29Si NMR Model Dissolution Study of the Degradation of Reversed Phases for High-Performance Liquid Chromatography

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^{29}Si NMR Model Dissolution Study of the Degradation of Reversed Phases for High-Performance Liquid Chromatography

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In order to simulate aging under chromatographic conditions, mono-, di-, and trifunctional octyl and octadecyl derivatized reversed-phase high-performance liquid chromatography silicas were exposed to the very aggressive mobile phase-like medium of 6 M NaOH in MeOH/H_2O (50/50 v/v). ^{29}Si NMR was used to study the dissolution characteristics of these phases by monitoring the concentrations of the dissolving silane and silica structures. Dissolution products of alkylchlorosilanes were used as model compounds for assignment purposes. Octadecyl phases appeared to degrade by dissolution of the silica substrate; octadecylsilane structures were shown to be insoluble under the experimental conditions. Monofunctional octyl phases were shown to deteriorate through initial dissolution of monomeric ligand silane particles, whereas for difunctional octyl phases, the silica backbone appeared to dissolve with the silane ligands still attached. The latter mechanism was also observed for the trifunctional octyl phases, but these phases resemble octadecyl phases, probably because the free ligand silane particles are almost insoluble and the major cause of phase degradation is dissolution of the silica substrate.

Silica gel is by far the most widely used substrate for stationary phases in reversed-phase high-performance liquid chromatography (RP-HPLC). The most important disadvantage of silica gel in liquid chromatography is its hydrolytic instability both at low and especially at high pH values of the mobile phase. Therefore, there has been a substantial effort to synthesize new chromatographic substrates, mostly organic polymers (in their pure form or immobilized on silica supports), that are more resistant to the extreme mobile phase compositions sometimes necessary to achieve a successful separation of, for example, basic drugs.[1]

Despite several successful results in this area,[2-5] the overall merits of these polymer substrates do not fully counterbalance the well-known favorable properties of silica gel, i.e., its large surface area which is chemically fairly easy to modify, its high pressure resistance, and, finally, the fact that silica substrates do not suffer from swelling effects. Other research therefore concentrates on improving the hydrolytic stability of RP silicas by developing new derivatization materials and techniques.[6-9] The observed improved stability of these reversed phases is apparently based on the better shielding by the attached surface structures, which minimizes the interaction of the silica substrate with the surrounding mobile phase.

^{29}Si CP MAS NMR plays an important role in the characterization of the chromatographic silicas because of its surface selectivity. Since the introduction of this technique for silica NMR analysis,[10] much research has been done in which the relations between the NMR characteristics of the solid-state silica phases and the chromatographic performance were investigated.[11] An obvious characteristic of these studies is that properties of the silica surface in the dry solid state are related to chromatographic phenomena taking place under conditions in which the silica particles are suspended in the mobile phase. Previously, a combination of carefully controlled artificial aging of stationary phases for RP-HPLC and chromatographic and physicochemical tests, including solid-state NMR, were carried out by our group.[12] This, in turn, prompted us to perform experiments on silica phases that were dissolved in a very aggressive "mobile phase-like" liquid in order to study the dissolution behavior of RP-HPLC phases as a function of the length of the ligand alkyl chain.[13] This earlier study was confined to monofunctional reversed phases only.

It is the aim of the present study to elucidate the influence of the functionality (mono-, di-, or trifunctional) of the original silanizing agent on the dissolution rate and dissolution mechanism of a chromatographic phase at high pH. It is also checked whether a surface treatment of the native silica material prior to derivatization has any measurable effect on the dissolution behavior. Metal impurities are suspected to be a major cause of the imhomogeneity of silica surfaces, leading to highly acidic residual surface silanols and unwanted chromatographic peak broadening and tailing.[14] Leaching procedures reduce the impurity concentration, thereby providing a more homogeneous silica surface which should also enhance the stability of the chemically bonded phase.

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Table 1. List of the Stationary Phases Under Study

notation	silica substrate	silane reagent	% carbon (w/w)	surface ligand density (μmol/m^2)
U-1/8	untreated	methoxydimethyl-*n*-octylsilane	10.0	2.61
U-2/8	untreated	dimethoxymethyl-*n*-octylsilane	12.6	3.82
U-3/8	untreated	trimethoxy-*n*-octylsilane	12.0	4.06
U-1/18	untreated	methoxydimethyl-*n*-octadecylsilane	18.1	2.65
U-2/18	untreated	dimethoxymethyl-*n*-octadecylsilane	20.4	3.27
U-3/18	untreated	trimethoxy-*n*-octadecylsilane	20.0	3.37
T-1/8	treated	methoxydimethyl-*n*-octylsilane	10.7	3.39
T-2/8	treated	dimethoxymethyl-*n*-octylsilane	11.6	4.18
T-3/8	treated	trimethoxy-*n*-octylsilane	11.9	4.85
T-1/18	treated	methoxydimethyl-*n*-octadecylsilane	18.5	3.27
T-2/18	treated	dimethoxymethyl-*n*-octadecylsilane	20.7	4.01
T-3/18	treated	trimethoxy-*n*-octadecylsilane	20.3	4.13

Furthermore, some conclusions from our earlier investigations are reconsidered on the basis of new information obtained from the present study in which model experiments play an essential role.

EXPERIMENTAL SECTION

Materials and Methods. The noncommercial silica phases, made as research samples (Lichrospher 100, $d_p = 5$ μm), were obtained from E. Merck (Darmstadt, Germany). Some surface properties are summarized in Table 1. The specific surface areas of the treated (T-0) and untreated (U-0) native silica substrates were 309 and 371 m^2/g, respectively. The pore volume of both substrates was 1.2 mL/g.[15] (The exact nature of the surface treatment is confined to the manufacturer's knowledge only.)

