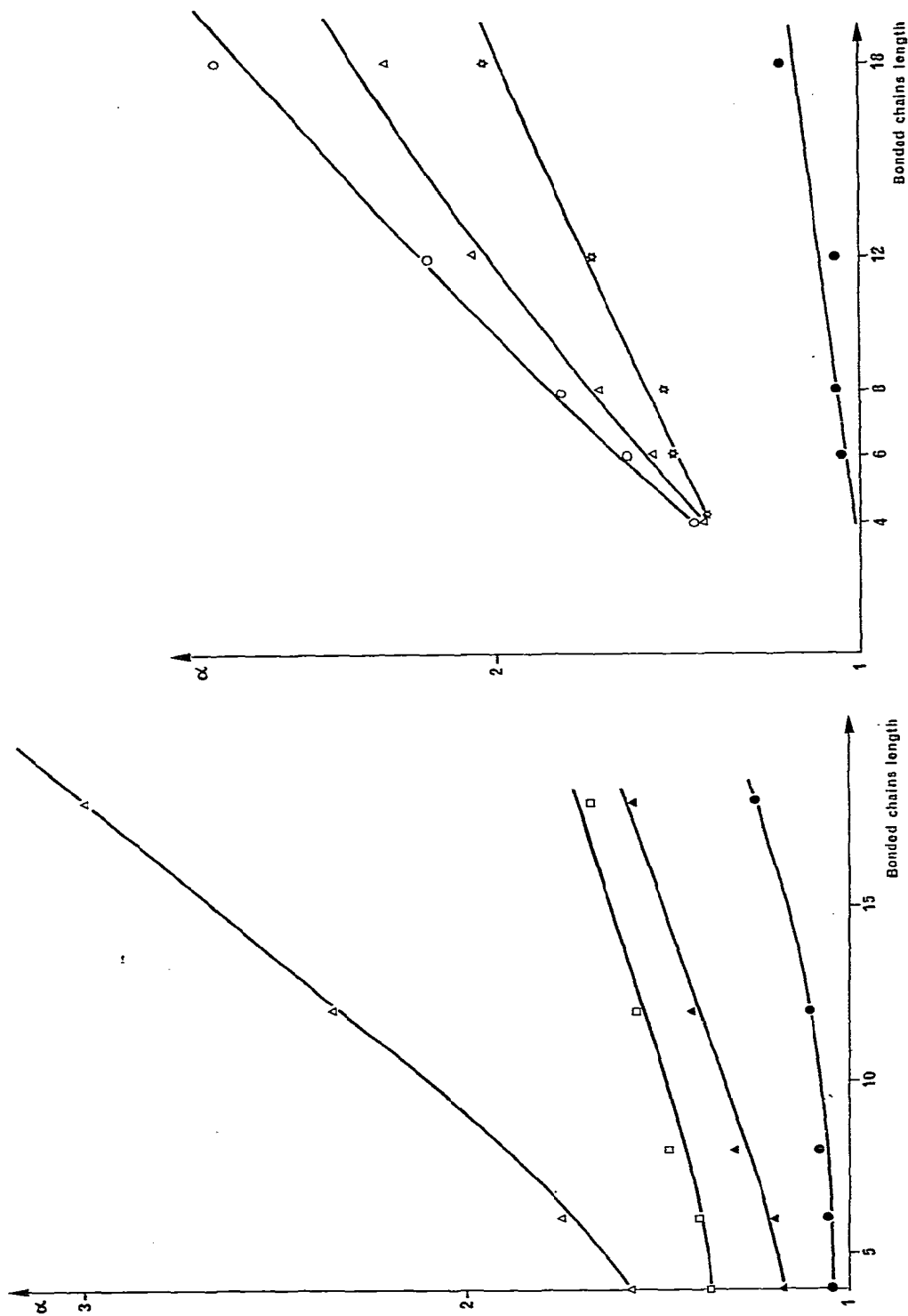


Fig. 9. Variation of the selectivity factor,  $\alpha$ , for various couples of hydrocarbon compounds with the length of the bonded chain (surface coverage  $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ ). Mobile phase: methanol-water (70:30). ●, Anthracene-pyrene; □, triphenylene-pyrene; ▲, anthracene-phenanthrene; △, pyrene-anthracene; ○, naphthalene-benzene.

