Journal of Chromatography, 364 (1986) 25–37
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 904

CONFORMATIONAL BEHAVIOUR OF ALKYL CHAINS OF REVERSED PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ERNST BAYER*, ARAN PAULUS, BERNADETTE PETERS, GABRIELE LAUPP, JÜRGEN REINERS and KLAUS ALBERT
Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-7400 Tübingen (F.R.G.)

SUMMARY

Monofunctional reversed-phase materials with different carbon contents were prepared and their chromatographic behaviours examined. Using NMR techniques, the mobility of the alkyl chains of these materials was determined. It was found that a relationship exists between the retention data of a given test mixture and the mobility of the alkyl chain, which is influenced by both carbon content and solvent composition of the mobile phase.

INTRODUCTION

Although reversed-phase chromatography enjoys high popularity, our understanding of the separation process involved is still low. According to the solvophobic theory[1,2], the main reason for retention and selectivity in reversed-phase liquid chromatography is the solubility of the solute in the mobile phase. The stationary phase is assumed to have a non-polar surface, formed by hydrocarbonaceous ligands. In the discussion of the separation process, the influence of stationary phase properties such as surface concentration and dynamic behaviour of alkyl chains as well as residual silanol groups have to be considered.

From practical experience, it is well known that the mobile phase is easier to reproduce than the stationary phase[3]. The reasons for differing chromatographic behaviour of stationary phases from different manufacturers can be attributed partly to the different properties and pretreatments of the original silica and partly to the method and extent of modification. Even phases modified with the same hydrocarbonaceous ligand, *e.g.* octadecyl phases, are different if different modifying agents (mono-, di- or trifunctional alkylsilanes) or surface coverage and a possible post-treatment ("endcapping") is used[4–8].

Information about stationary phase properties under chromatographic conditions have been derived from chromatographic measurements[2,4,6,7]. Surface structures of reversed-phase material were examined by IR spectroscopy[9], differential scanning calorimetry[10], by studying the luminescence of pyrene silanes bound to silica[11,12] and recently by NMR measurements[13–23]. We have continued our work in the latter field[23], in which we used ^{13}C and ^{29}Si-cross-polarization magic angle spin-

0021-9673/86/$03.50

ning (CP-MAS) NMR spectroscopy for surface investigations of reversed-phase materials, by further NMR studies of monoalkylfunctional silicas under more real chromatographic conditions in the presence of mobile phase.

Whereas ^{13}C and ^{29}Si CP-MAS-NMR spectroscopy has been proved to be an excellent method for the characterization of the solid-state structure of the reversed-phase material and allows us to distinguish between different binding sites of the hydrocarbonaceous ligands, it is of limited value to investigate the dynamic behaviour in the presence of mobile phase and solute. Therefore, we investigated suspensions of reversed-phase materials in solvent mixtures normally used in chromatography, like acetonitrile–water. An advantage of such ^{13}C-NMR measurements in suspension is the possibility of obtaining information about the mobility of the ligands in the presence of solvents by determining ^{13}C-spin lattice relaxation times, which is not possible by CP-MAS-NMR spectroscopy. Gilpin and co-workers reported data with ^{13}C-enrichment at the terminal C-atoms. However, we were interested to avoid the laborious preparation of ^{13}C-enriched reversed-phase material, and rather to use readily available reversed-phase material with the natural ^{13}C-abundance. In this case, also, the dynamic properties of the medium, main part of the alkyl chain can be determined, *e.g.* C_4–C_{15} of an octadecyl phase. The relaxation time of a terminal C_{18}-atom is not as characteristic for the medium segment mobility of the alkyl chain, because its dynamic properties are dominated by rotation about the end bond.

Three reversed phases with varying carbon contents were prepared, and their ^{13}C-NMR spin lattice relaxation times in various acetonitrile–water mixtures were investigated. In this way, we obtained data relevant to the average segment mobility of the alkyl chain. This, we believe, is important for chromatographic process.

Simultaneously, retention and selectivity data of these phases were examined by chromatographic measurements, and correlated to the NMR data.

EXPERIMENTAL

Preparation of C_{18} reversed-phase supports

A 5-g quantity of Nucleosil 100, 7 μ (Macherey & Nagel, Düren, F.R.G.), 3.5 mmol OH/g, was dried at 150–200°C under vacuum for 24 h and then suspended in 250 ml of 1,2,4-trichlorobenzene. In a nitrogen atmosphere, octadecyldimethylmethoxysilane (20 mmol, resulting in chemically bonded silica gel phase LAB I; or 13 mmol, resulting in phase LAB II) was added and the mixture refluxed for 24 h. The reaction product was filtered off, washed with several portions of 1,2,4-trichlorobenzene and methanol and dried under vacuum for 30 h (0.05 Torr = 6.6 Pa).