Mobile phase-like alkaline solutions (2, 4, and 6 M NaOH) were prepared by adding respectively 4, 8, and 12 g of sodium hydroxide (99.9%, Boom B.V. Meppel, The Netherlands) to 20 mL of deionized water (Milli-Q, Millipore Corp.) in a Teflon measuring jug, with cooling in a water bath at ambient temperature. After dissolution of the sodium hydroxide, 20 mL of methanol (Lichrosolv, Merck AG, Darmstadt, Germany) was added, and finally the solution was filled up to 50 mL using a previously prepared mixture of methanol/water (50/50 v/v). This MeOH/H_2O ratio was chosen for practical reasons; the solution contains enough methanol to assure appropriate wetting of the derivatized silica gels. (In the Conclusions section, the influence of mobile phase composition on bonded phase structure is briefly discussed.) Furthermore, MeOH/H_2O (50/50 v/v) is a reasonable "average" chromatographic mobile phase composition.

To suspend the silica phases, 700 μL of sodium hydroxide solution was added to an exactly known amount of stationary phase in a glass vial. To assure good mixing of the liquid and solid, the vial was shaken vigorously. After the suspension was degassed by a short ultrasonic treatment (5–10 s), approximately 350 μL was transferred with a syringe through a small opening in the Kel-F cap of a 7 mm o.d., double air bearing type, zirconia spinner (Bruker GmbH, Rheinstetten, Germany). The spinner cap was previously glued to the inside spinner wall, and the opening was closed with a conical Kel-F stopper. This construction showed no leakage of fluid which could otherwise easily damage the NMR probe head. The total amount of suspension in the NMR sample holder was weighed and then transferred to the NMR spectrometer as quickly as possible.

NMR Experiments. ^{29}Si NMR measurements were performed on a Bruker MSL-400 NMR spectrometer at a Larmor frequency of 79.4 MHz. The samples were spun at a frequency of 800 Hz to minimize any broadening due to dipolar interactions in the viscous liquids. Flip angles of 45° were applied with an acquisition time of 40 ms, and the pulse interval time was set to 5 s. This relatively short pulse delay time was chosen to ensure that only dissolved silica particles (with small T_1 values) are detected, thus avoiding disturbing signals from any remaining solid phase or precipitated particles. A total of 360 FIDs were accumulated, resulting in a time resolution of 0.5 h per spectrum. Typically, 40 spectra were recorded per dissolution experiment. The raw FIDs were zero-filled to 8K, and prior to Fourier transformation a line broadening of 20 Hz was applied. Unless stated otherwise, intensity scales of all spectra were normalized to the scale of the spectrum of the native silica gel U-0 in 6 M NaOH in MeOH/H_2O (50/50 v/v) after 20 h of dissolution. Reported chemical shifts are referenced to liquid tetramethylsilane, using Q_8M_8 (the trimethylsilyl ester of cubic octameric silicate) as an external reference.

Optimization of the Dissolution Conditions. In order to obtain a useful NMR signal to noise ratio in combination with a solvent composition that as closely as possible approximates true chromatographic mobile phases, the sodium hydroxide concentration and the Na:Si ratio of the suspensions had to be optimized. As ^{29}Si NMR generally lacks high sensitivity, a high pH will be necessary to promote dissolution of a large quantity of reversed-phase material. However, if the sodium hydroxide concentration is too high, dissolution will be very fast and the time required to obtain a single NMR spectrum will be too long to allow observation of the mechanism through which the dissolution has proceeded. On the other hand, a too low Na:Si ratio allows only large polymeric silica structures to be dissolved, and then little can be concluded about the initial monomeric molecular structures leaving the reversed-phase surface. Therefore, several experiments were carried out in which the sodium hydroxide concentration as well as the amount of reversed-phase material was varied before the real dissolution experiments were conducted. To illustrate the considerations above, Figure 1 displays ^{29}Si NMR spectra of suspensions of U-1/8 in which the sodium hydroxide

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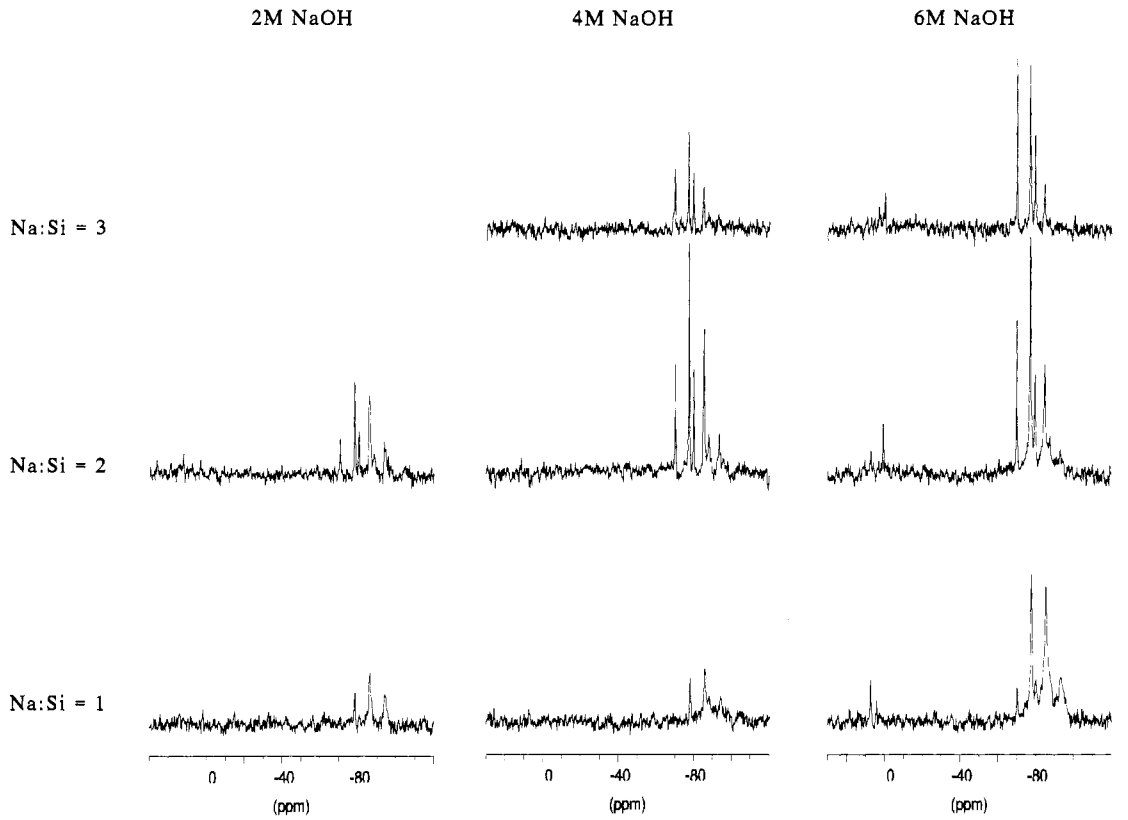

