Influence of Alkyl Chain Length on the Stability of n-Alkyl-Modified Reversed Phases. 1. Chromatographic and Physical Analysis

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The influence of the ligand alkyl chain length, chemically bonded at the surface of a silica substrate, on stationary phase stability in liquid chromatography practice is reviewed. Several factors affecting long-term stability of modern reversed-phase high-performance liquid chromatography (RP-HPLC) phases are considered and their individual contributions are evaluated in this paper and the following paper in this issue. The stationary phases under study were identically modified on the same batch of silica substrate to eliminate differences in substrate properties and synthesis conditions. Modifications with ligand alkyl chain length between C_1 and C_{18} were performed such that an approximately equal ligand density was obtained for the seven RP-HPLC phases studied. These n-alkyldimethylsiloxysilane bonded phases were exposed to simulated aging experiments. A subsequent chromatographic characterization regarding changes in capacity, lipophilic and polar selectivity, and silica degradation related separation performance was carried out. Comparison with results determined by other characterization methods, like bulk analysis, elemental analysis, and solid-state ^{29}Si cross-polarization magic angle spinning NMR revealed that with longer n-alkyl ligands gradually better substrate shielding properties were obtained. The ligand alkyl chain length affects the stability of reversed phases to a large extent. Short ligand modified phases changed drastically using relatively aggressive eluents. Organic modifier rich eluents combined with longer ligand modified phases are preferable for a longer lifetime of RP-HPLC columns, especially when aggressive eluents are used.

INTRODUCTION

Modified silica-based packings most commonly used as reversed phases in high-performance liquid chromatography (RP-HPLC) are n-octyl and n-octadecyl bonded phases. Usually commercially available RP-HPLC phases are synthesised with monofunctional reagents like n-alkyldimethylmonochlorosilane, to prevent additional hydroxyl groups due to hydrolysis of remaining Si–Cl groups when di- and trifunctional silanes are used (2, 3). Most RP-HPLC separations in laboratory practice are performed with n-octadecyl modified silica substrates. These phases are better defined than the corresponding di- and trifunctional modifications and have the advantage of a large stationary phase volume and the possibility of adequate selectivity for the

separation of many organic compounds. Until recently, only separations of large organic molecules with molecular weights between 1000 and 10 000 were carried out on short alkyl ligand phases, e.g. n-butyldimethylsiloxysilane modified silica. With the better understanding of the phenomenon "selectivity" in modern RP-HPLC practice (4–8) and the need for sophisticated and tailor made solutions of separation problems in the last years, the interest in stationary phases with various alkyl chain length ligands has grown (2, 9–13). With the introduction of commercially available expert systems in HPLC (14), the popularity of all kinds of stationary phases with different selectivity will probably increase even more. Furthermore, introduction of silica substrates with a very large specific surface area (>500 m^2 g^{-1}) will promote the application of short alkyl ligand bonded phases. However, relatively high ligand surface concentrations and superior substrate shielding properties by secondary groups (e.g. isopropyl) or multidentate surface attachments, with varying numbers of chemical bonds with the surface, are needed (3, 15). The alkyl chain length and alkyl surface concentration are two critical parameters that determine the hydrophobicity of a packing material and thence retention and selectivity characteristics (9, 10). However, the same two parameters also affect the useful lifetime of RP-HPLC columns to a large extent. Long-term stability of chemically bonded stationary phases in laboratory practice is limited by hydrolysis and dissolution of the ligands from the silica surface and of the silica substrate itself, especially when aggressive eluents or conditions are used (3, 15). Although hydrolysis is a slow process, in practice, a stationary phase alters after a period of intensive use. This can be observed by changes in capacity factors, selectivity, and separation performance (3, 16). Although these changes can be determined, the factors causing the process of stationary phase degradation are not completely clear. The influence of ligands anchored at the silica surface, their functionality, chain length, and surface concentration on the process of hydrolysis, has been partially reported previously (3, 15–19). However, the specific effect of the ligand alkyl chain length of chemically bonded phases on stability and ligand and/or substrate hydrolysis and dissolution has not yet been systematically studied. In this and the subsequent paper (1), the emphasis is on the process of stationary phase deterioration, correlated with the shielding effect by the anchored ligands at the silica surface. At the same time the influence of various eluents on this process was studied. Combined with chromatographic data, factors affecting stationary phase aging in laboratory practice are reviewed.

The stability of seven RP-HPLC stationary phases modified on the same batch of silica substrate with various n-alkyl ligands between n-C_1 and n-C_{18} was studied by controlled aging of these phases under simulated routine use conditions.

The silica substrate used for modification possessed a very large specific surface area (S_{BET} = 579 m² g⁻¹). Modification of this silica substrate was performed such that an approximately equal ligand density was obtained for all seven phases. After the modified phases were subjected to artificial aging, changes were investigated in detail by chromatography, analysis of various bulk properties, elemental analysis, and high-resolution solid-state ²⁹Si NMR. In this way, the influence of the n-alkyl chain length on the stability and the resistance toward hydrolysis of the stationary phases under study could be determined.

EXPERIMENTAL SECTION

Materials. The test components used for chromatographic characterization were all reference grade. n-Alkylbenzenes (test mixture 1) were benzene, methylbenzene, ethylbenzene, n-propylbenzene and n-butylbenzene (Pierce Chemical Corp., Rockford, IL). n-Alkyl aryl ketones (test mixture 2) used as test compounds were ethanophenone, n-propanophenone, n-butanophenone, n-hexanophenone, and n-octanophenone (Pierce). p-Hydroxybenzoic acid n-alkyl esters (test mixture 3) were methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, n-propyl p-hydroxybenzoate, and n-butyl p-hydroxybenzoate (Sigma Chemical Co., St. Louis, MO). All other solvents and chemicals were analytical reagent grade (E. Merck, Darmstadt, F.R.G.). The deionized water exhibited a specific electric resistance >10 MΩ·cm⁻¹. (Milli-Q System, Millipore Corp., Bedford, MA). All eluents were freshly prepared and filtered over 0.22-μm membrane filters (Millipore) prior to use.