Phase LAB II (5 g) was endcapped in 250 ml of trichlorobenzene under nitrogen atmosphere with 8 mmol of trimethylchlorosilane and 8.7 mmol of hexamethyldisilazane. The mixture was worked up as above.

In order to prove whether the alkyl chains are really bound to the silica surface, the home-made phases were investigated by ^{13}C CP-MAS-NMR spectroscopy. Fig. 1 shows ^{13}C CP-MAS-NMR spectra of phase LAB II before (a) and after (b) endcapping. During the process of endcapping, free OH groups react with $(CH_3)_3SiCl$ and $[(CH_3)_3Si]_2NH$. Because of the increased concentration of $Si–CH_3$ groups after endcapping, the resonance at 0.3 ppm attributed to silicon-bound methyl groups

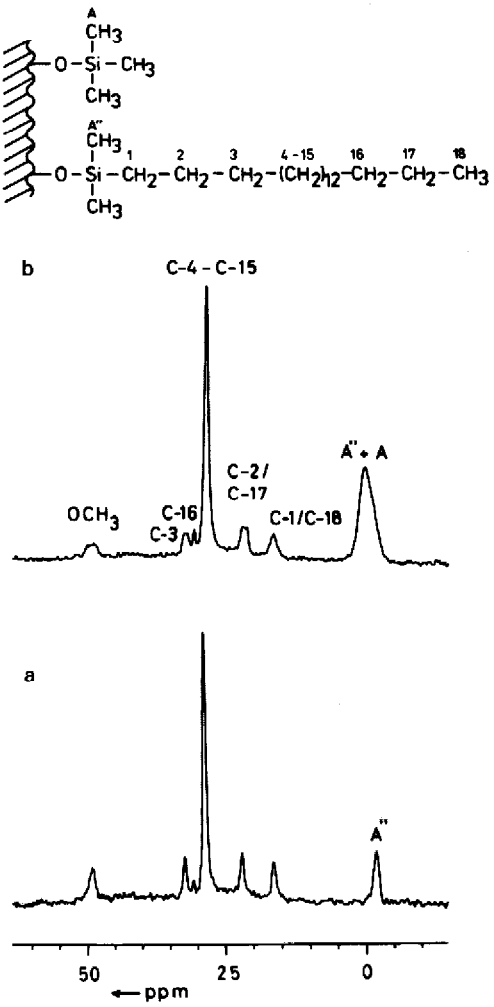

Fig. 1. ^{13}C CP-MAS-NMR spectra (75.46 MHz): (a) of the reaction product of *n*-octadecyldimethylmethoxysilane with silica gel Nucleosil 100, 7 μm (phase LAB II); (b) after endcapping of phase LAB II with hexamethyldisilazane–trichlorosilane.

$-Si(CH_3)_2R$ (A'') and $Si(CH_3)_3$ (A)[23] increases. Carbon atoms C_1 and C_{18} absorb at 16.6 ppm and the resonance of C_2 and C_{17} lies at 22.2 ppm. The strong signal at 28.7 ppm must be assigned to C_4–C_{15}, the peaks at 30.8 ppm and 32.5 ppm to C_{16} and C_3, respectively. At 49.7 ppm, the resonance of adsorbed methanol can be observed. These assignments were described earlier[23].

Elemental analysis of the stationary phase was performed on a Carlo Erba Model 1104 elemental analyser.

NMR studies

CP-MAS spectra. The ^{13}C NMR spectra were obtained on a Bruker CXP-300 Fourier transform NMR spectrometer with samples of 100–200 mg in 6.3 mm I.D.

boron nitride rotors. CP-MAS was used with alternating inversion of the 90° pulse phase. The proton 90° pulse length was 4.5 μs. The contact times were 3 ms and repetition times 4–6 s. The spinning speed was between 3.5 and 5.0 kHz; hence, side-bands do not appear for those compounds with a low anisotropic chemical shift tensor. The variation of the magic angle was checked with glycine between the experiments; the line width of the carbonyl signal never exceeded 30 Hz. The chemical shifts were referenced to tetramethylsilane, using the carbonyl signal of glycine as a secondary standard (179.09 ppm).

Suspension NMR spectra. ^{13}C NMR spectra were measured in 10-mm sample tubes on a Bruker WH 90 Fourier transform NMR spectrometer using proton broadband decoupling at 303 K. A 600-mg quantity of the silica gel phase was suspended in 2 ml of solvent and the resulting slurry was adjusted to a height of 10 mm in the NMR tube by means of a PTFE plug with an inner diameter of 6 mm.

For the relaxation time measurements, a 90° pulse of 16 μs at a measuring time of 0.68 s (6000 Hz/8 K) and a waiting time of 1 s was employed. Typical spectra represented the sum of 5000–10 000 scans.

Chromatographic studies

The chromatographic apparatus used was a Hewlett-Packard 1084 liquid chromatograph equipped with a high-speed UV diode-array detector (HP 1040). Chromatograms were recorded at 254 nm and stored on disk. Each chromatographic run was repeated at least three times; random standard deviation was in all cases better than 1%. Spectra of the solutes were taken to check the sequence of elution.