Figure 1. ^{29}Si NMR spectra of suspensions of the phase U-1/8 in alkaline MeOH/H_2O (50/50 v/v) after 5 h of dissolution. Horizontally, the sodium hydroxide concentration is varied, and in the vertical direction the Na:Si ratio is varied.

concentration was varied between 2, 4, and 6 M in combination with Na:Si ratios that were 1, 2, and 3, respectively. A compromise between signal intensity and dissolution rate was found with a combination of a 6 M NaOH in MeOH/H_2O (50/50 v/v) solution and a Na:Si ratio of 2. This combination will be used throughout this study. Such a highly alkaline solution obviously will not be useful as a mobile phase in everyday chromatographic practice. In the present study, rather bold circumstances are chosen on purpose, so as to obtain useful results within a reasonable time.

Model Experiments. The assignment of the observed ^{29}Si nuclear magnetic resonances to specific ligand silane structures was performed by using model experiments in which alkylchlorosilanes were used. For example, methyl-*n*-octyldichlorosilane was added to a 6 M NaOH in MeOH/H_2O (50/50 v/v) solution to obtain information on the ligand silane signals observed after dissolution of difunctional octyl phases in the same medium. The added amount of chlorosilane always corresponded to the total amount of ligand structures introduced by dissolving the original phase. To study the influence of the presence of silicate structures on the chemical shift and intensity patterns of the ligand signals, native silica gel was dissolved in an equilibrated solution of the corresponding model silane in 6 M NaOH in MeOH/H_2O (50/50 v/v). Chlorosilanes were purchased from different suppliers, used as received, and stored under an argon atmosphere.

RESULTS AND DISCUSSION

Silica Substrate. Table 2 lists the NMR chemical shifts of the various silicate species that occur in alkaline silicate solutions. Figure 2 displays the spectrum of a suspension of U-0 in a 6 M NaOH in MeOH/H_2O (50/50 v/v) solution after 20 h of dissolution. The relative signal intensities of the various silicate species are in good agreement with those reported in the literature for an equilibrium solution with a Na:Si ratio of 2.[17] In Figure 3, the signal intensities are plotted as a function of dissolution time for both native silicas U-0 (Figure 3a) and T-0 (Figure 3b). It can be seen that under our experimental conditions no effect from the surface treatment can be discerned. Actually, this effect could not be observed for any of the phases listed in Table 1. As a consequence, further results and discussion are focused on the phases bonded on the untreated silica substrate, while all conclusions are equally valid for the phases bonded on the

Table 2. Silicate Structures Present in 6 M NaOH in MeOH/H_2O (50/50 v/v) Solutions, Their Notation, and Their Chemical Shift Externally Referenced to Liquid Tetramethylsilane

silicate structure	notation[a]	δ (ppm)
tetrahydroxysilane	Q^0	−70.0
hexahydroxydisiloxane	Q_2^1	−77.0
hexahydroxycyclotrisiloxane	Q_T^2	−79.7
dihydroxydisiloxysilane (linear oligomer)	Q^2	−84.7
hydroxytrisiloxysilane (part of trisiloxane ring structure)	Q_T^3	−87.4
hydroxytrisiloxysilane	Q^3	−92.5

[a] From ref 16.

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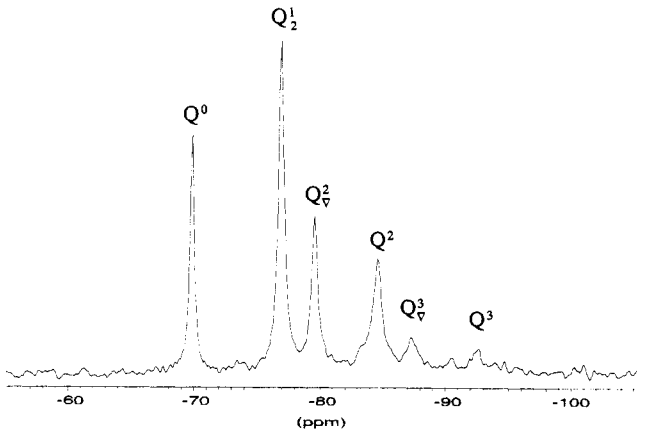

Figure 2. ^{29}Si NMR spectrum of U-0 after 20 h of dissolution in 6 M NaOH in MeOH/H_2O (50/50 v/v).

