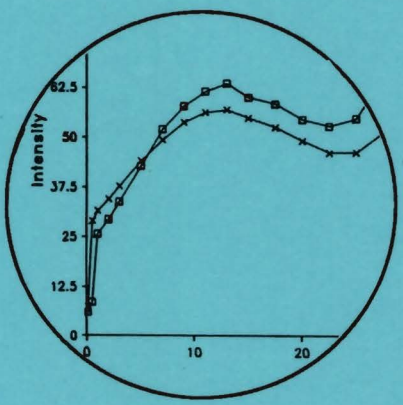
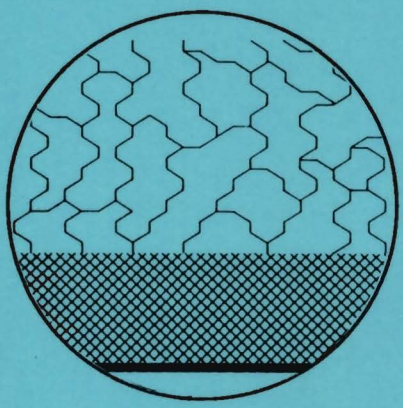
A fundamental study of chemically modified silica surfaces in chromatography

A FUNDAMENTAL STUDY OF CHEMICALLY MODIFIED SILICA SURFACES IN CHROMATOGRAPHY





MARTIN J.J. HETEM

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CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

In analytical chemistry chromatography has become the most widely used technique for identification and quantitative analysis of a wide range of analytes. In the past years the samples to be analyzed have become increasingly complex and the sample through-put has grown enormously.

The chromatographic separation process can be characterized by the resolution of a critical peak pair, which in turn is determined by the selectivity and separation efficiency, according to:

$$R_S = 1/4 * \underbrace{\frac{\alpha - 1}{\alpha} * \frac{k_2}{k_2 + 1}}_{\text{selectivity}} * \underbrace{\sqrt{N}}_{\text{efficiency}}$$

where α is the separation factor (the relative retention of two adjacent peaks in the chromatogram), k_2 is the capacity factor of the later eluting peak of a peak pair and N is the number of theoretical plates.

The separation efficiency is mainly determined by physical parameters, which affect the dispersion in the column. Physical parameters like particle size or capillary column inner-diameter, mobile phase velocity, phase ratio and diffusion constants in the mobile and stationary phase govern the maximal attainable number of plates [1,2]. However, extremely high separation efficiencies can only be obtained at the expense of long analysis times. In

gas chromatography (GC) at maximum 10^6 theoretical plates can be attained within a reasonable time [2]. In liquid chromatography (LC) or supercritical fluid chromatography (SFC) this plate number can only be reached at the cost of extremely large analysis times. This is caused by slower diffusion. Further enhancement of the resolution (R_S must be larger than 1.5 for an acceptable separation) can only be attained by increasing the selectivity of the phase systems. The selectivity of a chromatographic separation depends on the phase ratio and on specific interaction between solutes, mobile phase and stationary phase, e.i. the distribution constant.
The enhancement of the selectivity in GC depends mainly on the functional groups incorporated in the stationary phase [3,4]. In a liquid polar interactions between polarizable compounds and functional groups in the stationary phase can be very specific and pronounced. In LC the magnitudes of these interactions can be regulated by the mobile phase composition, either isocratically or with a solvent gradient [5,6]. In SFC only moderate selectivity can be attained, depending on functional groups incorporated in the stationary phase, the mobile phase nature and density and the modifier content in the mobile phase [7,8].

In GC most separations are performed with capillary columns. In (micro) LC mostly packed columns are used. Physically adsorbed or chemically bonded organic coatings are used in capillary GC and surface anchored ligands or chemically bonded polymers in (micro) packed column LC. The most favoured support material in chromatography today is the amorphous polymeric SiO_2: in capillary chromatography fused-silica capillary column material and in (micro) packed column chromatography porous silica particles. The advantages of silica are its well-defined geometry and the possibility to modify its surface in various relatively simple ways with an organic coating [9-14].
The analytes should preferably show only interaction with the organic coating layer, unwanted interactions with accessible active sites at the surface of the silica support must be avoided [15,16]. The accessibility of these

sites depend mainly on the micro-structure of the organic coating layer, which determines the penetrability for analytes. Another important factor that has to be taken into account is the stability of the stationary phase material, especially when data-base identification systems are used. In synthesizing stationary phases the mechanical, thermal and chemical stability are of major importance. Specially the attachment of the organic layer to the silica support and the stability of the substrate material are objectives that have to be considered [17-19]. Changes in characteristics of these materials during use affect the reproducibility of chromatographic separations. World-wide, large investments are done for stationary phase materials. Therefore, more stable stationary phases in chromatography will have considerable economical advantages for both manufacturers and users.

1.2. SCOPE OF THIS THESIS

The investigations described in this thesis are concerned with the chemical modification of various non-porous and porous silica support materials. Furthermore, the resistance towards deterioration of various modified silicas under conditions normal for daily chromatography practice will be studied in detail.

Deactivation and coating fused-silica capillaries for GC

The first purpose for modification of non-porous amorphous silicas like fused-silica capillary inner walls was deactivation of active sites at the surface by chemical reaction. This deactivation of fused-silica capillaries is usually performed with monomeric silane reagents or polymeric siloxanes [10,20]. Optimization of various deactivation procedures can only be checked by chromatography. However, differences in chemical and physical surface structures between various deactivating methods are preferably characterized by model experiments with fumed-silica materials like Cab-O-Sil. The surface

area inside fused-silica capillaries (inner diameter between 50 μm and 530 μm) is far too little to allow most physico-chemical characterization techniques. Model experiments enable characterization with solid state nuclear magnetic resonance spectroscopy, elemental analysis and determination of physical bulk properties. The presence of remaining active sites at the silica surface upon modification can be observed directly. Secondly, the chemical structure of the deactivating layer and the mobilities of the remaining polymer chains is revealed also. Subsequently, the effect of a typical coating on top of the deactivating layer on the structure of the latter is observed.
In fast GC analysis 50 - 100 μm I.D. columns are used. A coating procedure for a thick-film coating is optimized inside deactivated capillaries of 50 μm. Optimization of various deactivation procedures and subsequent coating of fused-silica capillary columns, together with model studies on fumed vitreous quartz will be discussed in chapters 2 and 3 of this thesis.

Stationary phases for reversed-phase LC

Chemically modified porous silica is a favoured stationary phase for reversed phase liquid chromatography (RP-HPLC) [9,14,21]. Various modified porous silica substrates with different physical and chemical properties have been investigated with the objective of understanding the changes in chromatographic behaviour that occur during use in laboratory practice. In addition, this understanding may suggest improvements in the synthesis of more stable reversed phases. Furthermore, a discriminating and relatively fast procedure is developed to reveal the stability of reversed-phases by chromatographic testing.
Specific stationary phase properties have been reviewed for their effect on the stability under varying conditions in chromatography. The specific effect of various bulk properties and the improvement of the resistance of reversed phases towards deterioration by an acidic pretreatment of the substrate has been regarded explicitly. Various ligand attachments to the substrate surface

and polymer coating with and without precapping are investigated with respect to their effects on stability. The surface shielding effect of ligands with various alkyl chain lengths, between C_1 and C_{18} is studied systematically. This was carried out in three different ways: directly with chromatography, indirectly by physico-chemical means including solid state ^{29}Si NMR and finally with an *in-situ* procedure involving dissolution of chromatographic packing materials taking place in a sample holder of the NMR spectrometer.
Systematic studies regarding the stability of various reversed-phase packing materials will be discussed in chapters 4, 5 and 6.

^{29}Si nuclear magnetic resonance spectroscopy

Various ^{29}Si NMR techniques will be mentioned in this thesis. Conventional liquid state ^{29}Si NMR was used to examine the content and purity of the various deactivating agents. ^{29}Si magic angle spinning (MAS) NMR was used for liquid samples with the *in-situ* dissolution study of the stationary phases modified with different alkyl chain lengths. Solid state ^{29}Si MAS NMR was used to investigate various native and modified porous silicas. Solid state ^{29}Si cross-polarization magic angle spinning (CP-MAS NMR) was applied for the characterization of modified silicas. Within these samples, various ranges of mobilities exist for the silanes and siloxanes at the silica surface after modification. Rigid monolayers of more or less densely stacked ligands are present after certain modifications. Advanced cross-linked polymers multi-anchored to the substrate underneath or various mobile polymers loosely attached or only physically adsorbed at the modified silica with liquid like mobilities are found in other cases.

Quantitative aspects of solid state ^{29}Si NMR

Besides qualitative research regarding the chemical structure of the native and modified silica surfaces, the dissolved silanes, siloxanes and silicates,

quantitative aspects of the various ^{29}Si NMR techniques is part of this thesis.

It is common knowledge, that quantitatively meaningful pulse-excited NMR spectra of mobile and rigid samples can be obtained by a judicious choice of pulse interval times [22]. For ^{29}Si MAS NMR of (derivatized) silicas see [23]. On the other hand, cross-polarization is in principle a selective form of excitation [24]. A specific example for silicas is given in [25]. In this thesis, experiments will be described to find the NMR signal response as a function of the excitation conditions: interval times and contact periods. The resulting "CP-curves" will be analyzed in terms of two (or sets of two) time constants. It is demonstrated that these constants contain useful information regarding sample mobilities for different parts of the sample molecules in the 10^2 and 10^5 Hz range [26].

Some quantitative and qualitative aspects of solid state ^{29}Si NMR will be outlined in chapter 7.

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CHAPTER 2

DEACTIVATION OF NON-POROUS SILICA BY POLYSILOXANE AND PHENYL CONTAINING DISILAZANE [1]

This is a ^{29}Si CP-MAS NMR study of the formation of polysiloxane chains at the surface of non-porous silica. Good deactivation of silica can be obtained with high temperature silylation. Deactivation of active sites is performed directly by chemical reaction. The remaining active sites should preferably be shielded by densely cross-linked polymers. The deactivation reactions involved are studied on Cab-O-Sil, a fumed-silica, which serves as a model substrate for the fused-silica, generally used as column material.

2.1. INTRODUCTION

A number of silylation reagents have been reported in literature for the deactivation of the inner wall of fused silica columns in gas chromatography under widely different conditions at temperatures above 300°C, resulting in deactivating layers of various nature [1,2].

Here, two different widely applied deactivation mechanisms are studied on Cab-O-Sil, a vitreous quartz. The two silylation methods compared are a deactivation by a phenyl containing disilazane, 1,3-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS), introduced by Grob et al. [3] and a

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deactivation by degradation of polydimethylsiloxane (PDMS), introduced by Schomburg et al. [4,5].

The mechanism of the high temperature silylation (HTS) with phenyldisilazanes was studied previously in this laboratory [6-8]. The main result was that at relatively mild, dry conditions (T ≈ 250℃) benzene was released selectively from the silane moieties at the surface.
In 1981, Li Y.F. et al. [9] studied the use of short (cyclic) alkyl polysiloxanes, which not only reacted with active silanol groups at the surface, but also masked the remaining silanol groups through the formation of short, bridged, surface anchored dimethylsiloxane chains. The formation of loop structures by the dimethylsiloxanes at the silica surface might therefore improve the deactivation.

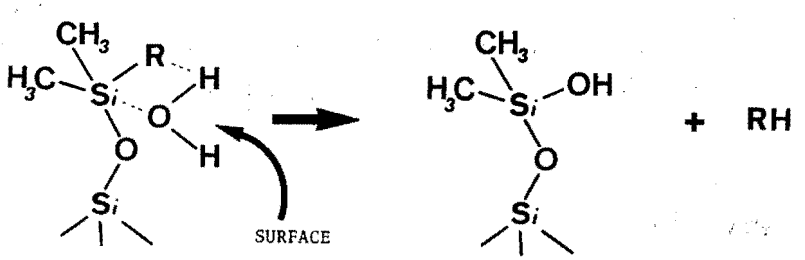

Figure 2.1

Aryldimethylsiloxysiloxanes react with water liberated from the surface according to [10].

Recently, Reiher [10] suggested that dimethylsiloxane chains were formed at the silica surface from phenyl containing silazanes by reaction of initially formed aryldimethylsiloxy silanes with water liberated from the surface, see Figure 2.1. The dimethylhydroxysiloxysilane would then react further with the excess of silazane forming short chains consisting of a few dimethylsiloxane units and terminated with an aryldimethylsiloxy silane. In

this way, the mechanisms of HTS with phenyl containing disilazanes and polysiloxanes would be intertwined.It was assumed that the presence of water at the surface favours the formation of these (short) dimethylsiloxane chains, which would explain both the relatively easy loss of benzene and the high critical surface tension using an excess of DPTMDS.
In order to test this hypothesis the silylation of Cab-O-Sil with DPTMDS was carried out at 377℃ for 16 h under different conditions of humidity and stoichiometry: two different Cab-O-Sil batches were used, an extremely dry Cab-O-Sil and a humid Cab-O-Sil conditioned over a saturated potassium bromide solution for a period of several months. The latter batch of Cab-O-Sil contained 5.5% (w/w) of water.
The amounts of DPTMDS added to both the Cab-O-Sil batches were either stoichiometric amounts of silylation reagents or ten times the stoichiometric ratio.

For reasons of comparison also silylation by polysiloxane degradation of PDMS at a number of temperatures, between 300℃ and 420℃ for 16 h, on dried Cab-O-Sil is studied.
With solid state ^{29}Si CP-MAS NMR and ^{29}Si MAS NMR techniques the anchoring of (short) dimethylsiloxane chains to the surface of the fumed-silica was studied with the explicit aim to investigate whether or not larger, more or less mobile chains are present at the surface of the silylated Cab-O-Sil.

2.2. EXPERIMENTAL

Materials

The Cab-O-Sil M5 (Carbot Corp., Tuscola, Ill, USA) was a gift from Heybroek & Co's Handelsmij (Amsterdam, NL). The specific surface area

of grade M5 is, according to the manufacturer, 200 ± 25 m^2/g. A value of 202 m^2/g was measured with BET measurements, 200 m^2/g will be used in this work.

1.3-Diphenyl-1,1,3,3-tetramethyldisilazane was obtained from Fluka A.G., 9470 Buchs, Switzerland (purum) and the polydimethyl siloxane, OV-101, was obtained from Ohio Valley Specialty Chem. (Ohio, USA). Other chemicals and solvents used were all analytical grade from Merck A.G. (Darmstadt, FRG).

Pretreatment of the Cab-O-Sil

The Cab-O-Sil M5 was ignited at 720℃, rehydrated as described before [6]. Part of the Cab-O-Sil was dried further over phosphorus pentoxide in a vacuum desiccator for several weeks. Part of the Cab-O-Sil was conditioned in air with 84% relative humidity over a saturated solution of potassium bromide. This Cab-O-Sil batch contained 5.5% (w/w) water. This equals 15.3 μmol m^{-2}.

Silylation of Cab-O-Sil

DPTMDS:

About 0.4 g of Cab-O-Sil was placed in a quartz glass reaction ampoule (length 20 cm, 1 cm I.D., wall thickness 1 mm). A constriction was drawn in the middle of the tube and the required amount of reagent was added with a syringe. The tube was evacuated twice (because the volatility of the deactivation reagents, the ampoule was meanwhile cooled in dry ice) and filled with nitrogen to atmospheric pressure. After the final evacuation the ampoule was sealed to a volume of about 8 ml.

The amount of silylation reagent was calculated according to the densest attainable surface concentration of trimethylsiloxy groups formed on

hydrated silica by Stöber [11]: 4.7 μmol m^{-2}. Therefore, 0.47 mmol DPTMDS for the stoichiometric reaction and 4.7 mmol DPTMDS for the excess reaction were added per gram dry Cab-O-Sil.

For the humid Cab-O-Sil batch these amounts were calculated to be 2.0 mmol DPTMDS for the stoichiometric reaction and 6.23 mmol DPTMDS for the excess reaction, assuming that 2 mol H_2O reacts with 1 mol DPTMDS.

PDMS:

Cab-O-Sil was coated with 1.96 g OV-101 per gram, which corresponds to a layer of 10 nm. The OV-101 was dissolved in *n*-pentane and the corresponding amount of Cab-O-Sil was added. The *n*-pentane was evaporated under reduced pressure in a rotary evaporator.

TABLE 2. I

Cab-O-Sil samples; experimental conditions and weight % carbon.

Sample No.	Pretreatment Cab-O-Sil	Reagents	Amount/g Cab-O-Sil	Reaction temp.(℃)	%(w/w) carbon [a]
1	dried over P_2O_5	DPTMDS	0.133	377	3.04
2	dried over P_2O_5	DPTMDS	1.342	377	5.33
3	above KBr	DPTMDS	0.57	377	4.30
4	above KBr [b]	DPTMDS	0.57	377	4.30
5	dried over P_2O_5	OV-101 [c]	1.96	110	
6	dried over P_2O_5	OV-101	1.96	300	10.26
7	dried over P_2O_5	OV-101	1.96	340	9.33
8	dried over P_2O_5	OV-101	1.96	380	6.50
9	dried over P_2O_5	OV-101	1.96	420	3.01

a %(w/w) Carbon determined after silylation and pentane washing.

b Samples conditioned over KBr contained 5.5% (w/w) of water.

c The PDMS used is OV-101, M_w = 30.000 $g.mol^{-1}$.

The resulting coated Cab-O-Sil was dried in a vacuum desiccator over phosphorus pentoxide for several weeks. About 0.4 g of the coated Cab-O-Sil

sample was placed in quartz glass reaction ampoules and vacuum sealed as described for DPTMDS.After sealing the ampoules were wrapped in aluminum foil, placed in a well ventilated oven and heated to the required temperatures for 16 h. After reaction the ampoules were opened and the contents washed twice with toluene and twice with *n*-pentane. The Cab-O-Sil samples were dried overnight in a vacuum oven at 110℃. Table 2.I lists the silylated Cab-O-Sil samples.

Elemental carbon analysis

The carbon content of the silylated Cab-O-Sil was obtained with a Perkin Elmer Analyzer model 240 (Perkin Elmer Corp., Avondale, CT, USA). Tungsten oxide was added to the deactivated silica as a catalyst.

Solid state NMR measurements

The ^{29}Si and ^{13}C solid state NMR spectra were obtained on a Bruker CXP 300 spectrometer at 59.63 MHz and at 75.48 MHz respectively. The samples were spun at ca. 3.5 kHz using aluminum oxide rotors of the standard Bruker double bearing type with 7 mm O.D.

In cross-polarization experiments variable contacts were used with contact times of 1, 2, 4 and 8 ms for ^{29}Si and 1 ms for ^{13}C spectra. Acquisition times of 10 ms (^{29}Si) and 29 ms (^{13}C) and pulse interval times of 1 s resp. 2 s were applied. Typically 12000 FIDs (free induction decays) of ^{29}Si and 3000 FIDs of ^{13}C were accumulated in 1K data points, zero-filled to 8K prior to Fourier transformation. The spectral width was 20 kHz. Line broadening used is 20 Hz prior to zero-filling and Fourier transformation.

2.3. RESULTS AND DISCUSSION

Earlier, models for silica deactivation by means of polysiloxane degradation and by reaction with e.g. D4 (octamethylcyclotetra-siloxane), HMDS, TPSA and DPTMDS have been studied on Cab-O-Sil and with GC in our laboratory [6-8]. All chemical shifts were assigned before. The chemical shifts most relevant to this paper are collected in Table 2.II. The corresponding cyclosiloxanes at the surface are presented in Figure 2.2.

TABLE 2. II

Siloxane/silane functionality, notation and typical ^{29}Si chemical shift.

Moiety	Chemical (spectral) funct.	Topological (network) funct.	Code	Typical shift [a]
Phenyldimethylsiloxysilaner	M	1	M_1P	+ 2
Dimethylhydroxysiloxysilane	D	1	D_1	- 4
Dimethyldisiloxysilane	D	2 [b]	D_2	- 8
Paired dimethyldisiloxysilane	D	2 [c]	$D_{2'}$	- 16
Poly(dimethyldisiloxysilane)	D	2	$D_{2''}$	- 22
Aminotrisiloxysilane	T	3	T_3A	- 88
Dihydroxysiloxane	Q	2	Q_2	- 91
Hydroxysiloxane	Q	3	Q_3	- 101
Tetrasiloxysiloxane	Q	4	Q_4	- 110

a ppm downfield from TMS.
b dimethyldisiloxysilane has a bidentate linkage to the surface forming a cyclotrisiloxane.
c paired dimethyldisiloxysilane forms a cyclotetrasiloxane with the surface siloxanes.

Unfortunately the solid state ^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR technique does not distinguish between cyclosiloxane structures with four or five units. From polysiloxane degradation experiments

at temperatures between 400℃ and 500℃, as published before [7], it can be concluded, largely on ^{29}Si NMR chemical shift arguments and on cross polarisation behaviour, that rather small fragments of polydimethyldisiloxysilanes are formed at the surface. However, no terminal groups were detected and only a relatively small, narrow signal in the dimethyldisiloxysilane region near -22 ppm was noticed, assigned on the basis of chemical shift arguments to longer, mobile polydimethylsiloxanes.

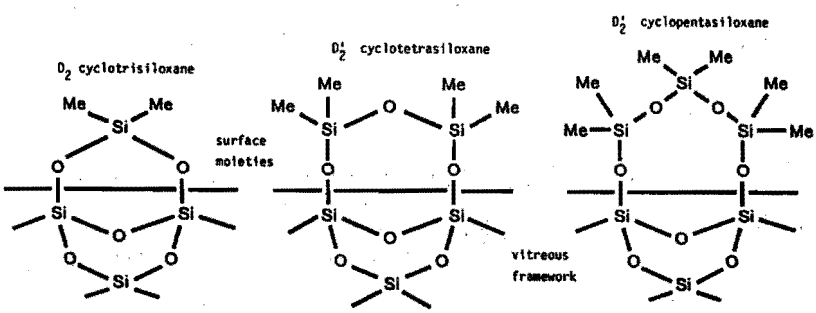

Figure 2.2

Small ring structures with dimethyldisiloxysilanes at the surface of the silica.

DPTMDS silylation

Samples 1-4, listed in Table 2.I, were prepared with DPTMDS at 377℃ and ^{29}Si CP-MAS NMR spectra were recorded, contact time 4 ms. From the ^{29}Si NMR spectra, depicted in Figure 2.3, it can be concluded that a variety of silylation products is formed at the silica surface with chemical shifts between +2 and -18 ppm from TMS. For sample 4 also a discernable signal at -88 ppm, assigned to aminotrisiloxysilane [8] is detected. This illustrates a large difference in product distribution influenced by reaction conditions similar to those used for DPTMDS deactivation of capillary columns. The primary coupling product of DPTMDS, phenyldimethylsiloxysilane (M_1P, +2 ppm) is found for all four samples, although the relative amounts vary.

The other silylation products in the region between -4 and -18 ppm are mainly assigned to bifunctional dimethyldisiloxysilanes as listed in Table 2.II.

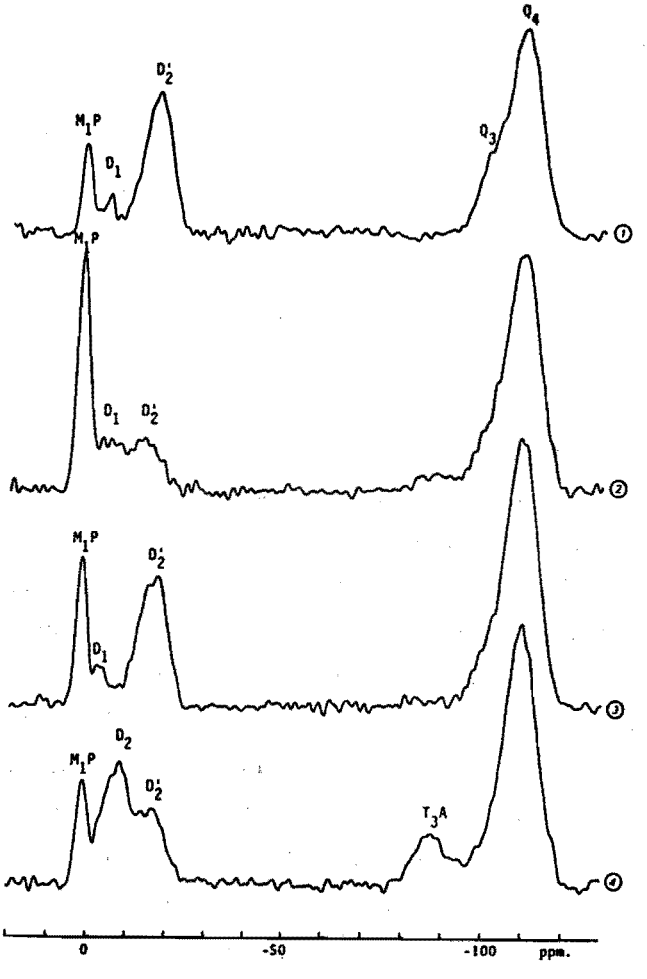

Figure 2.3

^{29}Si CP-MAS NMR spectra of DPTMDS silylated Cab-O-Sil. Samples 1 to 4; N_S = 12000; contact-time, 4 ms; line broadening, 20 Hz.

With a stoichiometric amount of DPTMDS (samples 1 and 3) relatively large amounts of D_2' cyclotetrasiloxane and possibly larger siloxane ring systems

are formed. When an excess of DPTMDS is added (samples 2 and 4) instead of these D_2' cyclosiloxane structures, substantial amounts of D_2 cyclotrisiloxanes are formed, especially in the presence of water. An explanation of this phenomenon could be that the silylation of the surface starts with covering the silica with the primary coupling product phenyldimethylsiloxysilane.
The primary phenyldimethylsiloxysilanes at the silica surface will than presumably react further with the remaining neighbouring silanol groups with formation of dimethyldisiloxysilanes (D_2 cyclotrisiloxane) and evolution of benzene. This reaction is promoted by the presence of water at the surface (sample 4).
An alternative reaction occurs between two neighbouring phenyldimethylsiloxysilanes resulting in a shared siloxane bridge (D_2' cyclotetrasiloxane). The latter reaction predominates, when a stoichiometric ratio of DPTMDS is used for deactivation at 377℃.
The loss of the phenyl group is confirmed by ^{13}C CP-MAS NMR, see Figure 2.4, and elemental carbon analysis, see also Table 2.I. Presumably, steric hindrance caused by a high concentration of primary attached phenyldimethylsiloxysilanes partly prevents formation of larger cyclosiloxane systems.The presence of aminotrisiloxysilane (T_3A) at the surface of the Cab-O-Sil sample 4 was discussed extensively by Van de Ven et al. [8]. Aminotrisiloxysilane is formed in the presence of water especially when an excess of DPTMDS is added to the silica. The changes of the critical surface tension reported [10], could very well be caused by the aminotrisiloxysilane groups formed at the fused silica surface. This amino group, however, adds an extra activity to the surface and decreases deactivation.

Variation of the contact times, for sample 4, see Figure 2.5, shows that none of the signals near -16 ppm is enhanced upon increasing contact time, only the signal at lowest field, assigned to phenyldimethylsiloxysilanes shows this effect slightly, in line with the known tendency of trialkylsilyl groups (alkyl ≤ ethyl). The growth of the quaternary signal at -110 ppm here is due to indirect

cross-polymerization via protons at least four bonds away [12], not to larger mobilities.

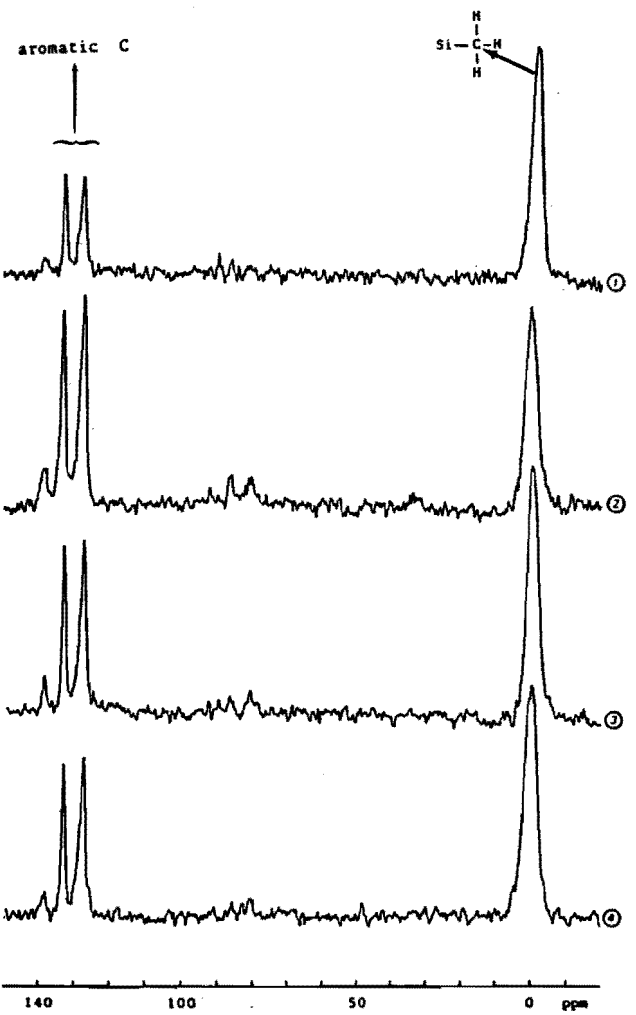

Figure 2.4

^{13}C CP-MAS NMR spectra of DPTMDS silylated Cab-O-Sil. Samples 1 to 4; N_S = 3280; contact-time, 1 ms; line broadening, 20 Hz.

The sharp signal at -22 ppm (see below), characteristic of dimethydisiloxysilane

chains anchored at the Cab-O-Sil surface is missing. It can be concluded that no mobile dimethyldisiloxysilanes are formed at the surface of the Cab-O-Sil. Dimethylhydroxysiloxysilanes (D_1) are probably present at the surface of all four samples, but no longer chains are formed even in the presence of water and large amounts of reagents added. This contradicts the reaction scheme proposed by Reiher, shown in Figure 2.1.

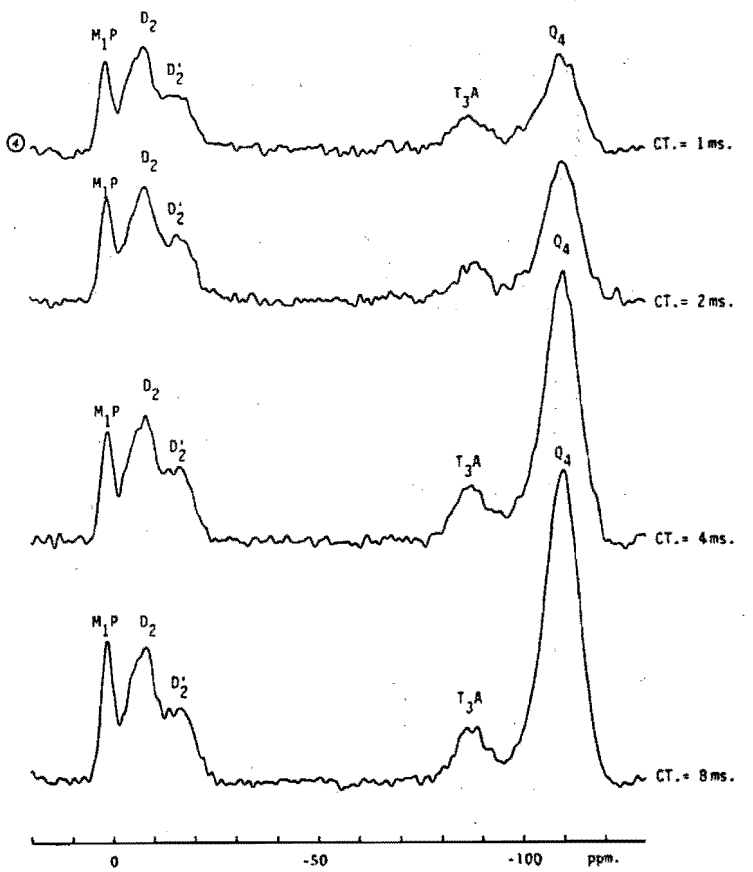

Figure 2.5

Varying contact times, ^{29}Si CP-MAS NMR spectra of DPTMDS silylated Cab-O-Sil. Sample 4; N_S = 12000; line broadening, 20 Hz.

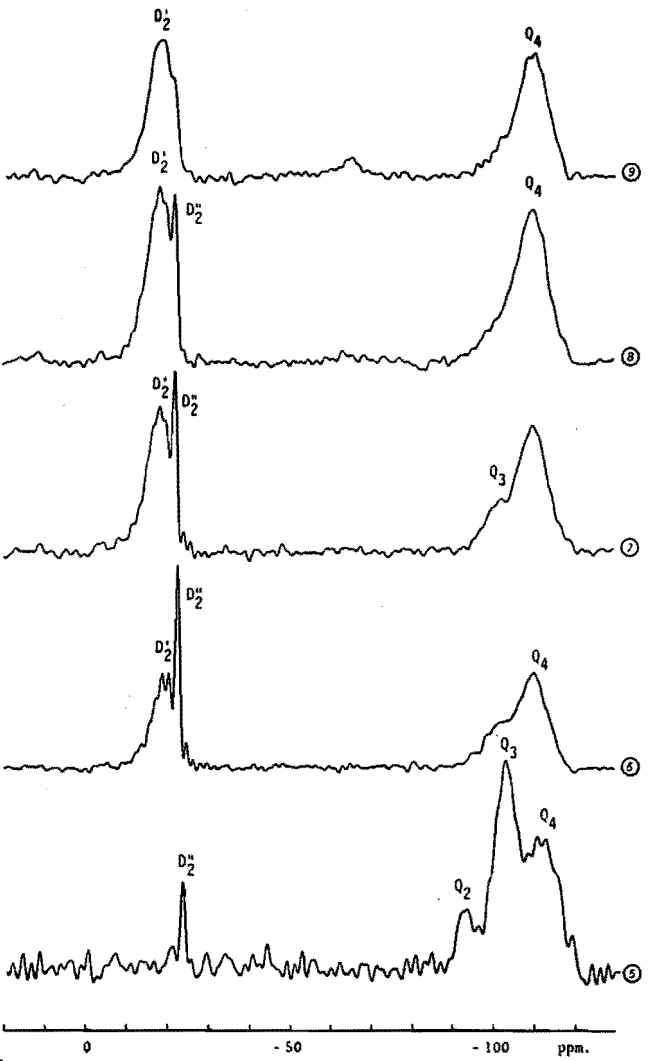

Figure 2.6

^{29}Si CP-MAS NMR spectra of PDMS silylated Cab-O-Sil at elevated temperatures. Sample 5-9; N_S = 2000. contact-time, 4 ms; line broadening, 20 Hz.

PDMS silylation

Samples 6-9, listed also in Table 2.I, were prepared with OV 101 at temperatures between 300℃ and 420℃. ^{29}Si CP-MAS NMR spectra of

these samples together with Cab-O-Sil coated with OV 101 and dried at 110℃ vacuum (sample 5), were recorded with a contact time of 4 ms, see Figure 2.6. In the ^{29}Si NMR spectra one sharp signal at -22 ppm occurs.
This signal, as mentioned before, is assigned to polydimethylsiloxane (D_2"). The signal at -18 to -20 ppm, which increases with increasing reaction temperatures up to 380℃, corresponds to the formation of degradation products of polydimethylsiloxane chains, existing of dimethyldisiloxysilanes surface paired (or D_2' cyclotetrasiloxane) and larger, anchored dimethylsiloxane ring structures.

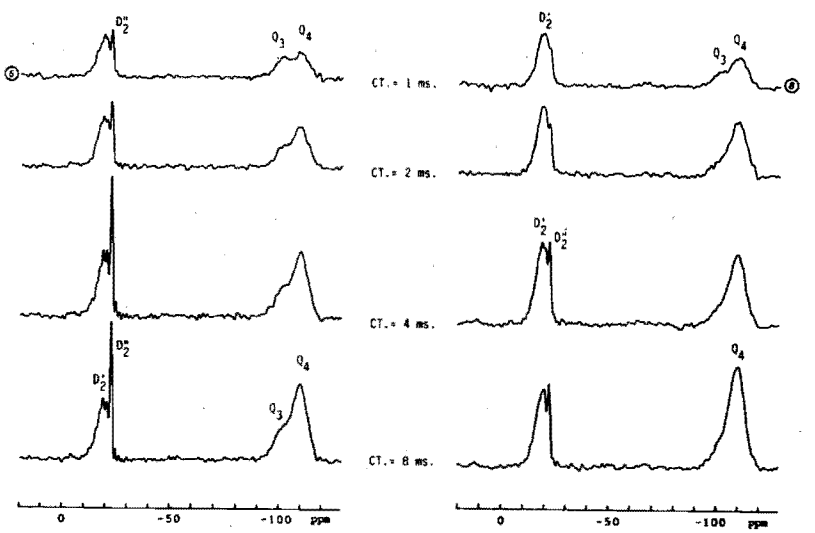

Figure 2.7

Varying contact-times, ^{29}Si CP-MAS NMR spectra of PDMS silylated Cab-O-Sil. Samples 6 and 8; N_S = 2000; line broadening, 20 Hz.

At temperatures between 300℃ and 340℃, practically no chain degradation is found. Decreasingly less polydimethylsiloxanes are found after silylation between 380° and 420℃. However, there is no indication that D_2

cyclotrisiloxanes or dimethylhydroxysiloxysilane end groups are formed. The loss of larger dimethylsiloxane chains is confirmed by elemental carbon analysis, see also Table 2.I.

A difference in the maximum for D_2' cyclosiloxane structures is recorded between PDMS and DPTMDS silylation. The maximum for the ^{29}Si NMR signal representing D_2' cyclosiloxane after PDMS deactivation is situated 3 ppm upfield from D_2' cyclosiloxane products after DPTMDS deactivation. This shift in maximum could be explained by deshielding (change of ^{29}Si NMR signal position towards lower field) of dimethyldisiloxysilanes in larger ring structures (up to five or six units) or can be caused by the first units of the polydimethylsiloxane chains close to the surface. These anchored units do not possess the high liquid mobility as observed for polymer chain dimethylsiloxane units, while their NMR shielding is still influenced by the chemical bonding at the surface.

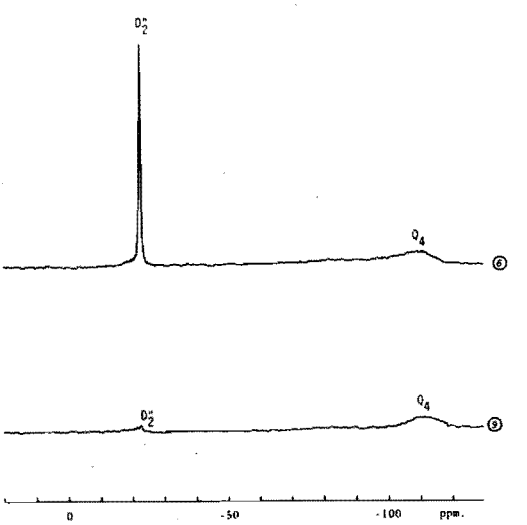

Figure 2.8

^{29}Si pulse excitation MAS NMR spectra of samples 6 and 9; N_S = 5500; line broadening, 1 Hz.

Variation of contact times clearly indicates that the signal at -22 ppm belongs to rather mobile silane groups (in the 10^4 Hz range), in contrast to other

signals in the same region, see Figure 2.7. This is in complete agreement with the earlier assignments of this signal (see above). The average mobility of the D_2" moieties at the surface of sample 8 is reduced compared to sample 6. This is shown by the signal at -22 ppm, which is less influenced by spectrometric contact time variation.The spectrum of sample 5, depicted in Figure 2.6, indicates that practically no surface bonding has taken place at 110℃, under vacuum. The corresponding ^{29}Si NMR signal could hardly be obtained by cross polymerization, but was relatively easy to excite by conventional pulse methods, see Figure 2.8, as usual in measuring NMR spectra for very mobile groups. By pulse excitation it is shown that at 420℃ less than 5 percent of the original polydimethylsiloxanes are left at the surface. At increasing temperatures the signals of hydroxysiloxane (Q_3) and dihydroxysiloxane (Q_2) are decreasing rapidly, see also Figure 2.5 and so is the silanol activity of the surface. The remaining silanol groups will be covered with anchored polydimethylsiloxane chains, and small cyclosiloxanes loops.