Chromatography. The n-alkyldimethylsiloxysilane bonded phases were all prepared on the same experimental spherical silica (d_p = 5 μm, S_{BET} = 579 m² g⁻¹, research sample, E. Merck) to eliminate substrate-dependent effects on the stability of the phases under study. The silica modification with methyl-, ethyl-, n-butyl-, n-hexyl-, n-octyl-, and n-dodecyldimethylmonochlorosilane was catalyzed with 2,6-lutidine to accelerate the kinetics of the silanization reaction. The modification with octadecyldimethylmonochlorosilane was catalyzed with imidazole (20, 21) in order to obtain a maximal ligand surface concentration. Still, the coverage was somewhat lower; see Table II. The seven n-alkyldimethylsilane-modified bonded phases were prepared at the R&D Chromatography Department of E. Merck, according to the optimized procedures reported earlier (20). Approximately 30 g of material was synthesized for each of the reversed phases. From each stationary phase seven identical columns (100 mm, 4 mm i.d.) (Knauer, Bad Homburg, F.R.G.) were packed according to a standard packing procedure. Due to the packing procedure, it was difficult to control variations in column efficiency. Variations up to 50% were measured between apparently identical columns. Regarding the objectives of the present study, the test procedures were focused on uniform capacity factors and selectivities of all columns packed with identical reversed phases. The test criterion was a deviation of ±10%, close to the analytical reproducibility. After this chromatographic test six columns were placed in an apparatus for simulated routine use experiments. The procedure and equipment for these experiments are discussed and described earlier (3, 6). Only the pulse damping system was modified with addition of a pulse damper (Waters, Millipore), a 3 m × 0.10 mm i.d. capillary and a permanent 0.2-μm filter in series to ensure a more constant flow through the columns during the aging experiments. The basic and acidic aqueous and aqueous–methanol buffers used as eluents for simulated routine use experiments are listed in Table I. Before and after the aging experiments, the columns were subjected to a chromatographic characterization, derived from retention models proposed earlier by Jandera (5, 6, 22). The aging experiment with eluent 4 appeared to be too destructive; therefore a subsequent chromatographic characterization was not meaningful. In an earlier study it was shown that eluent 4 was destructive due to the absence of the protecting organic modifier (3). In this study three series of homologues, n-alkylbenzenes, n-alkyl aryl ketones, and p-hydroxybenzoic acid n-alkyl esters, at suitable eluent composition were used. Chromatograms of the test mixtures were collected at minimal 4 different eluent compositions. By multiple linear regression of the logarithms of the capacity factors of a homologous

Table I. Eluent Compositions of the Experiments for Simulating Routine Use[a]

aging expt no.	buffer	pH	vol fraction of methanol in eluent	ion pairing agent, concn 5 mmol
1	0.05 M phosphate	3.0	0	
2	0.05 M phosphate	3.0	0.5	
3	0.05 M phosphate	3.0	0.5	hexylsulfonate
4	0.05 M carbonate	8.4	0	
5	0.05 M carbonate	8.4	0.5	
6	0.05 M carbonate	8.4	0.5	triethylamine

[a] Each column was purged by 7000 column volumes of a typical eluent; flow rate 0.5 mL/min; time 240 h; ambient temperature.

series at suitable eluent compositions, a set of characteristic parameters can be derived (3, 6, 22)

$$\log k' = a_0 + a_1 n_c - x(m_0 + m_1 n_c) \qquad (1)$$

further

$$p = m_1/a_1 \text{ and } q = m_0 - pa_0 \qquad (2a,b)$$

where x is the volume fraction of organic modifier in a binary eluent with water and n_c is the incremental carbon number of a homologous series of components. The characteristic parameters to be determined with regression are a_0, a_1, m_0, m_1, p, and q. The validity of this regression has been shown before (3, 16). Good correlation coefficients (r > 0.995) were obtained for eluent compositions containing 50% to 90% (v/v) methanol as modifier for all stationary phases in this study. The parameters m_0 and q in eqs 1 and 2b, respectively, characterize contributions to the selectivity (6): m_0 denotes mainly nonspecific, lipophilic selectivity and q represents specific, polar selectivity, as discussed extensively earlier (3).

The chromatographic characterization experiments of the stationary phases were performed with a Model 100A pump (Beckmann Instruments, Berkeley, CA), a Model CV-6-VHPa-N60 injection valve equipped with a 20-μL loop (Valco, Houston, TX), and a Model LC-3 variable-wavelength UV detector (Pye Unicam, Cambridge, U.K.) operated at suitable wavelengths for an optimal response: test mixture 1, 261 nm; test mixture 2, 241 nm; and test mixture 3, 257 nm. Typically, injections of 5–10 μL of the test mixtures were performed. The detector signal was sampled at 10 Hz and integrated with a Nelson 3000 data system (Nelson Analytical, Cupertino, CA).

Solid-State ²⁹Si NMR Measurements. The solid-state ²⁹Si NMR spectra were obtained on a Bruker CXP-300 Fourier transform nuclear magnetic resonance spectrometer at 59.63 MHz. Representative samples of 160–240 mg were spun at ca. 3.5 kHz using 7 mm o.d. aluminum oxide and zirconium oxide rotors of the Bruker double-bearing type. ²⁹Si Bloch decay magic angle spinning (MAS) NMR spectra of the original silica substrate were obtained with a pulse interval time of 90 s. If interval times exceed 5 times the T_1 relaxation time, the signal areas measured with this technique represent the relative amounts of silicon nuclei of different nature in the sample. Typically 1000 FIDs (free induction decays) with an acquisition time of 10 ms were accumulated in 1K data points and zero-filled to 8K prior to Fourier transformation. A line broadening of 10 Hz prior to zero-filling was used. ²⁹Si cross-polarization magic angle spinning (CP-MAS) NMR spectra of all modified stationary phases prior to and after aging experiments were obtained with a cross-polarization contact time of 6 ms. The pulse interval time was 1 s. Typically, 2000 FIDs with an acquisition time of 10 ms were accumulated in 1K data points and zero-filled to 8K prior to Fourier transformation. The line broadening used was 15 Hz prior to zero-filling and Fourier transformation. The spectral width for all spectra was 15 kHz.

²⁹Si CP-MAS NMR experiments with variable contacts of trimethylsiloxysilane silica (Si-C_1) before and after several aging experiments showed consistent CP characteristics. This pertains to all siliceous moieties at the silica surface. Different results were found for different signals in the ²⁹Si NMR spectrum. Thence, quantification of siliceous moieties at the surface with ²⁹Si CP-

Table II. Bulk Properties Prior to Aging of the Bare Substrate and the n-Alkyldimethylsiloxysilane Modified Phases Studied Here[a]

stationary phase	specific surface area, S_{BET}, m^2 g^{-1}	mean pore size, nm	pore volume, cm^3 g^{-1}	carbon content P_C, % (w/w)	ligand surface density, α_1 (see ref 2, eq 1), μmol m^{-2}
bare silica	579	6.0	0.82		
Si-C$_1$	522	5.7	0.68	6.69	2.67
Si-C$_2$	428	5.4	0.58	7.59	3.15
Si-C$_4$	410	5.2	0.53	10.53	3.03
Si-C$_6$	396	5.8	0.48	12.01	2.63
Si-C$_8$	345	4.8	0.41	13.96	2.50
Si-C$_{12}$	299	5.2	0.39	18.78	2.58
Si-C$_{18}$	357	5.4	0.48	18.06	1.69

[a] Mean particle size, $d_p = 5 \pm 1.3$ μm.

MAS NMR can be performed with a single contact time but with specific correction factors for different ^{29}Si NMR absorptions (23).