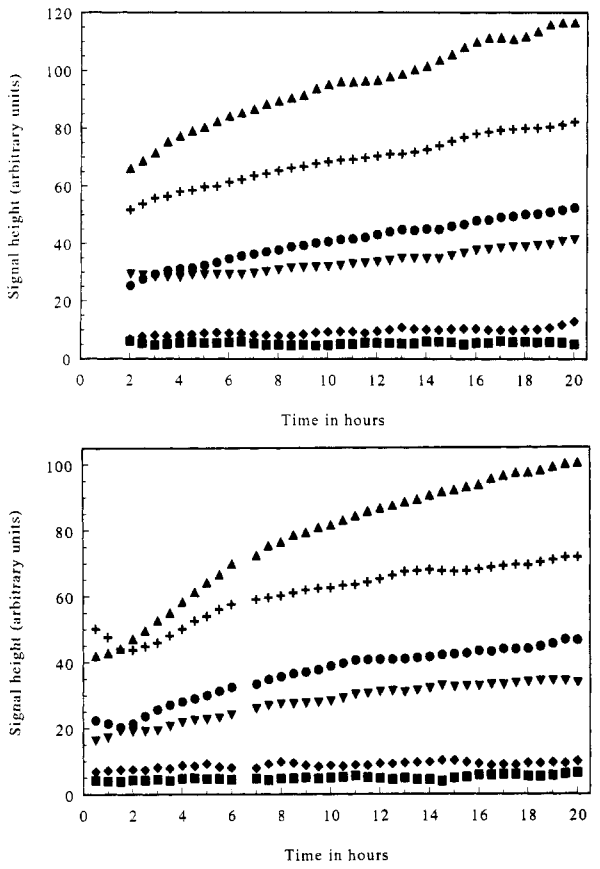

Figure 3. Heights of the ^{29}Si NMR signals of the silicate structures of the native silicas U-0 (a, top) and T-0 (b, bottom) as a function of dissolution time in 6 M NaOH in MeOH/H_2O (50/50 v/v). A 3-point moving average was used to smooth the curves (+, Q_0; ▲, Q^1_2; ●, Q^2_∇; ▼, Q^2; ◆, Q^3_∇; ■, Q^3).

treated silica substrate. The dissolution circumstances probably are too severe to allow detection of subtle differences in surface properties due to the pretreatment. (These subtle differences are present, because chromatographically the phases on the treated silica substrate were shown to be generally more stable.[15]) Due to the rapid dissolution and chemical exchange between the different silicate structures in solution,[18] any small initial difference in relative peak intensities is averaged out very quickly (at the most within a few minutes).

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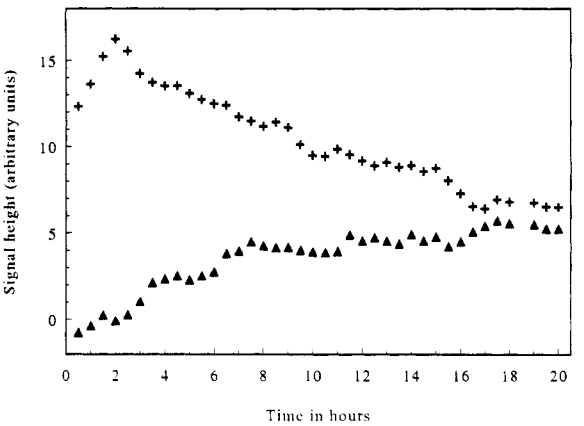

Figure 4. Heights of the ^{29}Si NMR signals of the U-1/8 ligand silane structures as a function of dissolution time in 6 M NaOH in MeOH/H_2O (50/50 v/v). A 3-point moving average was used to smooth the curves (+, −0.1 ppm; ▲, +7.3 ppm).

Monofunctional Phases. After suspension of the monofunctional C_8 phase U-1/8, two extra resonances could be observed in the ^{29}Si NMR spectra, apart from the silica pattern in the −70 to −100 ppm range which is similar to that of U-0. The chemical shifts of the corresponding ^{29}Si nuclei are +7.3 and −0.1 ppm. Figure 4 displays the peak heights of these signals as a function of dissolution time. The shape of both curves suggests that the species resonating at −0.1 ppm is formed immediately after suspension of the derivatized silica and then reacts to give the species resonating at +7.3 ppm. Figure 5 displays the ^{29}Si NMR spectra from which these two resonances can be assigned to specific silane structures. There is no doubt that the species detected in the solution of dimethyl-*n*-octylchlorosilane in 6 M NaOH in MeOH/H_2O (50/50 v/v) shown in Figure 5b is dimethyl-*n*-octylhydroxysilane (M^0), resonating at −1.6 ppm. It seems logical to ascribe the resonance at +7.3 ppm in Figure 5e and f to 1,1,2,2-tetramethyl-1,2-di-*n*-octyldisiloxane (M^1_2), but the hexamethyldisiloxane dimer is seen to hydrolyze to the hydroxy monomer in the alkaline environment (Figure 5c and d). From a comparison of Figure 5b and e, it must be concluded that when U-0 is added to a solution of dimethyl-*n*-octylchlorosilane in 6 M NaOH, a fast condensation reaction occurs between M^0 and silicate species, forming dimethyl-*n*-octylsiloxysilane (M^1) structures. The silane part of these structures then gives rise to a signal at +7.3 ppm.