From this it can be concluded that PDMS degradation at an optimal temperature gives a more effective diminution of the silane activity and adds no extra activity to the surface, contrary to DPTMDS silylation.

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CHAPTER 3

DEACTIVATION AND COATING OF NON-POROUS SILICA SURFACES WITH POLYSILOXANES IN CAPILLARY CHROMATOGRAPHY [1,2]

Deactivation of fused-silica capillary columns and of vitreous silica surface with polymethylhydrosiloxanes at temperatures between 240 and 360°C is investigated directly by gas chromatography and indirectly by solid state ^{29}Si NMR using a non-porous model substrate. Further modification of deactivated silica surfaces with a stationary phase coating containing polymethyloctadecylsiloxane is optimized and evaluated. The interactions between the deactivation and the coating layers are studied. Applications of short capillary columns, deactivated with PMHS and coated with a tick film of PMODS are shown.

3.1. INTRODUCTION

Substantial improvements in non-polar surface deactivation and modification of the inner wall of fused-silica capillary columns at lower temperatures have

1. M. Hetem, G. Rutten, B. Vermeer, J. Rijks, L. van de Ven, J. de Haan, C. Cramers, J. Chromatogr., 477 (1989) 3-24.
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been made in recent years by the use of several kinds of methylhydrosiloxanes [1,2]. Mostly, mixtures of linear and cyclic polymers are used.
The advantages of the use of polymethylhydrosiloxanes (PMHS) for modification of fused-silica capillary column walls are the lower silylation temperatures, the relatively short reaction times and the high degree of effective deactivation.
Narrow-bore fused-silica columns modified with PMHS coated with non-polar stationary phases have shown excellent deactivation also in capillary LC and SFC [3,4].
Although very effective deactivation of fused-silica capillary columns with PMHS was obtained at optimal silylation time and temperature, the nature of the deactivating film inside fused-silica capillaries is not yet exactly known. Some authors have explained the effective deactivation of methylhydrosiloxanes by the formation of a very thin film [2] (film thickness of a few monolayers), where all hydrogen groups have reacted with silanol groups into a dense network of cross-linked polymers bonded to the surface via siloxane linkages and with methyl groups protruding upwards from the surface. Although this explanation accounts for the character of the deactivated capillaries observed in GC tests, in view of the high original hydrogen content, it is bold to assume the silylated film to be void of hydrogen groups.

Here, deactivation with PMHS inside fused-silica capillaries and on Cab-O-Sil, a vitreous silica, as a model substrate for fused-silica material that allows spectrometric surface characterization is described. Fused-silica capillary columns deactivated with PMHS were tested on inertness, acidity and thermal and chemical stability after silylation at temperatures between 260℃ and 320℃ with various reaction times. Further, the influence of little water present at the inner wall before silylation was studied.
PMHS modified fused-silica capillaries were subjected to a censorious test for remaining silanol activity and acid or basic adsorption [5-7]. The

deactivated uncoated fused-silica capillaries were tested by a double column method [8], using a thick-film apolar precolumn as reference.
The use of fumed vitreous silica for model experiments in combination with solid state NMR has proved to be a powerful tool for the study of surface moieties after silylation [5,9]. Solid state ^{29}Si NMR provides information on the nature, the amounts and chemical properties of the groups formed at the surface of the silica. PMHS coated with different film thicknesses on both dried and wetted Cab-O-Sil was silylated between 240℃ and 360℃ for various reaction times. In this chapter the relative amounts of organo-siliceous surface moieties not directly connected to the surface were determined with ^{29}Si magic angle spinning (MAS) NMR. ^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR provided information about the mobility of the anchored and auto cross-linked PMHS chains by contact-time variation experiments. Also the relative amounts of the siliceous moieties directly attached to the silica surface were determined by ^{29}Si CP-MAS NMR.
The results of the measurements of silylated Cab-O-Sil match the retention behaviour and peak shape of the test compounds on the likewise deactivated fused-silica capillaries very well.

In capillary column preparation practice deactivation is followed by further modification with a suitable stationary phase. In this chapter a new stationary phase applied in narrow-bore columns (inner diameter $\leq 50\ \mu m$), suitable for fast gas chromatography (GC), capillary super-critical fluid chromatography (SFC) is discussed. The attention is focused on a relatively thick (phase ratio ≤ 50), stationary phase film containing polymethyloctadecylsiloxanes (PMODS). Interactions with the PMHS deactivating film underneath are improved when dried Cab-O-Sil is added to the coating. A relatively high ratio of the cross-linking agent benzoyl-peroxide improves the stability of the stationary phase as well. Again, model studies with solid state ^{29}Si NMR gave information on

chemical properties, rigidity and stability of the stationary phase and the interactions with the PMHS deactivating layer.

3.2. BACKGROUND

Capillary fused-silica columns in chromatography typically have inner diameters between 200 and 530 μm. At present only thin-film capillary SFC and fast GC columns are available with inner diameters down to 50 μm. However, because of the extremely low specific surface area of these non-porous capillaries solid state NMR and other spectroscopic analysis techniques can not be applied for surface characterization. Therefore, a non-porous fumed vitreous silica, Cab-O-Sil, with a large enough specific surface area and solid particles with a typical diameter of about 10 nm is chosen as a model substrate for surface deactivation and modification. One should consider, however, that for fused-silica capillaries deactivation and modification is performed at the inner wall, while for Cab-O-Sil the outer surface of the particles is used, see Figure 3.1.

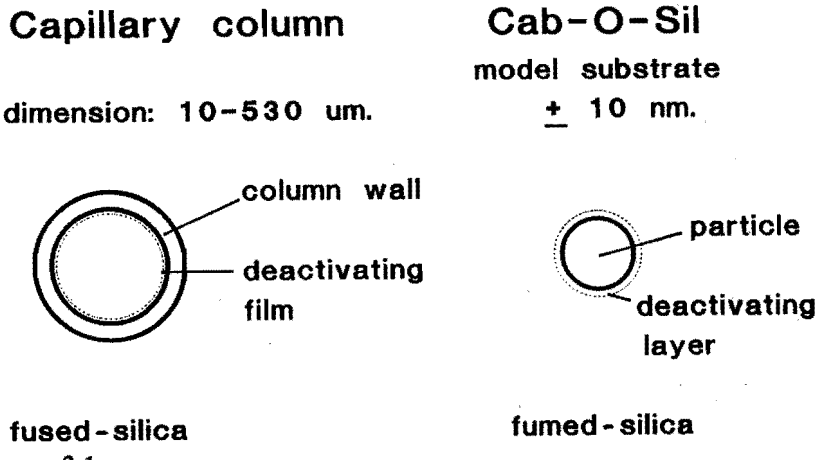
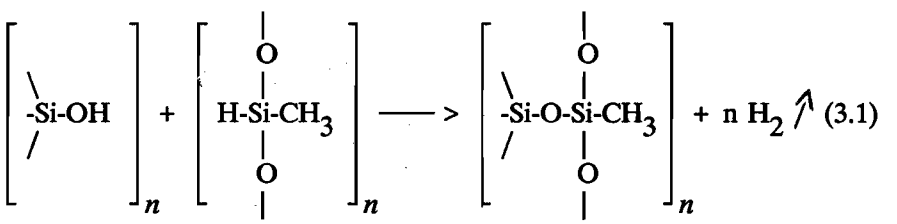


Figure 3.1

Comparison of the two non-porous silica surfaces used in this study.

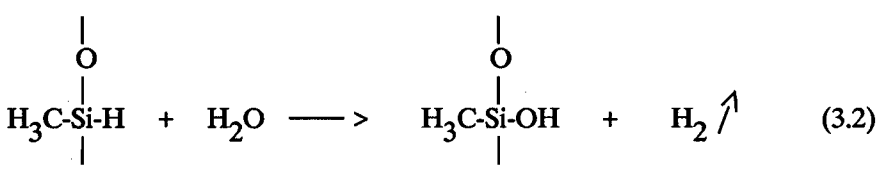
3.3. THEORY

In contrast to polydimethylsiloxanes, in which siloxane bonds are broken at high temperatures to react with surface silanols, see paragraph 2.1., the bonding of PMHS with surface silanols proceeds through condensation [10]:

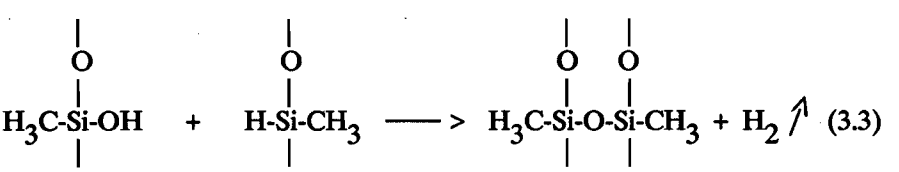


surface silica + PMHS ——> deactivated surface

This reaction proceeds rapidly at moderate temperatures (around 290°C). If some physisorbed water is present at the surface, this interferes by hydrolysis of the silicon-hydrogen bond and intra- or intermolecular cross-linking of PMHS can occur according to the following successive reactions:



methylhydrosiloxane ——> methylhydroxysiloxane



methylhydroxy- + methylhydro- ——> cross-linked PMHS
siloxane siloxane

The presence of water causes also extra silanol activity, which should be reduced by the deactivation of the siliceous surface. Another problem can be the hydrogen content of the deactivating film. Unreacted hydrosiloxanes could exhibit hydrogen bonding with eluting compounds and interfere with the chromatographic process.
In this chapter the effect of the reaction temperature, the film thickness and the water content of the surface are parameters which were considered.

3.4. EXPERIMENTAL

Materials

The PMHS material PS 122 (50% Si-H, η = 85 cS, liquid) and the PMODS material PS 130 (50% Si-C_{18}, M_W = 28000 g mol^{-1}, M_N = 7000 g mol^{-1}, solid) were obtained from Petrarch (Bristol, PA, U.S.A.). The amount of methylhydrodisiloxysilane groups present in PS 122, determined by high resolution (HR) ^{29}Si liquid NMR was 96% $\pm$ 1%. The only other significant signal in the ^{29}Si NMR spectrum was assigned to trimethylsiloxysilane end-groups (see below). The amount of methyloctadecyldisiloxysilane (D_2C_{18}) in the PS 130 determined by solid state ^{29}Si CP-MAS NMR was 95% $\pm$ 2%. Here also some trimethylsiloxysilane end groups, about 3%, together with unreacted methylhydrosiloxane groups, about 2%, were found (see below). The fused-silica capillary column material used was a gift from Chrompack (Middelburg, The Netherlands). The Cab-O-Sil M5 (Cabot Corp., Tuscola, IL, U.S.A.) was a gift from Heybroek & Co. Handelmij. (Amsterdam, The Netherlands). The specific surface area of grade M5 is, according to the manufacturer's specification 200 $\pm$ 25 m^2/g. A value of 202 m^2/g was determined after ignition and rehydration with BET-measurements. This value will be used in this chapter. The benzoyl peroxide was obtained from Aldrich (Beerse, Belgium). The solvents were all analytical grade from E. Merck (Darmstadt, FRG), except the demineralized

water, which was obtained with a Milli-Q System (Millipore, Bedford, MA, U.S.A.).

Deactivation and modification of fused-silica capillaries

A piece of 85 m * 0.25 mm I.D. fused-silica capillary column material was conditioned by flushing with helium at 290°C for 2 h. After drying 90% of the fused-silica capillary was filled with a 10% (w/w) HCl solution and flame sealed at both ends. Hydrothermal treatment was performed by heating at 120°C during 90 minutes. After this treatment, the column was rinsed with deionized water until neutral and with methanol for 30 min. It was then dried again by helium flushing at 140°C for 1 h and cut in five sections: four sections with a length of *ca.* 18 m, the fifth section (*ca.* 12.5 m) was rinsed with deionized water and dried at room temperature with helium flushing for 1 h.

These five capillaries were coated dynamically with a 1% (v/v) PS 122 solution in n-pentane. A coating speed of approximately 25 cm min^{-1} was applied. According to Bartle [11] this results in a film thickness of approximately 10 nm (assumed viscosity, 1.8 x 10^{-3} kg m^{-1} s^{-1} and surface tension, 2.5 x 10^{-2} N m^{-1}).

After coating with PMHS the columns were flushed with He for 15 min and flame sealed. Silylation was carried out at different temperatures and reaction times, see Table 3.I. Afterwards the capillaries were carefully rinsed to avoid plugging with 10 column volumes of dichloromethane and conditioned by purging helium at 290°C for 30 min.

For evaluation the deactivated fused-silica capillary columns were connected via a zero-dead volume connector (Valco, Houston, TX, U.S.A.), to a thick-film reference column, 24 m * 0.32 mm I.D., CP-Sil 5 CB (Chrompack) a chemically bonded polydimethylsiloxane (film thickness, d_f= 1.1 μm, phase

ratio = 72), showing as little adsorption as possible. In this way column evaluation can be performed with mixtures. It allows observation of peak shapes, measurements of column polarity by means of retention indices and quantitative comparison of column activity for differently deactivated capillaries.

TABLE 3. I

Deactivated fused-silica capillaries.

Column no.	Length (m) (I.D. 0.25 mm)	Silylation temp (℃)/time	Special treatment
1	17.90	260/2 h	
2	17.90	290/2 h	
3	17.70	310/2 h	
4	17.90	290/8 h	
5	12.65	290/2 h	rinsed with H_2O before coating
6	no. 2	--	reconditioned at 290℃ for 65 h

The connected columns were placed in a Carlo Erba (Milan, Italy) 5300 gas chromatograph equipped with split injection and flame ionization detection (FID). Helium was used as the carrier gas. The test runs were performed isothermally at 110℃, chromatograms were recorded with a SP 4290 integrator with a 256K data memory (Spectra-Physics, San Jose, CA, U.S.A.).The injector and detector temperature were each 250℃. A test mixture consisting of activity markers for various active sites on the column wall before or after deactivation or coating is specified in Table 3.II. It was used for determination of column wall activity with respect to adsorption.

The solute test mixture contained ten components, five normal alkanes C_{10} to C_{14} and five compounds susceptible to various column wall activities. The mean linear velocity of the carrier gas in the deactivated capillaries was kept between 30 to 35 cm s^{-1}. Carrier gas velocity in the reference column

was typically about 25 cm s^{-1}. The split ratio varied between 1:50 and 1:100. The injection volume was 1 μl, corresponding to an amount on the column of typically 1 - 3 ng for each component.

After the first evaluation column no. 2 was reconditioned for 65 h at 290°C under helium and tested again as column no. 6. The original fused-silica capillary column material was also tested, and for comparison the test mixture was also injected onto the reference column.

Kováts indices were calculated taking the elution time of methane as dead time. Peak areas were normalized to n-decane = 100% and corrected for relative weight, but not corrected for FID response factors.

After the optimization of the deactivation procedure a few capillary columns (*ca.*5 m * 50 μm I.D.) were deactivated with PMHS under optimal conditions, 290°C for 2 h, and tested. These columns were subsequently coated with various PMODS/dried Cab-O-Sil-M5/benzoyl-peroxide mixtures in tetrahydrofuran (THF)/ diethylether (DEE), 50:50, at a temperature of 55°C by solvent evacuation under reduced pressure. Benzoyl peroxide and dried Cab-O-Sil particles improved the chemical and mechanical stability of the stationary phase. After successful evacuation the columns were dried and subsequently cross-linked at 140°C for 2 h with helium purge and further conditioned at 200°C for 15 h. Optimization of this coating procedure was carried out using different stationary phase component ratios and stationary phase volumes. A final stationary phase component ratio of 72% (w/w) PMODS, 14% (w/w) dried Cab-O-Sil and 14% (w/w) benzoyl-peroxide was found optimal for stationary phase volumes between 2 and 6% (v/v) of the column.

Silylation and coating of Cab-O-Sil

The Cab-O-Sil M5 was pretreated by ignition at 720°C and rehydrated as described previously [9]. Part of the Cab-O-Sil was dried further over

phosphorus pentoxide in a vacuum desiccator for several weeks and will be referred to as "dry Cab-O-Sil". Another part of the Cab-O-Sil was conditioned in air over a saturated solution of potassium bromide (84% relative humidity). This batch of Cab-O-Sil contained 5.5% (w/w) water, which equals 15.3 μmol m^{-2} siliceous surface. This batch will be referred to as "wet Cab-O-Sil".

TABLE 3. II

Test mixture for evaluation of deactivated capillaries.

Elution order on CP Sil 5 CB	Compound	Symbol	Concentration ($\mu g.ml^{-1}$ cyclohexane)	Activity marker for
1	decane	10	119.0	-
2	1-octanol	C_8-OH	112.4	exposed siloxane bonds, weak
3	2,6 dimethylphenol	DMP	118.3	exposed basic sides, strong
4	undecane	11	116.6	-
5	2.6 dimethylaniline	DMA	114.7	exposed acid silanol, strong
6	dodecane	12	114.4	-
7	1-aminodecane	Am	116.4	shielded acid silanol, weak
8	tridecane	13	107.8	-
9	nicotine	Nic	121.0	acid silanol, weak
10	tetradecane	14	128.4	-

The dry Cab-O-Sil was coated with an equivalent of 0.98 g and 0.20 g PMHS per gram, resulting in a "thick-film" coating of 5 nm and a "thin-film" coating of 1 nm thickness, respectively. For this purpose 10% (v/v) PMHS was dissolved in n-pentane and added to the required amount of Cab-O-Sil. The n-pentane was evaporated slowly under reduced pressure in a rotary evaporator at room temperature. The coated Cab-O-Sil was dried further in an oven at 100℃ under atmospheric pressure.

The wet Cab-O-Sil was coated with 0.93 g and 0.19 g PMHS per gram

resulting in films of the same thickness as described above. After evaporation of the pentane, the coated Cab-O-Sil was conditioned in a GC oven with temperature programming from 40℃ to 80℃, at 5℃ per min followed by 15 min at 80℃, to prevent evaporation of adsorbed water.

About 0.4 g of a coated Cab-O-Sil was placed in a quartz glass reaction ampoule (20 cm * 1 cm I.D., wall thickness 1 mm). A constriction was drawn in the middle of the tube. The tube was evacuated twice while cooled in dry ice because of the volatility of some short linear and cyclic methylhydrosiloxane polymers and of the remaining water. It was filled with helium to atmospheric pressure both times. Finally, the ampoule was sealed to a volume of about 8 ml.

After sealing, the ampoule was wrapped in aluminum foil, placed in a well ventilated oven and heated to the required temperature for several hours. After silylation the ampoule was opened and the content washed twice with dichloromethane and dried overnight in a vacuum oven at 110℃. Table 3.III lists the PMHS silylated Cab-O-Sil samples. The carbon content of a few silylated Cab-O-Sil samples was obtained with a Perkin Elmer (Norwalk, CT, U.S.A.) Analyzer model 240.

A batch of optimally deactivated Cab-O-Sil, 290℃ for 4 h, with a film thickness of 3 nm was coated with a second layer of 3 nm containing PMODS. For this purpose an optimized coating mixture for coating of deactivated fused-silica capillaries (mentioned above) was solved in THF/DEE (50:50) and added to the PMHS deactivated Cab-O-Sil. The solvent was evaporated under slightly reduced pressure at 55℃. The PMODS coated Cab-O-Sil was dried and cross-linked in a glass roar, 10 cm * 4.6 mm I.D., under helium flushing at 140℃ for 2 h and conditioned overnight at 200℃. For comparison the pure PMODS coating was cross-linked identically.

With the deactivated samples various solid state ^{29}Si NMR measurements were carried out in order to gain information about the chemical properties, the nature and mobility of the deactivating film at the silicious surface.

Solid-state ^{29}Si NMR measurements

Solid state ^{29}Si NMR spectra were obtained on a Bruker CXP 300 spectrometer at 59.63 MHz. The samples were spun at ca. 3.5 KHz using zirconium or aluminum oxide rotors (7 mm O.D.) of the standard Bruker double bearing type.

TABLE 3. III

PMHS modified Cab-O-Sil samples and ^{29}Si solid state NMR measurements.

Sample	Film thickness (nm)	Silylation Temp.(℃)/Time	Water content %(w/w) to PMHS	^{29}Si NMR [a] measurements
1	5	240/12 h	-	MAS
2	5	280/12 h	-	MAS, CT
3	5	320/12 h	-	MAS
4	5	360/12 h	-	MAS, CT
5	5	290/ 4 h	5.9 [b]	MAS, CT
6	5	290/ 4 h	-	MAS, CT
7	1	280/ 8 h	-	CP-MAS,CT
8	1	300/ 8 h	-	CP-MAS
9	1	320/ 8 h	-	CP-MAS
10	1	360/ 8 h	-	CP-MAS
11	1	280/ 8 h	29.6 [b]	CP-MAS
12	1	320/ 8 h	29.6	CP-MAS
13	1	360/ 8 h	29.6	CP-MAS

a MAS = magic angle spinning, CT = contact-time variation experiments, CP-MAS = cross-polarization magic angle spinning.

b 5.5% (w/w) water with respect to Cab-O-Sil.

From the 5 nm PMHS-coated Cab-O-Sil samples, ^{29}Si MAS NMR spectra were obtained with a pulse interval of 10 s, an acquisition time of 100 ms and an accumulation of typically 256 FIDs (free induction decays) in 4K data points. Prior to Fourier transformation, the data files were zero-filled to 16K data points. The spectral width was 20 KHz, no line broadening was applied. From the 1 nm PMHS coated Cab-O-Sil samples, ^{29}Si CP-MAS NMR

spectra were obtained using a contact-time of 15 ms, a pulse interval of 5 s and an acquisition time of 25.6 ms. Typically 500 FIDs were accumulated in 1K data points, zero-filled to 8K prior to Fourier transformation. The spectral width was 20 KHz. A line broadening of 15 Hz was used prior to zero-filling and Fourier transformation.

Cross-polarization experiments, under the Hartmann-Hahn conditions [12], with variable contact-times show the transfer efficiency of magnetism between two nuclei involved. The cross-polarization dynamics, obtained by simulating the experimental curves with appropriate time constants, yield significant information regarding the mobilities of the sample in the 10^2 and 10^5 Hz range [13]. After optimal deactivation with PMHS or PMHS deactivation with subsequent PMODS coating, cross-polarization experiments with variable contact-times were performed. Identically cross-linked plain PMODS stationary phase was also examined. In this chapter contact-time variation experiments between ^1H and ^{29}Si nuclei are performed. For cross-polarization experiments with variable contact-times, a series of nineteen contacts ranging from 0.1 - 40 ms was applied. Typically 6 * 50 FIDs were accumulated for each contact. The other spectral parameters were the same as above, except for a line broadening of 10, 30 and 15 Hz prior to zero-filling and Fourier transformation for 5 nm and 1 nm PMHS coated Cab-O-Sil and the PMODS coated samples, respectively.

3.5. RESULTS AND DISCUSSION

Characterization of the polysiloxanes used

A HR ^{29}Si NMR spectrum of the PMHS reagent (PS 122) used is given in Figure 3.2. One sharp signal is detected at -34.7 ppm upfield from tetramethylsilane (TMS). This signal is assigned to the methylhydrodisiloxy-silane groups (D_2H or methylhydrosiloxanes) of the various polymers present

in this reagent [14].

The relative amount of trimethylsiloxysilane end-groups (M_1), signal at +9 ppm, is less than 5% in this sample. This indicates only a little amount of short linear polymers present in PS 122. A gas chromatographic-mass spectrometric analysis of PS 122 on a thin-film apolar column, showed an extremely large dispersion of both cyclic and linear polymethylhydrosiloxanes. The chromatogram of PS 122 recorded with a FID, shown in Figure 3.3, starts with cyclic and linear tatramers and continues up to 66 methylhydrosiloxysilane units. Probably polymers containing over 70 units are present in this sample.

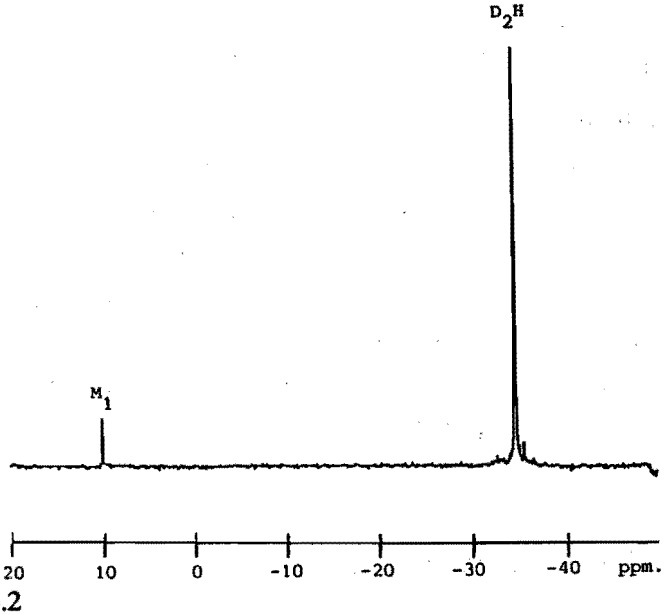


Figure 3.2

HR ^{29}Si NMR spectrum of the PMHS reagent, PS 122, used for deactivation in this study. N_S = 23000, pulse interval time: 10 s, acquisition time: 100 ms.

The amount of methyloctadecyldisiloxysilane (D_2C_{18}) in the PMODS determined by solid state ^{29}Si CP-MAS NMR was 95% $\pm$ 2%. Here also some M_1-end groups, about 3%, together with unreacted D_2H, about 2%,

were found. For an approximate quantitative analysis with ^{29}Si CP-MAS NMR and secondary information on the mobility of the polymer PMODS chains a cross-polarization contact-time variation curve is depicted together with the ^{29}Si NMR spectrum in Figure 3.4.

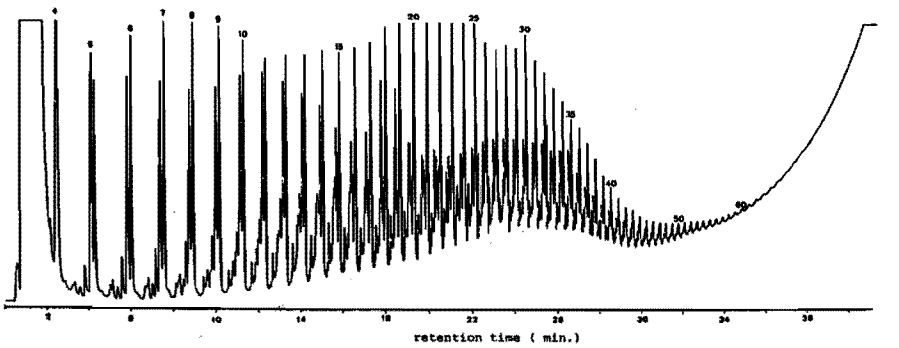


Figure 3.3

Chromatogram of the PMHS reagents PS 122. Chromatographic conditions: OV-1 column (25 m * 0.32 mm I.D.), d_f= 0.1 μm; carrier gas,helium; temperature program, initial temperature 80℃ for 2 min, increased at 10℃ min^{-1} to 380℃, then at 5℃ min^{-1} to 410℃. The peak numbers represent the methylhydrosiloxy-silane units in the various polymers.

Evaluation of the PMHS deactivated fused-silica capillaries

The recorded chromatograms of the reference column and the reference column connected to a piece of untreated fused-silica capillary column are represented in Figure 3.5. Corresponding Kováts retention indices and normalized peak areas for all evaluated fused-silica capillaries and the reference column are given in Table 3.IV.

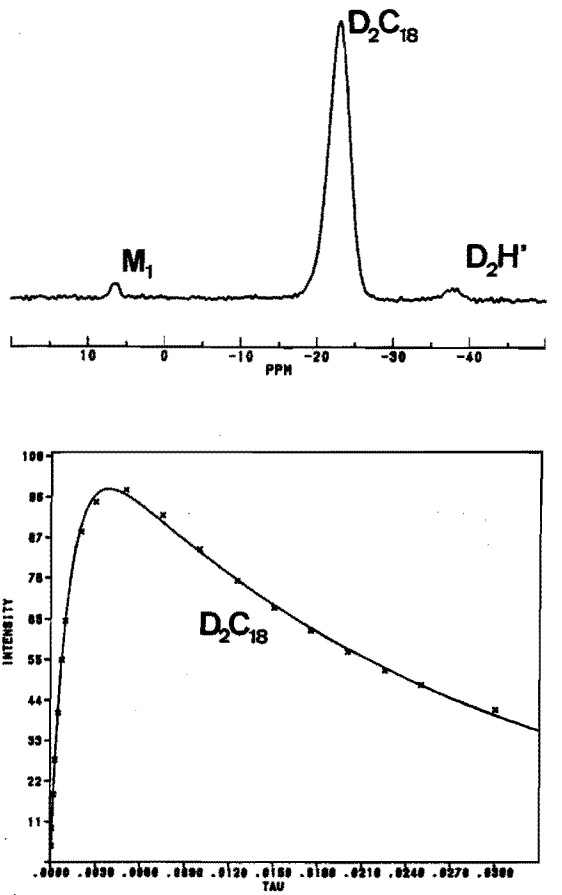


Figure 3.4

Solid state ^{29}Si CP-MAS NMR spectrum of the pure PMODS polymer with the contact-time curve of the D_2C_{18} groups. N_S = 960; pulse interval time, 7.5 s; acquisition time, 50 ms; contact-time spectrum, 5 ms.

The untreated piece of fused-silica capillary showed strong adsorption for all selective compounds in the test mixture. 1-Octanol (C_8-OH), 1-aminodecane (Am) and nicotine (Nic) did not elute at all, and 2,6-dimethylphenol (DMP) and 2,6-dimethylaniline (DMA) showed tailing and a decrease in the normalized peak areas, indicating strong acid silanol activity and the presence of basic sites.

TABLE 3. IV

Normalized peak area (NA) and retention index (I) for marker compounds in the test mixture specified in Table 3.II, determined with a dual column method. Deactivation conditions of the columns are outlined in Table 3.I.

Column no.	Marker compounds									
	Octanol-1		DMP		DMA		Aminodecane		Nicotine	
	NA	I	NA	I	NA	I	NA	I	NA	I
0	80	1050.2	94	1082.5	99	1139.2	96	1233.3	81	1312.6
1	66	1064.1	91	1076.3	74	1132.7	0	-	0	-
2	80	1050.3	94	1082.9	99	1139.8	95	1233.4	81	1313.0
3	65	1050.0	92	1080.4	96	1136.8	86	1235.5	75	1312.6
4	64	1049.2	93	1078.4	97	1134.8	88	1233.0	70	1309.2
5	43	1051.3	80	1083.3	84	1140.8	20	1239.4	26	1314.6
6(ex2)	79	1049.7	93	1076.6	98	1137.1	93	1234.5	80	1313.0
7	0	-	76	1082.5	85	1139.6	0	-	0	-

0 thick-film separation column.
7 untreated fused-silica capillary (L= 18.1 m).

Optimum deactivation was observed after silylation at 290℃ for 2 h (column no. 2, Figure 3.6), which is in agreement with results reported by Woolley [2,10]. The peak shapes of all components as eluted from the dual column system were similar on elution from the reference column alone. Deactivation at lower temperature (260℃, column no. 1) resulted in a substantial increase of the wall activity, especially toward Am and Nic, which did not elute and DMA, which partly eluted. This indicates that acid activity remained at the inner wall.

Deactivation took place but was not yet completed, which is also evident from adsorption of C_8-OH; interaction with exposed siloxane bridges [5,7] is responsible for this adsorption. Deactivation at higher temperature (310℃, column no. 3) or with a longer silylation time (8 h, column no. 4) demonstrated remaining, probably shielded, weak acid silanol activity. DMP and DMA showed no interactions with the column wall, but C_8-OH was adsorbed mildly, again indicating little activity of exposed siloxane bridges.

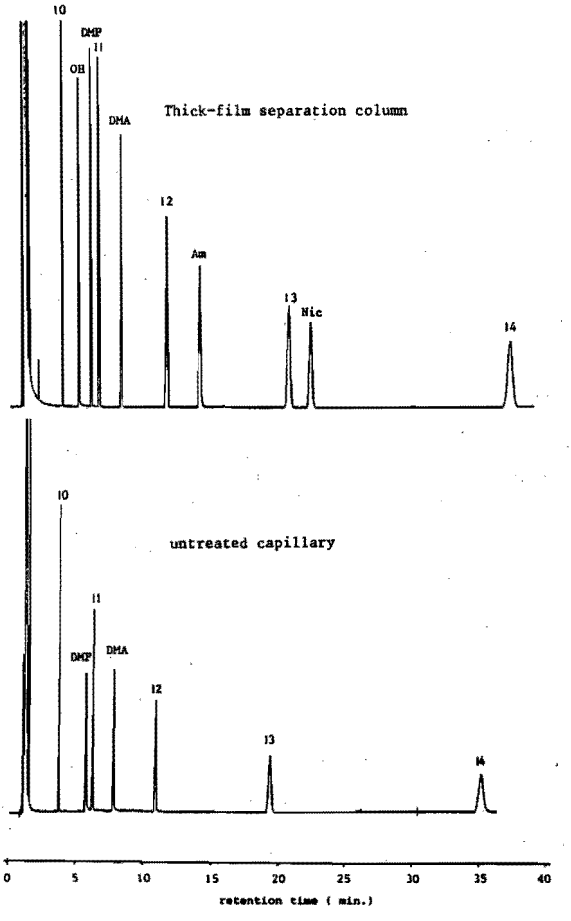


Figure 3.5

Representative chromatograms of the thick-film reference column and a non-treated capillary characterized with the double column system. Test conditions: helium carrier gas, isothermal operation at 110℃. For peak identifications of the test mixture, see Table 3.II.

A small amount of water on the inner surface prior to coating (column no. 5) is detrimental for the deactivating film formed during silylation, see Figure 3.6. The selective compounds were all partly adsorbed and showed increased retention indices. This deactivated capillary demonstrated the largest adsorption of C_8-OH and DMP.

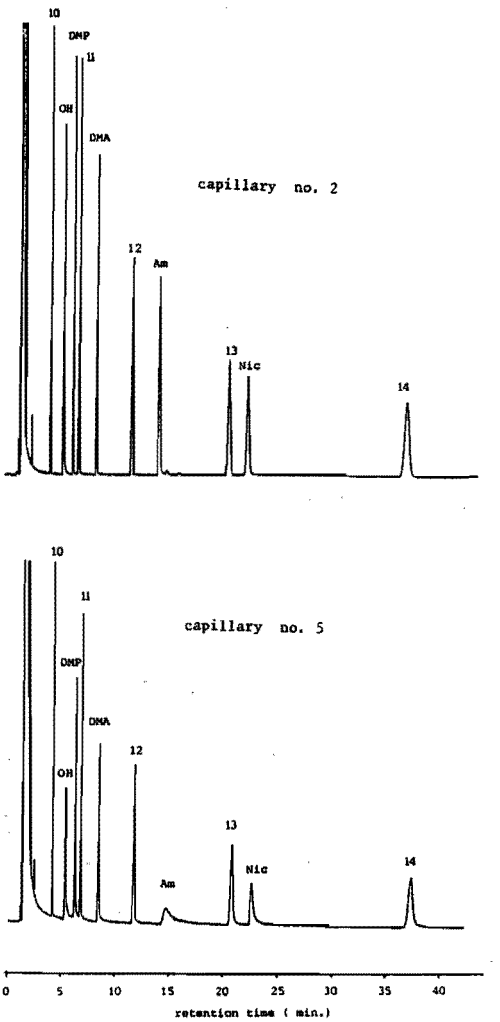


Figure 3.6

Representative chromatograms of the optimal deactivated capillary (no 2) and the pre-wetted capillary, deactivated under optimal conditions (no 5). Test conditions: helium carrier gas, isothermal operation at 110℃. For peak identifications of the test mixture, see Table 3.II.

Reconditioning column no. 2 at 290℃ for 65 h gave no increase of activity of the column wall (see column no. 6, Table 3.IV). All components eluted in the same way as before conditioning. The column showed a very good thermal stability, even after this long period of conditioning. The resistance

towards solvents was evaluated with pentane, dichloromethane and liquid carbon dioxide rinsing. No change of normalized peak area or retention indices was noticed.

^{29}Si NMR measurements of PMHS silylated Cab-O-Sils

The average carbon content determined after washing and drying of thick-film deactivated Cab-O-Sil was about 9.5% (w/w). Two exceptions were observed: the sample silylated at 240℃ showed a lower content, viz., 8.0% (w/w) carbon, probably caused by washing unreacted PMHS from the surface and the silylated wet Cab-O-Sil showed a 9.8% (w/w) carbon content owing to slightly more cross-linking.

TABLE 3. V

Siloxane/silane functionality notation, typical ^{29}Si NMR chemical and substituent induced shifts.

Compound	Chemical (spectral) functionality	Topological (network) functionality	Code	Chemical shift (ppm downfield from TMS)
trimethylsiloxy-silane	M	1	M_1	+9
methylhydrodi-siloxysilane [a]	D	2	D_2H	-36
methylhydroxydi-siloxysilane	T	2	T_2	-55
methyltrisiloxy-silane	T	3	T_3	-66
hydroxysiloxane	Q	3	Q_3	-101
tetrasiloxy-siloxane	Q	4	Q_4	-110

a Substituent-induced shift (ppm downfield from TMS) for methylhydrodisiloxysilane: $D_2H \cdot D_2H \cdot D_2H$, -35.3 ppm = D_2H; $D_2H \cdot D_2H' \cdot T_3$, -36.1 ppm = D_2H', one methyltrisiloxysilane on the α-position (see figure 3.7)

The formation of various siloxysilane surface moieties on Cab-O-Sil coated with a thick-film PMHS, silylated at temperatures ranging from 240℃ to

360℃ for 12 h, Cab-O-Sil samples 1 to 6, was investigated with ^{29}Si MAS NMR. The spectra are presented in Figure 3.8 and Table 3.V lists chemical shifts most relevant to this chapter. The relative ratio of the ^{29}Si MAS NMR signals, representing siliceous surface moieties, of Cab-O-Sil samples 1 to 6 are listed in Table 3.VI.

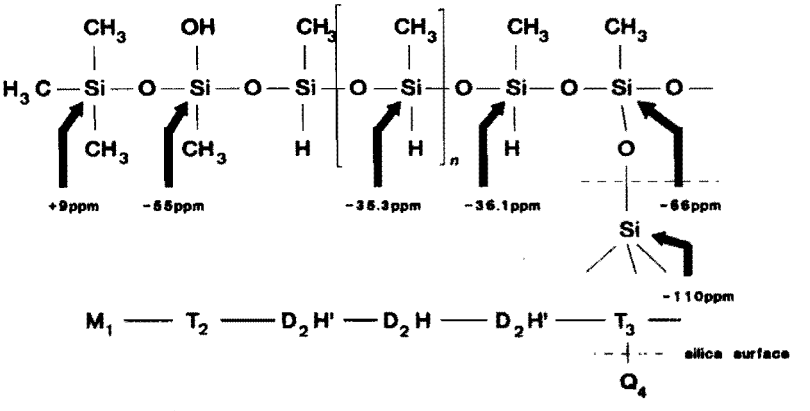


Figure 3.7

Chemical shifts of possible surface siloxane moieties after silylation with PMHS.

From these spectra it can be concluded that the silylation products have chemical shifts mainly in the -35 ppm region, indicating that only methylhydrosiloxanes are anchored to the surface. The narrow signals at -35.3 ppm and -36.1 ppm indicate that the methylhydroxysiloxane units are still part of mobile polymer chains now anchored to the silica surface. This was confirmed by contact-time variation experiments (see below). The methylhydrodisiloxysilane group with a chemical shift at -35.3 ppm has two identical unreacted neighbour groups, whereas the group with a chemical shift at -36.1 ppm has one methyltrisiloxysilane (T_3) on the α-position [14], see Figure 3.7.