Bulk Analysis. Surface area, pore size distribution, and pore volume were measured by the conventional Brunauer–Emmett–Teller (BET) method. The measurements were performed at the R&D Chromatography Department of E. Merck.

The bare silica and the derived modified phases were measured before the simulated aging experiments, results are listed in Table II. The measured drop in S_{BET} upon modification was in part caused by the increased density of the modified phases due to the added weight of the anchored ligands at the surface. Secondly, anchored ligands occupy part of the adsorption places. Hence, interpretation of the S_{BET} data will be rather complicated. The pore size and pore volume data are more clear.

After aging experiments only a selected group of stationary phases was submitted for BET measurements. The deviation of the surface area measurements varied by as much as 10–15%. The carbon contents of the modified stationary phases prior to and after each aging experiment were obtained with a Perkin-Elmer Model 240 Analyzer (Perkin-Elmer, Norwalk, CT). Tungsten oxide was added to the modified silica as a catalyst. The samples were periodically compared with materials of standard quality. The reproducibility of the elemental analysis measurements was within the 5% range. The carbon content, P_C, and the ligand surface concentration, α_1, calculated according to eq 1 (3), of the starting materials are listed in Table II.

RESULTS AND DISCUSSION

Chromatography. Fundamental chromatographic characterization of the n-alkyl modified reversed phases in this study was performed with three sets of homologous series of components with different molecular residues. The selectivity of a typical separation system can be described by the parameters m_0 for lipophilic and q for polar selectivity (see formulas 1 and 2b, respectively). The values of m_0 and q depend on the applied stationary phase, the type of organic modifier in the eluent, and the test solutes. The hydrophobic and hydrophilic properties of a stationary phase depend not only on the ratio of the number of μmol m^{-2} of ligands and residual silanol but also on the accessibility and type of silanol groups and the shielding factor of the ligands to that. Moreover, the distribution of the silanols and ligands across the silica surface may play an important role. Therefore it is difficult to give a completely satisfying explanation of the results when the test solutes carry both hydrophobic and hydrophilic functionalities. This is even true for the alkylbenzenes, applied to test the hydrophobicity of the stationary phases, where the benzene part is polarizable and probably interacts with the silanol groups present. Nevertheless, the presence of benzene was necessary in our experiment due to the detection limitations of the equipment. The lipophilic selectivity as a function of the n-alkyl chain length of the ligands for the test mixtures is depicted in Figure 1. As expected, increasing m_0 values as a function of longer n-alkyl ligands were determined for n-alkylbenzene test mixture, showing large lipophilic interactions/contribution during the
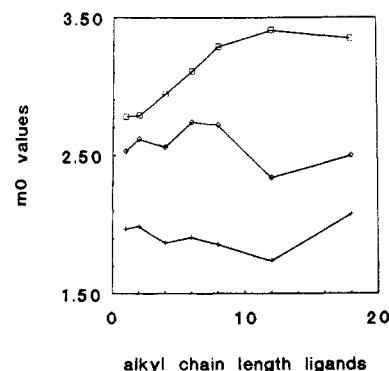

Figure 1. Chromatographically determined m_0 values (lipophilic selectivity) versus the ligand alkyl chain length of the reversed phases under study, before aging experiments: (□) n-alkylbenzenes, (+) aryl n-alkyl ketones, (◇) p-hydroxybenzoic acid n-alkyl esters. The lines drawn exhibit only an illustrative value.
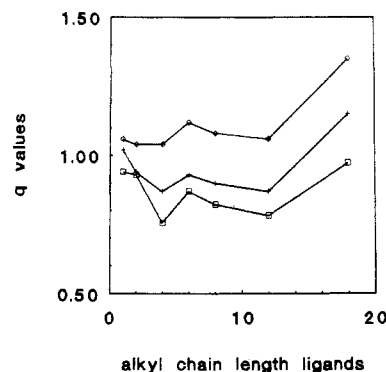
Figure 2. Chromatographically determined q values (polar selectivity) versus the ligand alkyl chain length of the reversed phases under study, before aging experiments: (□) n-alkylbenzenes, (+) aryl n-alkyl ketones, (◇) p-hydroxybenzoic acid n-alkyl esters. The lines drawn exhibit only an illustrative value.

elution. In contrast the m_0 values determined for the phenyl ketones and for the p-hydroxybenzoates showed less lipophilic interaction with the modified phases under study and a more or less capricious course, which can be attributed to the mixed retention mechanism discussed above. Thence, changes in lipophilic selectivity of a stationary phase may be monitored best by the n-alkylbenzene mixture.

The influence of the ligand alkyl chain length on the polar selectivity, q, for the three test mixtures is shown in Figure 2. With the exception of the C$_{18}$-reversed phase, which showed a smaller ligand density, the q values for the p-hydroxybenzoates were found constant to a certain amount. Due to the more or less equal ligand concentrations at the silica surface (except for the octadecyl bonded phase), the polar interactions between the p-hydroxybenzoate and the residual silanol groups at the surface are shown to be equal to a certain
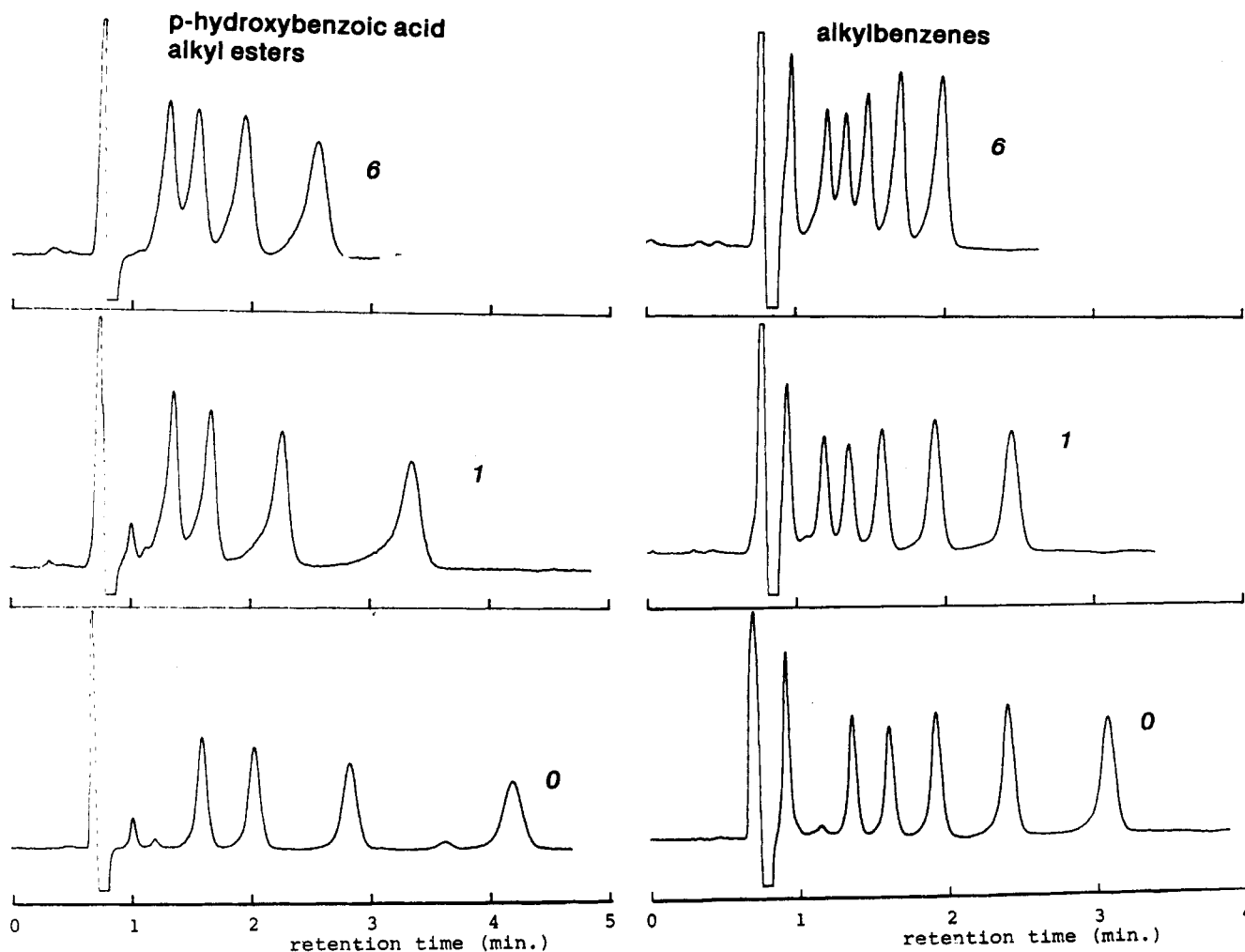
Figure 3. Chromatograms of the p-hydroxybenzoic acid n-alkyl ester (left) and the n-alkylbenzene (right) test mixtures eluted on n-butyldimethylsiloxysilane modified phase before and after aging experiments 1 and 6. Chromatographic test conditions were as follows: methanol–water (80:20 (v/v)); flow rate, 1.0 mL/min; UV detection, test mixture 1, 261 nm; test mixture 3, 257 nm.