Fig. 10. Variation of the selectivity factor,  $\alpha$ , for various couples of phenol compounds with the length of the bonded chain (surface coverage  $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ ). Mobile phase: methanol-water (40:60). ●, Phenol-*p*-methoxyphenol; ▲, methyresorcinol-resorcinol; ☆, *p*-cresol-*p*-methoxyphenol; ○, *p*-cresol-phenol.

## EFFECTS OF POLARITY OF MOBILE PHASE

Mobile phases commonly used in reversed-phase chromatography are mixtures of organic water-miscible solvents (especially methanol and acetonitrile) and water.

*Effect on capacity ratio*

Fig. 11 shows an exponential relationship between the capacity ratios of aromatic hydrocarbons and polarity of the mobile phase as measured by its water content. A solute will be eluted more quickly as the proportion of organic solvent in the eluent increases.

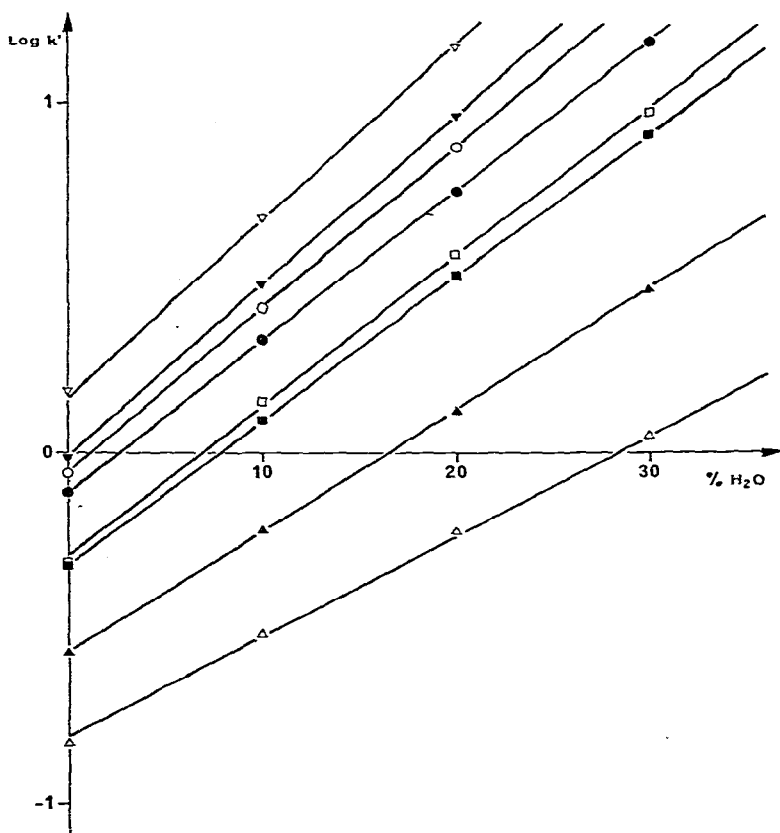


Fig. 11. Variation of the logarithm of the capacity ratio of aromatic hydrocarbons with the water content of the mobile phase ( $C_{18}$  bonded Partisil:11.5% C). Mobile phase: methanol-water mixtures.  $\Delta$ , Benzene;  $\blacktriangle$ , naphthalene;  $\blacksquare$ , phenanthrene;  $\square$ , anthracene;  $\bullet$ , pyrene;  $\circ$ , triphenylene;  $\blacktriangledown$ , chrysene;  $\nabla$ , 1,2-benzopyrene.

Much work has been carried out on this topic and different experimental relationships have been reported. However, it is frequently observed that  $\log k'$  varies linearly with the percentage of methanol when it is higher than 50% (v/v) for non-polar solutes<sup>6,11,23,30</sup>. For polar solutes, this applies for every percentage

except for pure water<sup>24,29</sup>. In fact, the rule is not simple and depends on the organic modifier<sup>24,29</sup> and the type of solute. We are studying the relationship between capacity ratio and mobile phase polarity.

#### *Effect on selectivity*

There is also an exponential relationship between the selectivity factor and the mobile phase polarity. However, it is impossible to increase the selectivity factor continuously by suitably adjusting the mobile phase composition. Indeed, an increase in the proportion of water in the mobile phase results in an increase in the capacity factor and consequently in the time of analysis. Moreover, an increase in column pressure drop occurs when the proportion of water in the eluent is increased, owing to the higher viscosity of the resulting water-methanol mixture. This may lead to sinking of the packing material in the column and consequently to irreversible degradation of the column. Therefore, a compromise between selectivity, time of analysis and pressure drop must be found.