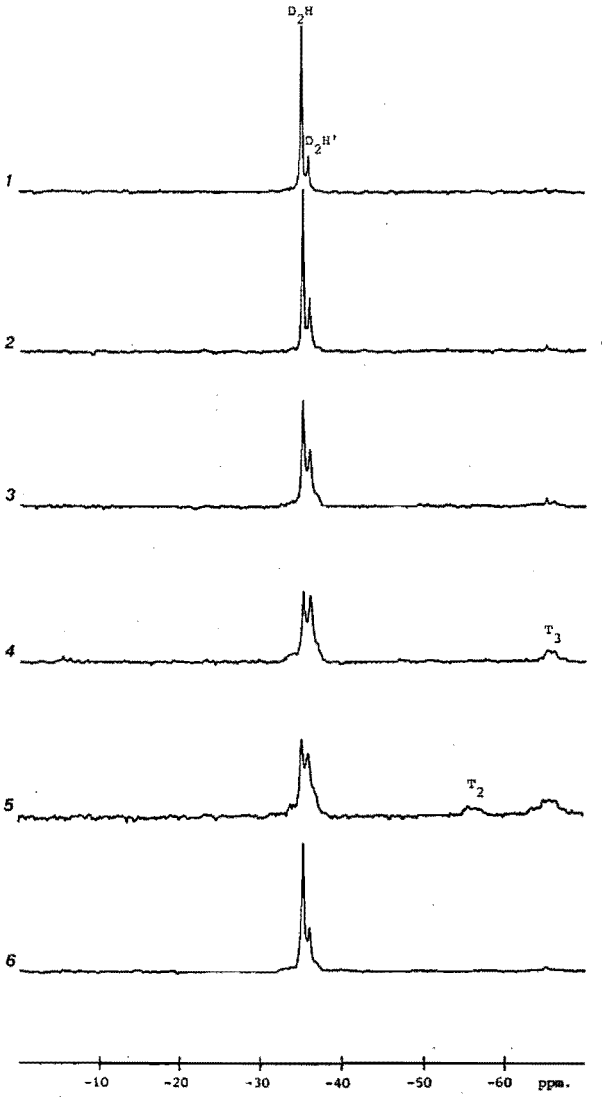


Figure 3.8

^{29}Si MAS NMR spectra of thick-film PMHS silylated Cab-O-Sil samples 1 - 6. N_S = 256; pulse interval time, 10 s; acquisition time, 100 ms.

TABLE 3. VI

Relative amounts of solid-state ^{29}Si NMR signals of surface moieties on silylated Cab-O-Sil samples.

Cab-O-Sil sample No.	^{29}Si NMR measurement	Relative ratio of ^{29}Si NMR signals			
		D_2H	D_2H'	T_2	T_3
1	MAS	0.82	0.18	-	-
2	MAS	0.71	0.29	-	-
3	MAS	0.54	0.43	-	0.03
4	MAS	0.39	0.49	-	0.12
5	MAS	0.40	0.44	0.05	0.11
6	MAS	0.71	0.29	-	-
7	CP-MAS	-	0.55	-	0.45
8	CP-MAS	-	0.44	-	0.56
9	CP-MAS	-	0.37	-	0.63
10	CP-MAS	-	0.18	-	0.83
11	CP-MAS	-	0.31	0.05	0.64
12	CP-MAS	-	0.24	0.06	0.70
13	CP-MAS	-	-	0.10	0.90

This T_3 group is a consequence of intra- and intermolecular cross-linking of the polymer chains (see reaction 3) or surface attachment at the silica (see reaction 1). Most of the T_3 groups on these silylated Cab-O-Sil samples were formed with the silica surface (see the appearance of a broad signal at -66.2 ppm). With increasing temperatures the polymer chains degrade and react with the surface. Using contact-time variation experiments in ^{29}Si CP-MAS NMR, the mobilities of the methylhydrodisiloxysilane groups (D_2H and D_2H') and, if present, of the methyltrisiloxysilane (T_3) groups have been determined. Contact-time curves (CP-curves) of Cab-O-Sil samples no. 2 (280℃/12 h) and no. 4 (360℃/12 h) are presented in Figure 3.9.

Solid state ^{13}C NMR contact-time variation experiments for the modified Cab-O-Sil samples showed curves similar to those obtained with octyl-modified reversed-phase high performance liquid chromatographic silica

in our laboratory [15], as shown in Figure 3.10. For the two methyl groups and C_1 the maxima in the CP-curves were between 5 and 10 ms, caused by the close connection to the rigid silica surface of these groups, resulting in only little mobility. For C_4 and C_5 the maxima were about 25 ms, indicating a higher mobility than for methyl and C_1 groups. The maximum for C_8 was above 30 ms indicating a large mobility owing to the large distance to the surface bond.

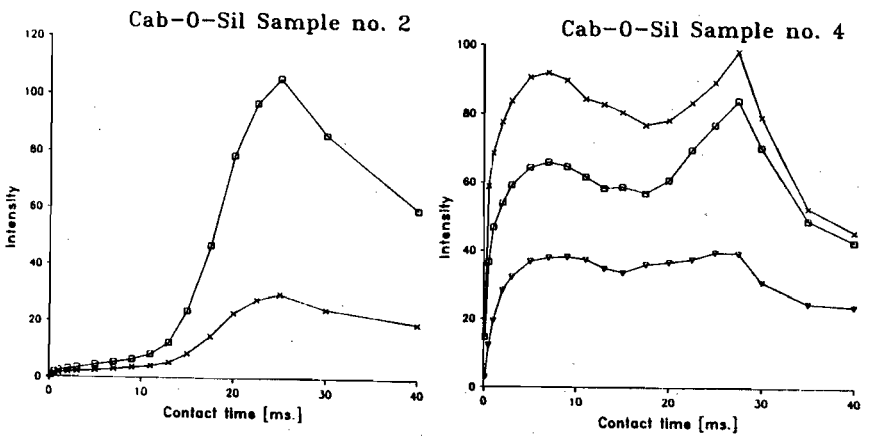


Figure 3.9

Contact-time variation curves with observed chemical shifts for surface siloxane moieties of PMHS silylated Cab-O-Sil samples 2 and 4. N_S = 300; pulse interval time, 10 s; acquisition time, 100 ms. Sample 2:□, -35.25 ppm; ×, -36.07 ppm. Sample 4:□, -35.45 ppm; ×, -36.27 ppm;▽, -66.3 ppm.

The CP-curves of sample 2 indicate that mobile methylhydrosiloxane chains are still present after silylation at 280℃. The maximum intensity for the signals in the -35 ppm region was observed for a contact-time of ca. 27 ms. This points to a mobility of the chain ^{29}Si, similar to that of a C_6 group in the octyl chain in Figure 3.10.

The deactivating film can be conceived of as two layers: a dense cross-linked

network near the surface anchored through methyltrisiloxysilane, only a few monolayers thick and with longer mobile methylhydrosiloxane polymers attached to this rigid layer, see Figure 3.11. The total number of methyltrisiloxysilane cross-linking groups is very small.

After silylation at 360℃ the CP-curves demonstrated at least two distinct maxima at contact-times of ca. 7 and 27 ms, indicating that two coherent methylhydro-siloxysilane systems are present in the deactivating film: a small amount of mobile, slightly cross-linked methylhydrosiloxane polymers, similar to silylation at 280℃, on top of a rigid layer with many immobile small chain segments anchored to the surface and intensely cross-linked polymers.

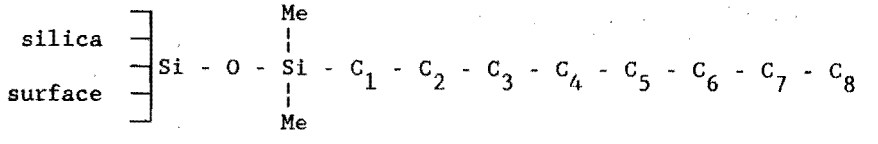


Figure 3.10

Octyl-modified reversed-phase HPLC silica.

Cab-O-Sil sample no. 5 was originally a wet Cab-O-Sil coated with 5 nm PMHS, silylated at 290℃ for only 4 h. As a reference also a dry Cab-O-Sil was coated and silylated in the same way (Cab-O-Sil no. 6). Their ^{29}Si MAS spectra are also shown in Figure 3.8. The ratio between the signals at -35.3 ppm and -36.1 ppm was influenced by the presence of water, more short chain segments were present in the silylated film of sample no. 5. The amount of methyltrisiloxysilane (T_3) was increased by enhanced cross-linking and surface attachments and an extra signal at -55 ppm, corresponding to not yet cross-linked methylhydroxy-disiloxysilanes (T_2), was detected (see reaction 2). These T_2 groups add extra silanol activity to the modified silica surface. From Cab-O-Sil sample no. 5 CP-curves were recorded as well.

They are presented in Figure 3.12. The CP-curves of Cab-O-Sil sample no. 6 were similar to sample no. 2 shown in Figure 3.9. From the ^{29}Si MAS NMR spectrum one should expect that a more rigid extensively cross-linked

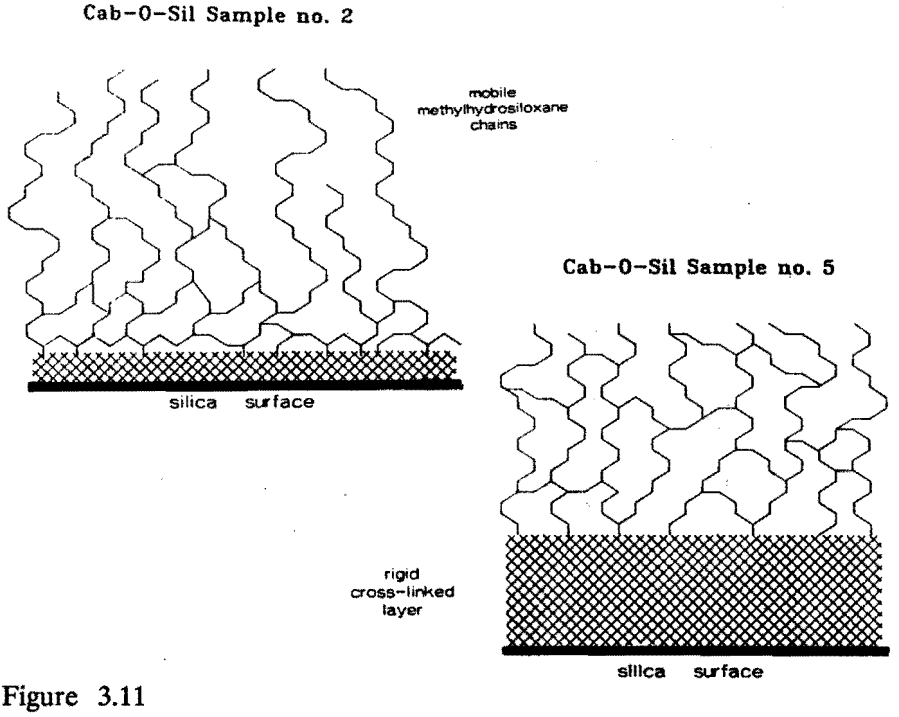


Figure 3.11

Models of the structures of the deactivating film after silylation of Cab-O-Sil samples 2 and 5.

layer was formed. This is shown by the CP-curves of the T_2 and T_3 groups, but the CP-curves of D_2H and D_2H' show a second maximum with an optimal contact-time larger than 40 ms indicating again flexible long methylhydrosiloxane chains, probably on top of the rigid layer, see Figure 3.11. In all thick-film deactivated Cab-O-Sil samples mobile methylhydrosiloxane chains were observed. This indicated the presence of exposed hydrogen groups as a part of the deactivating film.The carbon content of thin-film deactivated Cab-O-Sil silylated at 280℃ was about 4.4% (w/w). This

suggested that a deactivating film more than a few monolayers thick was formed. However, from the 1 nm coated Cab-O-Sil samples it was not possible to obtain ^{29}Si MAS NMR spectra within reasonable time, because of the long relaxation times and the low content of siliceous surface moieties. This also confirms the more rigid structure of the deactivating film.

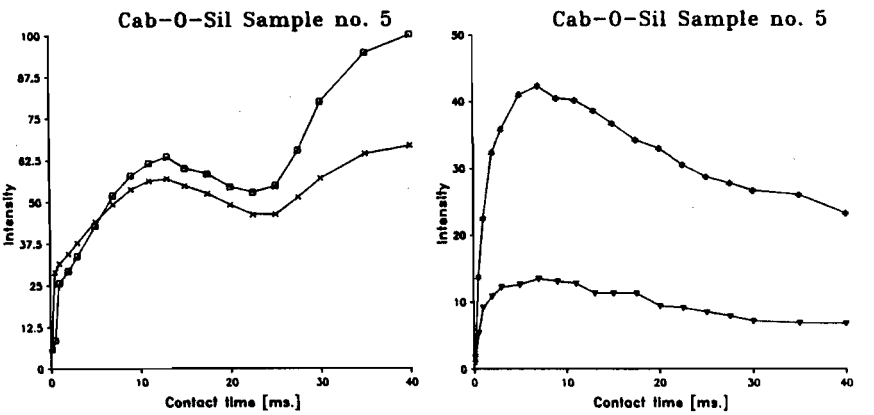


Figure 3.12

Contact-time variation curves with observed chemical shifts for surface siloxane moieties of PMHS silylated Cab-O-Sil sample 5. N_S = 300; pulse interval time, 10 s; acquisition time, 100 ms; line broadening 10 Hz. Sample 5:□, -35.3 ppm; x, -36.2 ppm;▽, -56.8 ppm; #, -66,1 ppm.

The ^{29}Si CP-MAS NMR spectra of silylated Cab-O-Sil samples no. 7 - 10 coated with a thin PMHS coating are given in Figure 3.13. These samples demonstrate a structure of the siloxane moieties different from the thick-film; the methylhydrosiloxane chains were degraded. The remaining methylhydrodisiloxysilanes (mainly D_4H') were anchored to the surface via methyltrisiloxysilanes and formed a rigid, fairly thin film as is demonstrated by the CP-curves in Figure 3.14.

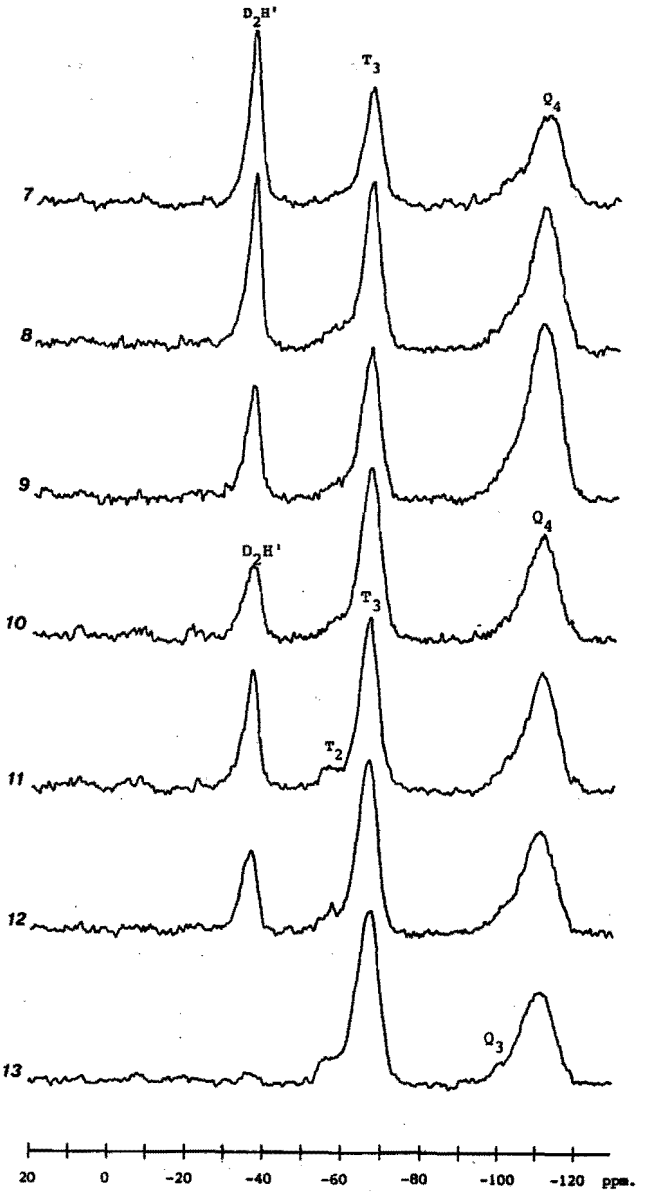


Figure 3.13

^{29}Si CP-MAS NMR spectra of thin-film PMHS silylated Cab-O-Sil samples 7 - 13. N_S = 500, pulse interval time, 5 s; contact-time, 15 ms; acquisition time, 25.6 ms; line broadening, 15 Hz.

Pronounced maxima for D_2H' and T_3 groups are observed between 10 and 20 ms. These groups exhibit approximately the same maximun as the C_3 group (see Figure 3.10), as an octyl string of a reversed phase C_8 silica. The silylated films consisted of a densely cross-linked network of D_2H' and T_3 groups anchored to the surface via T_3 groups.

As the temperatures increased from 280℃ to 300℃, the amount of D_2H and D_2H' groups decreased and the amount of T_3 groups increased. Above 300℃ no significant changes in the amount of T_3 groups were observed. Deactivation of wet Cab-O-Sil with 1 nm PMHS even caused a total loss of methylhydrodisiloxysilane moieties at a silylation temperature of 360℃, owing to conversion into methyltrisiloxysilanes (T_3) and to a smaller extent into methylhydroxydisiloxysilanes (T_2), see also Figure 3.13.

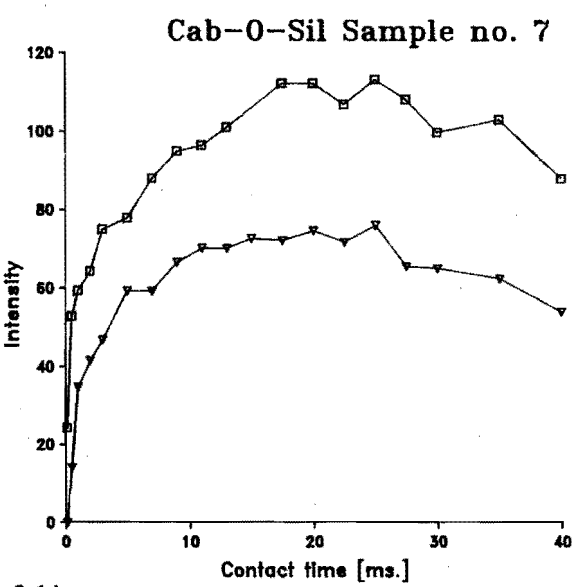


Figure 3.14

Contact-time variation curves with observed chemical shifts for surface siloxane moieties of PMHS silylated Cab-O-Sil sample no. 7. N_S = 300, pulse interval time: 5 s., acquisition time: 25.6 ms. Line broadening 30 Hz. Sample 7:□, -36,2 ppm; ▽, -66,1 ppm.

The total amount of T_2 groups did not change with increasing temperatures. These un-converted T_2 groups add extra silanol activity to the silylated surface as already mentioned above.

At silylation temperatures around 290°C a remaining substantial amount of methylhydrodisiloxysilanes was still detected (see Table 3.VI), although the broad signal at -36.5 ppm implied that most of these groups had converted neighbours and were part of a rather rigid and thus shielding film. The 1-nm PMHS films showed different physical and chemical properties to deactivating films silylated after coating with 5 nm PMHS. Relatively more T_3 groups were observed, causing a rigid and dense network. No long, flexible methylhydrosiloxane polymer chains were detected. The amounts of silicon hydride groups in the network were relatively low in comparison with thick films, but not negligible. At silylation temperatures below 300°C over 50% of the surface siloxane groups contained hydrogen when dry Cab-O-Sil was deactivated. Only part of these hydrogen groups will be shielded by the rigid structure.

Silylation of Cab-O-Sil as a model for fused-silica deactivation

In any comparison between results obtained with model chemistry, as introduced in this and the previous chapter and earlier papers of our laboratory [6,9] on one hand and actual deactivation of fused-silica columns as judged by GC, on the other hand, the thickness of the deactivating film plays a crucial role. It was shown above with solid-state ^{29}Si NMR that the natures of the films obtained on Cab-O-Sil upon deactivation with PMHS layers of 1 nm and 5 nm differ considerably.

With fused-silica columns, according to equations for dynamic coating as applied by Bartle [11], the thickness was ca. 10 nm. Consequently, one should compare these fused-silica capillaries with thick-film silylated Cab-O-Sil samples as prepared in the present study. After silylation of Cab-O-Sil with PMHS for several hours at 280°- 290°C, with a stoichiometry

corresponding to a film thickness of 5 nm, an anchored network was obtained close to the surface of Cab-O-Sil with only slight cross-linking (see above). Of the total methylhydrosiloxane units that were attached to the surface, only ca. 20% occupied α-positions with respect to surface attachment or cross-linking after silylation.

It proved difficult to detect with ^{29}Si CP-MAS NMR the methyltrisiloxanes (directly attached to the Cab-O-Sil surface), mainly because of their relatively low concentration of ca. 5 - 10%. In spite of this apparent low conversion of methylhydrosiloxanes to methyltrisiloxysilanes which attached the polymer chains tightly to the silica surface, the surface was adequately covered and deactivated, as shown by the GC experiments, see Figure 3.6. In earlier reports, the high deactivating efficiency of various PMHS forms and other organo-silicon hydride deactivating agents was attributed to the almost complete conversion of silicon hydride into siloxane bonds (reactions 1 - 3). In this chapter it is demonstrated that methylhydrosiloxanes show a high reactivity towards silanol groups, in particular to those which are Brønstedt acids. In order to achieve a more or less complete conversion of the silicon hydride groups, a temperature of ca. 360℃ is required. It seems that one of the main advantages of silylating agents containing silicon hydride moieties is connected with the small (Van der Waals) dimensions of these groups rather than with their allegedly high intrinsic reactivities [1,10,16]. The lack of reaction propensity of the chains towards the surface silanol groups, however, does not prevent a tight attachment of the chains to the surface (the surface silanol groups have disappeared). Furthermore, cross-linking of the chains near the surface, although of low overall concentration, provides additional surface screening. Remaining silicon hydride groups on the chains apparently do not interfere with the elution of the appropriate test components in GC experiments.
Deactivations under more severe conditions (higher temperatures and/or longer reaction periods) mainly caused an increase in the extent of

cross-polymerization near the surface. However, the mobilities of the chains protruding upwards from this layer were not affected significantly as was shown by variable contact-time ^{29}Si CP-MAS NMR (see above). An increase in adsorption of 1-octanol by cross-linking products became noticeable in GC experiments. Cross-linking serves to increase the total amount of siloxane bonds in the deactivating film. Intuitively, one would expect these siloxane moieties to be rather shielded (inaccessible), but this seems only partially true when coating a column wall with a rather thick film (ca. 10 nm) film of PMHS. Woolley et al. [2] reported an optimal silylation after 4 h at 300℃ with PMHS after which a very thin deactivating film was formed. In the determination of the capacity of these films, a film thickness of 1 - 4 monolayers was assumed. An exact experimental determination from chromatographic retention of such a film thickness is hardly feasible. Therefore, we assume here that a cross-linked layer near the surface is formed with a thickness similar to that postulated by Woolley et al. Moreover, on top of these initial layers we observed long, mobile methylhydrosiloxane chains. These chains could play a role in the anchoring of vinyl or phenyl containing stationary phases during radical induced cross-linking [17,18]. A complete rigid, dense cross-linked film exists only after silylation at optimal temperature of a thin-film coated silica.

The influence of water, present at the silica surface, upon the silylation is evident; advanced cross-linking occurs and a relatively thick rigid layer is formed near the surface containing methyltrisiloxysilanes, methylhydrodisiloxysilanes and a small amount of active, unreacted methylhydroxydisiloxysilanes. These hydroxyl groups caused strong adsorption of all critical components present in the test mixture with GC. When coated with a thick-film again a top layer of mobile methylhydrosiloxane chains appears after silylation at 280℃.

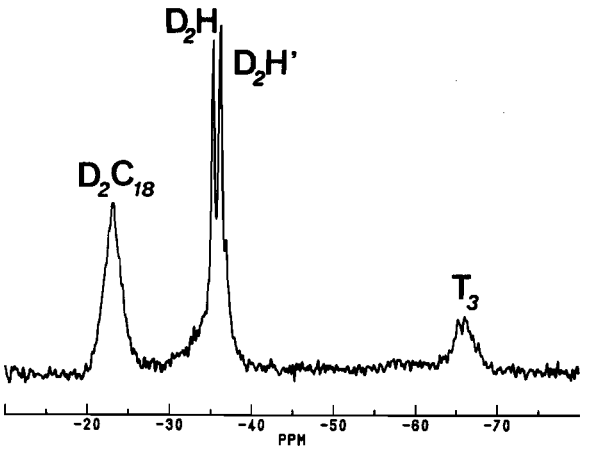
Very small amounts of water are necessary for cross-linking of a thin rigid layer near the surface and increase surface anchoring of the methylhydrosiloxane network. The appropriate amount of water seems to

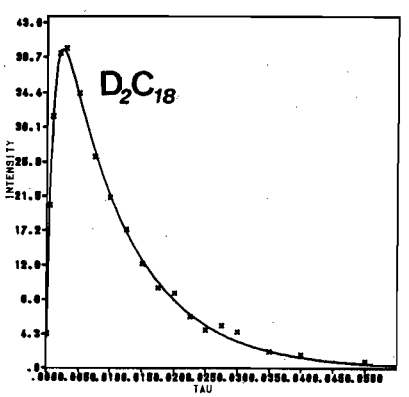
be present after severe leaching, extensive flushing with water and drying at a moderate temperature of 140℃. Remaining, probably physisorbed water, influences the structure and attachment of the deactivating film near the surface. A too high content of water yields unreacted hydroxyl groups and increased activity of the surface.
Further, the thickness of the dense cross-linked layer is increased proportionally with the amount of water present at the surface before silylation.

PMODS coating of deactivated silicas

Subsequent PMODS coating of optimally PMHS-deactivated Cab-O-Sil showed a changed D_2H to D_2H' ratio, relatively more D_2H' groups were found after stationary phase cross-linking and conditioning, see Figure 3.15. This indicates chemical interactions between the deactivating film and the stationary phase on top. Compared with the pure PMODS polymer the CP-curve of the cross-linked D_2C_{18} moieties point to decreased mobility, see also Figure 3.15. A similar CP-curve was determined for D_2C_{18} groups in plain cross-linked bulk stationary phase. The mobility of the D_2C_{18} groups in the stationary phase is decreased by dense cross-linking with benzoyl-peroxide and hardly influenced by interaction with the PMHS film or the substrate. The CP-curve for D_2H groups in the deactivating film though, is changed by subsequent coating and cross-linking with the PMODS stationary phase. The maximum in the CP-curve shifts from *ca.* 27 ms for optimally deactivated Cab-O-Sil to *ca.* 13 ms for deactivated and coated Cab-O-Sil. The mobility of the D_2H groups is decreased clearly.
The PMODS stationary phase used as a coating in deactivated fused-silica capillary columns showed good thermal and mechanical stability and solvent resistance. The amount of stationary phase as determined from the GC capacity factors, did not decrease after conditioning at 200℃ and intensive

solvent rinsing with various organic solvents or with high density CO_2.





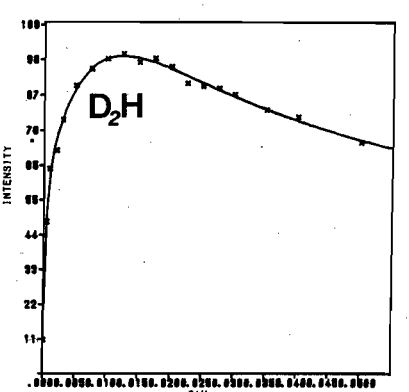


Figure 3.15

Solid state ^{29}Si CP-MAS NMR spectrum of the cross-linked PMODS polymer on top of the PMHS-deactivated Cab-O-Sil with the contact-time curve of the D_2C_{18} and D_2H groups. N_S = 1000, pulse interval time, 5 s; acquisition time, 50 ms; contact-time spectrum, 8 ms; Line broadening, 25 Hz.

Capillary columns with 50 μm I.D. and stationary phase volumes between

2% (v/v) and 6% (v/v) were prepared. A maximum separation efficiency of 12000 theoretical plates per meter (coating efficiency, $\eta \approx 60$ %) was obtained with a short 50 μm I.D. PMODS-coated column. Due to the viscosity of high concentration stationary phase solutions, preparation of longer 50 μm columns with high coating efficiencies seemed impossible. An example of the applications feasible with these columns, is a fast GC analysis of aromatic compounds, shown in Figure 3.16.

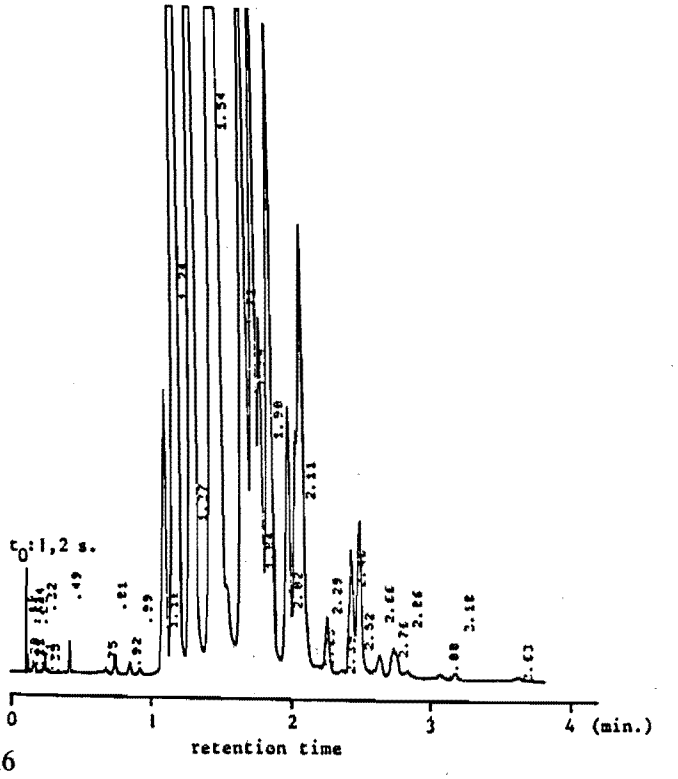


Figure 3.16

A fast GC analysis of a mixture of aromatic compounds separated with a 4 m * 50 μm I.D. PMODS-coated column, stationary phase volume 5% (v/v). Conditions: helium carrier gas, isothermal operation at 110℃, dead time, 1.2 s.

Characterization with the test mixture in Table 3.2 still showed small

adsorption for some of the sensitive compounds, owing to poor deactivation of the dried Cab-O-Sil incorporated in the stationary phase. Future research aimed at the preparation of mechanically stable thick-film narrow-bore columns with mixed polysiloxanes stationary phases will be necessary.

3.6. CONCLUSIONS

In conclusion, PS 122 is an efficient PMHS deactivating reagent for fused-silica capillary column walls for preparation of non-polar columns. The advantage of organo-silicon hydride agents for deactivation of fused-silica capillary columns is the small (van der Waals) dimension of the silicon hydride and their selective reactivity towards the Brønstedt acid silanol groups. The intrinsic reactivity of silicon-hydride to form siloxane bonds is not sufficient to provide total conversion of the silicon hydride groups. Important differences in nature and structure of the deactivating films on the silica surface have been established between deactivating films of 5 nm and 1 nm. When coated with a 5 nm film, the remaining methylhydro-siloxane groups are part of anchored mobile longer polymer chains protruding upwards on top of a dense cross-linked network of polymers near the surface. Physisorbed water present at the surface before silylation provides a good deactivating film on the fused-silica capillary column wall at an optimum silylation temperature of 290℃ for 2 h.

Too high content of water present before silylation leads to increasing activity caused by exposed siloxane bonds and methylhydroxydisiloxy silanes in the polymer network. The optimal deactivating film exhibits an excellent thermal stability and solvent resistance. No decrease of either film thickness or deactivation was observed on the silylated Cab-O-Sil samples or inside the deactivated capillaries after the completed deactivation of these surfaces. Subsequent coating with PMODS showed chemical interactions between the PMHS-deactivating film and the stationary phase on top. The mobility of

the D_2C_{18} groups in the stationary phase decreases due to cross-linking with benzoyl-peroxide. Interactions with the PMHS film or the substrate hardly influence the dynamics of the D_2C_{18} groups in the coating. However, the mobility of D_2H groups in the deactivating film decreased by subsequent coating and cross-linking with the PMODS stationary phase.
The PMODS stationary phase used as a coating in deactivated fused-silica capillary columns showed good stability and solvent resistance.

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CHAPTER 4

A STUDY OF THE CHANGES OF MONO-, DI- AND TRIFUNCTIONAL OCTADECYL-MODIFIED PACKINGS FOR RP-HPLC AT DIFFERENT ELUENT COMPOSITIONS [1]

Two different types of silica substrates have been modified into RP-HPLC phases by means of silylation with mono-, di- and trifunctional octadecylsilanes. Subsequently, these phases were subjected to artificial ageing under simulated routine conditions and were afterwards analyzed and evaluated. Changes in properties of the phases are correlated with selectivity, loss of silanes, gain in silanol and rearrangements of the silica to silane bondings. The substrate shielding of difunctional octadecylsilane-modified silicas is superior. The corresponding phases show significantly better behaviour towards stationary phase deterioration.

4.1. INTRODUCTION

The stability of chemically bonded phases on porous silica substrates employed in high performance liquid chromatography (HPLC) has been the issue of several recent studies [1-5].

1. M. Hetem, L. van de Ven, J. de Haan, C. Cramers, K. Albert, E. Bayer, J. Chromatogr., 479 (1989) 269-295.

The significance of these studies becomes clear considering the still increasing popularity of liquid-solid partition reversed-phase chromatography (RP-HPLC).

The wide variation in the properties of bonded phases commercially available today for RP-HPLC applications exist as a result of considerable differences in: the structure of the silica gel generally employed as the substrate for modification and the nature of the moieties at the surface like silanediol groups, and bonded and isolated silanols [3-9]; the chemical properties of the ligand moieties used for modification of the porous silica [8-15]; the number and nature of reactive groups involved in ligand surface attachment, like e.g. chloro and ethoxygroups [10,16]; and the reaction conditions for modifications [17].

These are also decisive factors with regard to the relative surface coverage of the bonded phase [13,17,18] and the type of anchoring of the ligands to the substrate [1,10,16].

It is obvious that dissimilarities in synthesis of chemically bonded phases not only determine the selectivity, but also influence their stability towards hydrolysis.

Aggressive eluents used as mobile phase in modern RP-HPLC practice cause hydrolysis of the bonded ligands at the silica surface followed by hydrolysis of the silica substrate [1-5]. Although this process normally proceeds rather slowly, after some time of use the stationary phase properties alter, indicated by changes in the capacity and eventually also in the selectivity of the reversed phase.

In this chapter, the interference of various eluents used in LC practice, with octadecylsilane RP-HPLC stationary phases modified on two different silica substrates and with various ligand anchorings, comprising six phases all together, were examined with chromatography, elemental analysis and high-resolution solid-state ^{29}Si NMR.

The two silica substrates with different physical properties and chemical nature of the silica surface were uniformly modified with mono-, di- and trifunctional octadecylsilanes (C_{18}) to obtain mono-, bi- and even tridentate linkage of the C_{18} ligands with the silica surface and neighbours (cross-linking). The reaction conditions employed for modification were such that for all six bonded C_{18} phases an equal relative coverage of the octadecylsilanes at the surface was obtained. Much to our surprise identical reaction conditions could be employed. The influence of both the properties of the substrate, the anchoring of the ligands on the stability of the ligand bonding to the surface and a further hydrolysis of the silica has been investigated by subjecting the six modified phases to artificial ageing under simulated routine conditions.

4.2. CHARACTERIZATION

In order to describe the qualitative and quantitative changes of the RP-HPLC stationary phase, one should include both bulk and surface properties related to solid-liquid interactions determining chromatographic behaviour, as follows:

a. Determination of bulk properties such as specific area, particle size, pore size distribution and total volume of the pores of the substrate [19-21]. Possible contaminations with traces of metals should be regarded as important for the stability of the stationary phase. With the determination of the carbon content of the bulk phase and the specific area of the substrate, the average ligand surface density (α_l) can be calculated with an equation derived by Berendsen [14]:

$$\alpha_l = \frac{P_c}{S_{BET}(M_c - P_c\,(M_l - 1))} \quad (\text{mol m}^{-2}) \qquad (4.1)$$

where P_c= the amount of carbon (g/g), S_{BET}= specific BET area of the substrate ($m^2\ g^{-1}$), M_c= the amount of carbon per mol bonded silane ($g\ mol^{-1}$), and M_l= molecular weight of the silane molecule ($g\ mol^{-1}$).

b. Characterization of surface structure elements should include the nature of various groups present at the modified surface. This surface contains a variety of siliceous groups, such as different types of silanol, siloxane bridges and octadecyl ligands. High-resolution solid-state ^{29}Si NMR proved to be a suitable technique for determining changes in ratios of these siliceous groups [1,3,7,10,18]. Solid state ^{29}Si NMR not only distinguishes the different types of surface modifications (mono-, di-, and trifunctional) under study, but also provides details concerning surface and neighbour attachments [22,23] via siloxane bonds, of the octadecylsilanes. This provides the possibility of studying the chemical reactions underlying the changes at different eluent compositions.

Until recently, only chromatographic experiments provided overall information of the changes in solid-liquid interactions between the stationary and the mobile phase after intensive use in laboratory practice. A more detailed chromatographic characterization of the subjected stationary phases should be aimed at in order to relate the results of other stationary phase characterization methods. In the present study the chromatographic experiments are focused on changes in the amount of bonded ligands and selectivity. Alternatively, attention can be focused on the determination of the influence of the silica substrate by applying polar test solutes like phenols, anilines, nitrobenzene, which are indicators for substrate interaction

[1,15,23]. However, a reliable determination of substrate interactions with polar components will be hindered by both a varying silanol activity and the "memory effect" of previous injections showing irreversible adsorption of the polar components. More fundamental chromatographic characterisations of RP-HPLC stationary phases were proposed by Smith [24] and Jandera [25-27] based on mainly solvophobic interactions of the eluting compounds with organic solvent-rich mobile phases.

The retention model proposed shows a linear relation between the logarithm of the capacity factors, k', of a homologous series and the eluent composition:

$$\log k' = a - m\,x \tag{4.2}$$

and

$$a = a_o + a_1\,n_c \tag{4.3a}$$

$$m = m_o + m_1\,n_c \tag{4.3b}$$

where x= the volume fraction of the organic part of the eluent (for binary eluents only), n_c is the incremental carbon number of a homologous series and a_o, a_1, m_o and m_1 are constants to be determined by regression. The validity of the regression has been extensively discussed [1,26,27] and linearity with respect to eluent composition was shown at least in the range 60-90% (v/v) of methanol for all octadecyl-modified stationary phases in this study. After elimination of the incremental carbon number of the eluting components from eqns. 4.3a and 4.3b, Jandera derived [25-26]:

$$m = q + p\,a \tag{4.4}$$

where

$$p = m_1/a_1 \tag{4.5a}$$

$$q = m_o - p\,a_o \tag{4.5b}$$

As already shown by Jandera, the value of the parameter p is independent

of the stationary phase. An approximate theoretical estimation for the value of p depends only on the type of eluent, especially the organic modifier [26]:

$$p \approx 2 \left(1 - \frac{I_{org}}{I_{H_2O}} \right) \qquad (4.6)$$

where I_{org} = interaction index of the organic component of the mobile phase, and I_{H_2O} = interaction index of water. For binary methanol-water eluents I_{org}/I_{H_2O} = 0.57. With eqn. 4.6, the theoretical value for p= 0.86. This value should be independent of the fraction organic modifier used. The constants m_0 and q in eqn. 4.5b describe the selectivity [26]: m_0 mainly non-specific, lipophilic selectivity and q specific, polar selectivity. The value of m_0 depends in practice on the character of the organic solvent in the mobile phase and the amount of bonded ligands of the stationary phase, whereas the value for q should depend more on the nature of the homologous series residue or on the stationary phase involved for characterization. The a_0 value equals log k' of the molecular residue, see eqns. 4.2 and 4.3a. The a_0 value of an essentially non-polar residue such as benzene should give a good indication for the phase ratio of the stationary phase used. A decrease of this a_0 value expresses the loss of attached ligands. The a_0 value of alkyl aryl ketones, include more specific interactions between the alkyl aryl ketone residue and the stationary phase. The nature of these interactions is more complex and includes also polar interactions with the substrate.

4.3 EXPERIMENTAL

Materials

The test components used for chromatographic characterization were all reference grade. Alkylbenzenes (test mixture 1) used were benzene, methylbenzene, ethylbenzene, propylbenzene and butylbenzene. Alkyl aryl ketones (test mixture 2) used as test compounds were ethanophenone, propanophenone, butanophenone, pentanophenone, hexanophenone and octanophenone (Pierce, Rockford, ILL, U.S.A.). All other solvents and chemicals used for simulating routine-use experiments and chromatographic characterization were analytical grade (E. Merck, Darmstadt, F.R.G.) and demineralized water (Millipore Milli-Q system, Millipore, Bedford, MA, U.S.A.). All eluents were freshly prepared and filtered over 0.22 μm membrane filters (Millipore) prior to use.