These findings differ from our earlier conclusions, which stated that the free ligand (M^0) resonates at +18 ppm.[13] This previous assignment was based on the chemical shift of trimethylethoxysilane (to be found in literature).[19] However, the hydroxy compound in a highly alkaline solution will ionize to a large extent, contrary to the alkoxy analogue. This ionization increases the electron density around the silicon nucleus, and thus the NMR signal is shifted to higher field (lower ppm value), from +18 to −0.5 ppm. This large upfield shift was observed earlier by Williams et al. in a study of the solvent effects on the chemical shifts of silanols.[20]

Our earlier conclusion that the +7.3 ppm signal is due to the dimeric ligand molecule M^1_2 was only partly correct. This

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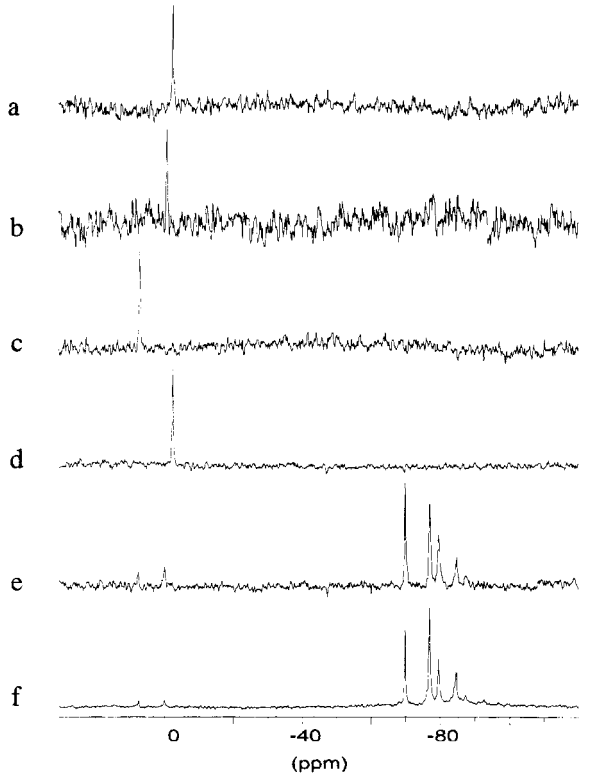

Figure 5. ^{29}Si NMR spectra of (a) trimethylchlorosilane; (b) dimethyl-*n*-octylchlorosilane; (c) hexamethyldisiloxane immediately after dissolution and (d) after 48 h of dissolution in 6 M NaOH in MeOH/H_2O (50/50 v/v); (e) dimethyl-*n*-octylchlorosilane 0.5 h after addition of U-0 in 6 M NaOH in MeOH/H_2O (50/50 v/v); and (f) an equilibrium solution of U-1/8 in 6 M NaOH in MeOH/H_2O (50/50 v/v). Intensity scales of the separate spectra are adjusted to fit the figure dimensions.

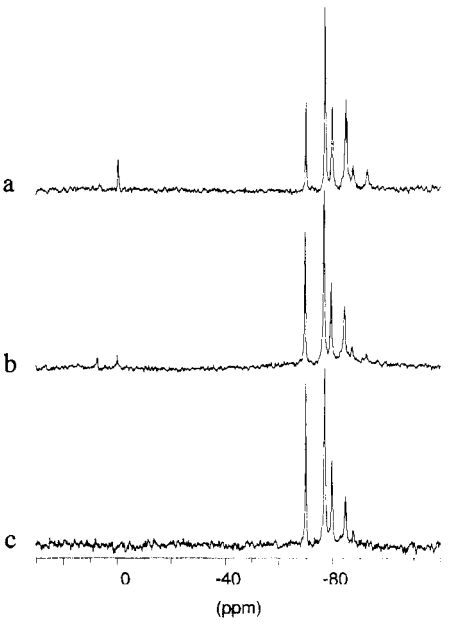

Figure 6. ^{29}Si NMR spectra of equilibrium solutions of (a) U-0 in 6 M NaOH MeOH/H_2O (50/50 v/v) after addition of trimethylchlorosilane; (b) U-1/8 and (c) U-1/18 in 6 M NaOH MeOH/H_2O (50/50 v/v).

study proves that in 6 M NaOH in MeOH/H_2O (50/50 v/v), dimeric monofunctional ligand particles (Figure 5c) and M^1 structures cannot be distinguished on the basis of ^{29}Si NMR chemical shifts. The major part of the +7.3 ppm signal must be caused by M^1 structures, because it is highly unlikely that dimeric ligand particles are formed, whereas siloxane silicate particles are by far the most abundant in solution. Thus the probability of two monomeric ligand species encountering each other before reacting with, for example, tetrahydroxysilane (Q^0) is remote.

Now, after the assignments made above, Figure 4 seems to indicate that deterioration of monofunctional C_8 phases occurs largely through initial dissolution of monomeric ligand silane species.

Upon dissolution of the monofunctional C_{18} phase (U-1/18), no ligand signals were observed. Most likely this is caused by the very low solubility of dimethyl-*n*-octadecylhydroxysilane species in the alkaline methanol/water solution. Figure 6 illustrates this by comparing respectively equilibrium solutions of trimethylchlorosilane in a U-0 suspension, a U-1/8 suspension, and a U-1/18 suspension. Trimethylhydroxysilane is very well soluble in the 6 M NaOH solution, whereas dimethyl-*n*-octylhydroxysilane and dimethyl-*n*-octylsiloxysilane (M^0 and M^1) are present in their equilibrium concentrations. Octadecyl species are apparently insoluble under the experimental conditions because of the long, apolar alkyl chain that cannot be accommodated well enough by the polar solvent medium. As a consequence, degradation of monofunctional C_{18} phases for RP-HPLC most likely takes place by dissolution of the underlying silica substrate and not by a mechanism in which octadecylsilanes are stripped off from the surface.

Interesting to note is the increasing Q^0/Q_2^1 ratio in the spectra of Figure 6 when going from the mimicked suspension of a monofunctional C_1 phase to the C_{18} phase suspension. Probably, the amount of dissolved ligand species in solution determines the Na:Si ratio expressed by the intensity distribution in the silicate signal region. No octadecyl ligands are observed in solution, and consequently the silicate pattern of Figure 6c is the one with the highest Q^0/Q_2^1 ratio.