extent. The values of q for the other two test mixtures were smaller but showed an identical course as the q values for the p-hydroxybenzoates. Changes in this type of selectivity may be monitored best by the p-hydroxybenzoic acid n-alkyl esters.

The effect of long-term exposure to relatively aggressive eluents on the performance of a stationary phase can be illustrated by chromatography. As an example Figure 3 depicts the chromatograms of the n-alkylbenzene and the p-hydroxybenzoic acid n-alkyl ester test mixtures eluted on the n-butyldimethylsilane modified phase before and after aging experiments 1 and 6. From the set of chromatograms collected of each combination of stationary phase, mobile phase, and test mixture, the capacity factors were calculated. Subsequently, typical diagrams of log k' versus the volume fraction methanol (x) in the test eluent and the incremental carbon number of the components of the homologous series (n_C) for each stationary phase could be derived, as was outlined previously (3). The values of m_0 (n-alkylbenzenes) were determined by multiple linear regression from these diagrams, eq 1, before and after each aging experiment. These m_0 values for the stationary phases under study are listed in Table III. As in earlier studies (3, 19) a good correlation was found between the lipophilic selectivity m_0 and a_0 (n-alkylbenzenes). The latter represents the logarithm of the capacity factor of the homologue residue extrapolated for pure water as the eluent and is strongly related to the amount of bonded ligands present at the surface.

For the short ligand phases considerable changes in lipophilic selectivity were determined, especially with the high

Table III. Lipophilic Selectivity, the m_0 (n-alkylbenzenes) Value Determined by Chromatographic Characterization for the Reversed Phases under Study, before and after Each Aging Experiment[a]

aging expt no.	m_0 for n-alkyldimethylsiloxysilane phases						
	Si–C_1	Si–C_2	Si–C_4	Si–C_6	Si–C_8	Si–C_{12}	Si–C_{18}
0	2.78	2.79	2.94	3.11	3.29	3.41	3.35
1		1.69	2.53	3.14	3.32	3.44	3.21
2	2.79	2.70	2.93	3.18	3.34	3.42	3.46
3	2.68	2.69	2.78	3.14	3.01	3.14	3.36
5		2.55	2.72	2.57	2.94	3.05	3.20
6		1.35	2.09	2.47	2.88	3.01	3.14

[a] The experimental conditions of the aging experiments are outlined in Table I. Experiment 0 denotes the stationary phase after packing/chromatographic characterization.

pH used in aging experiments 5 and 6. Chromatographic characterization of the Si–C_1 phase after these aging experiments was impossible; all components eluted shortly after the dead time. The drop in capacity factors (a_0 values) and lipophilic selectivity is related to intensive stripping of the anchored ligands from the silica surface. A gradually smaller decrease in lipophilic selectivity caused in the aging experiments was determined for phases containing longer alkyl chain lengths. From these data it can be concluded that shielding of the silica substrate surface depends largely on the ligand alkyl chain length. Short alkyl ligands showed inferior shielding properties in some of the aging experiments per-

Table IV. Polar Selectivity, the q (p-Hydroxybenzoates) Values Determined by Chromatographic Characterization for the Reversed Phases under Study, before and after Each Aging Experiment[a]

aging expt no.	q for n-alkyldimethylsiloxysilane phases						
	Si–C$_1$	Si–C$_2$[b]	Si–C$_4$	Si–C$_6$	Si–C$_8$	Si–C$_{12}$	Si–C$_{18}$
0	1.06	1.04 (0.94)	1.04	1.12	1.08	1.09	1.35
1		(1.23)	1.05	1.04	1.15	1.11	1.32
2	1.11	(0.90)	0.98	1.01	1.02	1.09	1.24
3	1.19	(0.95)	1.34	1.13	1.23	1.24	1.36
5		(1.52)	1.36	1.39	1.66	1.24	1.41
6		(1.48)	1.38	1.28	1.79	1.51	1.39

[a] The experimental conditions of the aging experiments are outlined in Table I. Experiment 0 denotes the stationary phase after packing/chromatographic characterization. [b] For the Si–C$_2$ phase the q (p-hydroxybenzoates) values were not determined after the aging experiments. Instead, the values of q (phenyl ketones) are listed in parentheses.

formed in this study. In earlier studies, we noticed also a great influence of the ligand surface concentration on the stability of modified phases (3, 16). In this study we concentrated on the role of the alkyl chain length in this respect. In the frame work of the experimental conditions it can be concluded that the minimum chain length of octyl ligands might be necessary to prevent monofunctionally n-alkylsilane modified stationary phases from deterioration. The q values of the p-hydroxybenzoates presented in Table IV support the conclusion that a substantial amount of the short ligands was hydrolyzed and dissolved from the silica surface. With decreasing m_0 (n-alkylbenzenes) values for these stationary phases the q (p-hydroxybenzoates) values increased. This indicates larger polar interactions between the p-hydroxybenzoates and the silica surfaces. The increased polar interactions can be explained by an increasing amount of silanol groups formed at the surface by hydrolysis of ligands and of silica.