Chromatography

The six octadecylsilane-modified bonded phases were prepared at the Institut für Organische Chemie of the University of Tübingen, F.R.G. The modification with monochlorooctadecyldimethylsilane, dichlorooctadecyl-methylsilane and trichlorooctadecylsilane on two different silica substrates, Hypersil (batch 5-192, Shandon Southern Products, Runcorn, U.K.) and

Nucleosil 100-7 (batch 5061, Macherey-Nagel & Co, Düren, F.R.G.) was catalysed with 2,6-lutidine [18]. The bulk properties of the two silica substrates are listed in Table 4.I.

TABLE 4. I

Bulk properties of the silica substrates.

	Hypersil	Nucleosil 100-7
Batch no.	5-192	5061
Mean particle size (μm)	5 $\pm$ 1.5	7 $\pm$ 1.5
Mean pore size (nm)	12	10
Specific area (m^2/g)	170	350
Pore volume (ml/g)	0.7	1.0
Iron content ($\mu g/g$)	377 $\pm$ 20	76 $\pm$ 10
Total silanol content *a* (ratio * 100%)	15.6	32.8
Relative silanol ratios (* 100%) *a*		
- dihydroxysiloxane	13.5	11.6
- hydroxysiloxane	86.5	88.4
Silanol density at the surface (contents * g m^{-2} *b*	0.092	0.093

a determined by deconvolution of the solid state ^{29}Si MAS NMR spectra of the substrates.
b calibrated dividing the total silanol content by the specific area.

A 25 g amount of silica substrate was activated at 160℃ under reduced pressure for 24h. A sample of 0.120 mole chlorodimethyl-, dichloromethyl- or trichlorooctadecylsilane was melted and dissolved in 100 ml dry methylene chloride together with 0.15 mole 2,6-lutidine. The mixture was added to the activated silica still under vacuum and with rigorous stirring to avoid clotting. The reaction mixture was refluxed for 5h. The silica was then filtered and thoroughly washed with dry methylene chloride, ethanol, ethanol-water mixture (50/50) and finally diethyl ether. The modified silicas were then dried at 50℃ for 72h.

Each stationary phase was packed in columns (100 mm * 4 mm I.D.) (Knauer, Bad Homburg, F.R.G.) according to a standard packing procedure.

From each series of seven columns of a typical phase, six columns were placed in an apparatus for simulating routine use after a chromatographic test to ensure reproducibility of the packing procedure and the remaining column was used as a reference column for initial chromatographic tests. The equipment for simulating intensive routine use consisted of a six-headed metering pump (Metering Pumps, London, UK) provided with laboratory constructed pulse dampers, allowing each column to be purged separately with a specific eluent, see Figure 4.1. The artificial, strictly controlled, ageing experiments consisted of continuous, separate exposure of columns packed with the C_{18} phases under well defined conditions to several eluent compositions for a period of 240h.

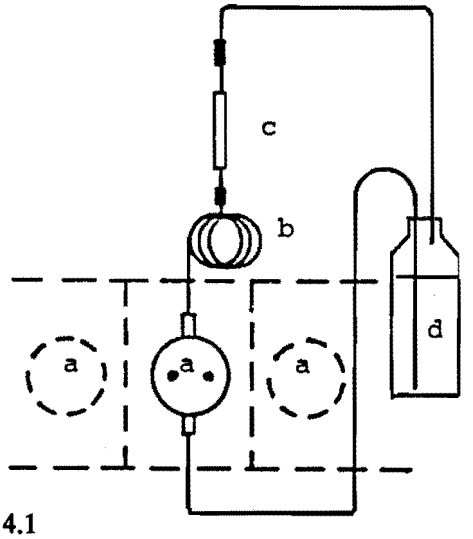

Figure 4.1

Schematic diagram of the equipment for simulating routine use of columns. a = pump head; b = pulse damper; c = column; d = eluent bottle. Flow-rate: 0.5 ml min^{-1}. Total purging time: 240 h (continuous); ambient temperature.

The basic and acidic aqueous and methanol/aqueous buffers used are listed in Table 4.II. The buffer solutions were prepared with equal amounts of buffer salt (calibrated for a total volume of 1 l aqueous or methanol-aqueous

solution), titrated in aqueous solution to the pH values 3.0 for acidic and 8.4 for basic solutions. Subsequently an equal volume of methanol was added for aqueous-methanol buffers.

TABLE 4. II

Treatments for simulating routine use experiments. Each column purged by 7000 column volumes of a typical purging eluent; flow-rate 0.5 ml/min; time 240 h; ambient temperature.

Ageing experim. no.	Buffer	pH	Volume fraction of methanol in the eluent	Ion pairing agent concentration 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	Hexylsulphonate
4	0.05 M Bicarbonate	8.4	0	-
5	0.05 M Bicarbonate	8.4	0.5	-
6	0.05 M Bicarbonate	8.4	0.5	Triethylamine

In order to include a study to the effect of additional ionic species on bonded phases, at high pH, a basic and at low pH an acid ion pairing agent were added to the methanol-water buffers. For economic and also practical reasons, these eluents were recirculated continuously in a closed system at a flow rate of 0.5 ml/min. Hence, during the purging process each column was purged with about 7000 column volumes of a specific eluent. Possible saturation of the eluent with dissolved silica and/or ligands could reduce the ageing effect of the experiment. The influence of multiple sample introduction to the column is also neglected. Therefore, it seems reasonable to consider the results of the present experiments as the minimum changes during normal laboratory use for the different combinations of reversed phases and eluents.

After finishing a typical series of purging experiments, the columns were carefully rinsed with water, aqueous-methanol mixtures and methanol to prevent deposition of the buffering salts. Subsequently, the columns were

subjected to chromatographic characterisation with the two series of homologues, alkylbenzenes and alkyl aryl ketones, at suitable eluent composition. These chromatographic experiments were performed with a Model 100 A pump (Beckman, Berkeley, CA, U.S.A.), a Model CV-6-VHPa-N60 injection valve equipped with a 20-μl loop (Valco, Houston, TX, U.S.A.) and a Model LC-3 variable wavelength UV detector (Pye Unicam, Cambridge, UK) operated at 254 nm. 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, U.S.A.).

Elemental Analysis

The carbon content of the modified C_{18}-stationary phases prior to and after ageing experiments was obtained with a Perkin Elmer Analyzer, model 240 (Perkin Elmer Corp., Norwalk, CT, U.S.A.). Tungsten oxide was added to the modified silica as a catalyst. The carbon content, P_c, and the ligand surface density, α_l, of the starting materials is given in Table 4.III.

Solid state ^{29}Si NMR measurements

The solid state ^{29}Si NMR spectra were obtained on a Bruker CXP 300 Fourier transform NMR spectrometer at 59.63 MHz. Representative samples of 150-200 mg were spun at *ca.* 3.5 KHz using aluminum oxide rotors (7 mm O.D.) of the standard Bruker double bearing type. ^{29}Si Bloch decay magic angle spinning (MAS) NMR spectra of the original silica substrates were obtained with a pulse interval time of 90 s. ^{29}Si MAS NMR is a rather time consuming technique but, when pulse interval times exceed the relaxation times by a factor of *ca.* 5, the signal areas measured represent the absolute amounts of silicon atoms of different nature present in the sample.

TABLE 4. III

Octadecylsilane modified RP-HPLC stationary phases under study, notation, carbon contents and ligand surface density.

Substrate	Modification	Code	Carbon content P_C %(w/w)	Ligand surface density α_l(eqn 4.1) (μmol.m^{-2})
Nucleosil 100-7 batch 5061	Dimethylchloro-octadecylsilane	NMO	15.9	2.38
	Methyldichloro-octadecylsilane	NDI	15.6	2.52
	Trichloro-octadecylsilane	NTR	15.6	2.62
Hypersil batch 5-192	Dimethylchloro-octadecylsilane	HMO	8.5	2.34
	Methyldichloro-octadecylsilane	HDI	8.7	2.55
	Trichloro-octadecylsilane	HTR	8.6	2.63

Typically 1000 FIDs (free induction decays) with an acquisition time of 10 ms were accumulated in 1K datapoints, zero-filled to 8K prior to Fourier transformation. A line broadening of 10 Hz prior to zero-filling was used. ^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR spectra of all modified C_{18} stationary phases prior to and after ageing experiments were obtained with a cross polarisation contact-time of 6 ms for the mono-functionally modified stationary phases and 2 ms for the other modified phases. These respective values were the optimum contact times for mono- and multidentate surface-linked groups. The pulse interval was 1 s. ^{29}Si CP-MAS NMR is confined to those silicon nuclei not too far from protons near the surface and facilitates fast acquisition. Typically 2000 FIDs with an acquisition time of 10 ms were accumulated in 1K datapoints, zero-filled to 8K prior to Fourier transformation. Line broadening used is 20 Hz prior to zero-filling and Fourier transformation. The spectral width for all spectra was 20 KHz.

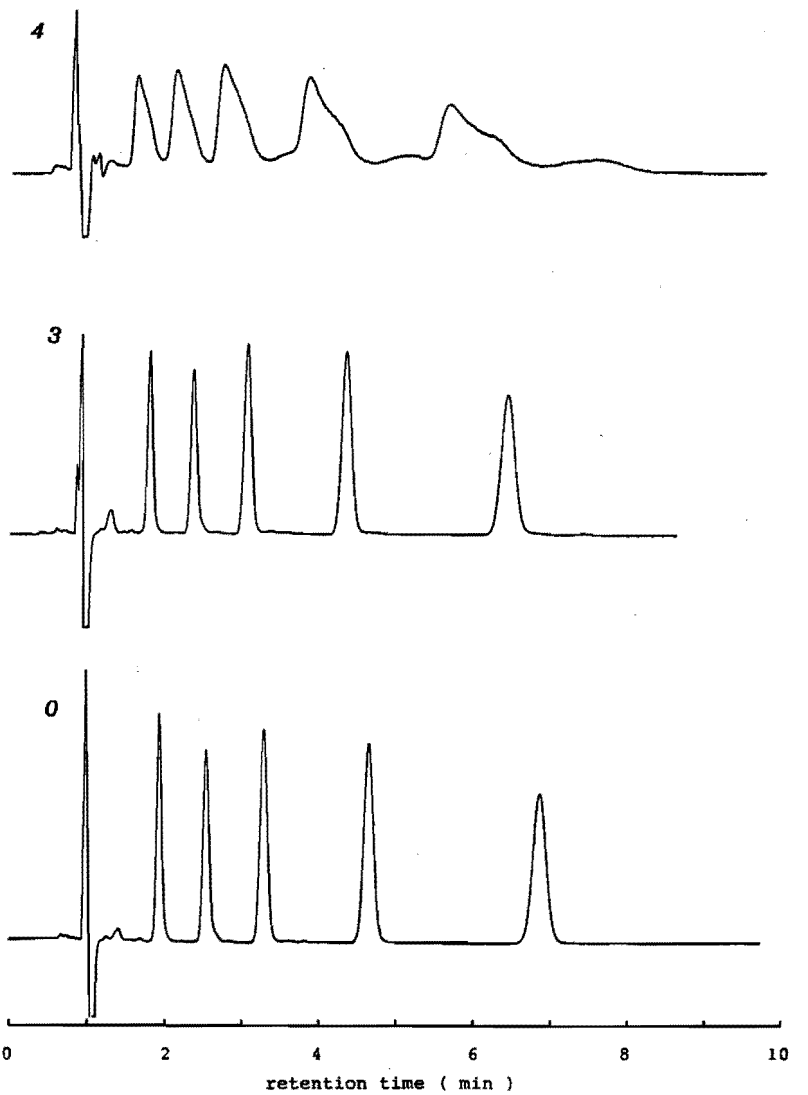

Figure 4.2

Chromatograms of the untreated difunctional octadecylsilane-modified Nucleosil silica, before (0) and after ageing experiment 3 and 4 according to Table 4.II. Chromatographic test conditions: test mixture 1, alkylbenzenes, water-methanol (30:70 v/v), UV-detection at 254 nm.

4.4 RESULTS AND DISCUSSION

The influence of long-term exposure to some aggressive eluents used in modern HPLC practice on the performance of a separation is illustrated best by chromatography itself. Figure 4.2 depicts the chromatograms of alkylbenzenes, test mixture 1, eluted on methyldichlorooctadecylsilane-modified Nucleosil phases (NDI), before (0) and after experiment 3 and 4.

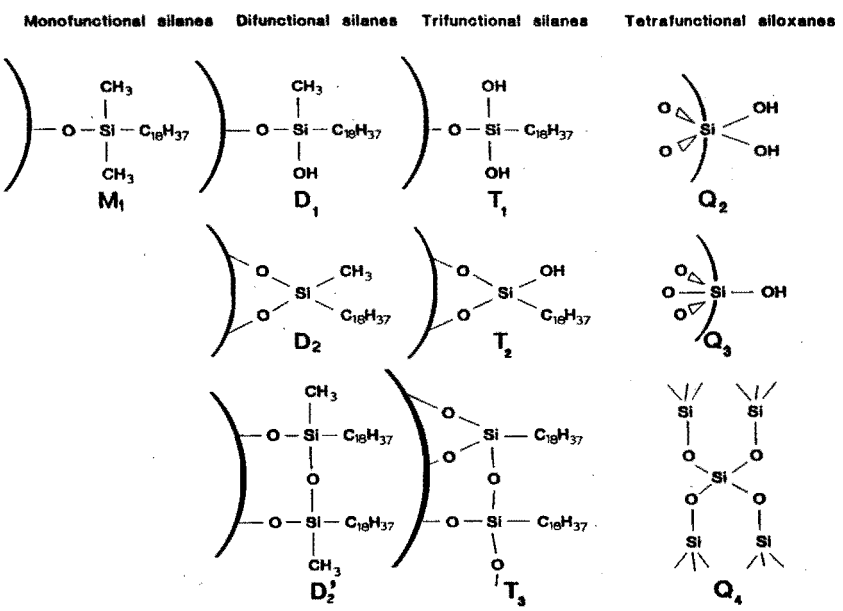

Figure 4.3

Structure of siliceous surface moieties most relevant to this study with their notations.

The characterization of the changes at the surface of the modified substrates after intensive use, the chemistry of the ageing process, the impact for chromatography and stationary phase modification are discussed below.

TABLE 4. IV

Most relevant surface silanes/siloxanes, their functionality, notation and typical ^{29}Si chemical shift.

Moiety	Chemical (spectral) function.	Topological (network) function.	Code	Typical [a] chemical shift
Dimethyloctadecyl-siloxysilane	M	1	M_1	+ 12
Methyloctadecylhydroxy-siloxysilane	D	1	D_1	- 3
Methyloctadecyl-disiloxysilane	D	2	D_2	-11
Paired Methyloctadecyl-disiloxysilane	D	2	D_2'	-16
Octadecyldihydroxy-siloxysilane	T	1	T_1	-48
Octadecylhydroxy-disiloxysilane [b]	T	2	T_2	-55
Octadecyltrisiloxysilane [b]	T	3	T_3	-66
Dihydroxysiloxane	Q	2	Q_2	-91
Hydroxysiloxane	Q	3	Q_3	-101
Tetrasiloxysiloxane	Q	4	Q_4	-110

a ppm downfield from TMS.

b paired T_2 and T_3 groups and double paired T_3 groups were not distinguishable.

Condensation of multifunctional silanes

The different types of surface silane modifications most relevant to this paper are collected in Table 4.IV with their ^{29}Si NMR chemical shifts. The corresponding structural elements at the surface of the silica gel are depicted in Figure 4.3.

The ^{29}Si MAS NMR spectra of both silica substrates are represented in Figure 4.4. The relative amount of silanol groups, mainly hydroxysiloxane (Q_3) groups, determined from these spectra is given in Table 4.I.

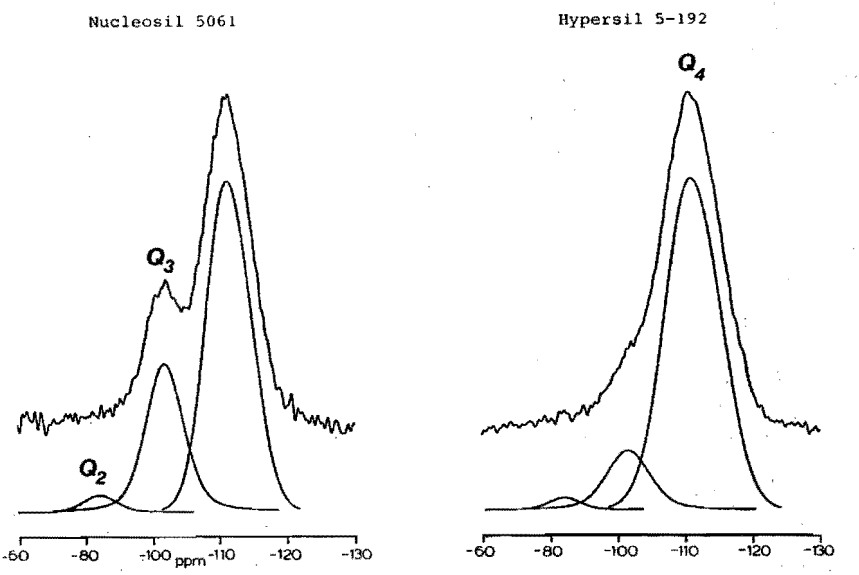

Figure 4.4

^{29}Si Bloch pulse MAS NMR spectra of the original Nucleosil and Hypersil silica substrates with the deconvoluted signals. N_S = 1000; pulse interval time, 90 s; acquisition time, 10 ms; line broadening, 10 Hz.

Owing to the large surface area of the Nucleosil substrate and assuming all silanol groups are located at the surface, an almost identical silanol density for both substrates was calibrated. In this study the total amount of geminal silanol, dihydroxysiloxane (Q_2) or silanediol groups, of both substrates before modification did not exceed 15%. Kohler et al. reported [3] a too large geminal silanol content of 32% relative to the total amount of silanol for various substrates, determined with ^{29}Si CP-MAS NMR.
For untreated substrates we measured a lower content of geminal silanols relative to the total silanol content, about 12% for both silica substrates with ^{29}Si MAS NMR.

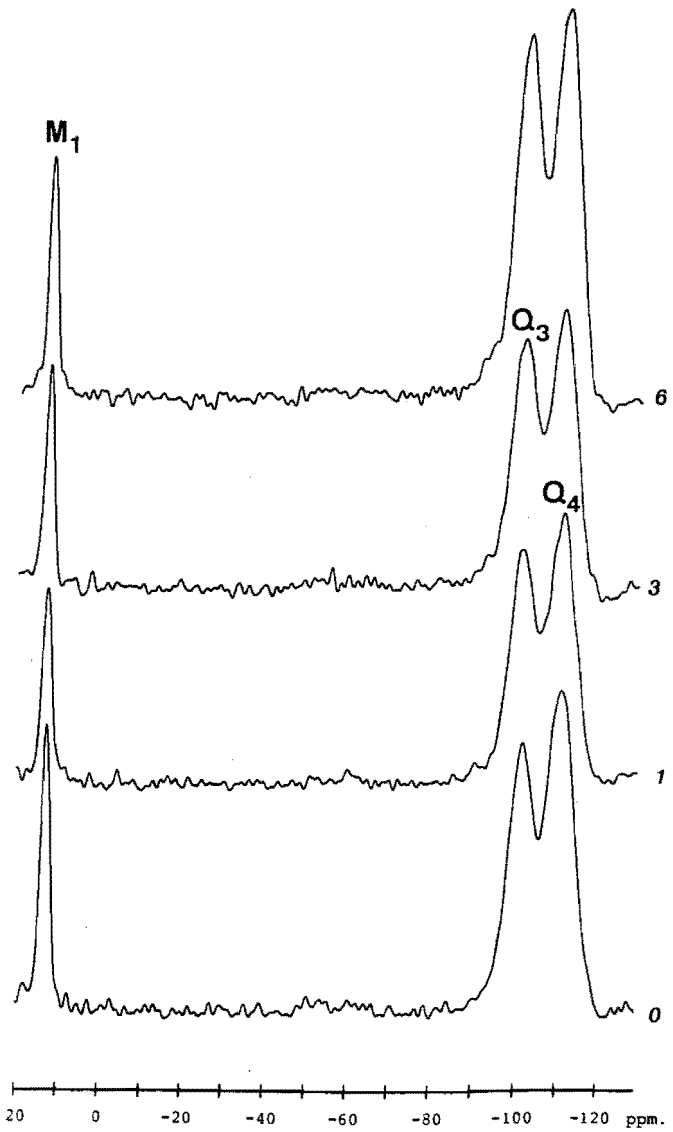

Figure 4.5

^{29}Si CP-MAS NMR spectra of monofunctional octadecylsilane-modified Nucleosil silica, before (0) and after ageing experiments 1, 3 and 6. N_S = 2000; contact-time, 6 ms; pulse interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz.

For relative comparison representative ^{29}Si CP-MAS NMR spectra of the monofunctional octadecylsilane-modified Nucleosil substrate, the difunctional octadecylsilane-modified Nucleosil substrate, the trifunctional octadecylsilane-modified Nucleosil, and the trifunctional octadecylsilane-modified Hypersil substrate before and after ageing experiments 1, 3 and 4 are shown in Figures 4.5, 4.6, 4.7 and 4.8, respectively.

The spectra of mono- and difunctional octadecylsilane-modified Hypersil were identical to the respective mono- and difunctional octadecylsilane-modified Nucleosil stationary phases. Only the spectra of the trifunctional modified Nucleosil and Hypersil stationary phases differed.
For a direct comparison between the depicted spectra, one should consider the different contact-times applied for mono- and multifunctionally modified phases, 6 and 2 ms, respectively [22]. A difference in contact-time will alter the ratio between Q_3 and Q_4 signals, which generally show different contact-time optima. To prevent the spectral difference interfering with the analysis all untreated stationary phases were also measured with a 2 ms contact-time. The Q_3/Q_4 ratio of all modified Nucleosil phases was found to be 1.22 $\pm$ 0.02 and for all modified Hypersil phases this value was 0.90 $\pm$ 0.04. The small spectral deviation indicated an equal residual silanol content after modification for all Nucleosil phases and also for all Hypersil phases.
The NMR spectra of the bonded phases indicate that the di- and trifunctional modified RP-HPLC phases under study tended to form more multidentate surface and neighbour linkages of the octadecylsilane ligands, when used intensively with plain aqueous buffer solutions of high and low pH as eluents. Methyloctadecylhydroxysiloxysilane (D_1) and methyloctadecyldisiloxysilane (D_2) moieties reacted to form methyloctadecyldisiloxysilane, especially paired (D_2') moieties, see also Figure 4.6).
A similar condensation reaction occurred with the trifunctional octadecylsilane-modified stationary phases. Octadecyldihydroxysiloxysilane (T_1) and octadecylhydroxydisiloxysilane (T_2) moieties formed on modification were

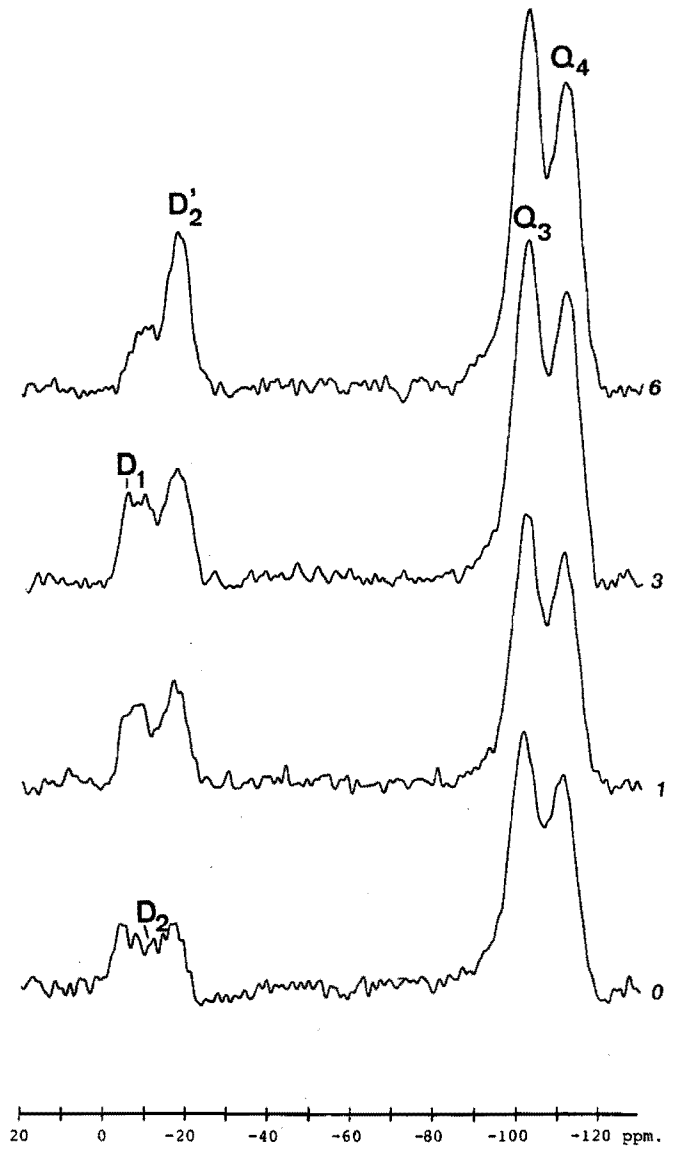

Figure 4.6

^{29}Si CP-MAS NMR spectra of difunctional octadecylsilane-modified Nucleosil silica, before (0) and after experiments 1, 3 and 6. N_S = 2000; contact-time, 2 ms; pulse interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz.

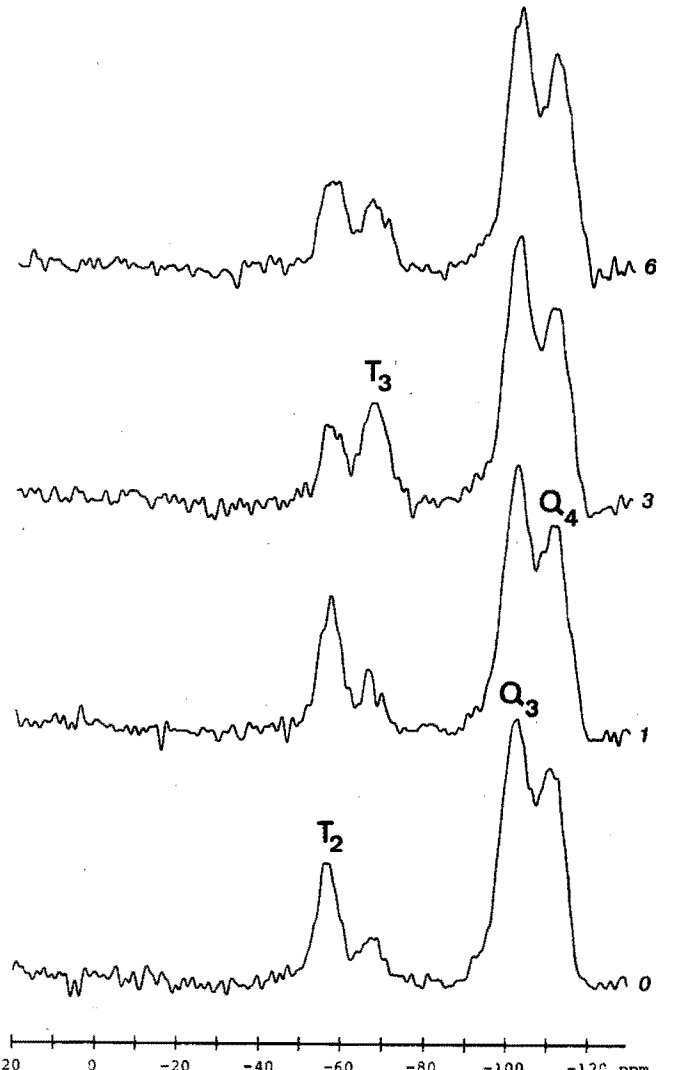

Figure 4.7

^{29}Si CP-MAS NMR spectra of trifunctional octadecylsilane-modified Nucleosil silica, before (0) and after treatments 1, 3 and 6. N_S = 2000; contact-time, 2 ms; pulse interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz.

dehydrated to form octadecylhydroxydisiloxysilane (T_2) and octadecyltrisiloxysilane (T_3) moieties respectively, see also Figure 4.7.

The amount of surface silanol groups did not alter accordingly. On the contrary, a slight increase of surface silanol groups was noticed. From this we can conclude that condensation of hydroxysilane moieties at the surface (D_1, T_1 and T_2) mainly occurred between anchored neighbours showing preferential polymerisation across the surface. This is in agreement with results of methyltrichlorosilane modifications of silica gels under conditions of different water content published by Sindorf and Maciel [7]. Our results indicate that multidentate linkage with other silanes at the surface prevented hydrolysis of the anchored ligands, this in contrast to the monofunctionally modified octadecyl phases. In the latter instance, a substantial gain in silanol content is found during ageing. Condensation of multifunctional silanes was more pronounced when high pH buffers were used as an eluent for simulation of intensive use.

TABLE 4. V

Results of elemental analysis, NMR experiments and chromatographic measurements of the monofunctional octadecylsilane-modified Nucleosil silica before and after experiments 1 to 6.

NMO ageing exp.no.	Ligand α_l (eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio		Chromatography: a_o	
		ligand M_1	silanol Q_3	alkyl-benzenes	alkyl aryl-ketones
0	2.38	0.34	0.66	2.22	0.81
1	2.32	0.24	0.76	2.31	1.01
2	2.31	0.32	0.68	2.34	0.41
3	2.26	0.23	0.77	1.80	0.32
4	2.06	0.23	0.77	1.73	0.25
5	2.13	0.32	0.68	1.76	0.32
6	2.11	0.28	0.72	1.91	0.21

Mean value for p (eqn. 4.5a) = 0.80.

The effect of ion pair agents added to aqueous-methanol buffers of low and high pH on the stationary phases under study here is not completely clear at this stage. All multifunctionally modified stationary phases except tri-functionally modified Nucleosil showed an enhanced condensation of surface

silanes upon addition. For trifunctional octadecylsilane Nucleosil condensation was reduced by the methanol content of the aqueous-methanol buffer.

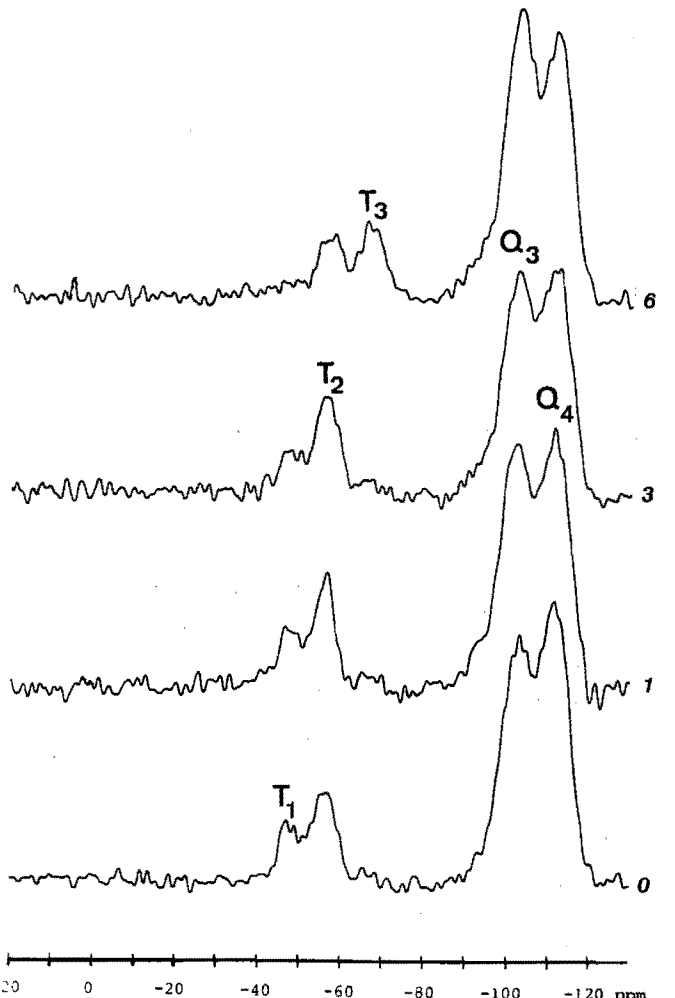

Figure 4.8

^{29}Si CP-MAS NMR spectra of trifunctional octadecylsilane-modified Hypersil silica, before (0) and after experiments 1, 3 and 6. N_S = 2000; contact-time, 2 ms; pulse interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz.

It should be noted that the trifunctionally modified Nucleosil used for the

simulation experiments already showed progress of condensation of octadecyldihydroxysiloxysilane (T_1) and octadecylhydroxydisiloxysilane (T_2) groups in the time elapsed between synthesis and start of the chromatographic experiments.

The concentration of T_1 groups was rather low and that of T_3 groups was much higher for the NTR phase than for the HTR phase used (see also Figures 4.7 and 4.8).

After a longer period no further changes at the surface of the trifunctionally modified Nucleosil were noticed. The results of the spectroscopic and the chromatographic measurements and the elemental analysis are summarized in Tables 4.V to 4.VII for modified Nucleosil silica and in Tables 4.VIII to 4.X for the modified Hypersil silica.

TABLE 4. VI

Results of elemental analysis, NMR experiments and chromatographic measurements of the difunctional octadecylsilane-modified Nucleosil silica before (0) and after experiment 1 to 6.

NDI ageing exp.no.	Ligand α_l(eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio ligand			silanol	Chromatography: a_o	
		D_1	D_2	D_2'	Q_3	alkyl-benzenes	alkyl aryl ketones
0	2.52	0.16	0.08	0.18	0.58	1.96	0.31
1	2.45	0.19	0.03	0.16	0.62	1.76	0.15
2	2.47	0.17	0.02	0.17	0.64	1.87	0.08
3	2.50	0.15	-	0.21	0.64	1.45	-0.05
4	2.32	0.12	-	0.22	0.66	1.36	-0.07
5	2.44	0.15	-	0.20	0.65	1.53	-0.06
6	2.37	0.08	-	0.24	0.68	1.46	-0.12

Mean value for p (eqn. 4.5a) = 0.65.

Stationary phase hydrolysis

The influences of ageing experiment 1 to 6 on the capacity factor values of the dichlorooctadecylmethylsilane-modified Nucleosil (NDI) stationary phase

for test mixture I are shown in Figure 4.9 with the eluent: methanol-water (80:20, v/v). The decreased capacity factor values after all experiments indicate a significant loss of ligands from the surface of the reversed phase. After extrapolation to $n_c = 0$ and $x = 0$ (methanol fraction in the eluent used for characterization), the differences in capacity were expressed in the a_0-values for alkylbenzene test mixture (see below).

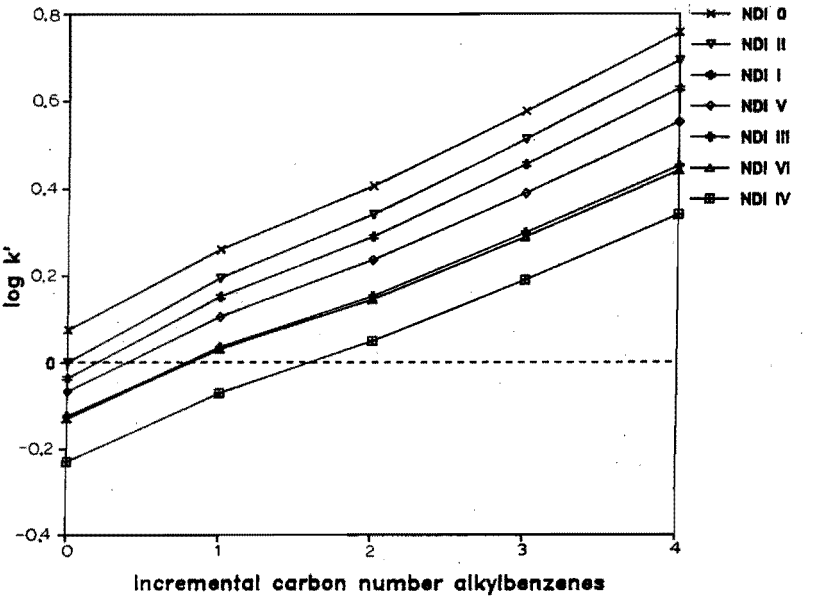

Figure 4.9

The influence of experiments 1 to 6 on the capacity factor k' determined with chromatography for alkylbenzenes, test mixture 1. Chromatographic conditions: eluent methanol-water (80:20 v/v).

A typical diagram of log k', determined by regression analysis from chromatographic results, *versus* the volume fraction methanol (x) and the incremental carbon number of the alkylbenzene (n_c) for the methyl-dichlorooctadecylsilane-modified Hypersil (HDI) stationary phase after ageing experiment 5 is shown in Figure 4.10, where the linear dependence

of the log k' values on both the eluent composition and the incremental carbon number is shown. According to eqns. 4.2 and 4.3, the a_0, m_0, a_1 and m_1 values were calculated by multiple linear regression. The validity of the regression was expressed by the correlation coefficient (r). All correlation coefficients had values between 0.995-0.999, indicating reliable values for all constants estimated by the regression model proposed by Jandera [25]. With eqn. 4.5a the matching values for p were calculated.

TABLE 4. VII

Results of elemental analysis, NMR experiments and chromatographic measurements of the trifunctional octadecylsilane-modified Nucleosil silica before (0) and after experiment 1 to 6.

NTR ageing exp.no.	Ligand α_1(eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio ligand			silanol	Chromatography: a_0	
		T_1	T_2	T_3	Q3	alkyl-benzenes	alkyl aryl ketones
0	2.62	0.02	0.24	0.10	0.64	2.06	2.44
1	2.57	-	0.22	0.11	0.67	2.38	-0.06
2	2.56	0.03	0.21	0.05	0.71	2.11	1.34
3	2.57	-	0.25	0.09	0.66	1.95	0.86
4	2.95	-	0.09	0.20	0.71	1.35	-1.25
5	2.58	-	0.11	0.15	0.74	1.72	-0.04
6	2.55	-	0.16	0.13	0.71	1.97	-0.83

Mean value for p (eqn. 4.5a) = 0.85.

Both elemental analysis and solid state ^{29}Si NMR measurements showed a significantly larger loss of ligands for monofunctionally modified stationary phases, especially for the Hypersil substrate, after simulation of intensive use. The effect of ligand loss was also registered by the a_0 values determined for the alkylbenzene and alkyl aryl ketone homologous series. From theory it can be concluded that there is a correlation between especially the a_0 values determined for alkylbenzenes and the ligand density α_1 calculated with eqn. 4.1 after elemental analysis, both indicating èventual stripping of ligands from the surface of the silica.

TABLE 4. VIII

Results of elemental analysis, NMR experiments and chromatographic measurements of the monofunctional octadecylsilane-modified Hypersil silica before (0) and after experiments 1 to 6.

HMO ageing exp.no.	Ligand α_l(eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio		Chromatography: a_o	
		ligand M_1	silanol Q_3	alkyl-benzenes	alkyl aryl ketones
0	2.34	0.29	0.71	2.37	0.99
1	1.45	0.11	0.89	1.89	0.71
2	1.81	0.22	0.78	2.28	0.34
3	1.82	0.18	0.82	1.97	0.47
4	1.55	0.16	0.84	2.01	-0.19
5	1.79	0.18	0.82	1.73	0.86
6	1.63	0.17	0.83	1.98	0.06

Mean value for p (eqn. 4.5a) = 0.84.

TABLE 4. IX

Results of elemental analysis, NMR experiments and chromatographic measurements of the difunctional octadecylsilane-modified Hypersil silica before (0) and after experiments 1 to 6.

HDI ageing exp.no.	Ligand α_l(eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio ligand			silanol	Chromatography: a_o	
		D_1	D_2	D_2'	Q_3	alkyl-benzenes	alkyl aryl ketones
0	2.55	0.07	0.20	0.05	0.68	2.09	0.49
1	2.39	0.05	0.15	0.12	0.68	1.91	-0.29
2	2.44	0.05	0.18	0.17	0.62	1.91	0.57
3	2.45	-	0.17	0.16	0.67	2.01	0.49
4	2.29	0.04	0.14	0.11	0.71	1.90	-0.36
5	2.36	0.04	0.12	0.14	0.70	2.01	0.14
6	2.21	0.04	0.10	0.17	0.69	1.60	0.32

Mean value for p (eqn. 4.5a) = 0.88.