#### SELECTION OF STATIONARY PHASE AND ELUENT

We have seen that for a given pair of solutes, the selectivity increases as the length of the bonded chains and their surface coverage increase. For instance, only the C<sub>18</sub> stationary phase with a surface coverage of 2.1  $\mu\text{mol}\cdot\text{m}^{-2}$  enables one to separate the three ethyltoluenes and to indicate by a slight shoulder in the graph the presence of *m*-xylene in the three xylenes.

However, such a phase with a high surface coverage leads to a prohibitively long time of analysis. To overcome this drawback, the polarity of the eluent can be decreased. Although this method inevitably leads to a decrease in selectivity, together with a shorter analysis time, it is nevertheless interesting to have a bonded stationary phase with a large surface coverage and long chains, as well as a low-polarity eluent. The practical advantage is a decrease in the high viscosity of the mobile phase (methanol has a viscosity of 0.6 N·sec·m<sup>-1</sup> and 70:30 water-methanol 1.5 N·sec·m<sup>-1</sup>) and consequently the pressure drop.

For instance, Fig. 12 shows two identical separations: one using as stationary phase a C<sub>18</sub> with a low surface coverage (carbon content 6.5% or 0.83  $\mu\text{mol}\cdot\text{m}^{-2}$ ) and as eluent a methanol-water (65:35) (pressure drop 80 bar), and the second using as stationary phase a C<sub>18</sub> with a high surface coverage (carbon content 14.4% or 2.1  $\mu\text{mol}\cdot\text{m}^{-2}$ ) and as eluent methanol-water (80:20) (pressure drop 60 bar). However, the resolution between the two peaks of phenanthrene and anthracene is better with the stationary phase of high surface coverage (14.5%) because the column efficiency is then at its maximum.

As far as possible, it is better to choose an octadecyl bonded stationary phase with a surface coverage of *ca.* 2  $\mu\text{mol}\cdot\text{m}^{-2}$ . With such a phase, it is possible to use a low-polarity eluent with a high methanol or acetonitrile content and thus to associate a minimal pressure drop with an optimal resolution. The decrease in the pressure drop should also improve the lifetime of the chromatographic columns.