Aging experiment 4 was found too destructive for subsequent chromatographic testing. Deterioration of the packed bed inside the column caused chromatograms with doubled peaks and bad peak shapes. However, the collapse of the particle bed may be independent of the chemical changes at the stationary phase surface; see the NMR section (below). Bulk analysis and solid-state NMR measurements on the stationary phases after aging experiment 4 were still possible.

Solid-State ^{29}Si NMR. The structures of siliceous moieties at the surface of the silica gel before and after modification, their notations, and typical ^{29}Si chemical shifts were presented earlier (3). The ^{29}Si MAS NMR spectrum, together with the deconvoluted signal areas, of the experimental silica substrate yield a silanol content of 43.9%. This points to an extraordinarily large specific surface area of the silica gel. By dividing the relative silanol content by the measured surface area (S_{BET}), one can estimate the silanol density at the silica surface, here 0.076 (content $\times$ g m^{-2}). This density was smaller than that reported for Hypersil and a Nucleosil silica earlier (3): 0.092 (content $\times$ g m^{-2}). We assume the geometry of the pore surface of the silica affects the density of the silanol groups. The small average pore size of 6 nm combined with the large surface area may point to an open silica structure with relatively thin pore walls/boundaries. Such an open structure increases the presence of siloxane groups at the pore surface, decreasing the silanol density. Of the total amount of silanol groups only 7.1% relative were geminal silanols, dihydroxysiloxanes (Q^2), and 92.9% relative were vicinal and/or "lone" silanol groups, hydroxysiloxanes (Q^3). Caused by the relatively low silanol density most of the hydroxysiloxanes will be "lone" silanol groups.

Before and after the aging experiments ^{29}Si CP-MAS NMR spectra were obtained. Figures 4, 5, and 6 show these spectra for Si–C$_1$, Si–C$_2$, and Si–C$_{12}$ phases, respectively. The trimethylsiloxysilane bonded phase showed an almost total loss of ligands after aging experiments 1, 4, 5, and 6. Concomitantly, the relative amount of hydroxysiloxanes was increased.

Especially after aging experiment 1, a pronounced amount of geminal silanol groups was determined. Secondly, we observed, that after the aging experiments with high pH eluents, the trimethylsiloxysilane ligands were stripped from the surface and parallel or subsequently the silica substrate was hydrolzyed and dissolved as well. Dissolution of the substrate caused a drop in surface area and subsequently a decreased amount of silanol groups relative to the nonaged trimethylsiloxysilane phase. A diminished surface area can be detected indirectly with ^{29}Si CP-MAS NMR by a decrease in total signal area and consequently a worse signal to noise ratio. Here, after aging experiments 4 to 6 the amount of silanol groups did not increase to the same extent as the decrease in trimethylsiloxysilane groups, indicating that substrate dissolution took place also. The spectra of the other two modified phases, Si–C$_2$ and Si–C$_{12}$, show again that the resistance toward ligand dissolution is generally superior with longer alkyl chain length ligands. With the Si–C$_2$ phase still only small amounts of ethyldimethylsilanes were detected after aging experiments 1, 4, and 6. On the other hand, the amounts of ligands anchored at the surface after aging experiment 5 were about half that of the nonaged phase.

The shielding effect of the anchored ligands and the dissolution of the substrate with some of the eluents is illustrated by comparison of the amounts of siliceous moieties present at the surface after a particular aging experiment, relative to their amounts at the nonaged silica surface. A comparison of the amount of ligands (M^1) and the total amount of silanol (Q^2 and Q^3) of all stationary phases after aging experiment 1, relative to the nonaged phases, is depicted in Figure 7. With short alkyl ligand modified phases large amounts, up to approximately 90%, of alkyldimethylsiloxysilanes were stripped from the surface by the low pH aqueous phosphate buffer. However, only a part of these dissolved ligands were replaced by silanol groups. The resulting vacancies at the silica surface were either condensed with formation of siloxane bridges or hydrolyzed/dissolved from the substrate resulting in less surface area. The substrate shielding effect by the long alkyl ligands was obvious. The n-octyl, n-dodecyl, and n-octadecyl ligands showed almost no significant reduction of the amount of anchored ligands and/or increased amount of silanol groups.