Stainless-steel Hyperchrome columns, 250 × 4.6 mm I.D. (Bischoff, Leonberg, F.R.G.), were packed using a Shandon packing pump (Shandon, Frankfurt, F.R.G.). The slurry of 3 g of stationary phase in 35 ml of isopropanol, shaken for 5 min in an ultrasonic bath, was filled into the columns using 150 ml of methanol as packing solvent.

The mobile phase used in the chromatographic experiments was acetonitrile–water (70:30), mixed by the two pumps. Acetonitrile was purchased from Riedel-de Haen (Hannover, F.R.G.) and water was prepared in high purity by a Milli-Q unit (Millipore, Neu-Isenburg, F.R.G.).

Samples of pro analisi grade were purchased from various suppliers (Merck, Darmstadt, F.R.G.; Fluka, Neu-Ulm, F.R.G.; Riedel-de Haen). Column dead volumes for the calculations of capacity factors (k') were measured using sodium nitrate[24].

RESULTS AND DISCUSSION

NMR investigations

Examination of the dynamic behaviour of the alkyl chains was accomplished by measuring spin lattice relaxation times of phases suspended in acetonitrile or other solvents. Since the stationary phase interacts with the solvent, these conditions seem to be a good approach to conditions in the column during the separation process. In spite of poorer resolution of suspension spectra compared to CP-MAS spectra, the characteristic signals of silica-bound octadecyl chains are well recognizable (Figs. 2–4). However, the chemical shift of the individual carbon atoms is the same in suspension NMR spectra as compared to ^{13}C CP-MAS-NMR spectra.

Spin lattice relaxation times (T_1) were measured by the inversion recovery method. The pulse sequence consists of a 180° pulse, a variable waiting time, τ, and a 90° pulse. In the case of longitudinal relaxation, stimulated nuclei lose energy to their surroundings (spin lattice relaxation) by interacting with local, fluctuating electromagnetic fields. The frequencies of these fields are identical with the larmor frequencies of the nuclei. Because the origin of the electromagnetic fields is due to movement of nuclei, one can examine the dynamic behaviour of molecules by measuring spin lattice relaxation times.

After having been inversed by the 180° pulse, magnetization M_z relaxes during the waiting time τ. Depending on the length of τ, the signal resulting after the 90° pulse is negative (inverted), zero (no absorption) or positive (absorption). In the case of no absorption, the waiting time, τ, is directly proportional to T_1:$T_1 = \tau/\ln 2$.

By measurements of temperature dependence, it could be proved that increasing T_1 values of the CH_2 groups correlate with increasing mobility.

Fig. 2 shows spin lattice relaxation time measurement of the phase with the lowest carbon coverage LAB II in [$^2H_3$]acetonitrile. In addition to the septet at 1.3 ppm of the C^2H_3 group of the solvent, a signal at 15 ppm attributed to the terminal methyl group is visible. The signal of the carbon atom C_{17} appears at 24 ppm, C_4–C_{15} absorb at 30 ppm and the resonance signal of C_3 and C_6 is recognizable as a shoulder on the resonance at 24 ppm. The signals of C_1 and C_2 are severely broad-

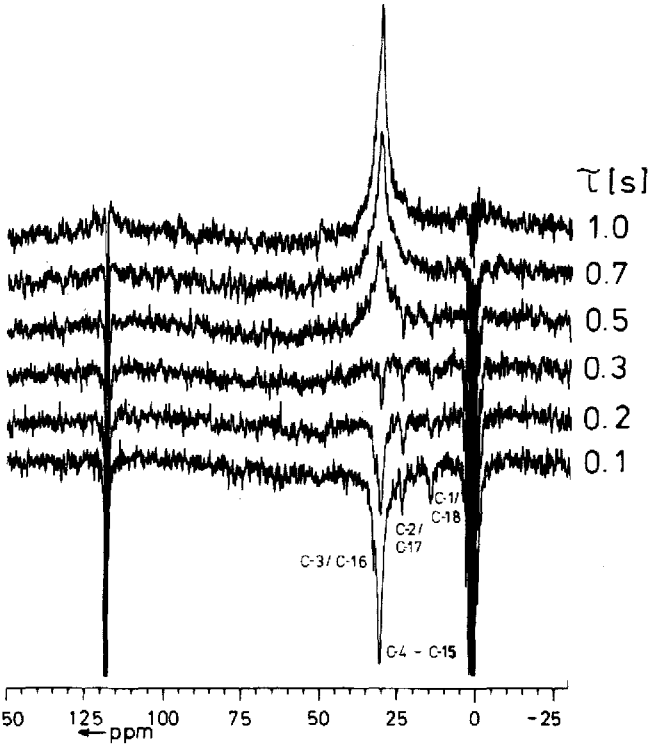

Fig. 2. Inversion recovery spectra (22.63 MHz) of the suspension of phase LAB II in [$^2H_3$]acetonitrile.