However, the observed correlation between α_l and a_o (alkylbenzene residue) for both stationary phases was rather poor, correlation coefficient values:

r(Nucleosil) 0.70-0.86 and r(Hypersil) 0.56-0.85. Plots of the α_l-values as a function of the a_o (alkylbenzene residue) values for both the Nucleosil and the Hypersil stationary phases are depicted in Figures 4.11 and 4.12, respectively.

TABLE 4. X

Results of elemental analysis, NMR experiments and chromatographic measurements of the trifunctional octadecylsilane-modified Hypersil silica before (0) and after experiments 1 to 6.

HTR ageing exp.no.	Ligand α_l(eqn.4.1) (μmol.m^{-2})	^{29}Si NMR ratio ligand			silanol	Chromatography: a_o	
		T_1	T_2	T_3	Q3	alkyl-benzenes	alkyl aryl ketone
0	2.63	0.18	0.18	-	0.64	1.97	0.51
1	2.51	0.07	0.22	-	0.71	1.76	0.31
2	2.54	0.10	0.22	-	0.68	1.82	0.48
3	2.57	0.10	0.23	-	0.67	1.80	0.41
4	2.69	-	0.12	0.15	0.73	1.23	-0.86
5	2.40	0.02	0.15	0.11	0.72	1.54	-0.06
6	2.38	-	0.12	0.14	0.76	1.37	-0.70

Mean value for p (eqn. 4.5a) = 0.84.

The a_o values fluctuated to a larger extent than the α_l values, especially for multifunctionally modified phases. The observed deviation between α_l and a_o values is due to the fact that α_l is an averaged bulk value determined from the probably changed specific surface area and the relative carbon content, which is also influenced by hydrolysis of the substrate. On the other hand the a_o value seemed to be more sensitive to irregularities in the packing, like local distortion of the ligand film and the packed bed, and chromatographic parameters.

New stationary phases with increasing ligand density showed a good correlation between the a_o (alkylbenzene residue) and α_l values. The bonded phases modified with multifunctional silanes were more stable, in contradiction with results reported earlier [1]. This earlier study already

contained a restrictive remark regarding the possible influence of a lower surface coverage of ligands for the trifunctional modified stationary phase studied. We can now conclude that the influence of different surface coverage is of major importance for the comparison of the stability of ligand anchoring on silica based substrates, at least for multifunctionally derivatized materials. For more stable stationary phases a full coverage of the surface with ligands is desirable. Hydrolysis of octadecyl ligands was reduced by eluents containing 50% (v/v) methanol. Most severe hydrolyses of octadecylsilanes were determined after use of high pH buffer eluents, especially ageing experiment 4.

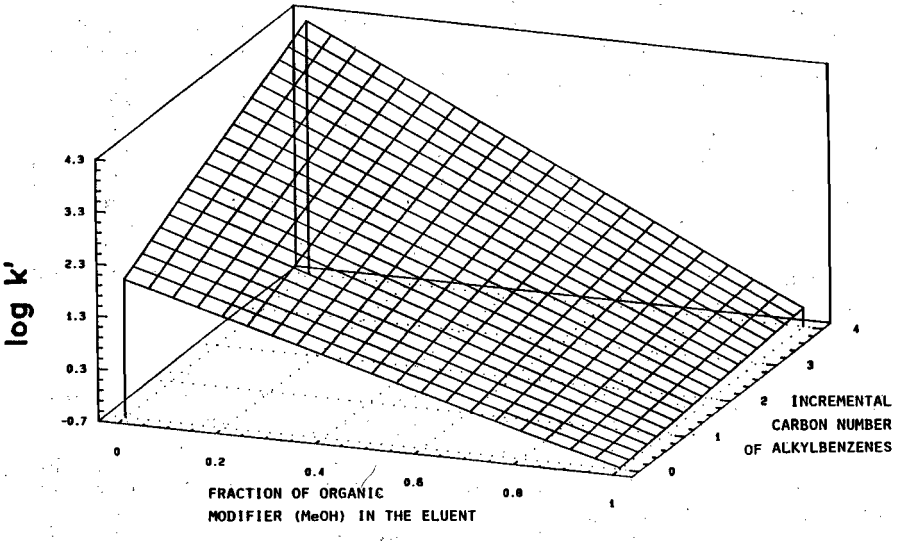

Figure 4.10

Graphical representation of the regression function for k' with the volume fraction organic modifier (here methanol) in the eluent measured between x= 0.6 and x= 0.9 and extrapolated for x between 0 and 1, and the incremental carbon number of the alkylbenzene homologue series for the difunctional octadecylsilane-modified Hypersil silica (HDI) after ageing experiment 5.

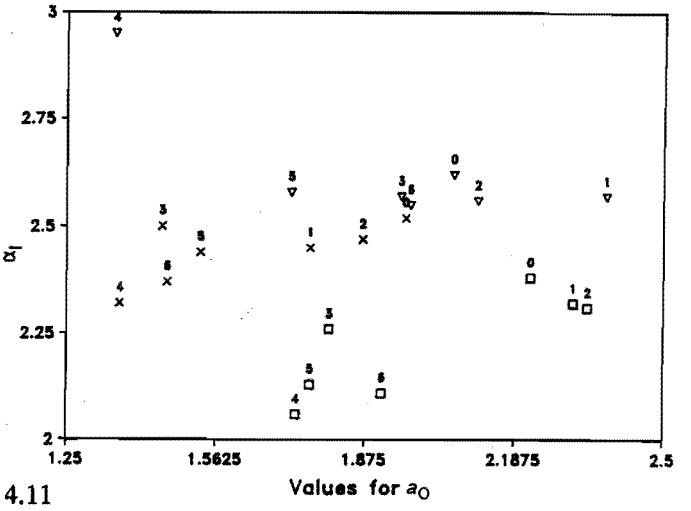

Figure 4.11

Octadecyl ligand density α_l as a function of the a_O(alkylbenzenes) value for modified Nucleosil stationary phases before (0) and after each ageing experiment (1 to 6). ▫, NMO; x, NDI; ▿, NTR.

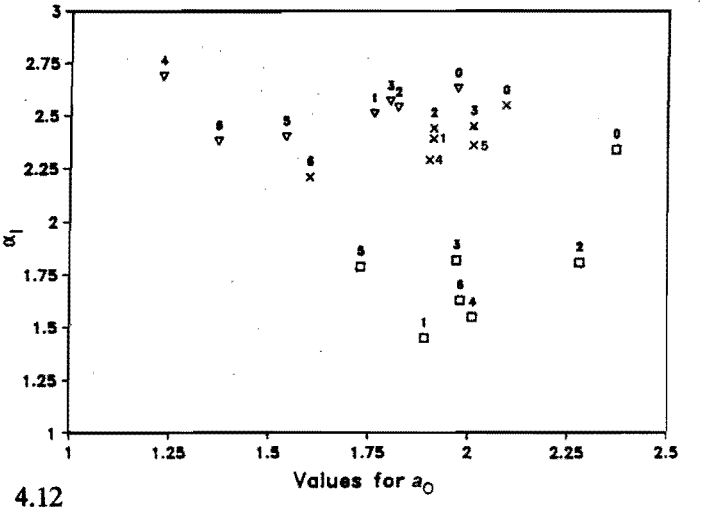

Figure 4.12

Octadecyl ligand density α_l as a function of the a_O(alkylbenzenes) value for modified Hypersil stationary phases before (0) and after each ageing experiment (1 to 6). ▫, HMO; x, HDI; ▿, HTR.

Elemental analysis sometimes showed an "increase" of the ligand density at the surface after ageing. This, however, is caused by rapid dissolution of the silica substrate than by extra hydrolysis of the silane ligands during ageing. The relatively higher hydrolysis of substrate material was in this study only observed for trifunctionally derivatized stationary phases.

The same results, mainly for trifunctional modifications, were reported earlier [1]. Ligands attached to silica with trifunctional silanes were the most resistant towards hydrolysis, when exposed to aggressive HPLC eluents compared with identically modified mono- and difunctional analogues. However, at high pH the shielding of the surface by polymerization did not prevent hydrolysis of the silica substrate, leading to a less preferable situation.

In a recent study, Kirkland et al. [28] used bridged "bidentates", containing two monofunctional silicon atoms with short alkyl ligands (C_1 to C_4 ligands) instead of difunctional alkyl silanes used for modification of the silica substrate in most other studies. They also noticed that these "bidentates" showed higher resistance towards hydrolysis of the anchored silanes. We have noticed before that the anchoring of longer alkyl silane modifications was more stable due to the shielding effect by these longer alkyl chains [1]. In the present study, difunctionally modified C_{18} phases the loss of ligands at the surface after the ageing experiments was limited to less than 10% relative to the initial carbon content, showing the highest stability towards hydrolysis by aggressive eluents. Compared with the short alkyl ligand modification described earlier [1], the multifunctional octadecylsilane-modified stationary phases studied here show extremely high stabilities. Therefore, in order to improve the stability of alkyl-modified reversed phases with alkyl ligands longer than C_4, the use of multifunctional alkyl silanes must be considered. This seems particularly true for bifunctional derivatized stationary phases.

Cracked particles and small channels formed through the packed column

caused double and split peaks in chromatograms, as shown for the NDI phase after ageing experiment 4 in Figure 4.2. These columns showed such a severe drop in column efficiency that any further use for chromatographic separations was inadvisable. For the trifunctionally modified phases more severe peak splitting occurred than for the difunctionally modified phases, indicating superior shielding in the latter case.

The relative deviations of the results determined by the characterization methods used in this study are listed in Table 4.XI. The relative deviation (RD) of the reported values were calibrated by comparing the determined values before and after ageing experiments relative to the value before ageing (see eqn. 4.7).

$$\text{Relative deviation: } RD = \sum_{i=1}^{n} \frac{\left| X_{after} - X_{before} \right|}{n} * \frac{1}{X_{before}} \qquad (4.7)$$

with X = the value determined with a specific characterization method and n = the number of ageing experiments included.
These relative deviation values give a summary of the results listed in Tables 4.V-4.X for comparison of the stability of the stationary phases examined. From the relative deviations for elemental analysis and solid-state ^{29}Si NMR experiments can be concluded, that on the Nucleosil substrate the di- and trifunctional octadecylsilane stationary phases were more stable than the monofunctional octadecylsilane phase for the same surface coverage. The relative deviations of a_o (alkylbenzene) did not give a clear indication of the stability of the Nucleosil stationary phases. However, the trifunctional octadecyl phase was already altered by condensation before characterization and ageing experiments. This alteration could possibly have had a positive influence on the stability compared with the trifunctionally modified Hypersil stationary phase. On the Hypersil silica, however, the difunctional

octadecylsilane modification manifested the highest stability after exposure to the HPLC eluents with the characterization methods used in this study.

TABLE 4. XI

Relative deviation of the results determined by elemental analysis, NMR experiments and chromatographic measurements for the stationary phases under study.

Stationary phase	Elemental Analysis	^{29}Si CP-MAS NMR		Chromatography: a_Q	
	α_1	total ligand	silanol Q_3	alkyl benzenes	alkyl aryl ketones
NMO	0.076	0.206	0.106	0.142	0.564
NDI	0.037	0.152	0.118	0.198	1.004
NTR	0.038	0.167	0.094	0.131	1.005
HMO	0.284	0.414	0.169	0.166	0.625
HDI	0.076	0.068	0.032	0.096	0.759
HTR	0.051	0.199	0.112	0.186	0.114

Compared with commercially available reversed-phase monofunctional octadecyl Hypersil phases the ligand density in this study was relatively low [α_1= 2.34 to α_1 (commercial Hypersil C_{18}) = 3.41]. This probably explains the diverged, rather poor stability of the HMO stationary phase compared to a previous study [1].

Selectivity

Selectivity in RP-HPLC depends on both the mobile and on the stationary phase used. To judge the selectivity of the mobile phase, the mean values for p are listed in Tables 4.V to 4.X. These p values were in agreement with the theoretical value for methanol-water eluents, p= 0.86 calculated by Jandera [26]. The only exception was found for the NDI stationary phase with a mean value for p= 0.65. This deviation was found after regression for both homologous test mixtures and was double checked. No reasonable explanation can be found for this deviation from the theoretical value.

More specific stationary phase-solute interactions were obtained by the values of m_0 for lipophilic and q for polar selectivity. The plots of m_0 values as a function of a_0 values for both homologue residues on the Nucleosil and Hypersil stationary phases are depicted in Figures 4.13 and 4.14, respectively.

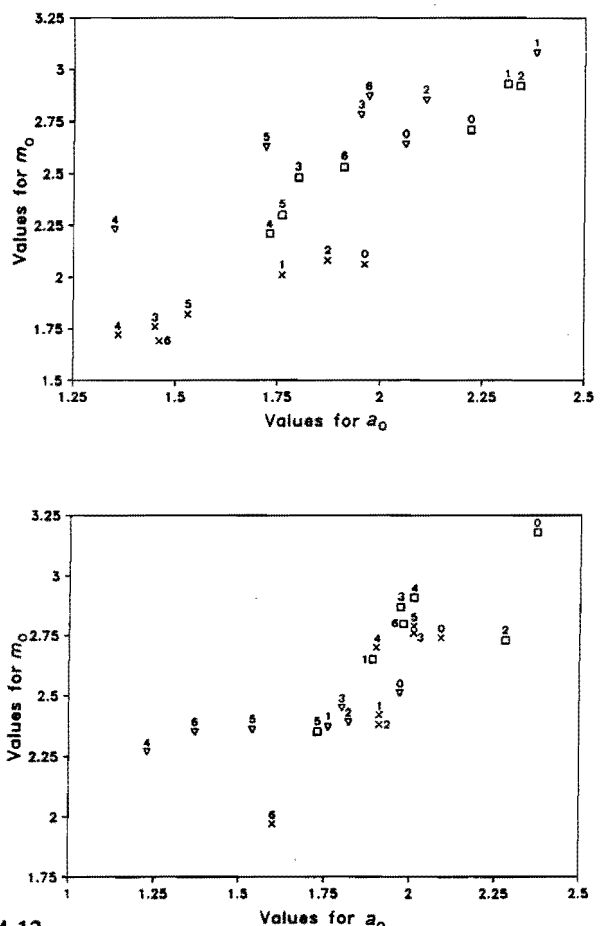

Figure 4.13

Chromatographically determined m_0 values (lipophilic selectivity) as a function of a_0 values for the alkyl benzene homologous series on modified Nucleosil and Hypersil stationary phases before (0) and after each ageing experiment (1 to 6). Top: Nucleosil; bottom: Hypersil; ▫ , monofunctional; x , difunctional; ▿ , trifunctional.

As already pointed out by Jandera [26,27], there is a good correlation between the non-polar contribution to selectivity m_0 and the capacity factor of the stationary phase represented by a_0 values.

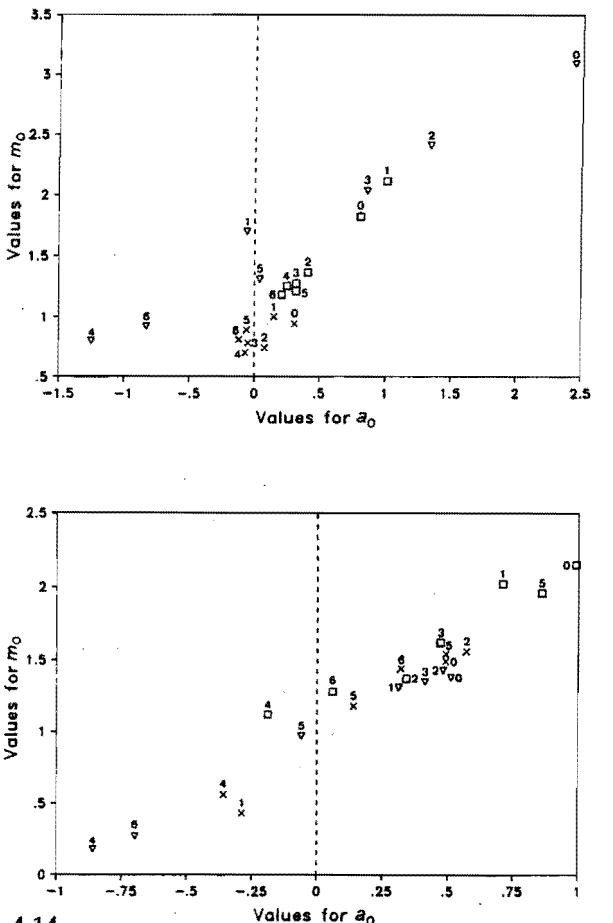

Figure 4.14

Chromatographically determined m_0 values (lipophilic selectivity) as a function of a_0 values for the alkyl aryl ketones homologous series on modified Nucleosil and Hypersil stationary phases before (0) and after each ageing experiment (1 to 6). Top: Nucleosil; bottom: Hypersil; ▫, monofunctional; x , difunctional; ▽, trifunctional.

The correlation coefficients were r(alkylbenzenes) = 0.92-0.98 and r(alkyl aryl ketones) = 0.97-0.996. As expected, a decrease in the amount of bonded ligands caused by intensive use caused a decrease in non-specific lipophilic selectivity.

Plots of the polar selectivity q *versus* a_0 values for the alkyl aryl ketone residue both on Nucleosil and Hypersil stationary phases are shown in Figure 4.15. The influence of the ageing experiments on the polar selectivity varied depending on the type of modification and the silica substrate. For the monofunctionally modified phases a decrease in the capacity factor and lipophilic selectivity implies also a decrease in polar selectivity for alkyl aryl ketones, especially for the HMO stationary phase an extreme drop of the q value occurred. This polar selectivity seemed to decrease with the loss of ligands and the gain in silanol groups at the surface, against expectation. Multifunctionally modified stationary phases, especially difunctionally modified phases, show an almost constant value for q after hydrolysis and condensation of the remaining ligands at the surface. It seems that the residual silanols are better shielded by the surface cross-linked ligands, except for the trifunctional octadecyl-modified Nucleosil phase. The substrate shielding of this stationary phase seems rather poor, indicated also by higher q values after aging experiments.

Substrate shielding by siloxane bridges directly at the surface, which occurs especially with bidentate, difunctional octadecyl-modified phases, D_2 and D_2' groups, prevent ligand and substrate hydrolysis and showed a remaining selectivity after the ageing experiments. Difunctionally modified stationary phases with bidentate linkage are preferable in RP-HPLC with aggressive eluents.

In contrast with results published by Jandera [27] on polar selectivity and the value of q for solutes, we noticed that the value of q (alkyl aryl ketone residue) did not indicate more specific polar interactions between the solute and the increasing amount of silanol groups measured with solid state ^{29}Si NMR.

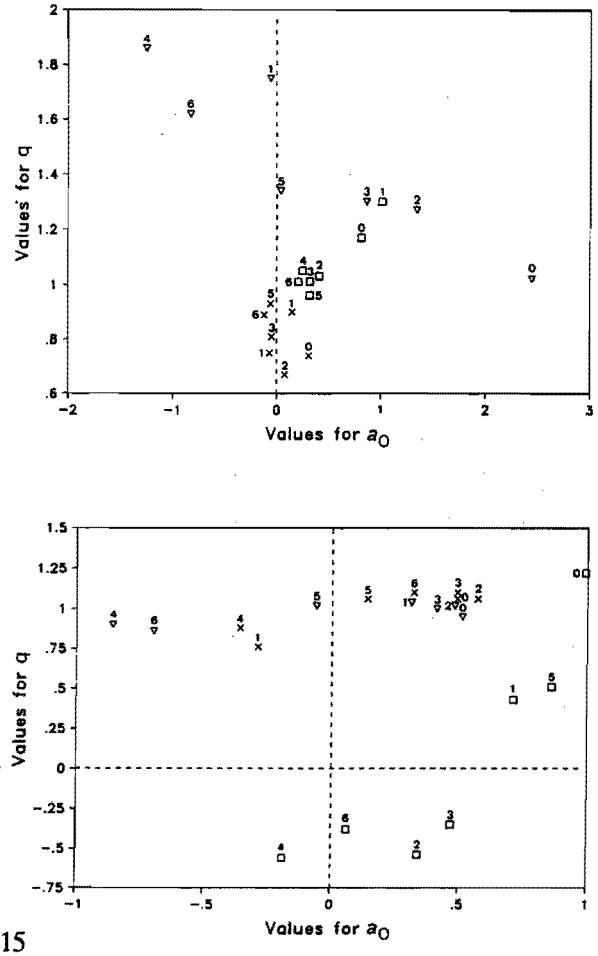

Figure 4.15

Chromatographically determined q values (polar selectivity) and a_O values for the alkyl aryl ketones homologous series on modified Nucleosil and Hypersil stationary phases before (0) and after each ageing experiment (1 to 6). Top: Nucleosil; bottom: Hypersil; ▫, monofunctional; x , difunctional; ▿, trifunctional.

Probably the "polarity" of the alkyl aryl ketone residue (steric hindrance by the benzene ring) and the shielding of the silanol groups formed, also due to a higher degree of mobility and irregularities in the octadecyl film [29]

after the ageing experiments, influenced these specific polar interactions negatively. More polar residues of homologous series are currently being investigated for future characterization of a more pronounced and discriminating value for the polar selectivity of different stationary phases before and after long-term exposure to aggressive eluents. As expected for the alkylbenzene residue, the value of q did not change much. More or less equal q values were determined even for the different silica substrates used for modification, due to the rather apolar residue of the alkylbenzene homologous series.

Ageing experiments, a practical guide

A comparison between the effect of different ageing experiments can be used to evaluate the choice of the eluents listed in Table 4.II.
From the results of the different characterisation techniques, it is obvious that both low and high pH eluents should be included in the series of ageing experiments. For the aqueous buffer with pH value 8.4, used in experiment 4, a severe degradation of the silica substrate was always observed. However, for more discriminating information about the stability of modified phases, the pH of the plain aqueous buffer solution should be selected at *ca.* pH = 7.5, especially for less stable RP-HPLC phases. Mixed eluents containing organic modifiers with buffers can be used with higher aqueous pH values, because the presence of modifier decreases with silica degradation. Although the effect of addition of ion pairing agents on the stability of the modified phases was moderate, the exclusion of these ionic species from the ageing experiments will deprive the experiments of additional, useful information.
For routine laboratory interest, a comparison of different stationary phases should be carried out with a more aggressive eluent than used in practice to decrease the exposure time. Especially when plain aqueous eluents with

abnormal pH values (below 3 and above 8) will be used, one should consider "ageing" of the stationary phases. Information about the stability, selectivity, capacity and silica degradation can be obtained by the chromatographic characterization method as described above combined with information on the column efficiency.

4.5. CONCLUSIONS

Condensation of the multifunctional octadecylsilanes at the surface of the silica substrate prevented hydrolysis of the anchored ligands even after long-term exposure to aggressive eluents. For stationary phases synthesized in order to achieve identical degrees of silylation, we conclude in this study that the bifunctional octadecylsilane stationary phases exhibited the best overall stability towards hydrolysis of the anchored ligands and hydrolysis of the silica substrate, especially when buffers of high pH were used as eluents. Difunctionally modified stationary phases with bidentate linkage are to be preferred in RP-HPLC with aggressive eluents. The decrease in capacity and lipophilic selectivity observed after long-term exposure was less pronounced for the multifunctionally derivatized stationary phases. Most multifunctionally modified octadecyl stationary phases showed an almost unchanged polar selectivity after intensive use. For the monofunctional phases a severe drop in polar selectivity was determined.

For Nucleosil and Hypersil silica, difference between the type of silica substrate used appeared to have much less influence on hydrolysis for multifunctionally modified stationary phases than for monofunctionally modified phases. Of the two monofunctional octadecylsilane reversed phases, the Nucleosil based phase seemed to be more resistant to aggressive eluents. The connection between the major substrate properties and stabilities of the stationary phases after modification will be further discussed in chapters 5 and 6.

4.6. REFERENCES

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CHAPTER 5

INFLUENCE OF ALKYL CHAIN LENGTH ON THE STABILITY OF n-ALKYL MODIFIED REVERSED-PHASES [1,2]

A stationary phase for PR-HPLC is usually synthesized by modifying silica substrate with a silane. Here, the influence of the ligand alkyl chain length, between C_1 and C_{18}, on stationary phase stability in LC practice is reviewed. An in-situ study of the hydrolysis and dissolution of n-alkyl modified phases was performed with ^{29}Si MAS NMR as a model for stationary phase ageing in laboratory practice. The substantial effect of the ligand length on the dissolution reaction of the stationary phases under aggressive eluent conditions is confirmed by both studies. On the basis of the in-situ dissolution study a model for ligand and substrate hydrolysis and dissolution with the reactions involved is presented.

5.1. A STUDY BY CHROMATOGRAPHY AND BY PHYSICAL ANALYSIS

5.1.1. 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.

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Usually, commercially available RP-HPLC phases are synthesized with monofunctional reagents like n-alkyldimethylmonochlorosilane, to prevent formation of additional hydroxyl groups by hydrolysis of remaining Si-Cl groups [1,2].

Most RP-HPLC separations in laboratory practice are performed with n-octadecyl modified silica substrates. These phases have the advantage of a large stationary phase volume and the possibility of adequate selectivity for the separation of most organic compounds. Until recently, only separations of large organic molecules with molecular weights between 1000-10000 g mol^{-1} were carried out with short alkyl ligand phases, e.g. n-butyldimethylsiloxysilane modified silica. With the better understanding of the phenomenon "selectivity" in modern RP-HPLC practice [3-7] 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 [1,8-12]. With the introduction of commercially available expert systems in HPLC [13] 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^2g^{-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. i-propyl) or multidentate surface attachments, with varying numbers of chemical bonds with the surface, are needed [2,14].

The alkyl chain length and alkyl surface concentration are two critical parameters which determine the hydrophobicity of a packing material and thence retention and selectivity characteristics [8,9]. However, the same two parameters also affect the useful life-time 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 [2,14]. 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 [2,15]. 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 functionalities, chain lengths and surface concentrations on the process of hydrolysis has been partially reported previously [2,14-18]. 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 chapter, 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 ageing 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 ageing of these phases under simulated routine use conditions. The silica substrate used for modification possessed a very large specific surface area (S_{BET} = 579 m^2g^{-1}). Modification of this silica substrate was performed such that an approximately equal ligand density was obtained for all seven phases. After subjecting the modified phases to artificial ageing, changes were investigated in detail by chromatography, by analysis of various bulk properties, by elemental analysis and by high resolution solid state ^{29}Si NMR. In this way, the influence of the n-alkyl chain length on the stability and the resistance towards hydrolysis of the stationary phases under study could be determined.

5.1.2. EXPERIMENTAL

Materials

The test components used for chromatographic characterization were all reference grade. n-Alkylbenzenes (test mixture 1) were benzene, methylbenzene, ethylbenzene, n-propyl-benzene and n-butylbenzene (Pierce Chem. Corp., Rockford, IL, U.S.A.). 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-hydroxy-benzoate, ethyl-p-hydroxybenzoate, n-propyl-p-hydroxybenzoate and n-butyl-p-hydroxybenzoate (Sigma Chem. Comp., St. Louis, MO, U.S.A.). All other solvents and chemicals were analytical reagent grade (E. Merck, Darmstadt, F.R.G.). The deionized water exhibited a specific electric resistance > 10 Mohm cm^{-1} (Milli-Q system, Millipore Corp., Bedford, U.S.A.). 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 m^2g^{-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 imidazole to accelerate the kinetics of the silanization reaction and to gain a higher ligand surface concentration [19]. The modification with monochlorooctadecyldimethylsilane was catalyzed with 2,6-lutidine [19,20] resulting in a somewhat smaller ligand surface

concentration. The seven n-alkyldimethylsilane-modified bonded phases were prepared at the R&D chromatography department of E. Merck, Darmstadt, F.R.G., according to the optimized procedures reported before [19]. 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 impossible to control variations in column efficiency. Variations up to 50% were measured between identical columns. Given the objectives of the present study, the test procedures were focused on uniform capacity factors and selectivity of all columns packed with identical reversed-phases. The test criterion was a deviation of $\pm$ 10%, close to the analytical reproducibility.

TABLE 5. I

Eluent compositions for experiments simulating routine use. Each column was purged by 7000 column volumes of a typical eluent; flow rate 0.5 ml/min.; purge time, 240 h; ambient temperature.

Ageing exper. no.	Buffer	pH	Volume fraction of methanol in the ageing eluent	Ion-pairing agent (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 Bicarbonate	8.4	0	-
5	0.05 M Bicarbonate	8.4	0.5	-
6	0.05 M Bicarbonate	8.4	0.5	Triethylamine

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 in chapter 4. 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

ageing experiments. The basic and acidic aqueous and aqueous-methanol buffers used as eluents for simulated routine use experiments are listed in Table 5.I.

Before and after the ageing experiments, the columns were subjected to a chromatographic characterization, derived from retention models proposed earlier by Jandera [4,5,21]. The ageing experiment with eluent 4 was found too destructive so that a subsequent chromatographic characterization was not meaningful. 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 series at suitable eluent compositions, a set of characteristic parameters can be derived, see chapter 4 (eqns. 4.2 - 4.5b) [5,21]:

$$\log k' = a_0 + a_1 * n_c - x (m_0 + m_1 * n_c) \qquad (5.1)$$

further: $p = {}^{m_1}/_{a_1}$ and $q = m_0 - p * a_0$ (5.2 a,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 in chapter 4. 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 eqns. 5.1 and 5.2b resp., characterize contributions to the selectivity [5]: m_0 denotes mainly non-specific, lipophilic selectivity and q represents specific, polar selectivity, as discussed extensively in chapter 4.

The chromatographic characterization experiments of the stationary phases

were performed with a model 100A pump (Beckmann Instruments, Berkeley, CA, U.S.A.), a model CV-6-VHPa-N60 injection valve equipped with a 20 μl loop (Valco, Houston, TX, U.S.A.) 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, U.S.A.).

Solid state ^{29}Si NMR measurements

The solid-state ^{29}Si 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. ^{29}Si 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. ^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR spectra of all modified stationary phases prior to and after ageing experiments were obtained with a cross-polarization contact-time of 6 ms. The pulse interval time was 1s. 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 prior to zero-filling and Fourier transformation was 15 Hz. The spectral width for all spectra was 15 kHz.

^{29}Si CP-MAS NMR experiments with variable contacts of trimethylsiloxy-silane silica (Si-C_1) before and after several ageing experiments showed consistent CP-characteristics. This pertains to all siliceous moieties at the silica surface. Different results were found for different signals in the ^{29}Si NMR spectrum. Thence, quantification of siliceous moieties at the surface with ^{29}Si CP-MAS NMR can be performed with a single contact-time but with specific correction factors for different ^{29}Si NMR absorptions [22].

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 under study here were submitted for measurements before the simulated ageing experiments, results are listed in Table 5.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. Secondary, 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 ageing 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 ageing experiment were obtained with a Perkin Elmer Model 240 Analyzer (Perkin-Elmer, Norwalk, CT, U.S.A.). 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 usually within the 5% range. The carbon content, P_C, and the ligand surface concentration, α_l calibrated according to eqn. 4.1,

of the starting materials are listed in Table 5.II also.

TABLE 5. II

Bulk properties prior to ageing of the bare substrate and the n-alkyldimethyl-siloxysilane modified phases studied here.
Mean particle size, d_p = 5 ± 1.3 μm.

Stationary phase	Specific surface area S_{BET} (m^2g^{-1})	Mean pore size (nm)	Pore volume (cm^3g^{-1})	Carbon content P_c %(w/w)	Ligand surf. density, α_l, see eqn.4.1 ($\mu mol.m^{-2}$)
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

5.1.3. 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 homologous residues involved were substituted benzenes (test mixture 1), phenyl ketones (test mixture 2) and p-hydroxybenzoates (test mixture 3). The influence of the molecular structures of these residues on the selectivity of a separation can be illustrated 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 also on the stationary phases used for the separation. The dependence of the lipophilic selectivity of these three test mixtures as a

function of the n-alkyl chain length of the ligands of the stationary phases studied here, is depicted in Figure 5.1.

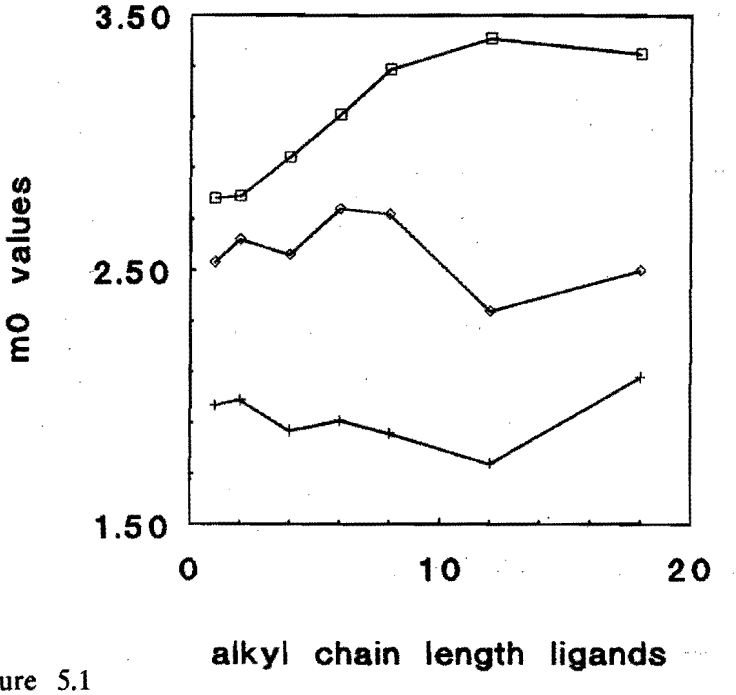


Figure 5.1

Chromatographically determined m_0 values (lipophilic selectivity) *versus* the ligand alkyl chain length of the reversed-phases under study, before ageing experiments. Test mixtures: , n-alkylbenzenes, +, aryl n-alkyl ketones, , p-hydroxybenzoic acid n-alkyl esters. The lines drawn exhibit only an illustrative value.

As expected, larger m_0 values were determined for the n-alkylbenzene test mixture, showing large lipophilic interactions during the elution. Thence, changes in lipophilic selectivities of the modified stationary phases caused by the ageing experiments can be monitored best by the n-alkylbenzene test mixture. Except for the Si-C_{18} phase the value of m_0(alkylbenzene) increased with longer n-alkyl ligands at the surface of the silica substrate.

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 were found almost independent of the ligand chain length.

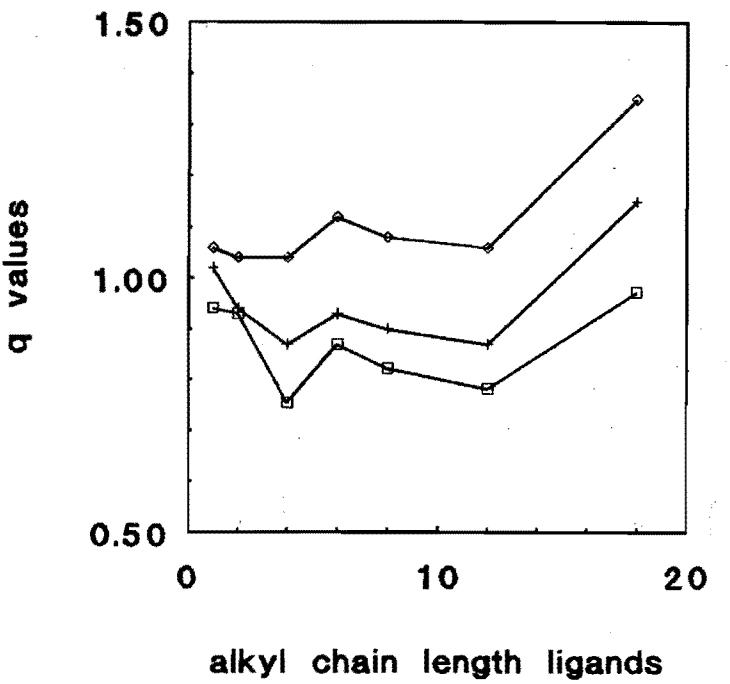


Figure 5.2

Chromatographically determined q values (polar selectivity) *versus* the ligand alkyl chain length of the reversed-phases under study, before ageing experiments. Test mixtures: , n-alkylbenzenes, +, aryl n-alkyl ketones, , p-hydroxybenzoic acid n-alkyl esters. The lines drawn exhibit only an illustrative value.

The influence of the ligand alkyl chain length on the polar selectivity q, for the three test mixtures is shown in Figure 5.2. Changes in this type of selectivity can be determined best by the p-hydroxybenzoic acid n-alkyl esters. With the exception of the C_{18}-reversed-phase, which showed a smaller ligand density, the values for q(p-hydroxybenzoate) were found more

or less constant. Due to almost equal ligand concentrations at the silica surface (except for the octadecyl bonded phase) the polar interactions between the p-hydroxybenzoate residue and the remaining silanol groups at the surface were equal. The values of q for the other two test mixtures were smaller, but showed an identical course as the q(p-hydroxybenzoate) values. However, the p-hydroxybenzoic acid n-alkyl ester test mixture is to be preferred for characterization of polar interactions in this study. The n-alkyl aryl ketone test mixture, used as indication for polar selectivity before [2,18], will be skipped in future research.

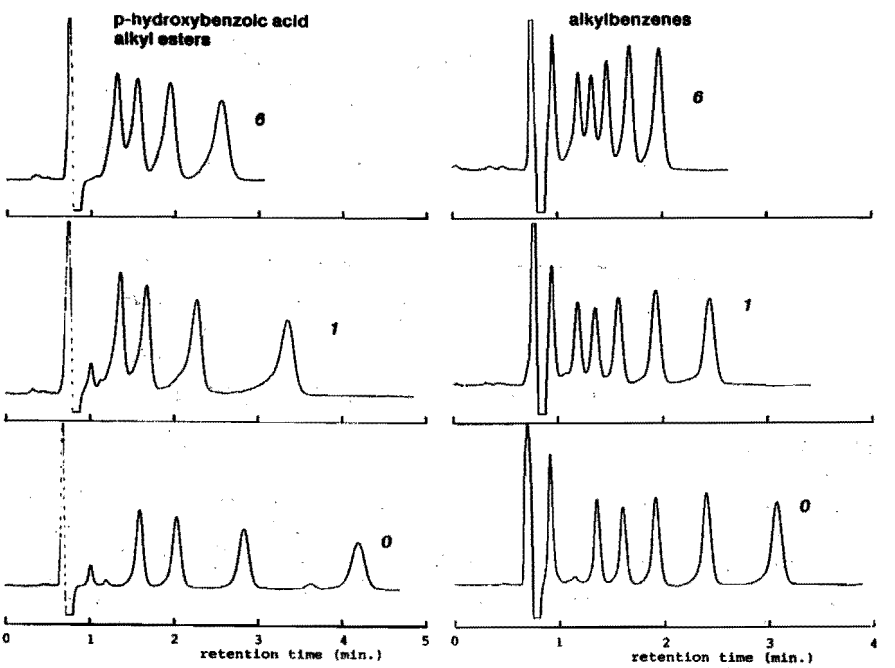


Figure 5.3

Chromatograms of the p-hydroxybenzoic acid n-alkyl ester (left) and the n-alkylbenzene (right) test mixtures eluted on n-butyldimethylsiloxysilane modified phase before (0) and after ageing experiments 1 and 6. Chromatographic test conditions: methanol-water (80:20, v/v), UV detection for test mixture 1: 261 nm, for test mixture 3 : 257 nm.
The effect of long term exposure to aggressive eluents on the performance

of a separation is best illustrated by chromatography. Figure 5.3 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 ageing experiments 1 and 6. From the set of chromatograms collected of each combination of stationary 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, see Figure 5.4. The values of m_0(n-alkylbenzenes) were determined by multiple linear regression from these typical log k' diagrams, see eqn. 5.1, before and after each ageing experiment. These m_0 values for all seven stationary phases under study here are listed in Table 5.III.

TABLE 5. III

Lipophilic selectivity, the m_0(n-alkylbenzene) value determined by chromatographic characterization for the reversed-phases under study, before and after each ageing experiment. The experimental conditions of the ageing experiments are outlined in Table 5.I.