A similar graph for the effect of the simulated routine use experiment with the low pH aqueous–methanol phosphate buffer, aging experiment 2, is depicted in Figure 8. Here, only relatively small fluctuations in ligand and silanol amounts were observed. The ligand shielding seemed independent of the alkyl chain length. Even the total amount of siliceous moieties remained constant. No intensive substrate hydrolysis or silanol condensation was detected. In sharp contrast, all high pH carbonate buffer eluents changed the substrate surface of the short alkyl ligand modified phases drastically; see Figure 9. Well advanced surface degradation by stripped ligands and dissolved silica was observed. Large amounts of both
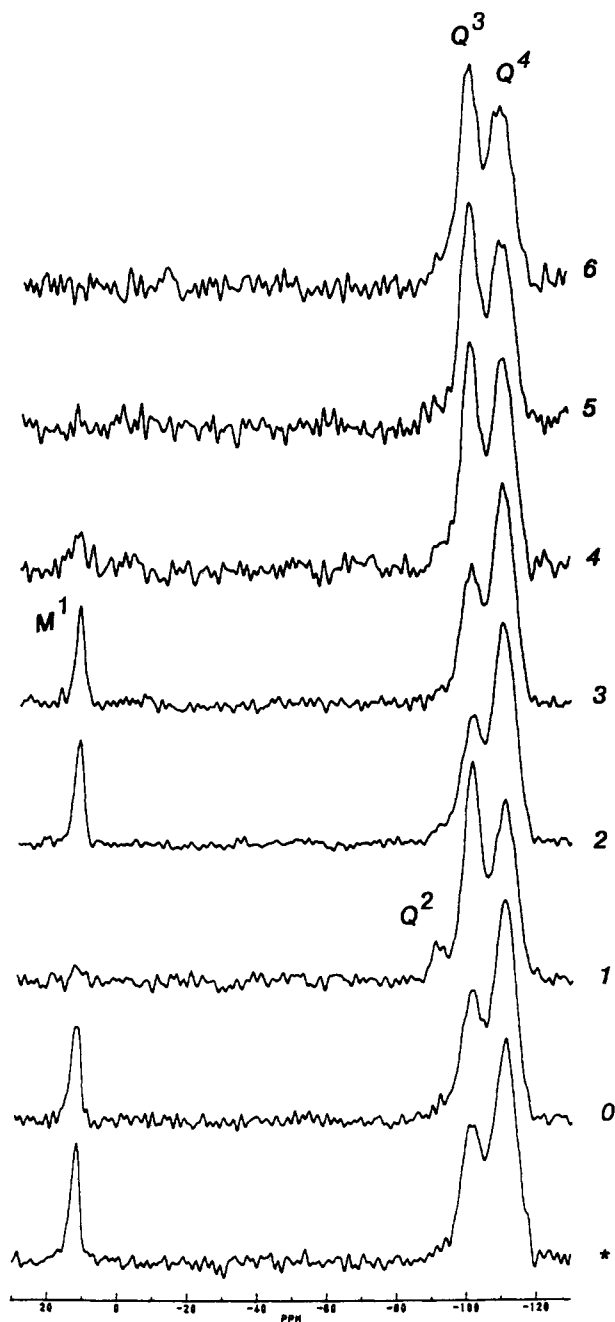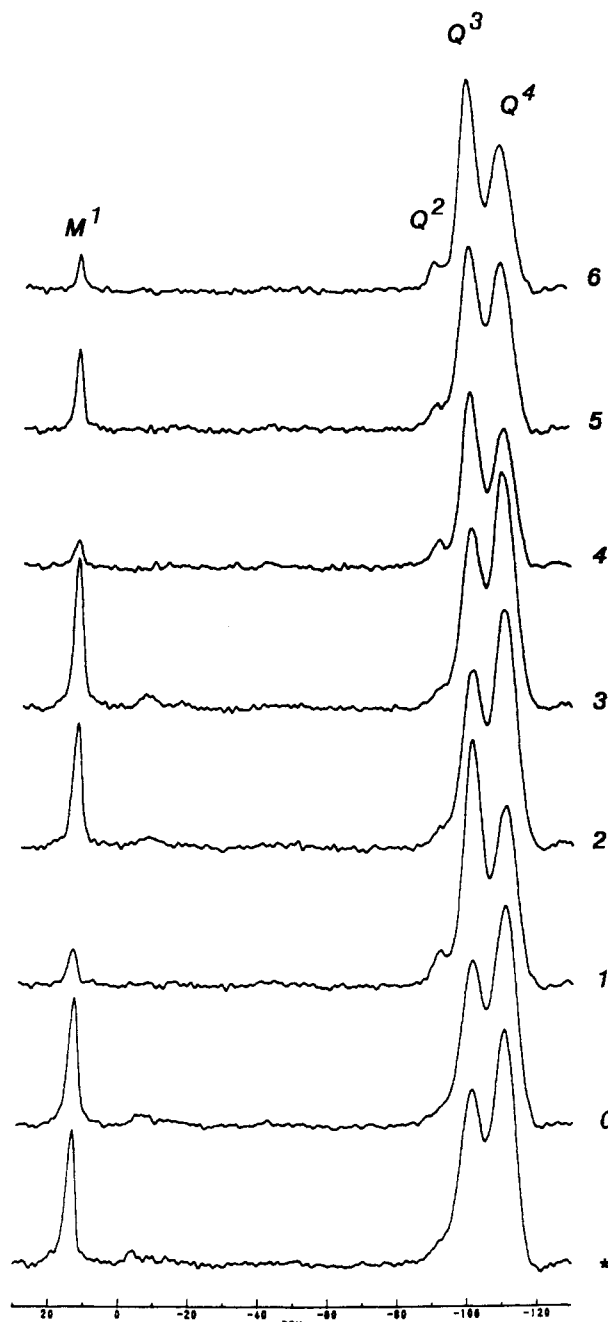
Figure 4. ^{29}Si CP-MAS NMR spectra of trimethylsiloxysilane modified silica (Si–C$_1$), before and after the aging experiments. The numbers indicate the typical aging experiments as outlined in Table I. Asterisk denotes the original stationary phase; 0, refers to the stationary phase after packing.

Figure 5. ^{29}Si CP-MAS NMR spectra of ethyldimethylsiloxysilane-modified silica (Si–C$_2$), before and after the aging experiments. The numbers indicate the typical aging experiments as outlined in Table I. Asterisk denotes the original stationary phase; 0 refers to the stationary phase after packing.

ligand silanes and hydroxysiloxanes disappeared from the surface. Only a small part of the silanol groups condensed to siloxane bridges, the major amount of silanol groups apparently dissolved in the aggressive buffer eluents. A worse signal to noise ratio of the Si–C$_1$ phases after high pH aging experiments indicated already a diminishing silica surface; see Figure 4. From the previous graphs it is clear that ligand hydrolysis was affected by the type of eluent used for the aging experiment.

The eluents used for the routine use simulations in this study can be ranked in order of increasing hydrolysis of ligands and silica: $2 \rightarrow 3 \rightarrow 5 \rightarrow 1 \rightarrow 6 \rightarrow 4$.

With the high pH eluents a large degree of ligand stripping, due to hydrolysis and dissolution, was observed, especially for short ligand phases. Addition of methanol (50% (v/v)) to the high pH buffer eluent only reduced the hydrolysis of long

ligand modified silica (n-alkyl chain length > n-octyl), probably due to solvatation of the long ligands by the organic modifier. For low pH buffered eluents, the addition of methanol reduced the hydrolysis of short ligands drastically. Long alkyl chain ligands were much less affected by low pH eluents.