ened as consequence of bonding to the rigid silica surface and therefore cannot be observed. Even if the C_1-position is ^{13}C-enriched, only a very weak and broad signal is obtained[14]. Chemical shifts were assigned by increment calculations, by comparison with the ^{13}C CP-MAS-NMR spectra, obtained from various reversed phases[23] as well as by comparison with the shift data of the soluble silane H_3CO–$Si(CH_3)_2$–$(CH_2)_{17}CH_3$. The latter were assigned by the use of fully coupled spectra and by the differences in relaxation times between C_1–C_{18} and C_2–C_{17}, respectively[25]. These data agree also with reported shift values[15].

In octadecyl phases, the relaxation time T_1 of the signal at 30 ppm represents the average mobility of the alkyl chain, namely of C_4–C_{15}. The relaxation of C_1, C_2, C_{17} and C_{18}, the dynamic behaviour of which differs from that of the intermediate main part of the alkyl chain, makes no contributions to this T_1 value, since they absorb at a higher field. The T_1 value of LAB II (carbon content 8.32%) in $[^2H_3]$acetonitrile was 0.48 s; after endcapping (carbon content increases to 10.83%), 0.29 s (Fig. 3). Thus, the process of endcapping reduces drastically the mobility of the alkyl chains. In all cases, mobility of the terminal carbon atoms C_{17} and C_{18} is greater than the average mobility of C_4–C_{15}, as can be seen by comparing zero tran-

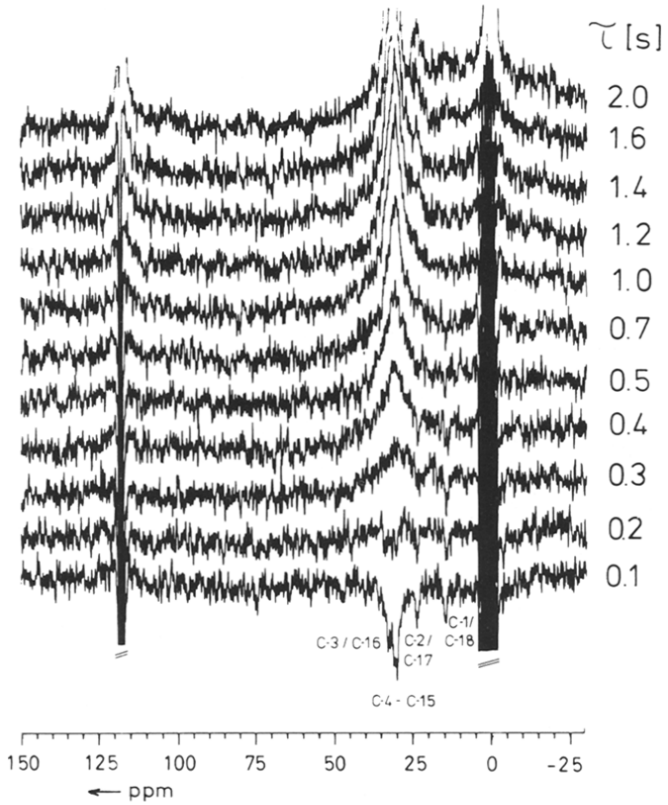

Fig. 3. Inversion recovery spectra (22.63 MHz) of the suspension of phase LAB II endcapped in $[^2H_3]$acetonitrile.

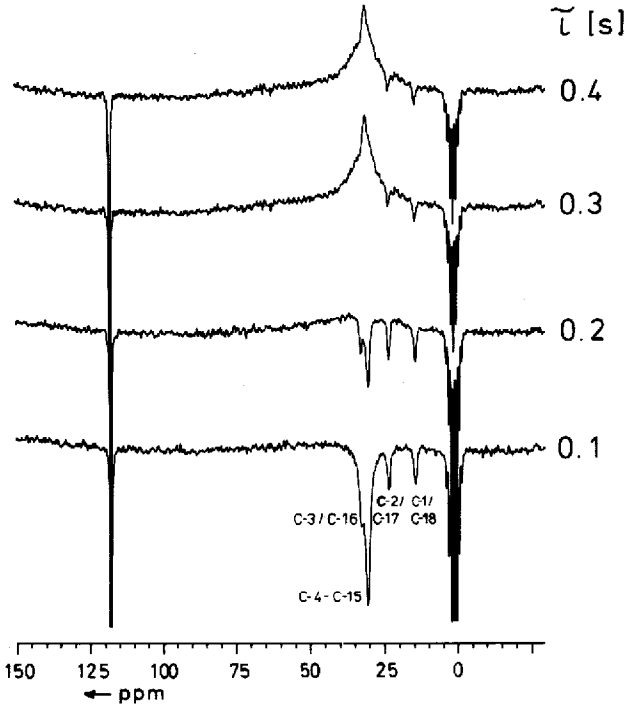

Fig. 4. Inversion recovery spectra (22.63 MHz) of the suspension of phase LAB I in [$^2H_3$]acetonitrile.

sition of the signals at 30 ppm (C_4–C_{15}), 17 ppm (C_{17}) and 15 ppm (C_{18}). Fig. 4 shows relaxation time measurement of LAB I in [$^2H_3$]acetonitrile.