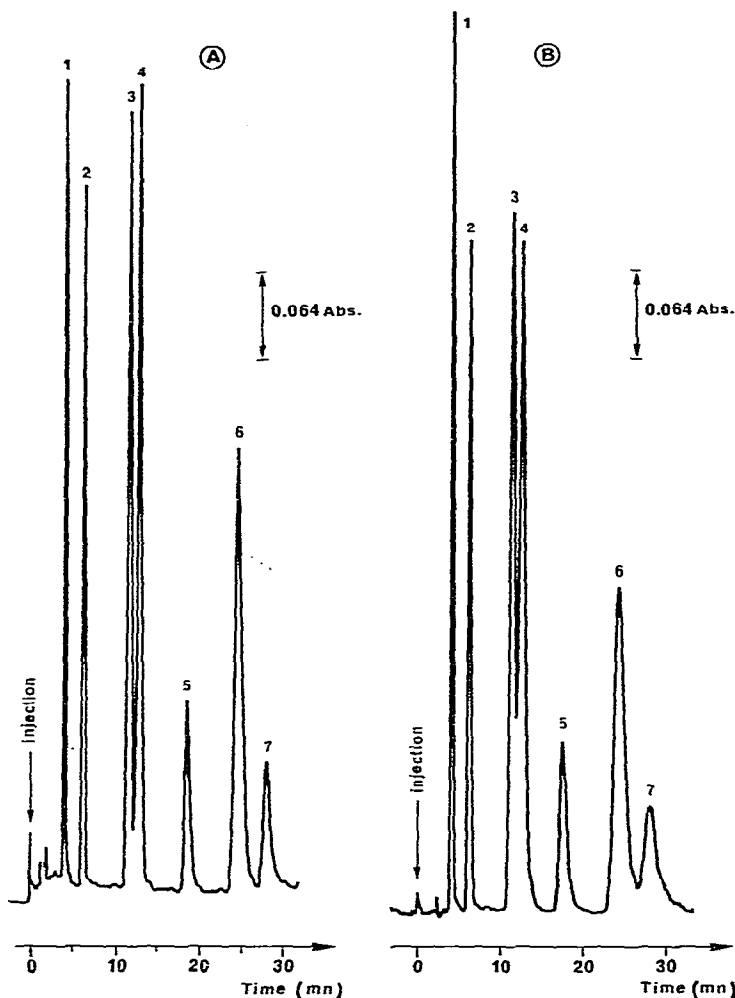


Fig. 12. Separation of aromatic hydrocarbons. Column: length 15 cm, I.D. 4.8 mm (O.D. 1/4 in.), packed with  $C_{18}$  bonded Partisil. A: carbon content, 14.5% ( $2.1 \mu\text{mol}\cdot\text{m}^{-2}$ ); mobile phase, methanol-water (80:20); pressure, 60 bar. B: carbon content, 6.5% ( $0.8 \mu\text{mol}\cdot\text{m}^{-2}$ ); mobile phase, methanol-water (65:35); pressure, 80 bar. Flow-rate,  $50 \text{ ml}\cdot\text{h}^{-1}$ ; detector, UV (254 nm). Solutes: 1 = benzene; 2 = naphthalene; 3 = phenanthrene; 4 = anthracene; 5 = pyrene; 6 = triphenylene; 7 = chrysene. Injection:  $3 \mu\text{l}$  of a solution about  $10^{-1} M$  in each hydrocarbon.

## CONCLUSION

We have demonstrated the relative importance of the three main parameters in reversed-phase liquid chromatography: the length of the chains bonded to silica, the surface coverage of these chains and the composition of the mobile phase.

The surface coverage and the length of the bonded chains play a similar role in reversed-phase chromatography to that played by the activity of silica in adsorption chromatography. It is well known that in order to obtain reproducible results

in adsorption chromatography it is necessary to control the water content of the mobile phase. In the same way, in reversed-phase chromatography it is necessary to determine the length and the surface coverage for each bonded stationary phase. We therefore think that the various manufacturers should state all of the characteristics of their bonded phases.

#### ACKNOWLEDGEMENTS

This work was supported by grant No. 76-7-1154 from the Délégation Générale à la Recherche Scientifique et Technique, which is gratefully acknowledged. The authors thank Rhône-Poulenc for the gifts of samples of experimental Spherosil and Professor Robert Rosset for reading the manuscript and helpful discussions.

#### EDITOR'S NOTE

During the 6-month period when this paper was being refereed and revised, a number of authors have dealt with most of the points covered in this paper. However, as the topic is of great interest at the moment, it was felt that results of several authors should be published.

#### REFERENCES

- 1 A. Pryde, *J. Chromatogr. Sci.*, 12 (1974) 486.
- 2 V. Rehak and E. Smolkova, *Chromatographia*, 9 (1976) 219.
- 3 P. A. Bristow, *Liquid Chromatography in Practice*, hept, Wilmslow, 1976, Ch. 6.
- 4 C. B. Cox, *J. Chromatogr. Sci.*, 15 (1977) 385.
- 5 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
- 6 R. E. Majors, *Analisis*, 3 (1975) 549.
- 7 R. E. Majors, *J. Chromatogr. Sci.*, 15 (1977) 334.
- 8 J. J. Kirkland, *Chromatographia*, 8 (1975) 661.
- 9 R. E. Majors and M. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.
- 10 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *Anal. Chem.*, 46 (1974) 1314.
- 11 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 9 (1976) 303.
- 12 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
- 13 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 14 E. J. Kikta and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
- 15 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
- 16 D. Aslin, *Int. Lab.*, July/August (1977) 59.
- 17 R. K. Gilpin and M. F. Burke, *Anal. Chem.*, 45 (1973) 1383.
- 18 D. G. I. Kingston and B. B. Gerhart, *J. Chromatogr.*, 116 (1976) 182.
- 19 B. B. Wheals, *J. Chromatogr.*, 107 (1975) 402.
- 20 J. M. Bather and R. A. C. Gray, *J. Chromatogr.*, 122 (1976) 159.
- 21 B. B. Wheals, C. G. Vaughan and M. J. Whitehouse, *J. Chromatogr.*, 106 (1975) 109.
- 22 K. O. Hiller, B. Masloch and H. J. Mockel, *Z. Anal. Chem.*, 283 (1977) 109.
- 23 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
- 24 B. L. Karger, J. Russel Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
- 25 R. P. W. Scott and K. Kucera, *J. Chromatogr.*, 142 (1977) 213.
- 26 M. J. Telepchak, *Chromatographia*, 6 (1973) 234.
- 27 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
- 28 C. McAuliffe, *J. Phys. Chem.*, 70 (1966) 1267.
- 29 C. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 30 K. Karch, I. Sebastian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.