Ageing exper.	n-alkyldimethylsiloxysilane phases						
no.	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

As in earlier studies [2,18] a good correlation was found between the lipophilic selectivity m_0 and a_0(n-alkyl-benzenes), the latter representing the logarithm of the capacity factor of the homologous residues extrapolated for pure water as a test eluent. In short, a_0(n-alkylbenzenes) represents the overall capacity of the stationary phase which is strongly related to the

amount of bonded ligands coverage at the surface.

For the short ligand phases big changes in lipophilic selectivity were determined, especially with the high pH used in ageing experiments 5 and 6.

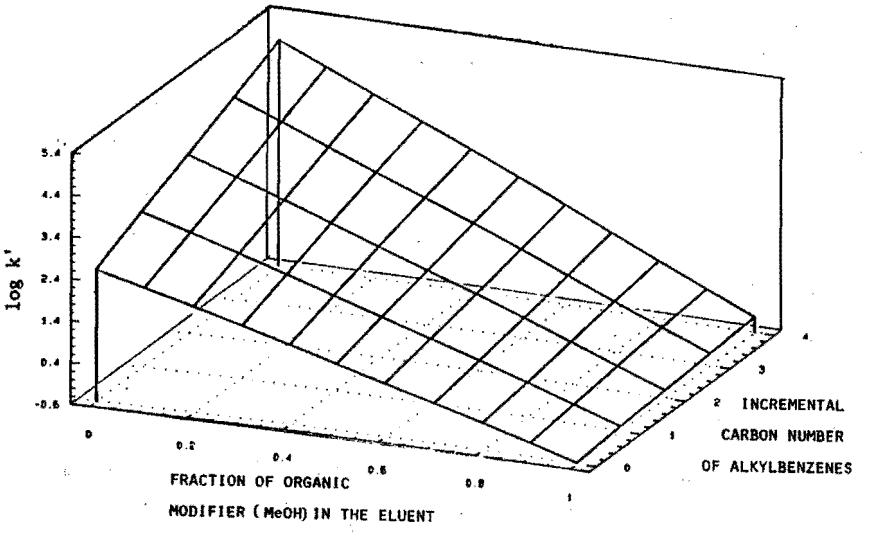


Figure 5.4

The elution surface of the n-alkylbenzene test mixture on n-hexyldimethylsiloxysilane modified silica (Si-C_6) after ageing experiment 2, graphically represented by log k'. The capacity factors are measured for an organic modifier content, x = 0.5 - 0.9 and extrapolated for x values between 0 and 1.

Chromatographic characterization of the Si-C_1 phase after these ageing 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 by the more aggressive ageing 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 ageing experiments performed in this study. In earlier studies, we noticed also a great influence of the ligand surface concentration on the stability of modified phases [2,15]. In this study, the influence of this particular parameter was not a topic of special concern. All n-alkyl-dimethylsilane phases (except the octadecyl bonded phase) were synthesized with almost equal ligand densities, see Table 5.II. The values of q(p-hydroxybenzoate) presented in Table 5.IV support the conclusion that a large 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-hydroxybenzoate) values increased. This indicates larger polar interactions between the p-hydroxybenzoate residue and the silica surfaces. The increased polar interactions could be explained by an increasing amount of silanol groups formed at the surface by hydrolysis of ligands and of silica.

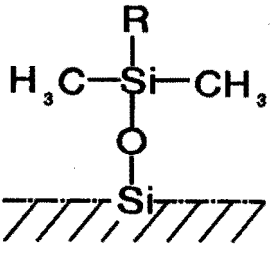
TABLE 5. IV

Polar selectivity, the q(p-hydroxybenzoate) values determined by chromatographic characterization for the reversed-phases under study, before and after each ageing experiment. The experimental conditions of the ageing experiments are outlined in Table 5.I.

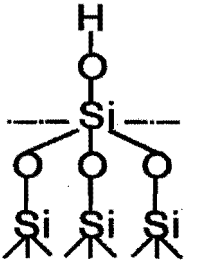
Ageing exper. no.	n-alkyldimethylsiloxysilane phases						
	Si-C_1	Si-C_2 [a]	Si-C_4	Si-C_6	Si-C_8	Si-C_{12}	Si-C_1
0	1.06	1.04 (0.94)	1.04	1.12	1.08	1.09	1.35
1	--	(1.23)	1.05	1.04	1.15	1.11	1.32
2	1.11	(0.90)	0.98	1.01	1.02	1.09	1.24
3	1.19	(0.95)	1.34	1.13	1.23	1.24	1.36
5	--	(1.52)	1.36	1.39	1.66	1.24	1.41
6	--	(1.48)	1.38	1.28	1.79	1.51	1.39

[a] For the Si-C_2 phase the q(p-hydroxybenzoate) values were not determined after the ageing experiments. Instead, the values of q(phenyl ketone) are listed between brackets.

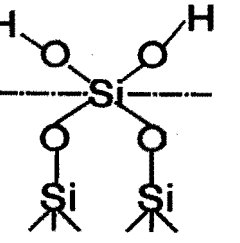
Ageing experiment 4 was too destructive for subsequent chromatographic testing. The chromatograms showed doubled peaks and bad peak shapes caused by degradation of the packed bed inside the column. Nevertheless, bulk analysis and solid-state NMR measurements on the stationary phases after ageing experiment 4 were performed.



M1:= n-alkyldimethyl-siloxysilane + 12 ppm



Q3:= hydroxytri-siloxysilane - 101 ppm



Q2:= dihydroxydi-siloxysilane - 91 ppm

Q4:= tetrasiloxy-silane -110 ppm

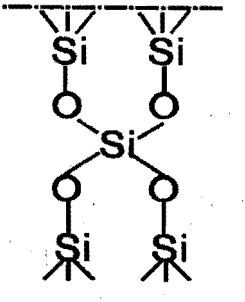


Figure 5.5

Structures of the siliceous surface moieties, their notation, and typical ^{29}Si NMR chemical shifts.

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 are

presented in Figure 5.5. The ^{29}Si MAS NMR spectrum, together with the deconvoluted signal areas of the experimental silica substrate are presented in Figure 5.6. Due to an extremely large specific surface area of the silica gel a total silanol content of 43.9% of all silicon nuclei present was calculated from the ^{29}Si MAS NMR spectrum.

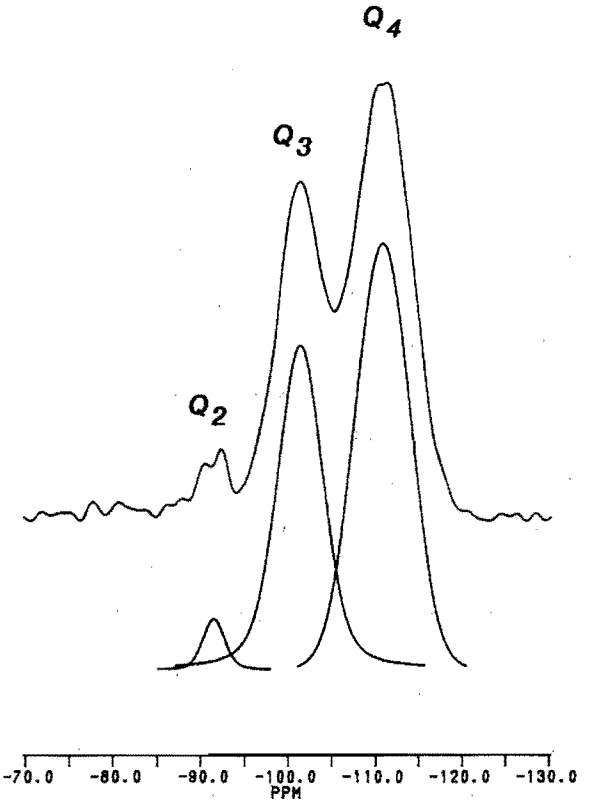


Figure 5.6

^{29}Si pulse excitation MAS NMR spectrum of the native silica substrate with the deconvoluted signals. N_S = 1000; pulse interval time, 90 s.; acquisition time, 10 ms.; line broadening, 10 Hz.

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 * g m^{-2}). This density was smaller than reported for Hypersil and Nucleosil silicas see chapter 4: 0.092 (content * 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. 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 percent were geminal silanols, dihydroxysiloxanes Q_2, and 92.9 percent 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 ageing experiments ^{29}Si CP-MAS NMR spectra were obtained. Figures 5.7, 5.8 and 5.9 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 ageing experiments 1, 4, 5 and 6. Concomitantly, the relative amount of hydroxysiloxanes was increased. And, especially after ageing experiment 1, a pronounced amount of geminal silanol groups was determined. Secondly, we observed, that after the ageing experiments with high pH eluents, not only the trimethylsiloxysilane ligands were stripped from the surface: parallel or subsequently the silica substrate was hydrolyzed 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 non-aged trimethylsiloxy-silane 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 ageing experiments 4 to 6 the amounts of silanol groups did not increase to the same extent as the decrease in trimethylsiloxy-silane groups, indicating that substrate dissolution took place also.

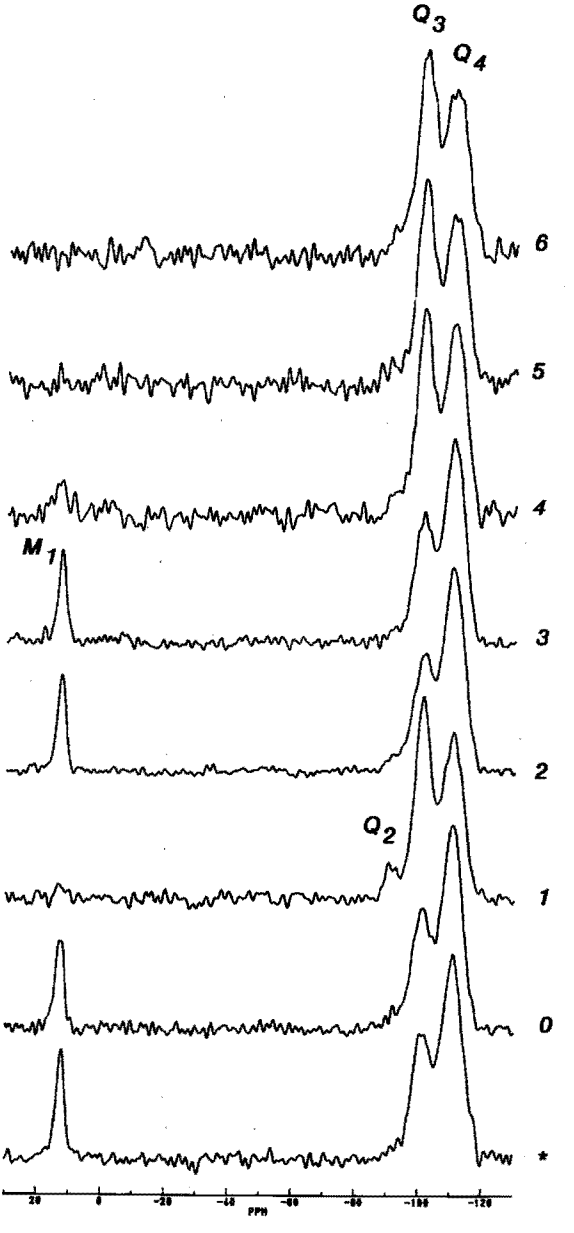


Figure 5.7

^{29}Si CP-MAS NMR spectra of trimethylsiloxysilane modified silica (Si-C_1), before and after the ageing experiments. The numbers indicate the typical ageing experiments as outlined in Table 5.I.

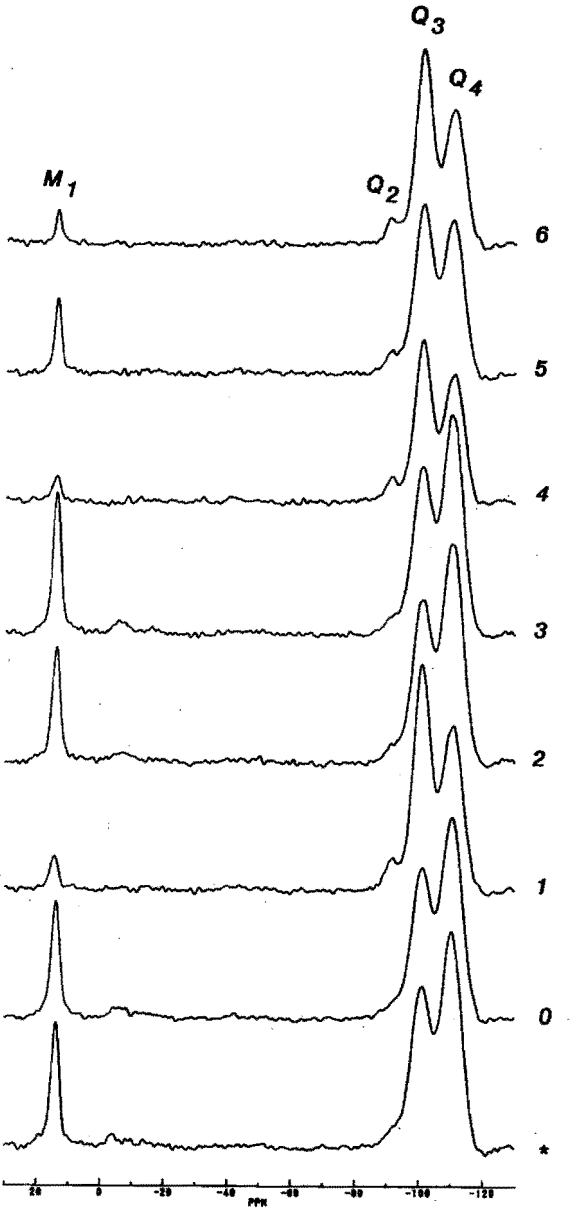


Figure 5.8

^{29}Si CP-MAS NMR spectra of ethyldimethylsiloxysilane modified silica (Si-C_2), before and after the ageing experiments. The numbers indicate the typical ageing experiments as outlined in Table 5.I.

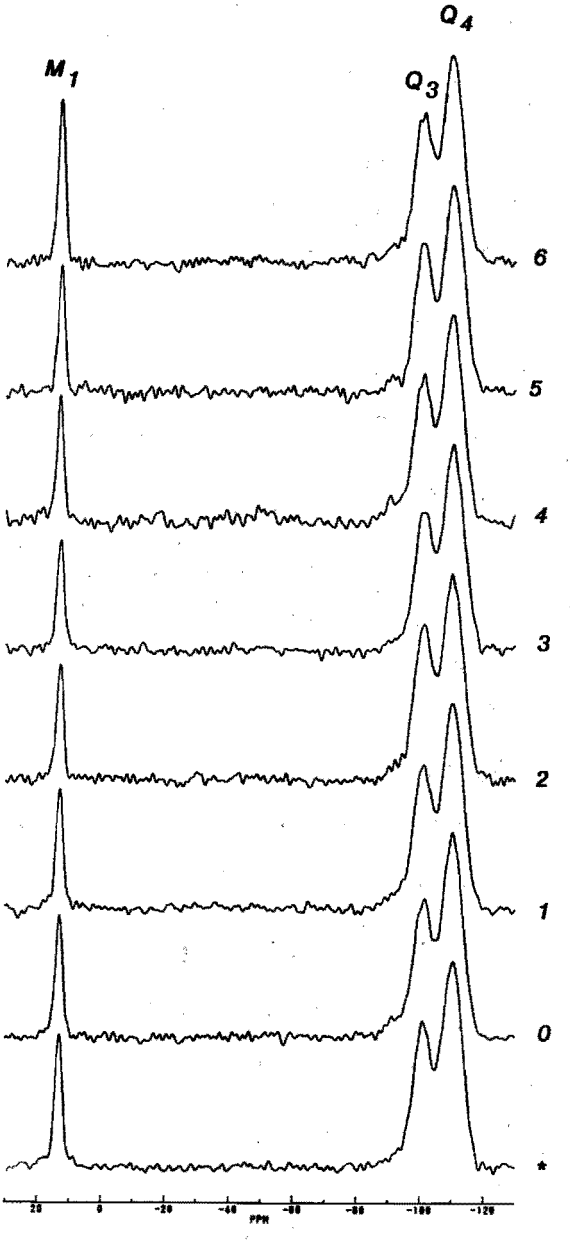


Figure 5.9

^{29}Si CP-MAS NMR spectra of dodecyldimethylsilane modified silica (Si-C_{12}), before and after the ageing experiments. The numbers indicate the typical ageing experiments as outlined in Table 5.I.

The spectra of the other two modified phases, Si-C_2 and Si-C_{12}, showed again that the resistance towards 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 ageing experiments 1, 4 and 6. On the other hand, the amounts of ligands anchored at the surface after ageing experiment 5 were about half that of the non-aged phase.

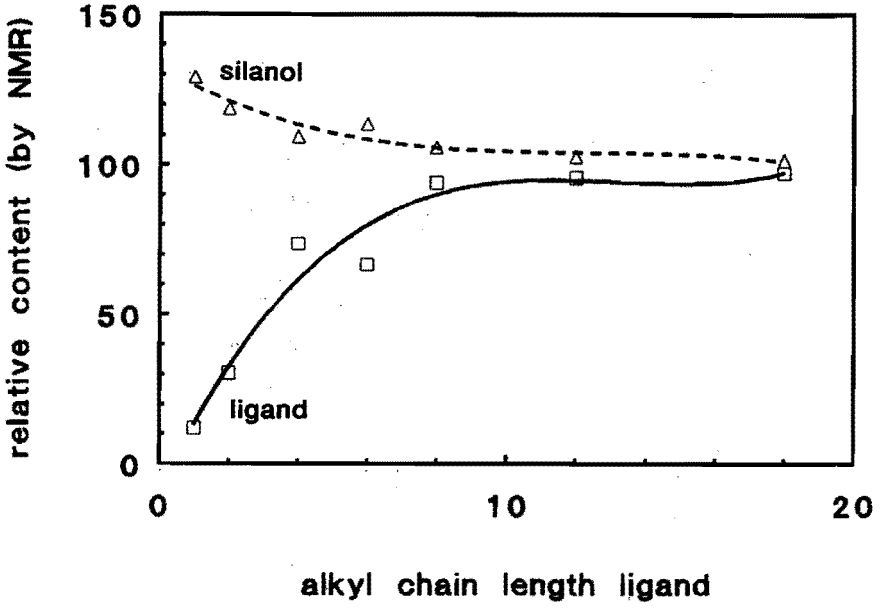


Figure 5.10

Percentage ligand, M_1 (□), and total silanol, Q_2 and Q_3 (Δ), present after ageing experiment 1, relative to the originate reversed-phases. Measured by ^{29}Si CP-MAS NMR.

The shielding effects 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 ageing

experiment, relative to their amounts at the non-aged 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 ageing experiment 1, relative to the non-aged phases, is depicted in Figure 5.10. With short alkyl ligand modified phases large amounts, up to approximately 90%, of alkyldimethyl-siloxysilanes 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 and subsequently dissolved 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, ageing experiment 2, is depicted in Figure 5.11. 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 5.12. Well advanced surface degradation by stripped ligands and dissolved silica was observed. Large amounts of both 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 ageing experiments indicated already a diminishing silica surface, see Figure 5.7. From the previous graphs it is clear that ligand hydrolysis was affected by the type of eluent used for the ageing experiment.

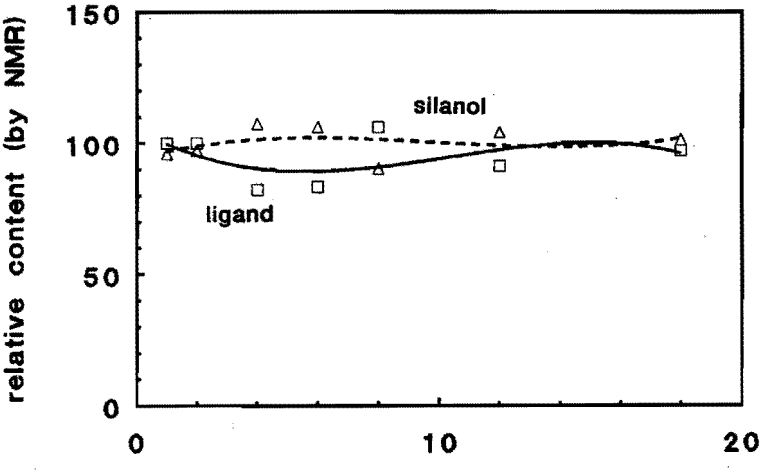


Figure 5.11

Percentage ligand, M_1 (□),and total silanol, Q_2 and Q_3 (Δ), present after ageing experiment 2, relative to the originate reversed-phases. Measured by ^{29}Si CP-MAS NMR.

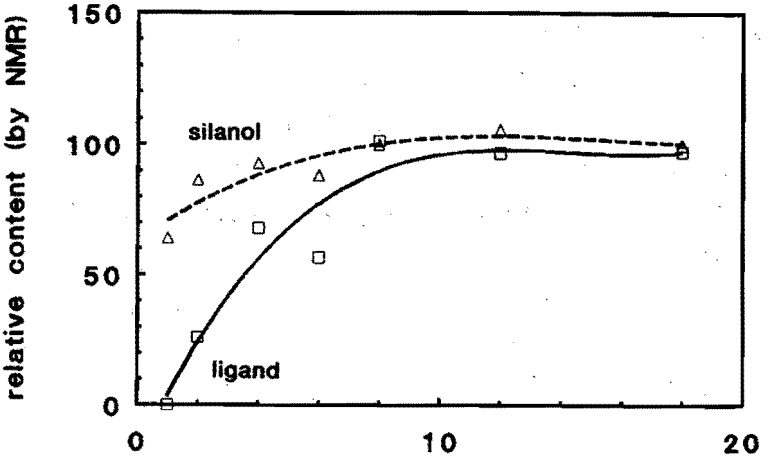


Figure 5.12

Percentage ligand, M_1 (□),and total silanol, Q_2 and Q_3 (Δ), present after ageing experiment 6, relative to the originate reversed-phases. Measured by ^{29}Si CP-MAS NMR.

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

With the high pH eluents a large degree of ligands 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 solvation of the long ligands by the organic modifier.
For low pH buffer eluents 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 contents after three different ageing experiments for all modified phases relative to that of the originate phase are depicted in Figure 5.13. The longer alkyl ligands inhibited dissolution of surface anchored n-alkyldimethyl-siloxysilanes by aggressive eluents. These curves reflect the ligand concentration at the silica surface. As such, the shape of the curves was similar to the relative ligand contents as measured with ^{29}Si CP-MAS NMR depicted in Figure 5.10 to 5.12.
However, in contrast with the results of the solid state ^{29}Si NMR measurements, here we observed substantial changes in the carbon content for the n-octyl bonded phase after ageing experiments 1 and 6. After ageing experiment 6 in total 12 percent 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 ageing experiment are listed in Table 5.V. This table reports two specific area values: measured with S_{BET} and the value corrected for the ligand content. 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 the pore diameter. As expected, increased pore sizes and pore diameters were noticed for short ligand phases after ageing experiments with high pH buffer solutions and low pH aqueous buffer solutions, indicating severe substrate hydrolysis.

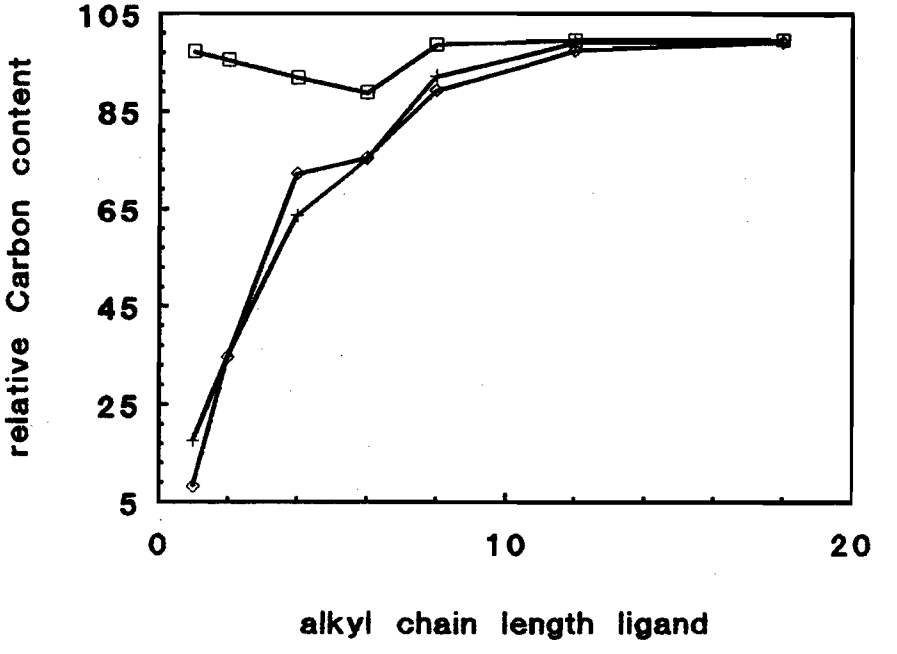


Figure 5.13

The carbon content relative to the originate reversed-phases after ageing experiment 1 (+), 2 (□), and 6 (◇). Measured with elemental analysis. The lines drawn exhibit only an illustrative value.

Changes in pore size and pore volume decreased gradually with larger alkyl chain lengths 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 hydrolysis by aggressive eluents.

TABLE 5. V

Bulk properties prior to and after ageing experiments of the n-alkyldimethylsiloxysilane modified phases studied here. The experimental conditions of the ageing experiments are outlined in Table 5.I.

Stat.phase /ageing exp.no.	Specific surface area S_{BET} (m^2g^{-1})	Mean pore size (nm)	Pore volume (cm^3g^{-1})	Carbon content P_C %(w/w)	Spec.surface area,corrected for ligand content(m^2g^{-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

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, thus enlarging the specific surface area. The short n-alkyl bonded phases showed an enlarged surface area after ageing experiments 5 and 6. Presumably, silica hydrolysis and dissolution resulted in small dissolved silica particles and coarsened pore surfaces.

On the other hand, direct silica dissolution results in a smaller specific surface area. Ageing 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.

5.1.4. CONCLUSIONS

The trimethylsiloxysilane, ethyl-, n-butyl-, and n-hexyldimethylsiloxysilane silica phases show a rather poor resistance towards ligand hydrolysis and dissolution when aggressive low and high pH buffer solutions are used as an eluent. The stabilities of these reversed-phases increase gradually with larger alkyl chain lengths 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 ageing. 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 [2,14-18], 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 solvation 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 larger alkyl chain length modifications are preferable for a longer life time of the RP-HPLC columns. Concurrent hydrolysis and dissolution of silica and ligands 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 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 life time of a typical RP-HPLC column. In the next paragraph 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.

5.2. A MODEL DISSOLUTION STUDY

5.2.1. INTRODUCTION

In the foregoing paragraph (5.1) it is made clear that one of the most important reasons for instability of stationary phases for RP-HPLC is concerned with unwanted chemical interactions between the mobile phase and the stationary phase, see paragraph 5.2. A similar conclusion was drawn in earlier publications from this [2,15] and other [14] laboratories. Long term stability of chemically bonded phases in laboratory practice is limited by hydrolysis and dissolution of ligands and silica substrates, especially when aggressive eluents or conditions are applied [2,14]. 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.
There are, on the other hand, only a few techniques that enable *in situ* studies of such reversed-phase dissolution. ^{29}Si NMR may provide useful detail concerning the molecular structures of species leaving the substrate surface and forming intermediate and final reaction products [23-26]. To the best of our knowledge, these studies have been confined to silicagel 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 paragraph 5.1. The stationary phases under study were modified on the same batch of silica substrate using various ligands of increasing n-alkyl chain lengths. These stationary phases and the native substrate were hydrolyzed and dissolved in an aqueous-methanol or aqueous sodium-hydroxide solution, while monitored with ^{29}Si MAS NMR.

With this study, information on the substantial effect of the ligand alkyl chain length on the various dissolution reactions was obtained. Thence, this paper emphasis on the process of stationary phase deterioration, correlated with the shielding effect of the anchored ligands at the silica surface. At the same time the influence of the eluent composition is considered.
As expected, longer n-alkyl ligands gradually showed better substrate shielding properties. The type of solvent system used mainly affects the proportions of dissolved silicates in the eluent.

5.2.2. EXPERIMENTAL

The synthesis and physical characterization of the RP-HPLC phases with varying n-alkyl chain length are described in paragraph 5.1.

Preparation of solutions

The dissolutions of the reversed-phases or the native silica were performed by adding *ca.* 350 μl of a 6 molar 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 MAS 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 molar aqueous sodium-hydroxide solution in order to obtain an overall, molar composition of 1 Na_2O : 3 SiO_2. The well mixed suspension was introduced into the NMR spectrometer as quickly as possible.

^{29}Si NMR measurements

The solid-state ^{29}Si NMR spectra of the suspension were obtained on a Bruker CXP Fourier transform nuclear magnetic resonance spectrometer at 59.63 MHz. Dissolution studies were performed with ^{29}Si MAS NMR to average any chemical shift anisotropies, which might occur in viscous fluids. The well mixed suspensions were introduced in the zirconium-oxide rotor with an outside diameter of 7 mm. The spin frequency was *ca.* 1.5 kHz. The spectra are collected at ambient temperature and atmospheric pressure. A 45° flip angle ^{29}Si NMR pulse was applied for excitation. Typically, a series of 30 sequential blocks of 360 accumulated FIDs (free induction decays) with 50 ms acquisition time and 5s pulse decay were collected in 1K datapoints each and zero-filled to 8K prior to Fourier transformation. A line broadening of 15 to 25 Hz was applied prior to zero-filling.

5.2.3. RESULTS AND DISCUSSION

The molar Na_2O/SiO_2 ratios applied for dissolution of chemically bonded phases in aqueous-methanol or aqueous solvent systems, were selected for practical reasons. The relatively high sodium content ensured hydrolysis and dissolution of the modified silicas within a reasonable time. With the simulated ageing experiments 1 litre of low pH buffer (pH= 3.0) or high pH buffer (pH= 8.4) was recycled. Thence, possible saturation of the eluent with dissolved ligands and/or silica could occur, reducing the ageing effect of the experiments. In RP-HPLC practice, chromatographers commonly use freshly prepared eluents. We will return to this point later.

The reactions involved in the dissolution of the n-alkyldimethylsiloxysilane bonded silica substrate were observed with ^{29}Si MAS NMR, by sequential blocks of accumulated acquisitions. Each block represents 30 min of reaction time. For example, the dissolution of the n-butyldimethylsiloxysilane phase in a well mixed suspension with an aqueous-methanol sodium-hydroxide solution is depicted in Figure 5.14. The ^{29}Si NMR spectra of the silica solution exhibit several resonances corresponding to structurally different sites of the ^{29}Si nuclei.

In this paragraph a deviating nomenclature code will be used. In the previous chapters a subscript code was used to indicate the topological funtionality of siliceous moieties present in and at silica surfaces. In this paragraph the topological functionality is indicated by a superscript code, according to Engelhardt et al. [24], normal for NMR studies of dissolved silicates. The chemical shifts of resonance peaks in the ^{29}Si NMR spectra of aqueous-methanol or aqueous sodium-hydroxide silicate solutions are presented in Table 5.VI. The differences in resonance frequencies are mainly caused by the local environments of the silicon nuclei. The ^{29}Si NMR spectra yield the proportions of mono- and tetrafunctional silicon atoms present in the solution.

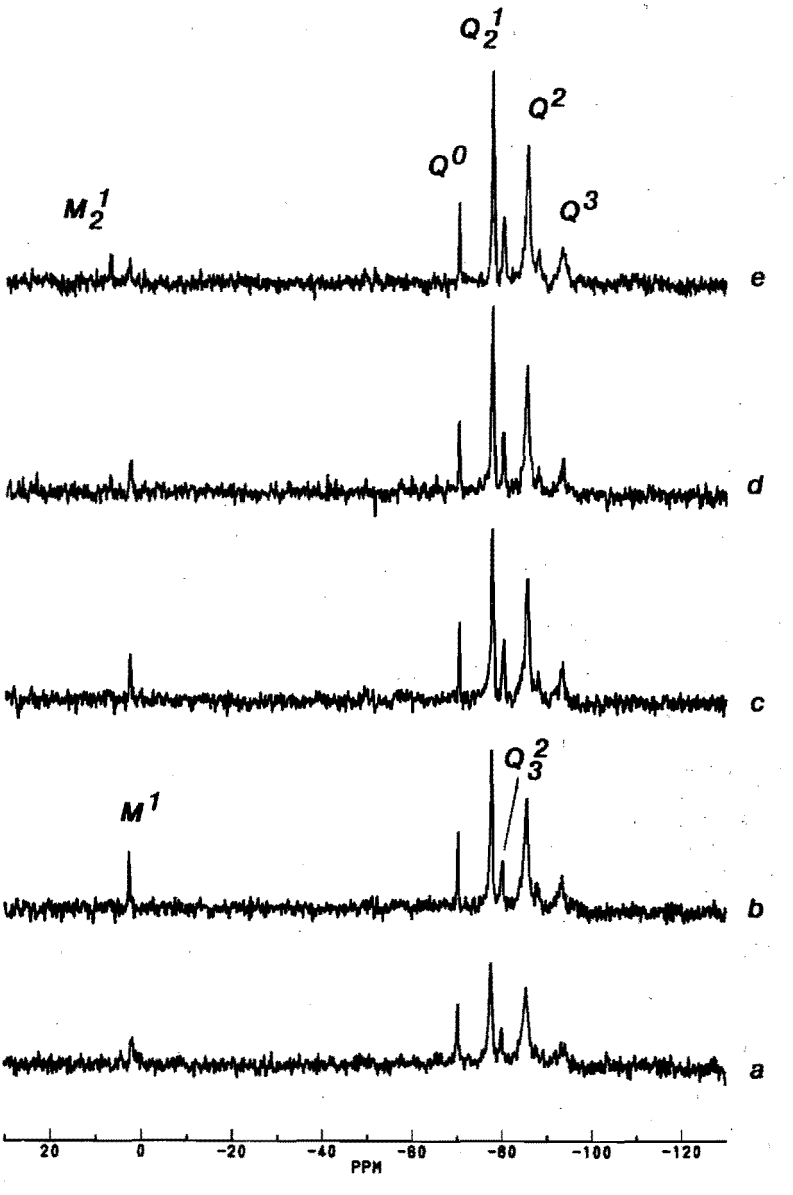


Figure 5.14

Dissolution of Si-C_4 in an aqueous-methanol sodium-hydroxide solution observed with ^{29}Si MAS NMR after (a) 0.5, (b) 1.5, (c) 3, (d) 6 and (e) 15h. Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

The silicon atoms, M^n (n = 0,1) and Q^n (n = 0,1,2,3,4) can be bonded through oxygen to n other silicon atoms. In this way, the structure of the silicon atoms present in the solution can be revealed. A subscript for a particular silicon nucleus refers to the number of identical sites within a single silicate species. For example, Q_2^1 and Q_3^2 represent the ^{29}Si nuclei present in hexahydroxydisiloxane (a linear dimer) and hexahydroxy-cyclotrisiloxane (a cyclic trimer), respectively.

TABLE 5. VI

Most relevant dissolved silane/siloxane species, their functionality, notation and typical ^{29}Si NMR chemical shift in the aqueous-methanol sodium-hydroxide solution and the aqueous sodium-hydroxide solution. If not mentioned, the species are part of small oligomers.

Silane/siloxane species in solution	Chemical (spectral) function.	Topological (network) function.	Code	Typical chemical shift (ppm down field from TMS) H_2O/MeOH	water
n-alkyldimethyl-hydroxysilane	M	0	M^0	+ 16	+ 15
n-alkyldimethylsiloxy-silane, dimer *a*	M	1	M_2^1	+ 7	--
n-alkyldimethyl-siloxysilane	M	1	M^1	+ 3	--
tetrahydroxy-siloxane	Q	0	Q^0	- 70.4	- 71.9
trihydroxysiloxane, dimer *a*	Q	1	Q_2^1	- 77.9	- 79.9
dihydroxysiloxane, cyclic trimer *a*	Q	2	Q_3^2	- 80.3	- 81.6
dihydroxysiloxane	Q	2	Q2	- 85.4	- 88.4
hydroxysiloxane	Q	3	Q^3	- 93.7	- 96.9
tetrasiloxysilane	Q	4	Q^4	--	- 106

a The subscript refers to the number of particular sites within a dissolved silicate species. In the nomenclature these silicates are also referred to as one species: M_2^1, di-n-alkyltetramethyldisiloxane; Q_2^1, hexahydroxydisiloxane; Q_3^2, hexahydroxycyclotrisiloxane.

The ^{29}Si NMR chemical shifts of quaternary siloxanes are in agreement with previous assignments for dissolution of aqueous silica gel systems [23,27]. The M^0 and M_2^1 ^{29}Si NMR chemical shifts were derived from ethoxytrimethylsilane and hexamethyldisiloxane, respectively [27]. The ^{29}Si NMR chemical shift of the oligomeric n-alkyldimethylsiloxysilane M^1 as assigned in the present study should be considered as preliminary. Shielding by chemical bonding to an oligomeric silicate species in solution could be responsible for a shift of approximately 4 ppm to higher field. This ligand attached silicate species was formed by direct dissolution of oligomeric silicates species from the silica gel.

Silica can be dissolved in highly alkaline media (pH > 11), where the hydroxyl anion is considered to be a catalyst for the dissolution reaction [23,28]. Hydroxyl anions increased the coordination number of a surface silica atom to more than 4, thus weakening the oxygen bonds with silicon atoms underneath. As such, silica dissolves in the form of monomeric tetrahydroxysiloxane, $Si(OH)_4$ or Q^0. Presumably, formation of monomeric silicate anions in the solution leads to oligomerisation of the monomers to hexahydroxydisiloxanes Q_2^1, hexahydroxycyclotrisiloxanes Q_3^2, polymeric dihydroxysiloxanes Q^2 and polymeric hydroxysiloxanes Q^3. Only recently, chemical exchange between various dissolved silicates (e.g. between Q^0 monomer and Q_2^1 dimer or Q_3^2 trimer) was demonstrated elegantly by Knight et al. [25].

In the present study, ^{29}Si NMR spectra of the dissolution and subsequent reactions of the n-butyldimethylsiloxysiloxane modified silica substrate (Figure 5.14) revealed a fast dissolution of the reversed-phase material with formation of monomeric tetrahydroxysiloxane followed by oligomerisation to polymeric silicate species. The tetrahydroxysiloxane Q^0 level in the methanol-water solution seemed to be rather low compared with results of other silica dissolution studies [23,29]. Possibly, the subsequent oligomerisation reaction proceeded relatively fast compared with monomer

dissolution under the given conditions. Already after half an hour substantial amounts of various oligomers were determined. The dissolution of the reversed-phase material starts with substrate dissolution to form monomers and subsequently to oligomeric silicate species. An equilibrium was reached after approximately 2h. The ratios of the various dissolved silicates remained almost constant with prolonged measurements. Only the signal areas of the oligomers increased slightly with time.

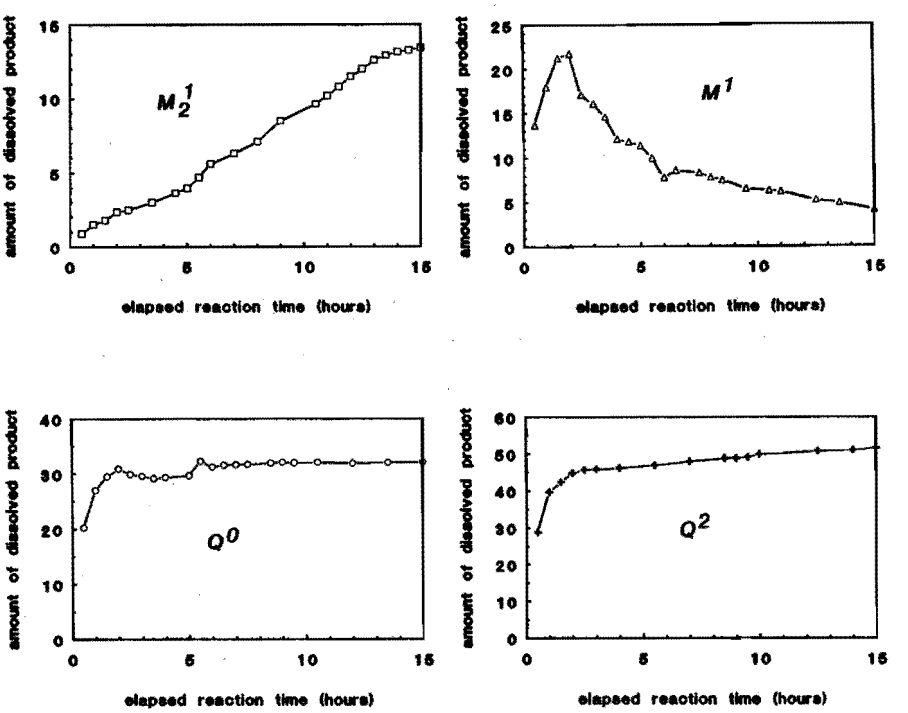


Figure 5.15

Curves of the relative signal intensities observed with ^{29}Si MAS NMR of several dissolved products from Si-C_4 in the aqueous-methanol sodium-hydroxide solution with elapsed reaction time.
Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

After one hour a significant amount of n-butyldimethylsiloxysilane M^1, as part of small oligomer species was detected in the solution. After approximately 2h this started to decrease. It seemed that a secondary product, a di-n-butyltetramethyldisiloxane dimer M_2^1, was formed by continued hydrolysis and condensation. Figure 5.15 depicts curves of the signal intensities of several dissolved products Si-C_4 in the aqueous-methanol solution with elapsed reaction time.