Bulk Analysis. The carbon content after three different aging experiments for all modified phases relative to that of the originate phase is depicted in Figure 10. The longer alkyl ligands inhibited dissolution of surface-anchored n-alkyldimethylsiloxysilanes by aggressive eluents. These curves reflect the ligand length and concentration at the silica surface. As such, the shape of the curves was similar to the ^{29}Si CP-MAS NMR measured relative ligand content depicted in Figures 7 to 9. However, in contrast with the results of the solid-state ^{29}Si NMR measurements, here we observed substantial
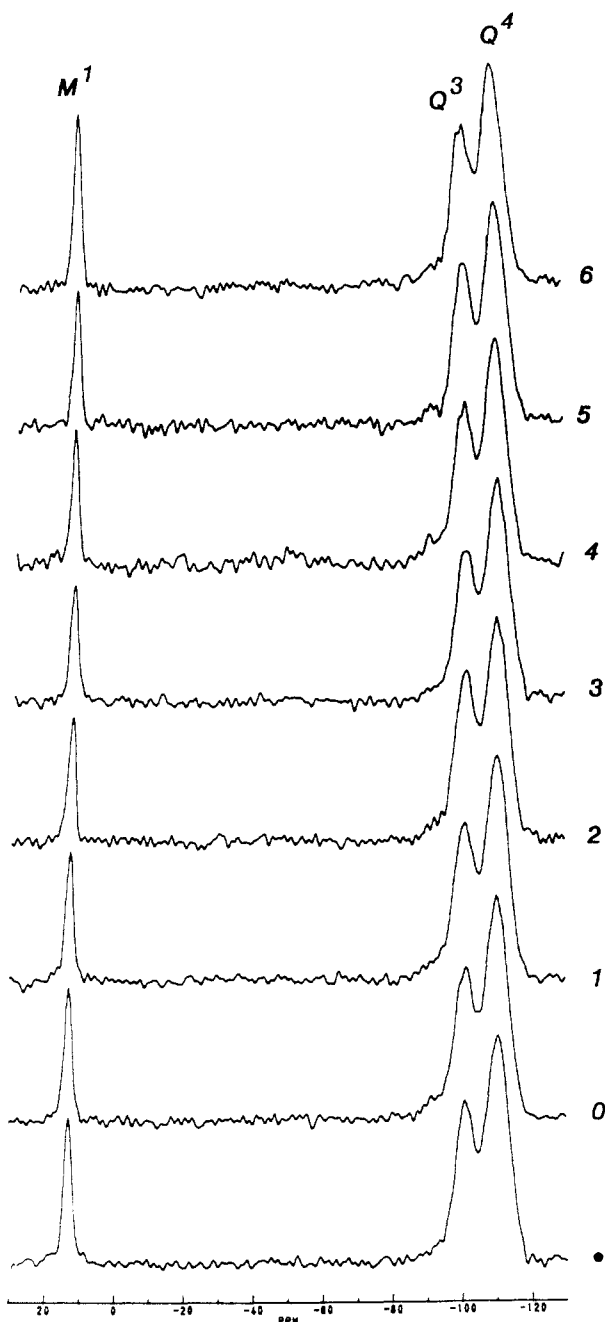
**Figure 6.** ^{29}Si CP-MAS NMR spectra of dodecyldimethylsilane modified silica (Si–C$_{12}$), before and after the aging experiments. The numbers indicate the typical aging experiments as outlined in Table I. Asterisk denotes the original stationary phase; 0 refers to the stationary phase after packing.
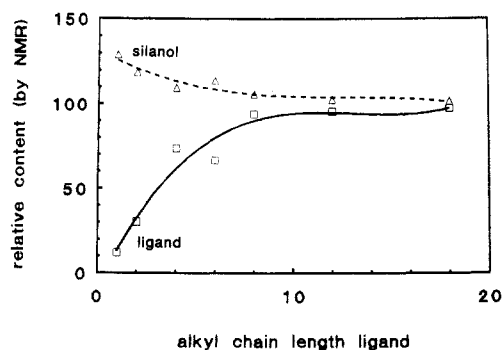

Figure 7. Percentage ligand, M^1 (□), and total silanol Q^3 and Q^2 (△), present after aging experiment 1, relative to the originate reversed phases. Measured by ^{29}Si CP-MAS NMR.
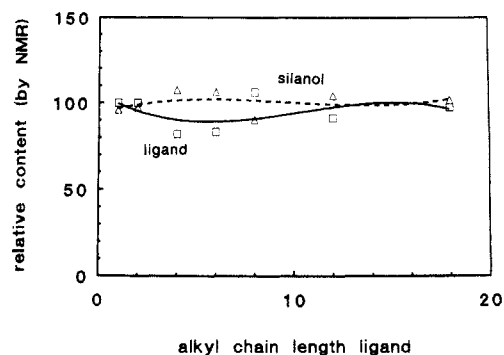
Figure 8. Percentage ligand, M^1 (□), and total silanol, Q^3 and Q^2 (△), present after aging experiment 2, relative to the originate reversed phases. Measured by ^{29}Si CP-MAS NMR.
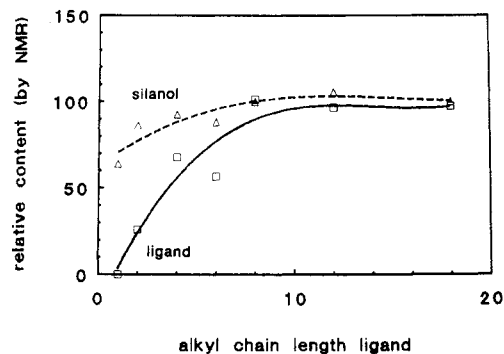
Figure 9. Percentage ligand, M^1 (□), and total silanol, Q^3 and Q^2 (△), present after aging experiment 6, relative to the originate reversed phases. Measured by ^{29}Si CP-MAS NMR.
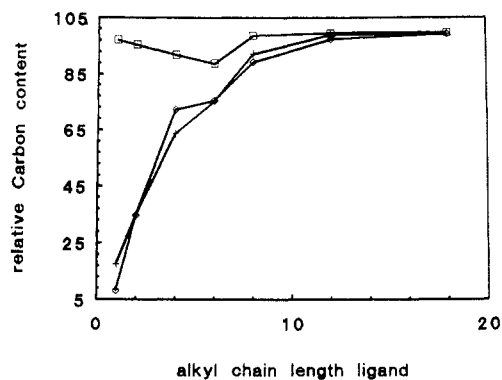
Figure 10. Carbon content relative to the originate reversed phases after aging experiment 1 (+), 2 (□), and 6 (◇). Measured with elemental analysis. The lines drawn exhibit only an illustrative value.

changes in the carbon content for the *n*-octyl bonded phase after aging experiments 1 and 6. After aging experiment 6 in total 12% of the carbon was removed from the silica substrate. Results of the BET measurements of a selected group of stationary phases before and after a specific aging experiment are listed in Table V. This table reports two specific area values: S_{BET} measured and the for the ligand content corrected value. The mass of the ligands affects the density of the substrate. Major changes in substrate properties were determined from the values of the pore size and pore diameter. As expected, increased pore size and pore volume were noticed for short ligand phases after aging experiments with high pH buffer solutions and low pH aqueous buffer solutions, indicating severe substrate hydrolysis. Changes in pore size and pore volume decreased gradually with longer alkyl chain length of the ligands. Although only a selected amount of BET measurements were performed, it can be concluded that longer alkyl ligands isolated the silica surface better from substrate

Table V. Bulk Properties before and after Aging Experiments of the *n*-Alkyldimethylsiloxysilane Modified Phases Studied Here[a]

stat phase/ aging expt	specific surface area, S_{BET}, m^2 g^{-1}	mean pore size, nm	pore volume, cm^3 g^{-1}	carbon content P_C, % (w/w)	spec surface area, corrtd for ligand content, m^2 g^{-1}
Si–C$_1$					
*	522	5.7	0.68	6.69	592
4	482	9.2	1.10	0.87	488
Si–C$_2$					
*	428	5.4	0.58	7.59	487
2	448	5.5	0.63	7.11	505
4	417	8.4	1.14	2.58	434
5	517	6.2	0.82	4.38	558
6	570	6.8	0.92	2.31	593
Si–C$_4$					
*	410	5.2	0.48	10.53	484
2	470	4.9	0.58	10.29	547
4	289	8.3	0.60	9.97	335
6	630	5.6	0.88	6.92	699
Si–C$_{12}$					
*	299	5.2	0.39	18.78	375
2	300	5.5	0.41	18.38	375
5	232	6.9	0.40	18.00	288
Si–C$_{18}$					
*	357	5.4	0.48	18.06	441
5	314	5.4	0.40	17.98	387

[a] The experimental conditions of the aging experiments are outlined in Table I. Experiment * denotes the original stationary phase.

hydrolysis by aggressive eluents.