Difunctional Phases. Dissolution of the difunctional C_8 phase (U-2/8) gives rise to two ligand signals at −14.0 and −22.8 ppm. The assignments of these resonances to specific silane structures can be made in analogy with the strategy described for the monofunctional phases. This is illustrated in Figure 7. Clearly, the resonance at −14.0 ppm in Figure 7a stems from dimethyldihydroxysilane, while the −22.8 ppm signal in Figure 7b is due to the presence of 1,2-dimethyl-1,2-di-*n*-octyl-1,2-dihydroxysiloxane (D_2^1). Apparently, the difunctional ligand species with the longer alkyl chain is more likely to dimerize in solution (compare Figures 7a and 7b). In Figure 7c, however, the −14.0 ppm signal must belong to the molecule methyl-*n*-octylhydroxysiloxysilane (D^1) because of the abundance of silicate structures in solution. This assumption is supported by the fact that after addition of U-0 to a suspension of methyl-*n*-octyldichlorosilane, only the −22 ppm signal initially is broadened and sharpened again after a few hours, while the −14 ppm signal remains unaffected. This successive broadening and sharpening (not shown in the figures) can be explained by the conversion of the dimer D_2^1 into D^1 species as soon as silicate particles interfere with the dynamic equilibrium between mono- and dimer ligand particles.

Figure 8 illustrates that, after a difunctional C_8-phase is suspended, equilibrium between the two ligand species is attained very fast. After 2 h of dissolution, the ratio of the

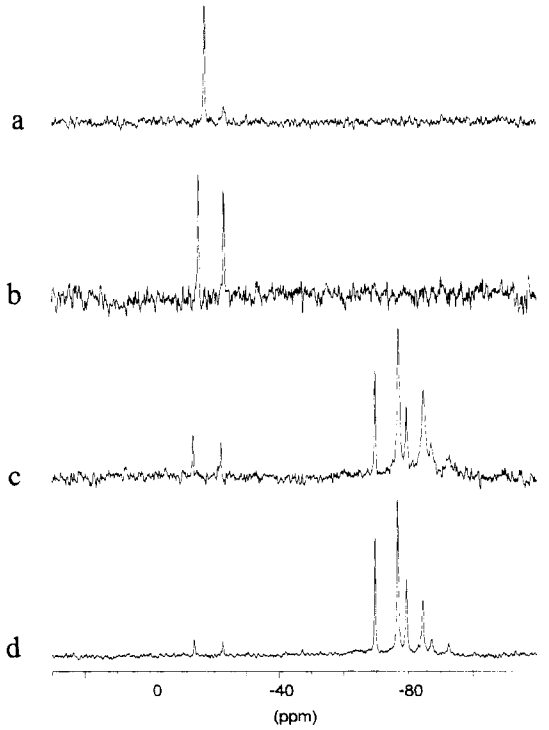

Figure 7. ^{29}Si NMR spectra of (a) dimethyldichlorosilane and (b) methyl-*n*-octyldichlorosilane in 6 M NaOH in MeOH/H_2O (50/50 v/v); (c) methyl-*n*-octyldichlorosilane 12 h after addition of U-0 in 6 M NaOH in MeOH/H_2O (50/50 v/v); and (d) equilibrium solution of U-2/8 in 6 M NaOH in MeOH/H_2O (50/50 v/v). Intensity scales of the separate spectra are adjusted to fit the figure dimensions.

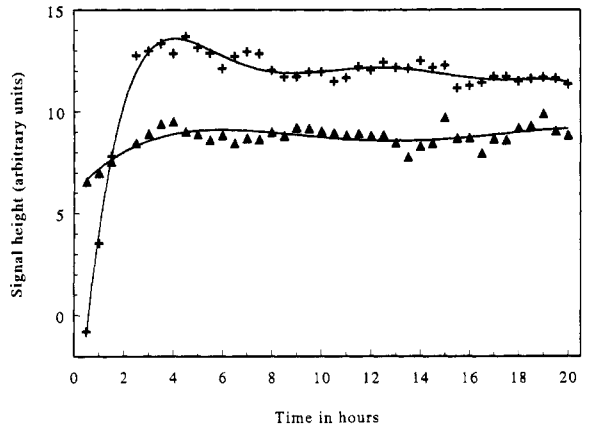

Figure 8. Heights of the ^{29}Si NMR signals of the U-2/8 ligand silane structures as a function of dissolution time in 6 M NaOH in MeOH/H_2O (50–/50 v/v). A 3-point moving average was used to smooth the curves (+, D^0; ▲, D^1, D_2^1). The lines drawn are only a guide to the eye.

signal heights of the two ligand species remains constant. Important to note is that during the first hour, the D^1 signal intensity is higher than that of D^0. This implies that the initial dissolution mechanism proceeds through hydrolysis of oligomeric (or at least dimeric) silane structures. Unfortunately, a ligand dimer D_2^1 cannot be distinguished from a D^1 structure (vide supra), and thus it cannot be stated whether the alkaline solution in the first instance strips off only ligand dimers, ligand structures attached to a small silica backbone particle, or a combination of both. However, difunctional derivatization agents are not known to form extensive polymer layers on

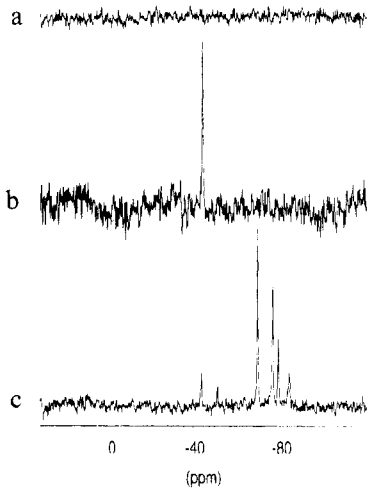

Figure 9. ^{29}Si NMR spectra of (a) *n*-octyltrichlorosilane in 6 M NaOH in MeOH/H_2O (50/50 v/v); number of scans, 360; (b) number of scans, 5000; (c) U-3/8 0.5 h after dissolution in 6 M NaOH in MeOH/H_2O (50/50 v/v). Intensity scales of the separate spectra are adjusted to fit the figure dimensions.