Almost identical spectra as shown were obtained from the dissolution of the trimethylsiloxysilane bonded silica (Si-C_1) in the aqueous-methanol solution under identical conditions, see Figure 5.16.

In this case, however, both dissolved ligand-silicates M^1 and M_2^1, were formed directly from the start. An equilibrium between these two dissolved species was attained after about two hours. Up to n-hexyldimethylsiloxysilane modified silica analogous ligand-silicates were observed upon dissolution. It can be concluded that the apparent detachment of short ligands from the modified silica surface was in fact caused by hydrolysis and dissolution of the underlying substrate silica. Thence, we conclude that with advanced monomer dissolution small oligomeric silicate species were concurrently dissolved with monomeric silicates in our aqueous-methanol solvent system. This concurrent dissolution of oligomeric silicates could be influenced by the open structure of the silica gel studied here. The rather thin pore walls could enhance dissolution of small silicate pieces.

The ^{29}Si NMR spectra following the dissolution of the n-octadecyldimethylsiloxysilane bonded phase in the aqueous-methanol solution revealed a pattern rather similar pattern to that for n-butyldimethylsiloxysilane bonded phase dissolution. Monomeric silicate dissolved and subsequently oligomerization progressed similar to the n-butyl reversed-phase, see Figure 5.17.

However, the dissolution rate of tetrahydroxysiloxane monomer was clearly decreased by the longer octadecyl ligands attached to the silica surface.

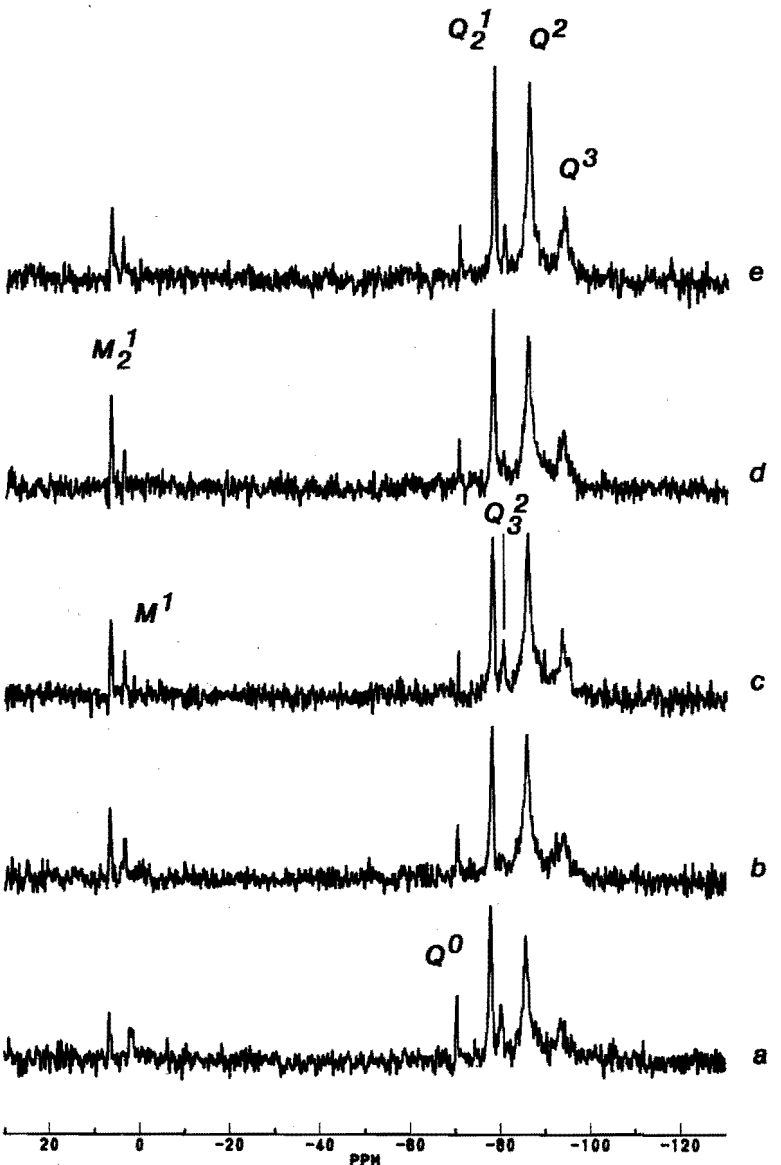


Figure 5.16

Dissolution of Si-C_1 in an aqueous-methanol sodium-hydroxide solution observed with ^{29}Si MAS NMR after (a) 0.5, (b) 1.5, (c) 3, (d) 6 and (e) 15h. Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

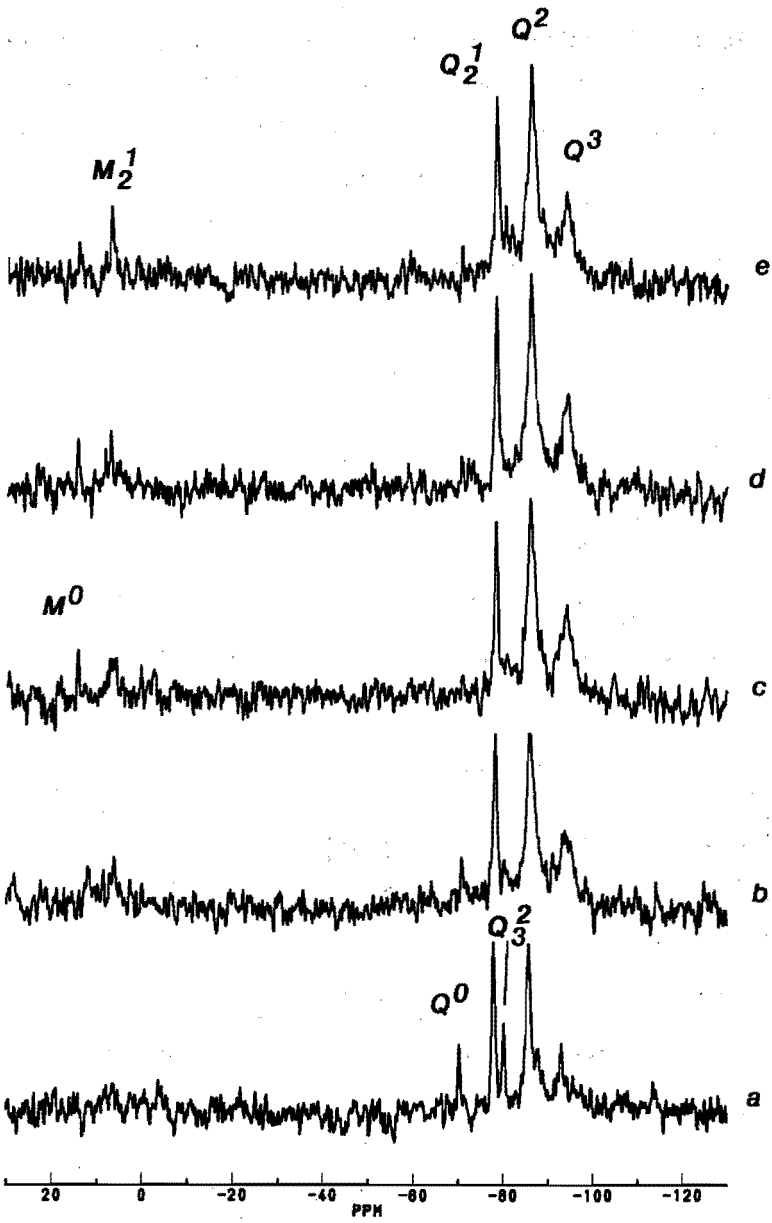


Figure 5.17

Dissolution of Si-C_{18} in an aqueous-methanol sodium-hydroxide solution observed with ^{29}Si MAS NMR (a) 0.5, (b) 1.5, (c) 6, (d) 15 and (e) 30h. Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

Oligomerisation of the dissolved monomeric silicate reduced the amount of monomer in the solution to a very small value. This smaller monomer content subsequently also affected the amounts of dissolved linear dimer and cyclic trimer. Through continued oligomerisation, relatively more polymeric dihydroxysiloxanes Q^2, and hydroxysiloxanes Q^3, were observed after 15h of dissolution of silica substrate modified with the octadecyl ligand.

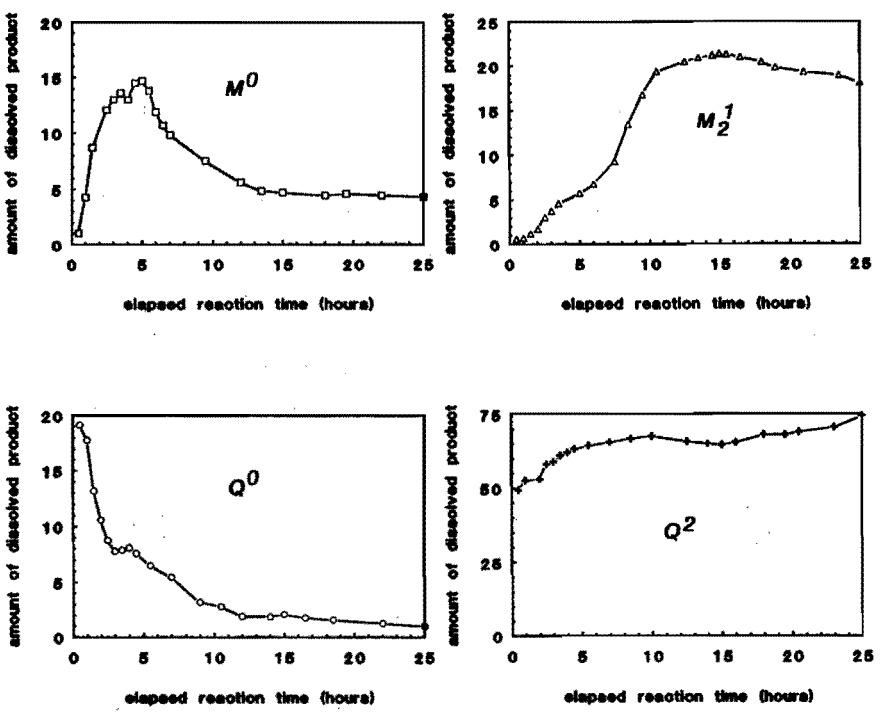


Figure 5.18

Curves of the relative signal intensities observed with ^{29}Si MAS NMR of several dissolved products from the Si-C_{18} phase in the aqueous-methanol sodium-hydroxide solution with elapsed reaction time.
Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

Here again, an equilibrium for the dissolved silicates was attained after approximately 2h. The ^{29}Si NMR resonances of the dissolved ligand

revealed smaller and broader signals. In fair agreement with all other evidence produced in this study, the amount of dissolved ligand-silicates was relatively small for longer ligands. Furthermore, the removal of longer ligands from the silica surface occurred mainly by direct hydrolysis to form dimethyloctadecylhydroxysilanes, M^0.

Subsequent condensation with dissolved monomeric silicates resulted in di-octadecyltetramethyldisiloxane dimers, M_2^1. Similar ligand silicate dimer was also observed after prolonged hydrolysis of short n-alkyl ligand modified phases. Figure 5.18 depicts curves of the signal intensities of several dissolved products Si-C_{18} in the aqueous-methanol solution with elapsed reaction time. Presumably, steric hindrance and boundaries caused by Van der Waals interactions between neighbouring ligands inhibited direct dissolution of small ligand-silicate oligomeric species from the silica surface.

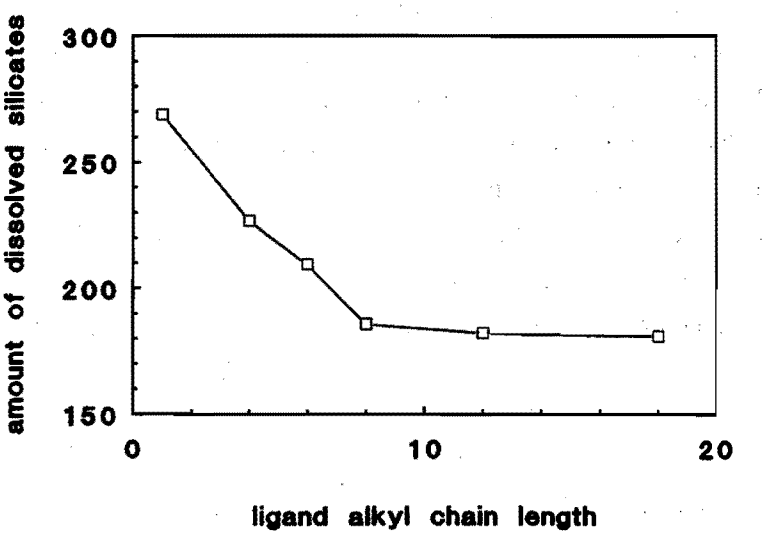


Figure 5.19

Total amount of dissolved silicates determined by ^{29}Si MAS NMR after 15 h reaction time for the n-alkyl modified phases in an aqueous-methanol sodium-hydroxide solution. Molar ratio 1 Na_2O : 1 SiO_2, methanol content 50% (v/v).

It seemed that dissolution of oligomeric silicate species was reduced likewise. Analogous dissolution behaviour was observed for the Si-C_8 and Si-C_{12} phases. The total amount of dissolved silicate species determined after Si-C_1, Si-C_4 and Si-C_6 dissolution in the aqueous-methanol sodium-hydroxide solution was significantly higher than after Si-C_8, Si-C_{12} and Si-C_{18} dissolution with a corresponding amount of stationary phase under identical conditions, see Figure 5.19. The n-octyldimethylsiloxysilane phase obviously possessed a kind of critical ligand chain length for the modified silicas studied here. Shorter ligand modified phases showed concurrent oligomer dissolution (partly influenced by the silica gel structure), whereas longer ligand modified phases mainly dissolved to form monomeric silicates.

The model dissolution studies were performed as a batch process in limited volume rotors (350 μl) with rather high pH solvents (pH > 12). The relatively high sodium content ensured hydrolysis and dissolution of the modified silicas within a reasonable time. In this small volume under the experimental conditions, saturation of the solvent may well affect the further dissolution of the reversed-phase materials. A comparison of dissolution and stationary phase hydrolysis in daily chromatography practice is, therefore, subject to certain restrictions.

The oligomerisation reactions observed in the sodium-hydroxide solution may be promoted as a consequence of a high concentration of the primary dissolution products. Probably, when freshly prepared eluents are used, only hydrolysis and dissolution of ligands and silica without subsequent oligomerisation will be the main causes of stationary phase degradation. Thence, the primary dissolution reactions observed with ^{29}Si NMR spectroscopy here, reflect the main course of the dissolution process that occurs under RP-HPLC conditions in practice. Factors affecting this process should be considered for stationary phase hydrolysis and dissolution.

The ^{29}Si NMR spectra showing the dissolution characteristics of the native silica in the aqueous-methanol sodium-hydroxide solution are depicted in Figure 5.20. The equilibrium was attained rapidly. The proportions of

dissolved silicates agreed with those of the short alkyl ligand modified phases, only the amount of dissolved monomer silicates observed was much less.

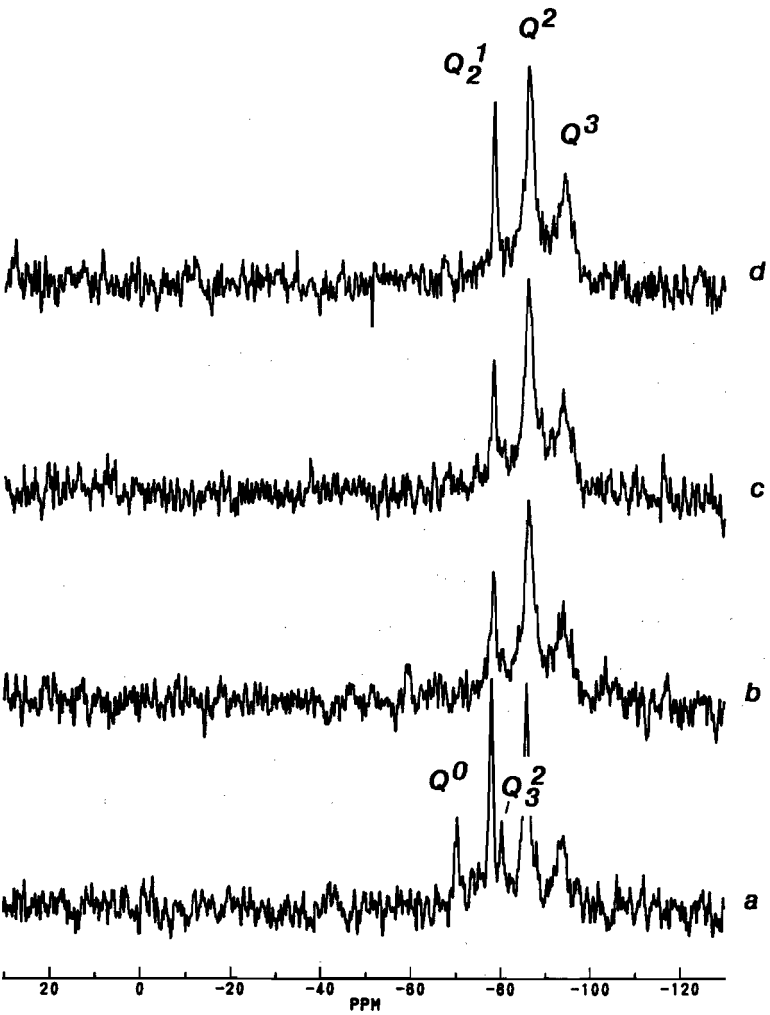


Figure 5.20

Dissolution of native silica gel in an aqueous-methanol sodium-hydroxide solution observed with ^{29}Si MAS NMR (a) 0.5, (b) 1.5, (c) 3 and (d) 15h. Molar ratio 1 Na_2O : 1 SiO_2 , methanol content 50% (v/v).

Major concurrent dissolution of small oligomeric silicate pieces probably affected the monomer content by chemical exchange with already dissolved monomeric silicates [25].

Dissolution studies of other n-octadecyl modified silicas revealed that the proportions of dissolved silicates depended primarily on the hydrolysis and dissolution rate of tetrahydroxysiloxanes, Q^0. Furthermore, the proportions of the oligomeric silicates in particular were affected by the type of solvent used for dissolution studies as is the case with silica substrates [23,29].

As an example of this sensitivity towards solvents, the ^{29}Si NMR spectra of the dissolution of the n-butyldimethylsiloxy-silane modified silica in a plain aqueous sodium-hydroxide solution with an molar composition of 1 Na_2O : 3 SiO_2 are depicted in Figure 5.21.

Spectra almost equal to those of the previous examples were collected from the dissolution of Si-C_1 and Si-C_{12} under these conditions. Due to a smaller sodium-hydroxide content the hydrolysis and dissolution of monomeric silicate started more slowly.

The smaller oligomerisation rate at the start of the experiment allowed a higher content of tetrahydroxy-siloxanes to be formed in the aqueous solution. However, with progressing oligomerisation the amounts of monomer, linear dimer and cyclic trimer decreased in favour of the large oligomeric dihydroxysiloxane, hydroxysiloxane and even tetrasiloxysilane silicates. After 15h of dissolution mainly broad Q^2, Q^3 and Q^4 signals were observed. Substantial amounts of n-alkyldimethylhydroxy-siloxysilanes were only observed with the dissolution in the aqueous buffer solution (pH > 12) of modified silicas. Condensation of this monomer to dimeric silicate was not detected. Presumably, oligomerisation with large silicate oligomers results in less mobile ligands, that are hardly detectable. Figure 5.22 depicts the curves of the signal intensities of several dissolved products from Si-C_4 in the aqueous sodium-hydroxide solution with elapsed reaction time. In the aqueous sodium-hydroxide solution dissolution proceeded mainly by monomeric silicates.

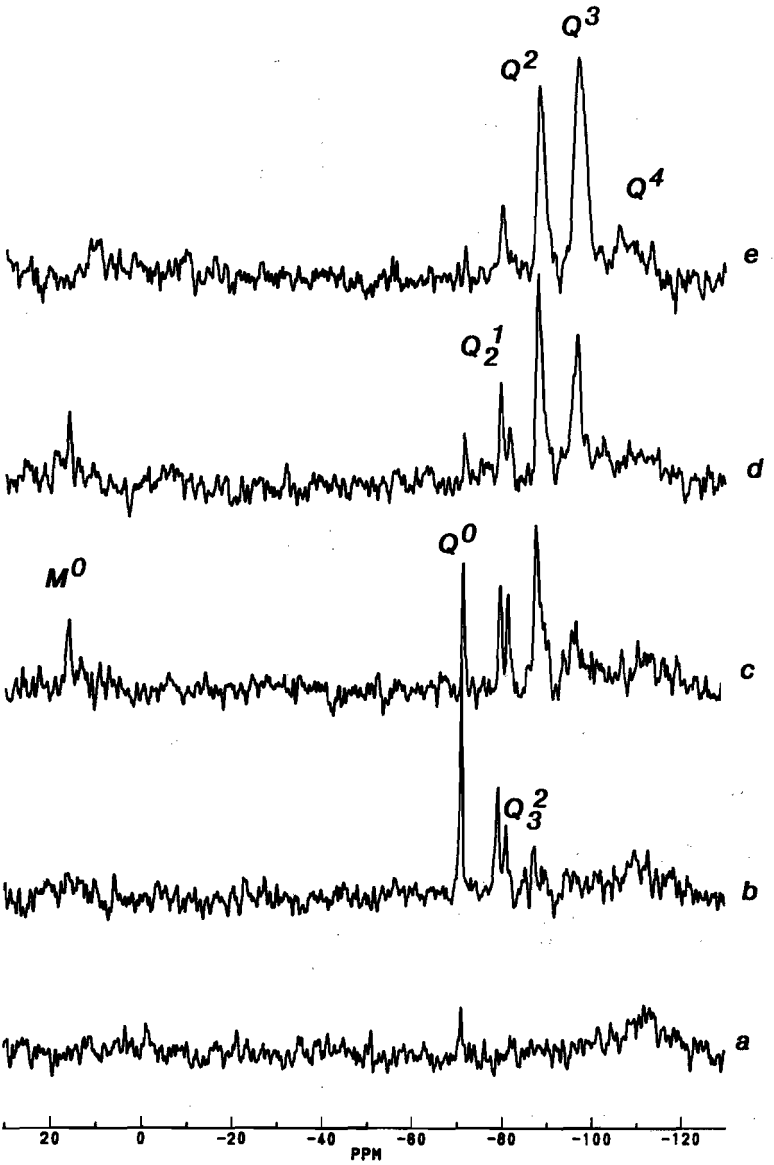


Figure 5.21

Dissolution of Si-C_4 in an aqueous sodium-hydroxide solution observed with ^{29}Si MAS NMR after (a) 0.5, (b) 1.5, (c) 3, (d) 6 and (e) 15h.
Molar ratio 1 Na_2O : 3 SiO_2.

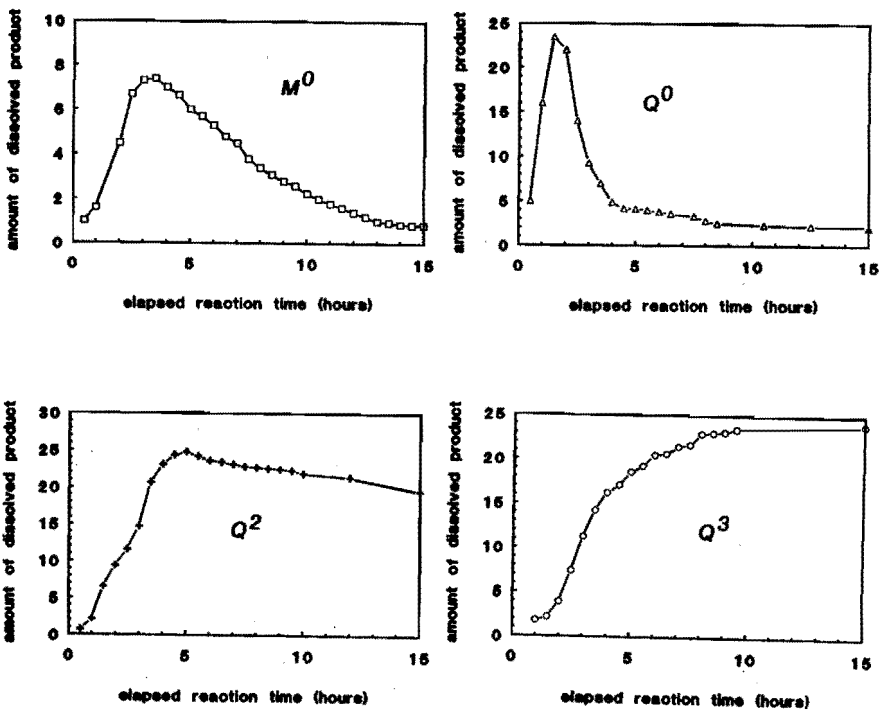


Figure 5.22

Curves of the relative signal intensities observed with ^{29}Si MAS NMR of several relevant dissolved silicates and ligand attached silicates from Si-C_4 in the aqueous sodium-hydroxide solution with elapsed reaction time. Molar ratio 1 Na_2O : 3 SiO_2.

5.2.4. CONCLUSIONS

Two fundamentally different ligand dissolution processes are observed in an aqueous-methanol solution. Short alkyl ligand modified silica gels degrade by concurrent dissolution of monomeric tetrahydroxysiloxane and small oligomeric silicate particles of sub-colloidal level (< 1-3 nm) [23]. The

process of oligomer dissolution enhances the degradation rate of short alkyl modified silicas drastically.

Longer alkyl ligand modified silicas degrade mainly by monomeric dissolution of silica and ligands. Surface attached ligands are hydrolyzed and dissolved as n-alkyldimethylhydroxysilanes. The use of water as a solvent promotes monomeric dissolution, also with short alkyl ligand modified silicas. The n-octyl bonded phase exhibited the critical ligand alkyl chain length upon modification of the silica substrate used in this study, with respect to direct oligomer dissolution in aqueous-methanol solvents.

For short alkyl ligand bonded phases multidentate surface attachment will probably not prevent dissolution of the ligands, due to oligomer dissolution. Polymer coated stationary phases cross-linked across the silica surface may prevent severe dissolution of the short ligands by oligomer dissolution. Specific surface shielding groups (e.g. pre- or end-capping with trimethyl-siloxysilanes) may contribute also to prevent degradation of the stationary phases. On the other hand, longer n-alkyl ligands with large enough surface concentration should be preferred with aggressive eluents.

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CHAPTER 6

NOVEL CONCEPTS IN RP-HPLC STATIONARY PHASE MATERIALS[1,2]

A hydrothermal hydrofluoric acid treatment of the silica substrate prior to modification with a dimethyloctadecylsilane is described. This treatment improves the surface coverage and the stability of the resulting stationary phase compared with that on the original substrate. The effect of this hydrothermal acid treatment on relevant substrate parameters including formation of crystalline regions at the surface is also discussed.

Improved surface shielding properties are often claimed advantages of cross-linked polymer coated stationary phases. Precapping of the substrate with trimethylsilanes prior to polymer coating increases the stability of these stationary phases further. This precapped polymer coated stationary phase reveals an almost consistent separation performance after ageing experiments with high pH buffer solutions. A small shift in selectivity is, however, observed.

6.1. INTRODUCTION

One of the major problems yet to be solved with chemically modified stationary phases for use in reversed-phase liquid chromatography (RP-HPLC) is the deterioration of these phases in the course of routine use

1. M. Hetem, J. de Haan, H. Claessens, Ph. Mussche, C. Cramers, Chromatographia, in press.
2. M. Hetem, J. de Haan, H. Claessens, C. Cramers, A. Deege, G. Schomburg, J. Chromatogr., submitted for publication.

with relatively aggressive eluents. The long term stability of these stationary phases in laboratory practice is limited by hydrolysis of the silica substrate. Currently, some manufacturers apply special hydrothermal treatments to silica substrates, thus giving the surface new physical or/and chemical properties in order to improve their stationary phases. In the first part of this chapter, paragraph 6.3, changes in surface properties brought about by a hydrothermal acidic treatment of the silica substrate before modification are studied in detail.

The two different octadecylsilane RP-HPLC stationary phases discussed were prepared from a single starting silica substrate. Part of this substrate was pretreated with a 0.1% (w/w) hydrofluoric acid solution in order to increase the reactivity of the silanol groups at the surface towards modification with octadecyl silanes. This stationary phase will be referred to as pretreated octadecyl Chromspher silica (PODCS). The second stationary phase was synthesized on the original substrate (ODCS). The hydrothermal acidic treatment altered some important surface properties as witnessed by an increased silanediol/silanol ratio and ligand density after subsequent modification. Furthermore, inhomogeneous areas of small crystalline micro-structures at the surface of the pretreated substrate were observed. In the present case, the pretreatment with hydrofluoric acid resulted in an increased ligand density of about 30 percent.

It may be concluded that the resistance to ligand hydrolysis of the pretreated octadecyl reversed-phase (PODCS), when exposed to ageing, was superior to that of the non-pretreated stationary phase (ODCS).

In the second part of this chapter, paragraph 6.4, two polymer coated silicas are compared with an octadecyl ligand modified silica, modified in the classical way, for their resistance against deterioration. The polymer coated silicas are extensively characterized with chromatography, adsorption measurements, elemental analysis and solid state ^{29}Si NMR.

The three RP materials have been synthesized from one batch of silica

(Nucleosil). One of the RP materials is silanized in the classical way, by silica modification with octadecyl ligands (C_{18}). The second stationary phase is modified by polymer coating and subsequent immobilization by cross-linking and chemical bonding with the silica surface. The polymer used as a coating is an octadecyl incorporating polymethyloctadecylsiloxane [1-4]. This polymer coated silica ($PMSC_{18}$) was not meant to be an advanced stationary phase. It was synthesized in order to enable an estimate of the stability offered by the polymer chain itself. Part of the polymers were chemically anchored at the silica surface by chemical reaction, as will be shown below.

The third "RP-18 phase" has been synthesized by pre-silanization with trimethylsilane-enolate [4] and subsequent coating with the same methyloctadecylsiloxane polymer under conditions identical to the previous phase. This latter type of RP material ($C_1/PMSC_{18}$) is well-known for its low concentration of residual silanol groups and a favourable surface shielding by cross-linked polymer [4]. In essence, this polymer is only physically adsorbed at the presilanized silica surface, as will be shown in paragraph 6.4. The emphasis of this paragraph is a comparison of shielding effects and stabilities between classically modified ligands and siloxane polymer coated directly on porous silica and on hydrophobic precapped silica.

6.2. EXPERIMENTAL

Materials

The test components used for chromatographic characterization were all of reference grade. n-Alkylbenzenes (test mixture 1), n-alkyl aryl ketones (test mixture 2) and p-hydroxybenzoic acid n-alkyl ester homologous series,(test mixture 3) are used for determination of the chromatographic lipophilic and

polar selectivity of the reversed-phases, discussed in detail in chapters 4 and 5. A fourth test mixture containing components with various selectivities was used for comparison with previous papers [1-4]: acetophenone, benzophenone, benzoic acid benzoyl ester, ethylbenzene, 2-n-octylpyridine, n-butylbenzene and n-hexylbenzene all of reference quality. Test mixtures 1 and 2 are used for characterization of the ODCS and PODCS phases in paragraph 6.3 and the test mixtures 1, 3 and 4 are used for characterization of the modified silicas in paragraph 6.4. All organic solvents and chemicals used for simulating routine experiments and chromatographic characterization were of analytical grade (E. Merck, Darmstadt, F.R.G.). Deionized water was freshly prepared (Milli-Q system, Millipore Corp., Bedford, U.S.A.). The eluents used for HPLC were filtered over 0.22 μm membrane filters (Millipore).

Simulating routine use experiments

The equipment and procedure for simulated intensive routine use are described in detail in chapter 4. However, the pulse damping system was modified with a pulse damper (Waters, Millipore), a 3 m * 0.1 mm I.D. stainless steel capillary and a 0.2 μm membrane filter in-line between the pump head and the column. This was done in order to ensure a more constant flow through the columns and to avoid pump seal material at the top of the column that could effect the ageing experiments.
The basic and acidic aqueous and methanol/aqueous buffers used for ageing are listed in Table 6.I. In this study the eluent used in experiment 4 was changed, the aqueous sodium bicarbonate buffer (pH = 8.4) was replaced by an aqueous sodium phosphate buffer (pH = 7.5). The plain aqueous high pH sodium bicarbonate buffer was found too destructive for the stationary phases studied. Meaningful chromatographic characterization could not be performed after this specific ageing experiment.

TABLE 6. I

Eluent compositions for experiments simulating routine use.
Each column purged by 7000 column volumes of a typical eluent; flow-rate, 0.5 ml/min; purge time, 240 h; ambient temperature.

Ageing exper. no.	Buffer	pH	Volume fraction of methanol in the ageing eluent	Ion pairing agent concentration 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 Phosphate [a]	7.5	0	-
5	0.05 M Bicarbonate	8.4	0.5	-
6	0.05 M Bicarbonate	8.4	0.5	Triethylamine

[a] This buffer solution was used with the polymer coated phases in paragraph 6.4. In paragraph 6.3 still the 0.005 M aqueous bicarbonate buffer with pH = 8.4 was used with ageing experiment 4.

After ageing the columns were subjected to a chromatographic characterization, as discussed extensively in paragraph 4.2, with the homologue test mixtures n-alkylbenzenes, n-alkyl aryl ketones or p-hydroxybenzoic acid n-alkyl esters at suitable eluent compositions.

Chromatography

The chromatographic characterization is performed with a model 100 A pump (Beckman Instruments, Berkeley, CA, U.S.A.), a model CV-6-VHPa-N60 injection valve equipped with a 20 μl loop (Valco, Houston, TX, U.S.A.) and a model LC-3 variable wavelength UV-detector (Pye Unicam, Cambridge, UK) operated at 254 nm. Typically injections of 5-10 μl of the test mixtures are performed. The detector signal is sampled at 10 Hz and integrated with a Nelson 3000 data system (Nelson Analytical, Cupertino, CA, U.S.A.).

Solid state ^{29}Si NMR measurements

The solid-state ^{29}Si NMR spectra were obtained on a Bruker CXP 300 Fourier transform nuclear magnetic resonance spectrometer at 59.63 MHz. Representative samples of 190-230 mg were spun at *ca.* 3.5 kHz using 7 mm O.D. aluminum or zirconium oxide rotors of the Bruker double-bearing type. ^{29}Si Bloch decay magic angle spinning (MAS) NMR spectra of the three native silicas and the polymer coated silicas were obtained with $\pi/2$-pulses and a pulse interval time of 90 s. ^{29}Si MAS NMR spectra were also collected of the $C_1/PMSC_{18}$ phases after the ageing experiments. If interval times are at least 5 times the T_1 relaxation time, the signal areas measured with this technique adequately 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 are accumulated for the Chromspher substrates (chapter 6.3); 620 FIDs with an acquisition time of 10 ms for the Nucleosil substrate and the octadecyl silanized silica (C_{18}) and 100 ms for the polymer coated samples ($PMSC_{18}$ and $C_1/PMSC_{18}$) were accumulated in 1K resp. 2K datapoints (chapter 6.4). Prior to Fourier transformation the data files were zero-filled to 8K and line broadenings of 10 Hz or 1 Hz were applied for 10 ms and 100 ms acquisition time, respectively.

^{1}H-^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR spectra of all modified stationary phases prior to and after simulated routine treatments were obtained with a cross-polarization contact-time of 6 ms. For the CP-MAS NMR spectra of both Chromspher silica substrates a contact-time of 2 ms was applied. A spin-temperature-alternated CP sequence with quadrature cycling and flip-back of the ^{1}H nuclei was applied to eliminate experimental artifacts. The pulse interval was 1 s. Typically 2000 FIDs (free induction decays) with an acquisition time of 10 ms were

accumulated in 1K datapoints, zero filled to 8K prior to Fourier transformation. Line broadening used is 20 Hz prior to zero-filling and Fourier transformation. The spectral width for all spectra was 20 KHz.

Experiments with variable contacts measuring the characteristic CP-behaviour were obtained for both Chromspher silica substrates and the ODCS and PODCS phases, before and after ageing experiments. Series of nineteen contacts ranging from 0.1 to 40 ms were applied. To eliminate time dependent artifacts block averaging was used. Typically 16 * 64 FIDs were accumulated for each contact. The other spectral parameters were identical to the single contact ^{29}Si CP-MAS NMR measurements of these stationary phases (see above).

^{29}Si CP-MAS NMR experiments with variable contacts, between 0.1 ms and 50 ms, of the C_{18}/A, the C_1/$PMSC_{18}$ and the $PMSC_{18}$ phases before and after a specific ageing experiment showed almost equal CP-characteristics for the C_{18}/A and the C_1/$PMSC_{18}$ phases. This pertains to all siliceous moieties at the silica surface. Different results were found for different signals in the ^{29}Si NMR spectrum. Thence, quantification of siliceous moieties at the surface with ^{29}Si CP-MAS NMR can be performed with a single contact-time but with specific correction factors for different ^{29}Si NMR absorptions [5]. The CP-behaviours of the $PMSC_{18}$ phases before and after ageing experiment no. 5 were different, in particular for the polymeric moieties at the surface, see chapter 6.4.

Elemental analysis

The carbon content of all modified silicas prior to and after simulated routine use was obtained with a Perkin Elmer Analyzer, model 240 (Perkin Elmer Corp., Norwalk, CT, U.S.A.).

6.3. ACID PRETREATMENT OF SILICA SUBSTRATE

6.3.1. GENERAL

The two octadecyl stationary phases subject of this study were produced by modification of the same substrate, an experimental Chromspher silica (Chrompack, Middelburg, The Netherlands). Part of the silica that had been hydrated according to standard procedures was dehydrated at 450°C.

TABLE 6. II

Bulk properties of both silica substrates previous to silane modification; A is untreated Chromspher silica, B is Chromspher silica after the hydrothermal acidic treatment.

	silica A	silica B
Mean particle size (μm)	5 $\pm$ 1.5	5 $\pm$ 1.8
Mean pore size (nm)	7	7.5
S_{BET}, specific area (m^2/g)	152	141
Pore volume (cm^3/g)	.51	.50
Ion contents (ppm) [a]		
- Sodium :	4200	1500
- Aluminum :	400	300
- Iron :	190	130
- Nickel :	20	50
Relative silanol ratio [b]		
- vicinal/"lone" :	1.0	0.88
- geminal :	--	0.12

a determined with atomic absorption spectrometry (AAS), calcium and copper content below 5 ppm.

b determined with solid state ^{29}Si MAS NMR.

Subsequent hydrothermal treatment with a 0.1% (w/w) solution of hydrofluoric acid in deionized water at 95°C for 2 h, increased the amount of more reactive, geminal silanol groups, Q_2 groups, at the silica surface and drastically decreased the amount of ionic impurities present in the substrate,

see Table 6.II. The two substrates, untreated and pretreated, were modified with dimethylamine-dimethyloctadecylsilane (DMA-DMODS) [6,7] under identical experimental conditions. An excess of the mono-reactive silane was dissolved in n-pentane and added to the silica substrate under an atmosphere of nitrogen.

TABLE 6. III

Bulk properties of the octadecylsilane modified RP-HPLC stationary phases under study; ODCS synthesized on silica A; PODCS synthesized on silica B.