Despite the higher carbon content of LAB I (12.53% C), T_1 of this phase in [$^2H_3$]acetonitrile–[$^2H_2$]water (70:30, Table I) is higher than that of endcapped LAB II (0.35 and 0.29 s, respectively), corresponding to lower mobility. Therefore, we conclude that a lower mobility of the alkyl chains and a more rigid conformation is not merely dependent on the bulk carbon content. Also, a reaction of the silanol groups with the short trimethylsilyl chains causes a lower mobility of the long octadecylsilyl chains. It could be concluded that the characterization of reversed phases by the carbon content is too simple if endcapped and non-endcapped materials are

TABLE I

SPIN LATTICE RELAXATION TIMES, T_1, OF THE ALKYL CHAIN OF LAB I, LAB II AND LAB II ENDCAPPED IN [$^2H_3$]ACETONITRILE AND [$^2H_3$]ACETONITRILE–[$^2H_2$]WATER (70:30)

	LAB II	*LAB I*	*Endcapped LAB II*
Carbon content	8.32% C	12.53% C	10.83% C
Functionalized silanol groups	0.35 mmol	0.52 mmol	1.04 mmol
T_1 values, 100% [$^2H_3$]acetonitrile	0.48 s	0.35 s	0.29 s
T_1 values, [$^2H_3$]acetonitrile–[$^2H_2$]water (70:30)	0.46 s	0.34 s	0.28 s

TABLE II

SPIN LATTICE RELAXATION TIMES, T_1, OF THE ALKYL CHAIN OF LAB I IN DIFFERENT SOLVENTS

Solvent	T_1 *value (s)*
100% [$^2H_3$]Acetonitrile	0.35
[$^2H_3$]Acetonitrile–[$^2H_2$]water (70:30)	0.34
[$^2H_3$]Acetonitrile–[$^2H_2$]water (50:50)	0.32
[$^2H_3$]Acetonitrile–[$^2H_2$]water (10:90)	0.29

compared. The mobility of the alkyl chain is dependent upon the degree of functionalization of the silanol groups. However, it can be seen from Table I that the molarity of functionalization corresponds nicely with increasing mobility.

In order to investigate the influence of different solvents on the dynamic behaviour of the alkyl chains, we measured spin lattice relaxation times in mixtures of [$^2H_3$]acetonitrile and [$^2H_2$]water. In all cases, and independent of the degree of functionalization, T_1 decreases with increasing water content of the mobile phase (Table II), indicating that water reduces the mobility of the alkyl chains on the silica surface. Thus, the chains become more rigid and spin lattice relaxation times decrease.

HPLC experiments

In order to test the chromatographic properties of stationary phases, the test mixture of solutes should fulfil several requirements. According to solvophobic theory, the interaction between non-polar hydrocarbonaceous ligands and solute molecules should increase with increasing contact area. We have taken four homologous alkylbenzenes (toluene, ethylbenzene, *n*-propylbenzene and *n*-butylbenzene), the retention times of which should be an indication of the non-polar surface area of the stationary phase. On the other hand, polar solutes show the so-called silanophilic interactions between the polar groups of the sample molecules and accessible silanol groups at the stationary phase surface[26]. A stationary phase with a high accessible silanol concentration should show strong interaction with the polar moiety of the solute molecule and therefore result in a higher retention in comparison to a phase with a low accessible silanol concentration. Four polar components which can be derived from toluene (benzamide, benzylalcohol, benzaldehyde, methylbenzoate) were chosen. Absolute retention and the range of retention of these four polar solutes give chromatographic information about the influence of silanol groups. Test chromatograms obtained with this test mixture on phases LAB II, LAB I and LAB II (endcapped) are shown in Figs. 5–7.

In Table III, the important chromatographic data and some stationary phase characteristics are summarized. The stationary phases are arranged according to increasing k' values for the alkylbenzenes, which does not necessarily correspond with the carbon content of the stationary phases, but with the degree of functionalization of silanol groups.