The alternating changes in measured and corrected specific surface areas for short ligand modified silica were indistinct. It may well be that substrate degradation into small sub-colloidal level (<1–3 nm) silica/silicate particles occurred, enlarging the specific surface area. The short *n*-alkyl bonded phases showed an enlarged surface area after aging experiments 5 and 6. Presumably, silica hydrolysis and dissolution resulted in small dissolved silica particles and coarsened pore surfaces (1).

On the other hand, direct silica dissolution results in a smaller specific surface area. Aging experiment 4 reduced the specific surface area drastically due to severe silica dissolution. However, both hydrolysis and subsequent dissolution processes resulted in a significant larger pore size and pore volume.

CONCLUSIONS

The trimethylsiloxysilane, ethyl-, *n*-butyl-, and *n*-hexyl-dimethylsiloxysilane silica phases show a rather poor resistance toward ligand hydrolysis and dissolution when aggressive low and high pH buffer solutions are used as eluent. The stability of these reversed phases increases gradually with longer alkyl chain length of the ligands. This is confirmed by our chromatographic characterization method, the ^{29}Si CP-MAS NMR measurements, and the elemental analysis of the stationary phases after aging. The longer alkyl ligands inhibit the attack of the surface by aggressive buffer solutions, under the experimental conditions and time schedule here.

Even if other stationary phase properties will affect the stability of modified stationary phases as well, see refs 3 and 15–19, the ligand alkyl chain length seems of major influence. Short *n*-alkyldimethylsilane ligand modified silicas will deteriorate when low or high pH buffer solutions are used as an eluent in practice, even over a short period of time.

Due to better solvatation of the ligands by organic modifiers, e.g. methanol, longer ligands presumably are more effective in shielding the substrate. Consequently, severe degradation of the stationary phases is inhibited to a large extent. Thence, as experienced from daily chromatographic practice, organic modifier rich eluents combined with longer alkyl chain length modifications are preferable for a longer lifetime of the RP-

HPLC columns. Concurrent hydrolysis and dissolution of silica and ligands (1) affects the chromatographic performance and alters the selectivity of reversed-phase columns. Changed capacity factors and lipophilic selectivity indicate that these processes are going on, causing increased specific polar interactions. For sensitive compounds large shifts in retention data may be observed, spoiling the easy life of the LC chromatographer and his data system.

As illustrated in this study a set of selective test components, like the homologous series of *n*-alkylbenzenes and *p*-hydroxybenzoic acid *n*-alkyl esters, can be used to adequately examine the actual status of the RP-HPLC column. The derived parameters m_0 and q adapted by an expert (or expert system) can easily be used to correct the eluent composition for a typical separation. The values for m_0 and q also support the decision about the practical useful lifetime of a typical RP-HPLC column. In an accompanying, subsequent paper some of the important interactions between the RP-HPLC phases and the eluents will be reported in some detail. This pertains in particular to the dissolution of the substrate into mixtures of water and methanol at pH >12, selected to mimic the situation prevailing in RP-HPLC using basic eluents.

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Influence of Alkyl Chain Length on the Stability of n-Alkyl-Modified Reversed Phases. 2. Model Dissolution Study

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A direct, in situ study of the hydrolysis and dissolution of various n-alkyl modified phases was performed with ^{29}Si magic angle spinning NMR spectrometry as a model for stationary phase aging in laboratory practice. With this study, the substantial effect of the ligand length on the dissolution reaction of the alkyl modified phases under aggressive eluent conditions is confirmed. As expected, longer n-alkyl ligands gradually show better substrate shielding properties. Above a certain critical ligand length, shielding by longer ligands inhibit the dissolution rate drastically by affecting the hydrolysis and dissolution processes. The type of solvent system used, in liquid chromatography practice or for dissolution studies as reported here, mainly affects the proportions of dissolved silicates in the eluent. On the basis of this in situ dissolution study a model for ligand and substrate hydrolysis and dissolution with the involved reactions is presented.

INTRODUCTION

In the foregoing, accompanying paper it is made clear that one of the most important reasons for instability of stationary phases for reversed-phase high-performance liquid chromatography (RP-HPLC) is concerned with unwanted chemical interactions between the mobile phase and the stationary phase (1). A similar conclusion was drawn in earlier publications from this (2, 3) and other (4) laboratories. Although hydrolysis and dissolution are rather slow processes in LC practice, the RP-HPLC phase deteriorates after a period of intensive use. This is observed by changes in capacity factors, selectivity, and column efficiency.

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There are only a few techniques that enable in situ studies of such reversed-phase dissolution. ^{29}Si NMR may provide useful details concerning the molecular structures of species leaving the substrate surface and forming intermediate and final reaction products (5-8). To the best of our knowledge, these studies have been confined to silica gel only, e.g. the substrate part of the stationary phases for RP-HPLC. This prompted us to perform a ^{29}Si NMR study of the dissolution characteristics of several phases for RP-HPLC, investigated in the accompanying paper (1). The stationary phases under study were modified on the same batch of silica substrate using various ligands of increasing n-alkyl chain length.

With this study, information on the substantial effect of the ligand alkyl chain length on the various dissolution reactions was obtained. Thence, this paper emphasizes the process of stationary phase deterioration, correlated with the shielding effect of the anchored ligands at the silica surface. The influence of the eluent composition is also considered.

EXPERIMENTAL SECTION

The synthesis and physical characterization of the RP-HPLC phases with varying n-alkyl chain length are described in the accompanying paper (1).

Preparation of Solutions. The dissolutions of the reversed phases or the native silica were performed by adding ca. 350 μL of a 6 M solution of sodium hydroxide in methanol-water (50:50 (v/v)) to approximately 70 mg of stationary phase (this amount was corrected for the ligand content) in a zirconium oxide rotor of the Bruker double air bearing type. In this way a well mixed suspension of Na_2O-SiO_2 with an overall, molar composition of 1:1 was obtained. Similar dissolutions were performed in a 2 M aqueous sodium hydroxide solution in order to obtain an overall, molar composition of 1:3 Na_2O-SiO_2. The well-mixed suspension was introduced into the solid-state NMR spectrometer as quickly as possible.

^{29}Si NMR Measurements. The ^{29}Si NMR spectra were obtained on a Bruker CXP Fourier transform nuclear magnetic