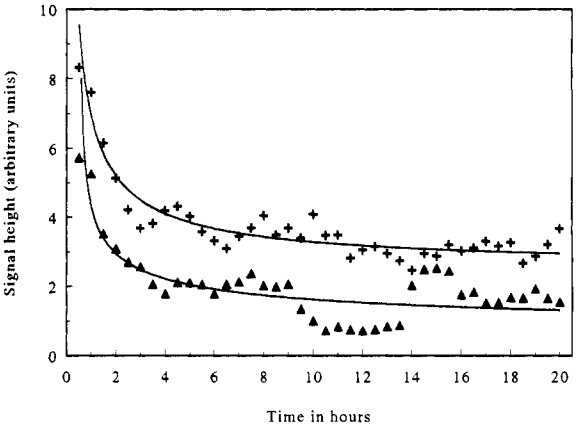

Figure 10. Heights of the ^{29}Si NMR signals of the U-3/8 ligand silane structures as a function of dissolution time in 6 M NaOH in MeOH/H_2O (50/50 v/v). A 3-point moving average was used to smooth the curves (+, T^0; ▲, T^1, T_2^1). The lines drawn are only a guide to the eye.

silica surfaces,[21] and therefore it seems most likely that the silica backbone is dissolved with the silane ligand still chemically attached. As already stated, no ligand species can be observed after dissolution of the difunctional octadecyl phase U-2/18.

Trifunctional Phases. In Figure 9c, the ^{29}Si NMR spectrum of the trifunctional phase U-3/8 is shown after 0.5 h of dissolution. Again, clearly two ligand signals are observed. Figure 10 shows the rapid disappearance of these signals. The peak assignment can again be made by dissolving the appropriate derivatizing agent in the 6 M NaOH in MeOH/H_2O (50/50 v/v) solution (see Figure 9). After addition of *n*-octyltrichlorosilane to a 6 M NaOH in MeOH/H_2O (50/50 v/v) solution, immediately a white precipitate is formed; after 360 scans, no significant NMR signal can be observed. After 5000 scans, a signal at –44.2 ppm is apparent which should be ascribed to the monomer *n*-octyltrihydroxysilane (T^0). This assignment is in accordance with results of Hasegawa et al., who found the corresponding methyl analogue to resonate at –42.1 ppm in an aqueous 2 M sodium hydroxide

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Table 3. Chemical Shifts of Ligand Silane Species Detected after Dissolution of Mono-, Di-, and Trifunctional Octyl Phases for RP-HPLC in 6 M NaOH (MeOH/H_2O (50/50 v/v), Externally Referenced to Liquid Tetramethylsilane (Na:Si = 2)

ligand silane species	notation[a]	δ (ppm)
dimethyl-*n*-octylhydroxysilane	M^0	−1
dimethyl-*n*-octylsiloxysilane	M^1	+7
1,1,2,2-tetramethyl-1,2-di-*n*-octyldisiloxane	M_2^1	+7
methyl-*n*-octyldihydroxysilane	D^0	−14
methyl-*n*-octylhydroxysiloxysilane	D^1	−23
1,2-dimethyl-1,2-di-*n*-octyl-1,2-dihydroxydisiloxane	D_2^1	−23
n-octyltrihydroxysilane	T^0	−44
n-octyldihydroxysiloxysilane	T^1	−52
1,2-di-*n*-octyl-1,1,2,2-tetrahydroxydisiloxane	T_2^1	−52

[a] See also Figure 11 for structural illustration.

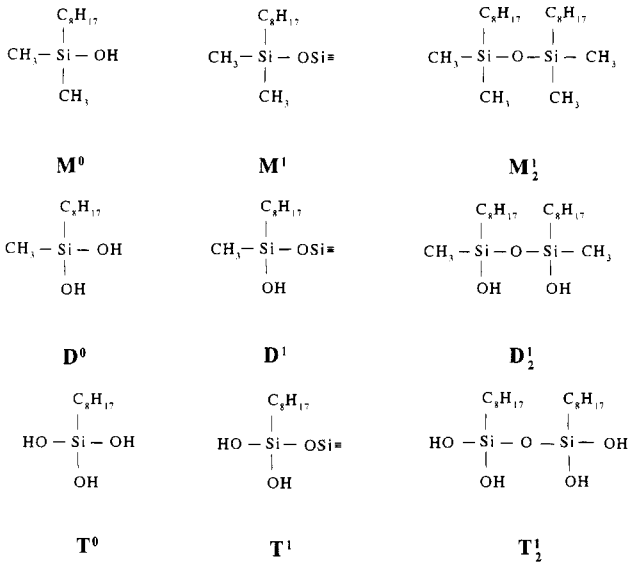

Figure 11. Molecular structures of the detected ligand species.