The k' values of the alkylbenzenes range from 1.31 to 2.69 on the non-endcapped phase LAB II with 8.32% C, from 1.91 to 3.92 on phase LAB I with 12.53% C, and from 2.24 to 5.18 on the endcapped phase LAB II with 10.83% C. The relative

TABLE III

k' VALUES OF HOME-MADE PHASES LAB II, LAB I AND ENDCAPPED LAB II

Solute	*Non-endcapped LAB II (8.32% C ≡ 0.35 mmol ODS)*		*Non-endcapped LAB I (12.53% C ≡ 0.52 mmol ODS)*		*Endcapped LAB II (10.83% C ≡ 1.04 mmol ODS)*	
	k'	α	k'	α	k'	α
Benzamide	0.69		0.63		0.56	
		1.16		1.27		1.41
Benzylalcohol	0.80		0.80		0.79	
		1.36		1.46		1.52
Benzaldehyde	1.08		1.16		1.20	
		1.21		1.28		1.29
Methylbenzoate	1.31		1.48		1.55	
		1.18		1.29		1.44
Toluene	1.54		1.91		2.24	
		1.17		1.23		1.28
Ethylbenzene	1.80		2.34		2.86	
		1.22		1.29		1.34
n-Propylbenzene	2.19		3.02		3.84	
		1.23		1.30		1.35
n-Butylbenzene	2.69		3.42		5.18	

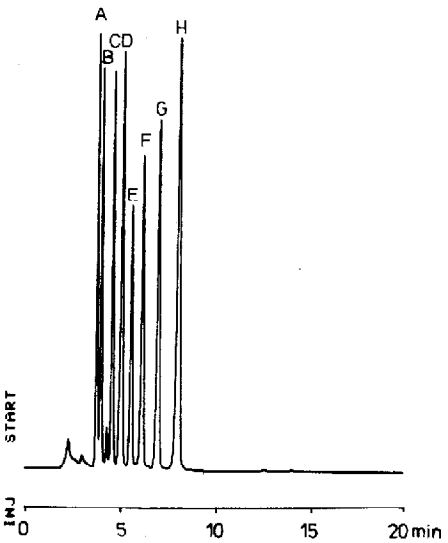

Fig. 5. Test chromatogram on reversed-phase LAB II, non-endcapped. Conditions: column, 250 × 4.6 mm I.D.; mobile phase, acetonitrile–water (70:30); flow-rate, 1.0 ml/min; detection, UV 254 nm; solutes: (A) methylbenzoate, (B) benzylalcohol, (C) benzaldehyde, (D) methylbenzoate, (E) toluene, (F) ethylbenzene, (G) *n*-propylbenzene, (H) *n*-butylbenzene.

retention times (α) of ethylbenzene to toluene on these phases are 1.17, 1.22 and 1.28, respectively. For the *n*-propylbenzene–ethylbenzene pair, α values of 1.22, 1.29 and 1.34 are found; the corresponding values for the *n*-butylbenzene–*n*-propylbenzene pair are 1.23, 1.30 and 1.35.

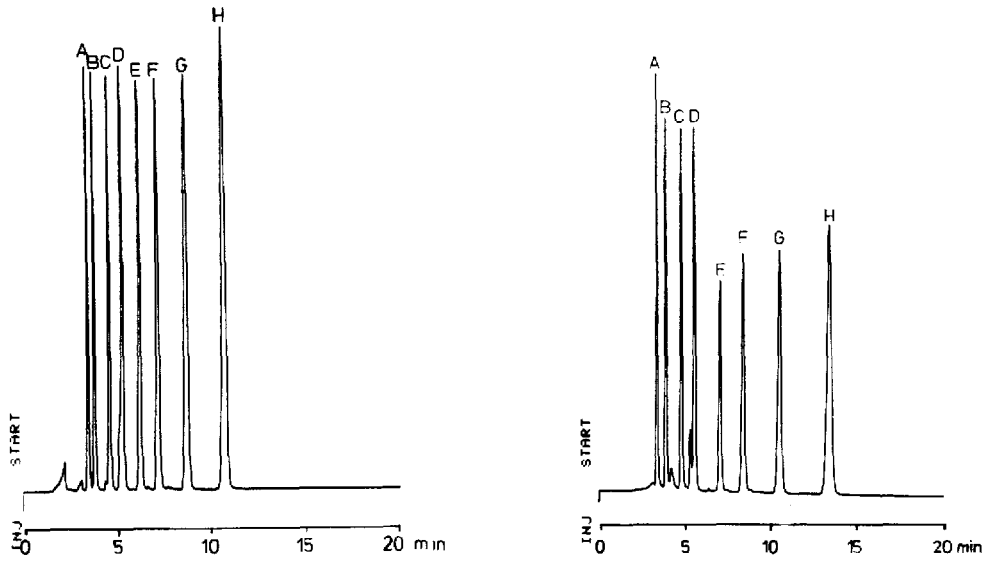

Fig. 6. Test chromatogram on reversed-phase LAB I, non-endcapped. Conditions and abbreviations same as in Fig. 5.

Fig. 7. Test chromatogram on reversed-phase LAB II, endcapped. Conditions and abbreviations same as in Fig. 5.

The fact that the α values of the homologous pairs of alkylbenzenes is the same for each phase and also that the differences in selectivity of each solute pair are identical for all phases, thus indicates a separation mechanism according to solvophobic interaction.

Retention and selectivity of the polar solutes are influenced by both hydrophobic and silanophilic interactions. The effect of reducing the silanophilic interaction by endcapping is evident upon comparison of the endcapped phase LAB II with the two others. The retention of the most polar solute (benzamide) is reduced on the endcapped LAB II. For the same reason, k' values for benzamide depend on the carbon content of non-endcapped phases. Retention decreases when carbon content, and therefore in this case surface concentration of alkyl chains, increases (see LAB II with 8.32% C *vs.* LAB I with 12.53% C). The k' value of benzylalcohol is the same for all stationary phases because the reduction of silanol groups is compensated by an increasing hydrophobic surface area. Retention of the benzaldehyde and methylbenzoate seems to be governed by the same hydrophobic interactions as the alkylbenzenes.

Correlation of HPLC experiments and NMR measurements

As discussed above, retention of all solutes, except the most polar (benzamide), is governed by their hydrophobic interaction with the alkyl chains of the stationary phase. Therefore, one would expect an increase of retention with an increase of the accessibel hydrophobic compartment. However, in spite of the higher carbon content (and therefore more alkyl chains bound to the silica surface) of LAB I compared to endcapped LAB II, retention on endcapped LAB II is higher than on LAB I. This

TABLE IV

k' VALUES OF SOME PHENYLALKANES, OBTAINED WITH DIFFERENT ACETONITRILE–WATER MIXTURES

Stationary phase, LAB I; flow-rate, 1 ml/min.

Solute	*Amount acetonitrile in mixture (%)*			
	100	*70*	*50*	*30*
Toluene	0.75	1.91	3.50	13.6
Ethylbenzene	0.75	2.34	4.67	23.8
n-Propylbenzene	0.81	3.02	6.46	>30
n-Butylbenzene	0.96	3.42	9.02	>30

means that the accessible hydrophobic compartment increases from LAB I to endcapped LAB II and is the reason for the difference in mobility of the hydrocarbonaceous ligands of both phases. The mobility of bonded ligands is a function of surface coverage, since the available space of a single chain decreases with increasing coverage (see LAB II *vs.* LAB I). Endcapping also decreases mobility, because the trimethylsilyl groups favour a more rigid conformation of the carbon atoms near the silica surface, the concentration of silanol groups is reduced and therefore the hydrophobic compartment of the stationary phase is increased.

There is a distinguished correlation between the mobility of the alkyl chains and the retention. Higher mobility, corresponding to more extented conformation, results in lower k' values (Table III). Low mobility, corresponding to a more collapsed conformation, correlates with larger k' values. Therefore, the measurement of T_1 values seems to be a reasonable parameter to characterize the hydrophobicity of bonded phases. This is important for evaluation of commercial stationary phases usually differing in surface coverage and number of endcapped silanol groups. The mobility of the alkyl chains would be a more meaningful parameter than carbon content to allow for prediction of retention behaviour.

Retention and selectivity also depend upon the interaction of the mobile with the stationary phase, as is exemplified by measurements on phase LAB I. A significant reduction of T_1 from 0.35 to 0.29 s is found when the solvent is changed from pure acetonitrile to 10% acetonitrile in water (see Table II). With increasing water content in the mobile phase, an increase in retention time is observed. This can be explained by a more hydrophobic compartment in the stationary phase.

In a mobile phase with a high water content, there is only a small number of acetonitrile molecules at the interface of mobile and stationary phases. By hydrophobic interaction, the alkyl chains tend to aggregate and, in the NMR experiments, this is measured as a reduced mobility. For the same reason, the absorption of a solute molecule is, at high water content, much stronger and retention time increases. In Table IV, k' values of some phenylalkanes are listed for several acetonitrile–water mixtures. The increase of the k' values is reciprocal to the decrease of the spin lattice relaxation times of the alkyl chains in Table II.

From our measurements, it is apparent that the formerly proposed "brush-model" for covalently bound alkyl chains on silica surfaces is a rather crude model. The alkyl chain is not a fixed unit "stick"[2,4] but rather a flexible chain, one end of