Table 4. Comparison of Chemical Shifts of the Ligand Silane Monomers in 6 M NaOH in MeOH/H_2O (50/50 v/v) and Literature Values of the Corresponding Alkylmethoxysilanes (from Ref 25)

methoxysilane	δ (ppm)	hydroxysilane	δ (ppm)	Δδ
$(CH_3)_3SiOCH_3$	+17	$(C_8H_{17})(CH_3)_2SiOH$	−1	−18
$(CH_3)_2Si(OCH_3)_2$	−2	$(C_8H_{17})(CH_3)Si(OH)_2$	−14	−12
$(CH_3)Si(OCH_3)_3$	−41	$(C_8H_{17})Si(OH)_3$	−44	−3
$Si(OCH_3)_4$	−79	$Si(OH)_4$	−70	+9

solution.[22] The small upfield shift of T^0 under our experimental conditions probably is caused by an increased ionization in the more alkaline solution. The signal at −51.6 ppm in Figure 9c indicates the presence of *n*-octyldihydroxysiloxysilane (T^1) moieties. It cannot be ruled out, however, that the oligomeric ligand particle T_2^1 also contributes to the NMR signal, because trifunctional silanizing reagents are known to form polymeric surface layers through cross-linking. This could result in an initial dissolution of dimeric ligand particles. Nevertheless, both the very low solubility of *n*-octyltrihydroxysilane and the rapidly decreasing ligand signals after dissolution of a trifunctional octyl phase support the idea that deterioration of trifunctional phases takes place mainly through dissolution of the underlying silica substrate, while ligand silane moieties remain attached to the dissolving silicate particles. If dissolution of the silica substrate is the main cause of stationary phase degradation, carbon contents are seen to increase upon aging.[23,24] This was actually observed after chromatographic aging experiments with the trifunctional C_8 phase discussed here.[15]

Chemical Shifts of the Detected Silane Species. Table 3 summarizes all ligand species that were detected after dissolution of the mono-, di-, and trifunctional C_8 phases, and Figure 11 gives a structural illustration. A remarkable feature is observed when comparing the chemical shifts of the mono- and difunctional monomeric ligand silane molecules with their respective dimeric species. The monomeric *mono*functional ligand silane particle M^0 has a decreased chemical shift compared to its dimer M_2^1, while for the monomeric *di*functional ligand silane particle D^0, an increase is observed. When the chemical shifts of the corresponding methoxysilanes are considered, the chemical shifts of the hydroxysilanes in alkaline solution can be understood by taking into account an ionization and a hydrogen-bonding contribution to the NMR chemical shift when a methoxy group is substituted for a hydroxyl group (Table 4). In their neutral form, hydroxysilanes have chemical shifts comparable to those of their alkoxy analogues. Ionization of trialkylhydroxysilane induces a shielding of about 18 ppm under the present experimental conditions. (Upon addition of the native silica to an equilibrium suspension of a model chlorosilane, the chemical shift of the hydroxysilanes is seen to shift by about +1.5 ppm, probably due to less extensive ionization as the dissolution of silicate particles "consumes" sodium hydroxide from solution and the base/silane ratio is decreased.) The dialkyldihydroxysilanes in 6 M NaOH in MeOH/H_2O (50/50 v/v) are shielded by about 12 ppm from their dialkoxy analogues, where one might expect a larger difference because of the difunctionality. We assume that an increased hydrogen-bonding capability of dialkyldihydroxysilanes can explain the smaller upfield shift. Kononov et al. state that the chemical shift of alkali metal organosilanols is largely determined by the polarity of the Si–O bond, giving rise to an upfield shift upon increasing polarity.[26] Coordination of water to the difunctional ligand silane particle (i.e., the formation of hydrogen bonds) weakens the polarity of both Si–O bonds, which explains the smaller upfield shift for the dihydroxysilane. Finally, *n*-octyltrihydroxysilane is shielded by 3 ppm from its trimethoxy analogue, and altogether the trend in the differences in chemical shift between the alkylalkoxy- and the alkylhydroxysilanes displayed in Table 4 is consistent, supporting the foregoing assignments.

CONCLUSIONS

From the results of this study it can be concluded that the mechanism through which a RP-HPLC phase in contact with basic solvent media deteriorates depends largely on the

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solubility of the individual silane structures at its surface. As shown, octadecyl ligand silanes are insoluble in the relatively polar 6 M NaOH in MeOH/H_2O (50/50 v/v) solution, and any octadecyl phase (mono-, di-, or trifunctional) therefore seems to deteriorate through dissolution of the underlying silica substrate. This conclusion is valid only for the MeOH/H_2O ratio used in this study, because there is ample evidence that the solvent composition has a great influence on the bonded-phase structure. Several authors postulated that the organization of alkyl ligands on a densely covered silica surface changes from a collapsed state at low percentages of organic modifier to an extended state at high percentages of organic modifier.[27-29] This should change the shielding properties of the alkyl chains at different solvent compositions which, in turn, could change the degradation mechanism. Generally, the solubility of octyl ligand silanes decreases in the order mono- > di- > trifunctional. This is reflected in the observed dissolution patterns of the corresponding silica phases: after monofunctional octyl phases are dissolved, mostly monomeric ligand silanes are observed; difunctional octyl phases initially appear to release ligand structures attached to a small silica backbone particle; and, finally, trifunctional octyl phases resemble octadecyl phases, probably because the great tendency of trifunctional silane particles to polymerize inhibits large-scale dissolution of monomeric ligand particles.

It should be borne in mind that the above results were obtained through a model dissolution study in which experimental circumstances differ considerably from those used in everyday chromatography. No difference could be observed between stationary phases stemming from the treated or the untreated silica substrate, nor were there any significant differences in the observed dissolution rates. Nevertheless, the results indicate that the use of alkylsilane reagents with bulky side groups or polymeric derivatization reagents may provide more stable RP-HPLC phases for two reasons. First, the solubility of the surface structures is decreased, and second, the silica surface is likely to be shielded more effectively from intense contact with aggressive eluents.

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