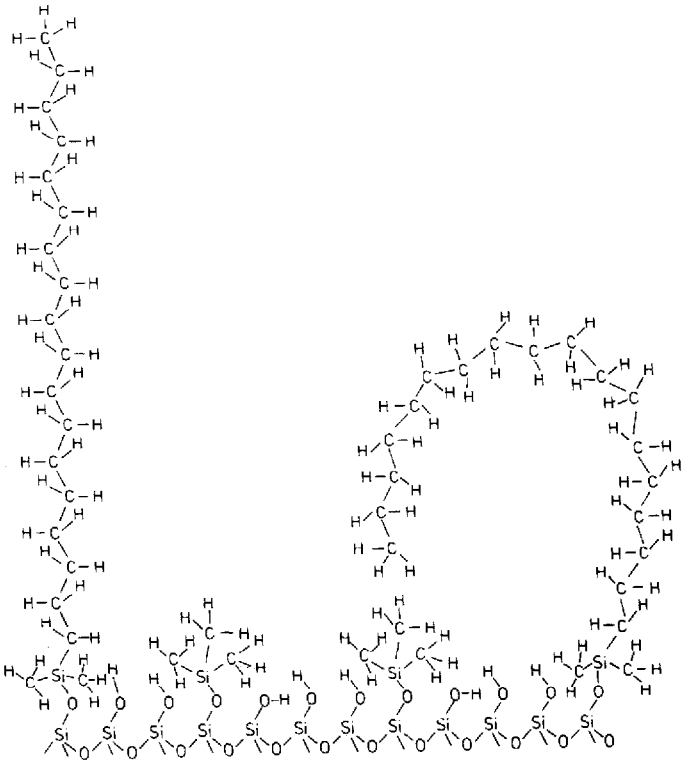

Fig. 8. Conformations of endcapped octadecyl reversed-phase material.

which is bound to the silica surface[27,28]. The momentary conformation is dependent not only upon the degree of functionalization of silanol groups but also upon solvent composition.

CONCLUSION

According to our results, the alkyl chains on the silica surface of a reversed phase under conditions typical for chromatographic separations assume different conformations, depending upon interaction with themselves, with the neighbouring chains, the trimethylsilyl groups, the mobile phase and the solutes (Fig. 8). With increasing polarity of the mobile phase or surface coverage of the silica gel, the alkyl chains assume a more rigid conformation, resulting in greater retention of more hydrophobic compounds. However, the increase of the more rigid conformation does not occur in distinctive steps, but rather continuously. Therefore, retention behaviour and NMR relaxation times do not increase step-wise, but rather continuous.

ACKNOWLEDGEMENTS

We are indebted to Bischoff Company, Leonberg, F.R.G., for providing us with Hyperchrome columns. We also thank Macherey & Nagel, Düren, F.R.G., for samples of Nucleosil.

REFERENCES

1 Cs. Horváth, W. R. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
2 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *Liquid Chromatography: Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, pp. 113.
3 I. Halasz, *Anal. Chem.*, 52 (1980) 1393 A.
4 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
5 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 9 (1976) 303.
6 H. Hemetsberger, M. Kellermann and H. Ricken, *Chromatographia*, 10 (1977) 726.
7 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.
8 K. K. Unger, *Porous Silica — Its Properties and Use as Support in Column Liquid Chromatography*, Elsevier, Amsterdam, New York, 1979.
9 R. G. Snyder, *J. Chem. Phys.*, 47 (1967) 1326.
10 S. J. Hansen and J. B. Callis, *J. Chromatogr. Sci.*, 21 (1983) 560.
11 C. H. Lochmüller, A. S. Colborn, M. L. Hunnicutt and J. M. Harris, *Anal. Chem.*, 55 (1983) 1344.
12 C. H. Lochmüller, A. S. Colborn, M. L. Hunnicutt and J. M. Harris, *J. Am. Chem. Soc.*, 106 (1984) 4077.
13 G. E. Maciel, D. W. Sindorf and V. J. Bartuska, *J. Chromatogr.*, 205 (1981) 438.
14 R. K. Gilpin and M. E. Gangoda, *J. Chromatogr. Sci.*, 21 (1983) 352.
15 R. K. Gilpin and M. E. Gangoda, *Anal. Chem.*, 56 (1984) 1470.
16 H. A. Claessens, L. J. M. van de Ven, J. W. de Haan, C. A. Cramers and N. Vonk, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 433.
17 M. E. Gangoda and R. K. Gilpin, *J. Magn. Reson.*, 53 (1983) 140.
18 D. Slotfeldt-Ellingsen and H. A. Resing, *J. Phys. Chem.*, 84 (1980) 220.
19 K. Tanaka, S. Shinoda and Y. Saito, *Chem. Lett.*, (1979) 179.
20 D. W. Sindorf and G. E. Maciel, *J. Am. Chem. Soc.*, 105 (1983) 1848.
21 D. W. Sindorf and G. E. Maciel, *J. Phys. Chem.*, 86 (1982) 5208.
22 D. W. Sindorf and G. E. Maciel, *J. Am. Chem. Soc.*, 105 (1983) 3767.
23 E. Bayer, K. Albert, J. Reiners and M. Nieder, *J. Chromatogr.*, 264 (1983) 197.
24 W. R. Melander, J. F. Erard and Cs. Horváth, *J. Chromatogr.*, 282 (1983) 211.
25 K. Albert, B. Peters and E. Bayer, *J. Magn. Reson.*, 62 (1985) 428.
26 Cs. Horváth, *LC, Liq. Chromatogr. HPLC Mag.*, 1 (1983) 652.
27 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
28 C. H. Lochmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.