	ODCS	PODCS
Mean particle size (μm)	5 $\pm$ 1.5	5 $\pm$ 1.8
Mean pore size (nm)	6	6
S_{BET}, specific area (m^2/g)	93	87
Pore volume (cm^3/g)	.285	.286
Ion contents (ppm) *a*		
- Sodium :	1500	200
- Aluminum :	200	100
- Iron :	120	110
- Nickel :	50	100
Carbon content P_c (%, w/w) *b*	10.06	11.59
Ligand surface density α_1 (see eqn.4.1; μmol.m^{-2})	3.16	4.03

a determined with AAS, calcium and copper content below 5 ppm.
b determined with elemental analysis.

The n-pentane was removed by evacuation and, subsequently, the reaction mixture was refluxed at 220℃ under nitrogen atmosphere for 18 h. The modified silicas were then filtered and thoroughly washed succesively with n-pentane, dry acetone and methanol. The bulk properties of the modified stationary phases are listed in Table 6.III.

From each stationary phase seven identical Swagelock columns, 100 mm *

4.6 mm. I.D. (Crawford Fitting Company, Solon, Ohio, U.S.A.) were packed according to standard packing procedures. After a chromatographic test to ensure reproducibility of the packing procedure, six columns were placed in an apparatus for simulated routine use (described in chapter 4), while the remaining column was used as a reference column for initial chromatographic characterization.

6.3.2. RESULTS AND DISCUSSION

Solid state ^{29}Si NMR

The solid state ^{29}Si CP-MAS NMR spectra of both Chromspher substrates and their modified C_{18} reversed-phases before and after simulated routine use are shown in Figure 6.1 and 6.2. The structure of siliceous moieties most relevant to this paper are depicted in chapter 4 (see Figure 4.3). Their ^{29}Si NMR chemical shifts are listed in Table 4.IV. From the spectra the relative surface concentration of all siliceous moieties can be calibrated. The results of spectroscopic measurements and elemental analysis are summarized in Tables 6.IV and 6.V for ODCS and PODCS, respectively.
Although the results from neither the elemental analysis nor the solid state ^{29}Si CP-MAS NMR showed a perfect match, the results for both methods before and after the ageing experiments show a fair agreement. It is clear that the density of ligands at the surface upon modification was increased due to the preceding hydrothermal hydrofluoric acidic treatment. The origin for this phenomenon is the formation of more reactive geminal silanol groups (Q_2, 12 percent more) by this pretreatment. These geminal silanols are formed when siloxane bridges at the surface of the silica substrate are extensively hydrolyzed.

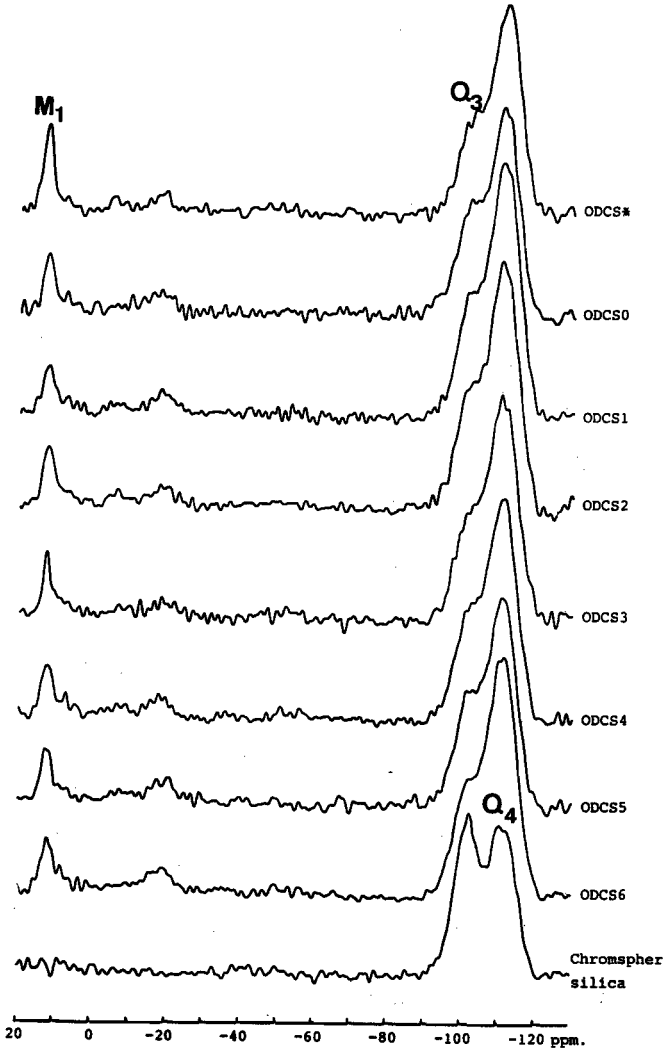

Figure 6.1

^{29}Si CP-MAS NMR spectra of the Chromspher silica substrate and ODCS phases before and after each treatment. N_S = 2000; contact-time, 6 ms; puls interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz. The roman numbers indicate the typical ageing experiments as outlined in Table 6.I.

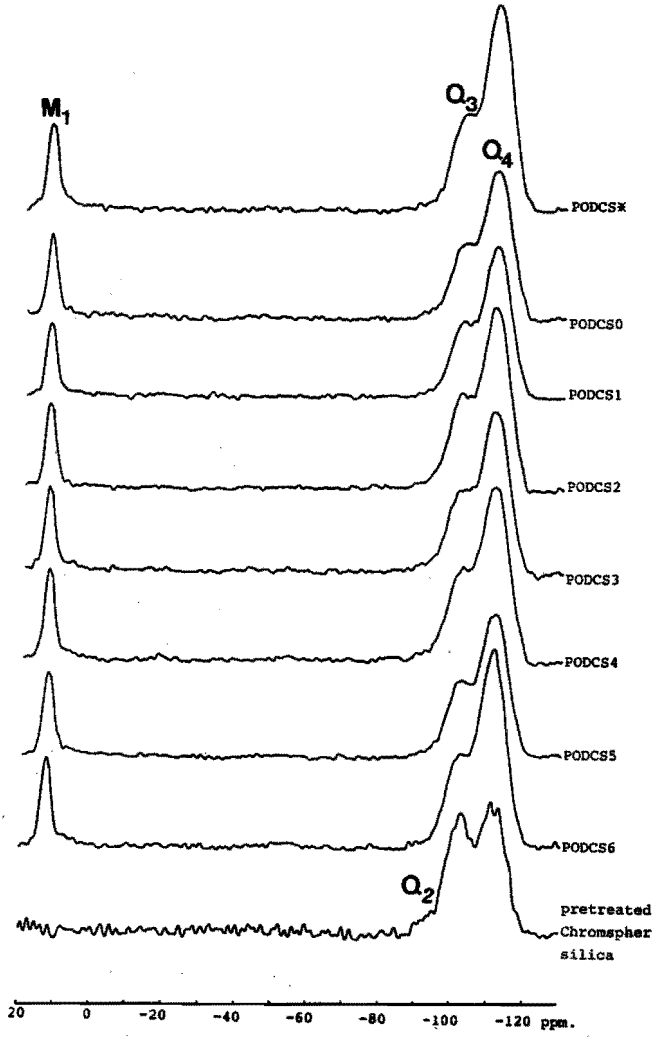

Figure 6.2

^{29}Si CP-MAS NMR spectra of the pretreated Chromspher silica substrate and PODCS phases before and after each treatment. N_S = 2000; contact-time, 6 ms; puls interval time, 1s; acquisition time, 10 ms; line broadening, 20 Hz. The roman numbers indicate the typical ageing experiments as outlined in Table 6.I.

TABLE 6. IV

Results of elemental analysis and solid state ^{29}Si CP-MAS NMR experiments of the **ODCS** stationary phase before and after simulating routine use experiments. The number indicates the typical ageing experiment as given in Table 6.I. The ODCS* and ODSC0 phase denote the original stationary phases before and after packing/chromatographic characterization, respectively.

Stationary phase/ ageing exp.	Carbon content P_C (%,w/w)	Ligand density α_l (eqn.4.1) (μmol.m^{-2})	NMR relative ratios	
			M_1	Q_3
ODCS*	10.06	3.16	0.44	0.56
ODCS0	9.07	2.77	0.37	0.63
ODCS1	8.74	2.69	0.23	0.77
ODCS2	8.79	2.71	0.32	0.68
ODCS3	8.84	2.73	0.33	0.67
ODCS4	8.65	2.66	0.25	0.75
ODCS5	8.46	2.60	0.31	0.69
ODCS6	8.51	2.62	0.33	0.67
R.D.(eqn.4.7):	3.67 %	20.3 %		

TABLE 6. V

Results of elemental analysis and solid state ^{29}Si CP-MAS NMR experiments of the **PODCS** stationary phase before and after each ageing experiment. Experimental conditions as outlined in Table 6.I.

Stationary phase/ ageing exp.	Carbon content P_C (%,w/w)	Ligand density αl(eqn 4.1) (μmol.m^{-2})	NMR relative ratios	
			M_1	Q_3
PODCS*	11.59	4.03	0.59	0.41
PODCS0	11.35	3.93	0.57	0.43
PODCS1	11.08	3.81	0.51	0.49
PODCS2	10.96	3.77	0.50	0.50
PODCS3	10.95	3.77	0.52	0.48
PODCS4	11.30	3.91	0.56	0.44
PODCS5	11.17	3.85	0.53	0.47
PODCS6	11.27	3.89	0.49	0.51
R.D.(eqn.4.7):	2.46 %	9.10 %		

As studied by Lork [8] hydrothermal acidic treatments of "soft" silica gels may very well introduce small hexagonal crystalline domains, most likely ß-tridymite, at the surface. In particular, a high level of ionic impurities, as in the present case, will enhance the formation and stability of the ß-tridymite domains. Feher et al. [9] reported the formation of ß-tridymite-like structures from heptameric trisilanol structures. The leaching of silicas with a relatively high impurity level could also lead to heptameric hexasilanol and consequently to ß-tridymite crystalline microstructures, at the surface. This phenomenon is also consistent with the CP-behaviour [11] of the ^{29}Si nuclei, as depicted in Figure 6.3 and Figure 6.4 for the substrates and the monofunctionally modified reversed-phases, respectively.

The initial rise on the left side of each plot is due to the growth of ^{29}Si magnetization due to cross-polarization by protons with a characteristic time T_{HSi}, and the subsequent decline is caused by relaxation of spin-locked protons with a spin-lattice relaxation time in the rotating frame, $T_{1\rho H}$. Both magnetization rise and decline of the "lone" and vicinal silanol groups, Q_3 and substrate siloxanes, Q_4 CP-curves, at -102 ppm. and -110.5 ppm. resp., of the Chromspher silica show an approximate single exponential behaviour [10,11]. The same is true for the dimethyloctadecylsiloxysilane, M_1 moiety (at +12 ppm.), Q_3 and Q_4 CP curves of the monofunctionally octadecyl-silane modified Chromspher silica.

The CP-behaviour of hydrothermal acid treated Chromspher silica and the derived octadecyl stationary phase is completely different. Here, shoulders or double maxima were determined, indicating surface areas with different cross-polarization behaviour. There coexist at least two different regions at the surface of the silica: amorphous silica, with relatively large T_{HSi} and $T_{1\rho H}$ values, and crystalline micro-structures, ß-tridymite like, with small T_{HSi} and $T_{1\rho H}$ values. This hypothesis is in agreement with the considerable wash out of strange ions in the silica substrate (mainly sodium ions) by the hydrothermal acidic treatment.

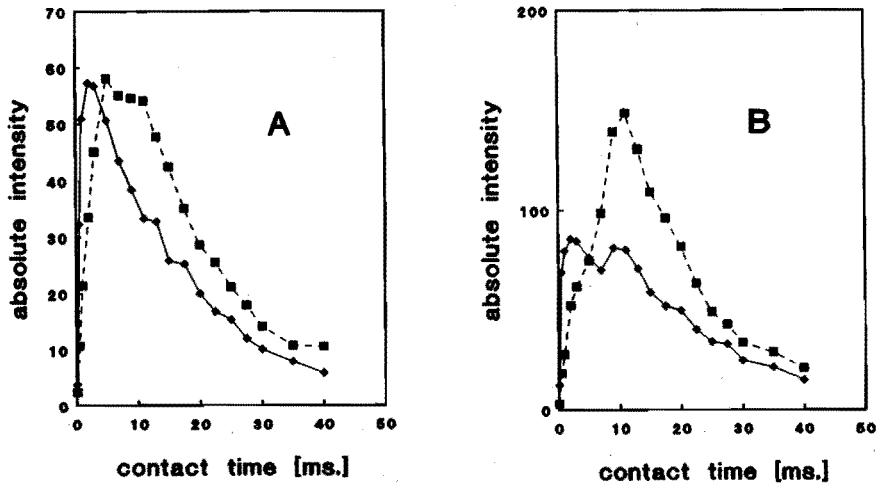

Figure 6.3

Characteristic CP-behaviour of ^{29}Si atoms in Chromspher silica [A] and hydrothermally acidic treated Chromspher silica [B]. ◆,Q_3 at -102 ppm; ■,Q_4 at -110 ppm.

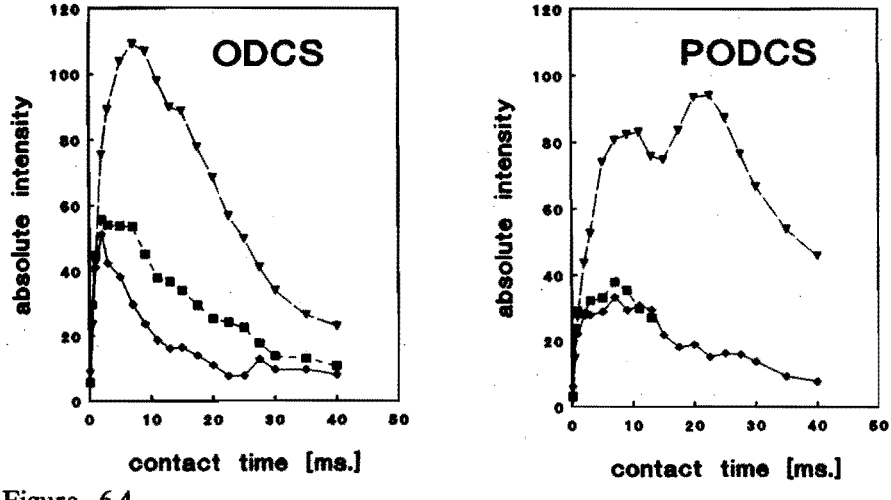

Figure 6.4

Characteristic CP-behaviour of ^{29}Si atoms in ODCS and PODCS stationary phases. ◆,M_1 at +12 ppm; ■ ,Q_3 at -102 ppm; ▼ ,Q_4 at -110 ppm.

Both elemental analysis and solid state ^{29}Si CP-MAS NMR measurements of the two stationary phases ODCS and PODCS showed a loss of ligands after exposure to the ageing eluents due to stationary phase stripping. However, the ODCS phase already lost a considerable amount of n-alkyl chains after the packing procedure and subsequent chromatographic characterization (before packing: ODCS* and after characterization: ODCS0). This drop in organic content is probably caused by the wash out of unreacted physisorbed silane ligands at the surface after modification with an excess of these silanes. Therefore, the concentration of ligands depicted for both phases after chromatographic characterization, treatment 0, should be taken as a reference for further comparison.
The relative deviation (R.D.) of the reported values, see Tables 6.IV and 6.V, were calibrated by comparing the values determined before and after ageing treatments relative to the value before, as calibrated by eqn. 4.7 in chapter 4. The R.D. values for both ligand density and relative M_1 ratio were higher for the ODCS phase than for the PODCS phase. The PODCS phase exhibited a better resistance towards hydrolysis of the chemically bonded ligands, especially for eluents with high pH values: ageing experiments 4 to 6.

The R.D. values calculated were smaller for elemental analysis than for ^{29}Si CP-MAS NMR. This is mainly caused by fundamental differences between the two methods. The determination of the amount of carbon relative to the amount of silica will be influenced by concurrent hydrolysis of the silica substrate. Surface analysis with ^{29}Si CP-MAS NMR includes also the analysis of the increasing amount of silanol groups after hydrolysis of ligands and substrate siloxane bonds. The large amount of silanol, mostly Q_3 sites, of course influenced the ratio of M_1 to Q_3 given in Tables 6.IV. and 6.V. In this case the R.D. value will be relatively large.

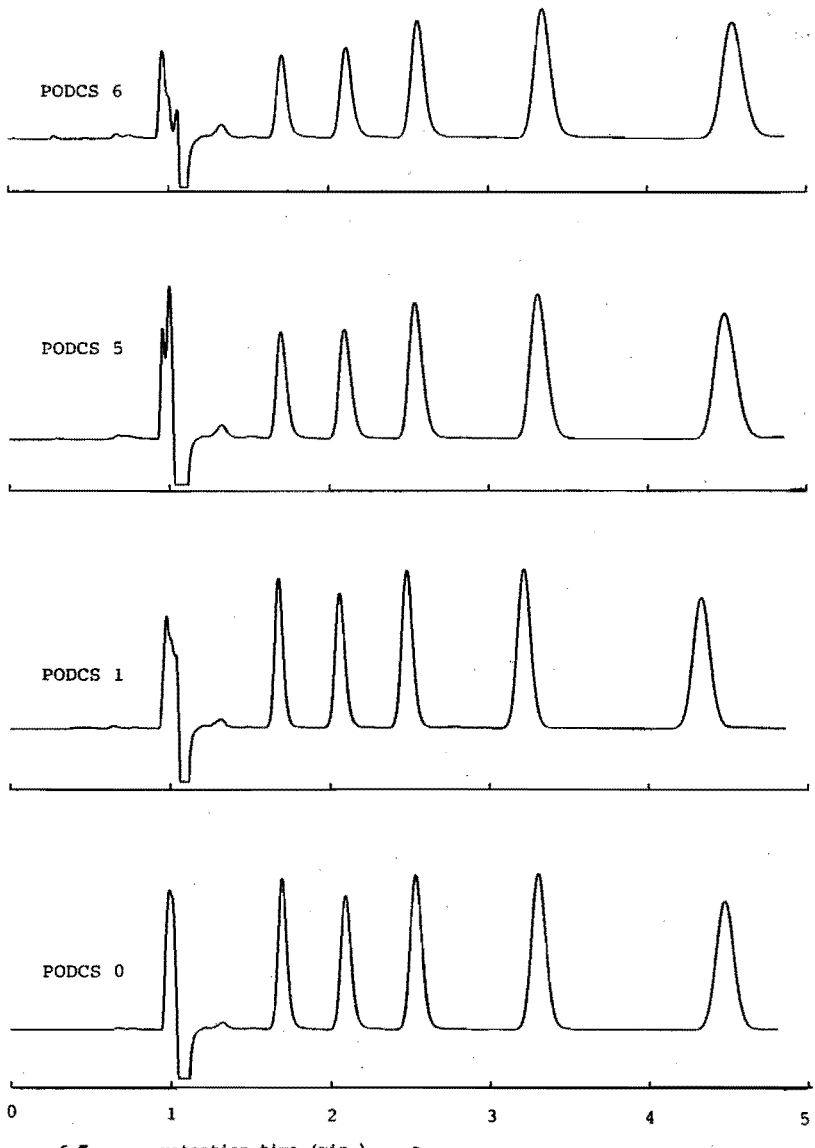

Figure 6.5

Chromatograms of the n-alkylbenzene test mixture on the pretreated octadecyl Chromspher phase before 0 and after ageing experiment 1, 5 and 6. Chromatographic conditions: methanol-water 80:20 (v/v); UV detection 254 nm.

From the chromatograms of n-alkylbenzenes, test mixture 1, and n-alkyl aryl ketones, test mixture 2, the capacity factors were determined before and after the ageing experiments. Also for each column the separation efficiency, N(sys), was calculated for butylbenzene eluted with an aqueous methanol eluent containing 80% (v/v) of methanol. The separation efficiency of all columns was determined at the start of the ageing experiments to check anomalous column packing. Due to more heterogeous size distribution of the PODCS particles the separation efficiency of all packed columns with the PODCS phase was 3 to 5 percent lower than that of the identically packed ODCS columns.

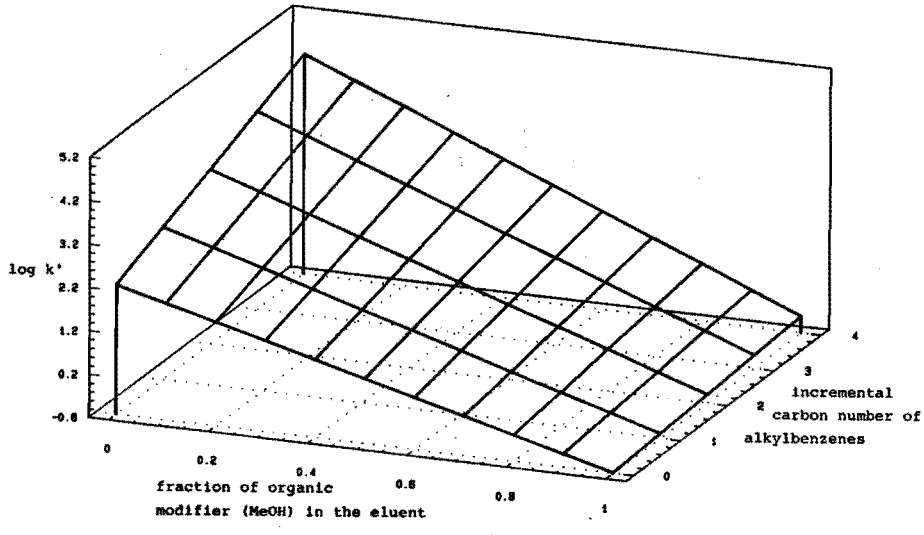

Figure 6.6

Graphical representation of the regression function for k' with the volume fraction (x) organic modifier (here methanol) in the eluent between x = 0.6 and 0.9 and extrapolated for x between 0 and 1. The incremental carbon number of the n-alkylbenzene homologue series for the PODSC phase after ageing experiment 3.

The chromatograms of the n-alkylbenzene test mixture before and after the simulated ageing experiments 1 , 5 and 6 of the PODCS reversed-phase are depicted in Figure 6.5.

A typical diagram of log k' versus the volume fraction methanol(x) and the incremental carbon number of test mixture 1 (n_c) is depicted in Figure 6.6. From these types of diagrams the linear dependence of the log k' values on the eluent composition and at the same time on the incremental carbon number of the solutes is clearly shown. According to eqn. 4.2 and 4.3, the a_0, m_0, a_1 and m_1 values were calibrated by multiple linear regression. The validity of this regression was controlled by the correlation coefficient (r). With eqn. 4.5a. and 4.5b. the derived values for p and q were calculated. Tables 6.VI and 6.VII summarize the values for a_0, m_0, p, q and r determined for both test mixtures used before and after each ageing experiment for the ODCS phase, respectively. Tables 6.VIII and 6.IX summarize these values for the PODCS phases. All correlation coefficients were between 0.995 and 0.998, indicating very reliable values for all constants estimated with the regression model used in this study.

TABLE 6. VI

Chromatographic characterization parameters, determined with regression and the correlation coefficient for regression for the **n-alkylbenzene** homologous series on the ODCS phase before and after each ageing experiment. Experimental conditions is outlined in Table 6.I.

Phase	a_0	m_0	p	q	r	N(Sys) [a]
ODCS0	2.11	2.84	.829	1.09	.996	7164
ODCS1	2.26	3.05	.841	1.15	.997	6670
ODCS2	2.23	3.00	.843	1.12	.996	6664
ODCS3	2.22	2.97	.850	1.08	.997	6022
ODCS5	2.04	2.76	.838	1.05	.996	5796
ODCS6	1.93	2.60	.852	0.96	.996	5122

a determined for butylbenzene eluted with an eluent containing 80% (v/v) of methanol, before the ageing experiments columns 1 to 6 showed separation efficiencies, N(sys) = 6800 $\pm$ 200.

An exception are the stationary phases after ageing experiment 4, the eluent used with this experiment (aqueous bicarbonate, pH = 8.4) apparently causes a severe loss of ligands, because of its aggressive nature. A reliable determination of k' values was impossible due to extreme band broadening and peak splitting. The value of a_0 of a relatively apolar residue like benzene, listed in Tables 6.VI and 6.VIII for ODCS and PODCS phases respectively, represents the capacity factor of these stationary phases.

TABLE 6. VII

Chromatographic characterization parameters, determined with regression and the correlation coefficient for regression for the **n-alkyl aryl ketone** homologous series on the ODCS phase before and after each ageing experiment. Experimental conditions as outlined in Table 6.I.

Phase	a_0	m_0	p	q	r
ODCS0	.362	1.54	.827	1.24	.996
ODCS1	.557	1.81	.828	1.35	.997
ODCS2	.414	1.61	.840	1.26	.995
ODCS3	.329	1.50	.852	1.22	.996
ODCS5	.183	1.33	.837	1.18	.995
ODCS6	.458	1.60	.833	1.22	.996

As expected the separation efficiency of both stationary phases decreased after long time exposure to relatively aggressive eluents. For the ageing experiments with low pH aqueous and aqueous-methanol buffer solutions the drop in separation efficiency indicates normal changes in structure of the packed bed. Nevertheless, the capacity factors of the test solutes increased, as will be discussed later (see "selectivity").

After ageing experiments of the ODCS phase with high pH eluents the capacity factors for all test solutes decreased and a severe drop in separation efficiency and resolution was found. On the other hand the PODCS phase exhibited almost constant capacity factors and separation efficiency under identical ageing conditions. It can be concluded from these data that the PODCS phase is more resistant towards prolonged exposure to relatively

high pH eluents. However, the CP-behaviour of the PODCS stationary phase was altered after ageing.

TABLE 6. VIII

Chromatographic characterization parameters, determined with regression and the correlation coefficient for regression for the **n-alkylbenzene** homologous series on the PODCS phase before and after each ageing experiment. Experimental conditions as outlined in table 6.I.

Phase	a_0	m_0	p	q	r	N(Sys) [a]
PODCS0	2.04	2.79	.834	1.09	.996	6946
PODCS1	2.02	2.80	.820	1.13	.997	6605
PODCS2	2.20	2.96	.824	1.14	.997	5427
PODCS3	2.19	2.98	.820	1.17	.997	6032
PODCS5	2.00	2.71	.842	1.03	.996	6384
PODCS6	2.06	2.84	.818	1.12	.997	6467

a determined for butylbenzene eluted with an eluent containing 80% (v/v) of methanol, before ageing experiments columns 1 to 6 showed separation efficiencies, N(sys) = 6600 $\pm$ 200, except the PODCS2 column with N(sys) = 5950.

TABLE 6. IX

Chromatographic characterization parameters, determined with regression and the correlation coefficient for regression for the **n-alkyl aryl ketone** homologous series on the PODCS phase before and after each ageing experiment. Experimental conditions as outlined in table 6.I.

Phase	a_0	m_0	p	q	r
PODCS0	.203	1.40	.833	1.23	.995
PODCS1	.330	1.61	.810	1.34	.997
PODCS2	.179	1.35	.836	1.20	.995
PODCS3	.490	1.74	.807	1.34	.996
PODCS5	.209	1.37	.838	1.19	.997
PODCS6	.309	1.58	.811	1.33	.996

The typical CP-curves of M_1, Q_3 and Q_4 ^{29}Si nuclei changed back to an almost normal CP-behaviour as given by the untreated Chromspher silica. Apparently the crystalline regions at the surface of the substrate were

destroyed by hydrolysis and only amorphous silica was left, although for aged PODCS phases the $T_{1\rho H}$ values were still larger than for ODCS phases (see also chapter 7).

Selectivity

Selectivity in RP-HPLC depends on both the mobile and the stationary phase. To judge the changes in selectivity of the mobile phase the values for p are listed in Tables 6.VI - 6.IX. These values were close to the theoretical value for methanol-water binary eluents calculated by Jandera, p = 0.86. More specific stationary phase solute interactions were estimated by the values of m_0 for lipophilic and q for polar selectivity. As expected, rather good correlations (correlation coefficient values 0.975 and 0.996 for ODCS and PODCS, respectively) were observed between the non-polar contribution to selectivity m_0 and the capacity factor of the stationary phase represented by a_0 values. This is especially the case for the most apolar residue of an homologous series of test solutes used in this study, the n-alkylbenzene test mixture.

However, some unexpected results should be noticed: with decreasing ligand density of the stationary phases (see also the results of elemental analysis and ^{29}Si CP-MAS NMR) the values of m_0 and a_0(n-alkylbenzenes) increased after all ageing experiments at high pH, indicating extended lipophilic selectivity. It may be concluded that the a_0 and m_0 values of the n-alkylbenzene test mixture were influenced by the high density of the stacked octadecyl ligand chains, especially at the surface of the PODCS phase. Probably due to less densely stacked ligand chains of the original stationary phases this phenomenon was not determined for octadecyl modified silicas in the chapters 4 and 5, where α_1 was between 1.7 - 2.6 μmol m^{-2}. With the PODCS phase discussed here, only part of the densely stacked C_{18} ligands of the stationary phase before ageing seemed accessible

for apolar interaction. After the ageing experiments with high pH aqueous-methanol buffer solutions the lipophilic selectivity decreased for the ODCS phase, indicating less resistance towards hydrolysis for the ODCS phases.

The fluctuation in polar selectivity q showed the limited influence of the silanol groups, present at the surface, probably due to steric hindrance by densely stacked ligand chains. For both test mixtures no serious increase of the q values was noticed. Even for the separation of more polar compounds, such as n-alkyl aryl ketones, the value of q did not change significantly after prolonged exposure to relatively aggressive eluents.

6.3.3. CONCLUSIONS

After the hydrothermal acidic treatment of a silica gel with a relatively high level of ionic impurities, such as the experimental Chromspher silica gel used in this study, small crystalline regions, ß-tridymite-like, will be present at the silica surface. After monofunctional modification with octadecyl silanes the pretreated silica still contained a considerable amount of crystalline regions and the CP-curves of the ^{29}Si nuclei involved showed the typical double domain behaviour. However, after simulated ageing experiments the crystalline regions disappeared. It can be concluded that the crystalline regions were formed close to the substrate surface and destroyed by hydrolysis.

After the hydrothermal treatment with a 0.1% (w/w) hydrofluoric acid solution the silica surface also contained about 12 percent more geminal silanol groups, which show a higher intrinsic reactivity upon modification with silanes. Modification of pretreated silica substrate with DMA-DMODS gave a 30 percent higher octadecylsilane ligand density, also due to a better accessibility of the silica surface inside the pores.

The resulting PODCS reversed-phase exhibited a better resistance towards

hydrolysis of the chemically bonded ligands, especially when aqueous-methanol buffer solutions with high pH were used with the ageing treatments. The higher ligand density on the PODCS phase remained more or less constant during simulated routine use and showed a significantly better performance in all characterization methods used in this study.

6.4. SILANIZED *VERSUS* POLYMER COATED OCTADECYL REVERSED PHASES

6.4.1. GENERAL

All stationary phases discussed in this paragraph, were prepared on the same Nucleosil silica (batch 7021, $d_{particle}$ = 5 μm, d_{pore} = 10 nm, S_{BET} = 360 m^2g^{-1}, Macherey, Nagel & Co., Duren, F.R.G.) to eliminate substrate dependent effects on the stability of the reversed-phases [12,13]. This silica was modified with dimethylamine-dimethyloctadecylsilane (DMA-DMODS) [7] to obtain a classical monodentate bonded n-octadecyldimethylsilane phase for comparison with the polymer coated phases. The two polymethyloctadecylsilane 0.25 molar ratio n-octadecyl groups, % C coating = 53.8%) coated phases, one precapped with trimethylsilane-2,4-pentadione-enolate [3], were prepared according to the procedures described before [2,4]. The non-precapped polymethyloctadecyl-siloxane coated stationary phase is not meant to be an advanced RP-HPLC phase. These three n-octadecyl modified stationary phases originated from the Chromatography Department of the Max-Planck Institut für Kohlenforschung (Mühlheim a/d Ruhr, F.R.G.) and will be referred to as a n-octadecyl-dimethylsiloxysilane bonded phase (C_{18}/A), a polymethyloctadecylsiloxane coated phase ($PMSC_{18}$) and a trimethylsilane-enolate precapped polymethyl-octadecylsiloxane coated phase (C_1/$PMSC_{18}$), respectively. The bulk properties of these modified stationary phases are listed in Table 6.X. The

trimethylsilane-enolate precapped Nucleosil-5-100 (C_1/E) was characterized for reference values of the polymer coated phase.

From each stationary phase seven identical columns (100 mm * 4 mm I.D., System B, Knauer, Bad Homburg, F.R.G.) were packed according to a standard packing procedure. After a chromatographic test to ensure reproducibility of the packing procedure, six columns were placed in an apparatus for simulated routine use. A 15 percent variation in column efficiency and a deviation of $\pm$ 5 percent in capacity factors and selectivities was allowed for a given series of columns.

In this study two series of homologues, n-alkylbenzenes and p-hydroxy-benzoic acid n-alkyl esters were used for fundamental chromatographic characterization of the phases for at least 4 different eluent compositions after each ageing experiment. By multiple linear regression of the logarithms of the capacity factors of a homologous series at as a function of different eluent composition, a set of characteristic parameters can be derived, as illustrated in the previous chapters 4 and 5. From this set of parameters the value of m_0 and q, calibrated by eqn. 4.2 and 4.3, provide the most relevant information on specific interaction between the stationary phase and solutes. These interactions depend largely on the type and actual condition of the stationary phase when identical chromatographic test conditions are applied. Under these restrictions these parameters characterize the contribution of stationary phase solute interactions toward an overall selectivity of a stationary phase. The parameter m_0 denotes mainly non-specific, lipophilic selectivity (interactions) and q represents polar selectivity (interactions).
For each column the separation efficiency, N_{sys}, was calculated for n-butylbenzene eluted with an aqueous-methanol eluent containing 80% (v/v) of methanol, before and after the ageing experiments for each column. In addition the condition of the stationary phases was also tested with test mixture 3, a mixture of compounds introduced by the

Chromatographic Department of the Max Planck Institute [2-4]. With this test mixture the selectivity factor α for the retention of 2-n-octylpyridine relative to ethylbenzene was determined.

For the C_{18}/A and C_1/PMSC_{18} phases adsorption isotherms of ethanol were determined to evaluate the amounts of accessible silanol groups left at the silica surface after modification [14]. Two columns (150 mm * 4.6 mm I.D.) were packed with these phases. The columns contained approximately 1.60 grams of stationary phase material. The breakthrough method was employed to quantify the modifier coverage at the stationary phase surface as a function of the mobile phase composition. Equipment and experimental design were similar as published [14].

6.4.2. RESULTS AND DISCUSSION

Chromatography

From adsorption isotherms, measured with the breakthrough method, the maximal adsorbed ethanol coverage of the C_{18}/A and C_1/PMSC_{18} phase surfaces is obtained. The columns packed with C_{18}/A and C_1/PMSC_{18} contain equal amounts of stationary phase material. The maximal amount of ethanol adsorbed at the C_{18}/A surface was 152 μmol on 1.60 grams of material, with a specific surface area, corrected for the ligand content, of 264 $m^2\ g^{-1}$. The maximal amount of ethanol adsorbed at the C_1/PMSC_{18} surface was 70 μmol on 1.60 grams, with a corrected specific surface area of 281 $m^2\ g^{-1}$. Thence, the adsorbed ethanol coverage at the surface can be calculated for both stationary phases. For the C_{18}/A phase the ethanol coverage was 0.360 μmol m^{-2} and for the C_1/PMSC_{18} phase it was 0.156 μmol m^{-2}. This difference in maximally adsorbed ethanol was partly due to the difference in ligand surface density: 3.31 μmol m^{-2} for the C_{18} ligands

versus 4.60 μmol m^{-2} for the precapped C_1 ligands, as given in Table 6.X. Ethanol adsorption by accessible residual silanol groups decreases with increasing amounts of chemically bonded ligands. However, the large difference in ethanol adsorption, more than twice as much for the C_{18}/A stationary phase, illustrates clearly the shielding effect of the polymethyloctadecylsiloxane polymer.

TABLE 6. X

Bulk properties of the native silica and the silanized and polymer coated phases. The silanol content (Q_2 and Q_3) is determined by ^{29}Si MAS NMR relative to the tetrasiloxane (Q_4) content.

Stationary phases	Carbon content P_C %(w/w)	Ligand surface density, α_l, eqn.4.1. (μmol.m^{-2})	Film thickness polymer coating d_f (nm)	29Si MAS NMR rel. silanol content
Nucleosil # 7021	--	--	--	0.311 *a*
C_{18}/A	20.48	3.31	--	0.186
$PMSC_{18}$	13.23	--	1.02	0.217
C_1/E	5.20	4.60	--	0.165
$C_1/PMSC_{18}$	16.18	4.60 *b*	0.80	0.163

a The relative silanol ratio of geminal silanol groups, Q_2 was 0.073 and vicinal/"lone" silanol groups, Q_3 was 0.927, see Figure 5.

b This are precapped trimethylsiloxysilane ligands, see C_1/E phase.

This latter polymer is hardly chemically bonded to the silica surface, only physically adsorbed at the trimethyl silanized surface by Van de Waals interactions between hydrocarbons. A pronounced shielding effect of the residual silanol groups by the polymer, even for small molecules like ethanol, is thus indicated.

The chromatograms of test mixture 4 eluted on the C_{18}/A, the $PMSC_{18}$ and the $C_1/PMSC_{18}$ stationary phases previously to and after some ageing

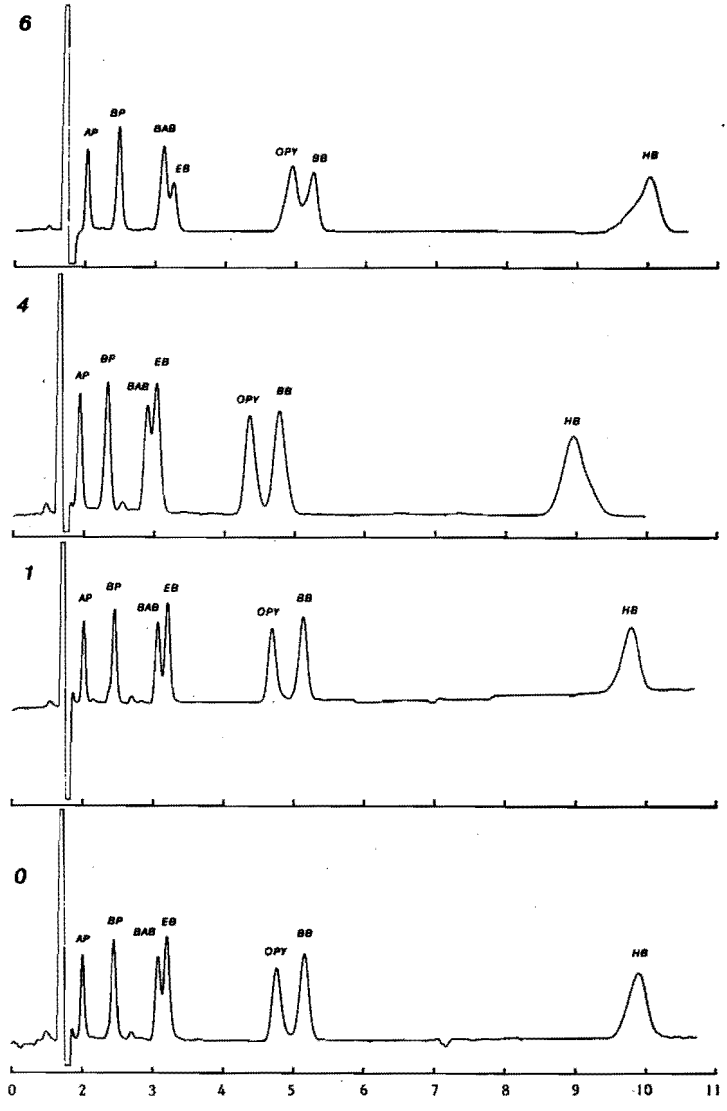

Figure 6.7

Chromatograms of test mixture 3 eluted from the C_{18}/A stationary phase before (0) and after ageing experiments 1, 4 and 6. Chromatographic test conditions: water-methanol (20:80, v/v); UV detection at 254 nm; Compounds: AP, acetophenone; BP, benzophenone; BAB, benzoic acid benzoyl ester; EB, ethylbenzene; OPY, 2-n-octylpyridine; BB, n-butylbenzene; HB, n-hexylbenzene.

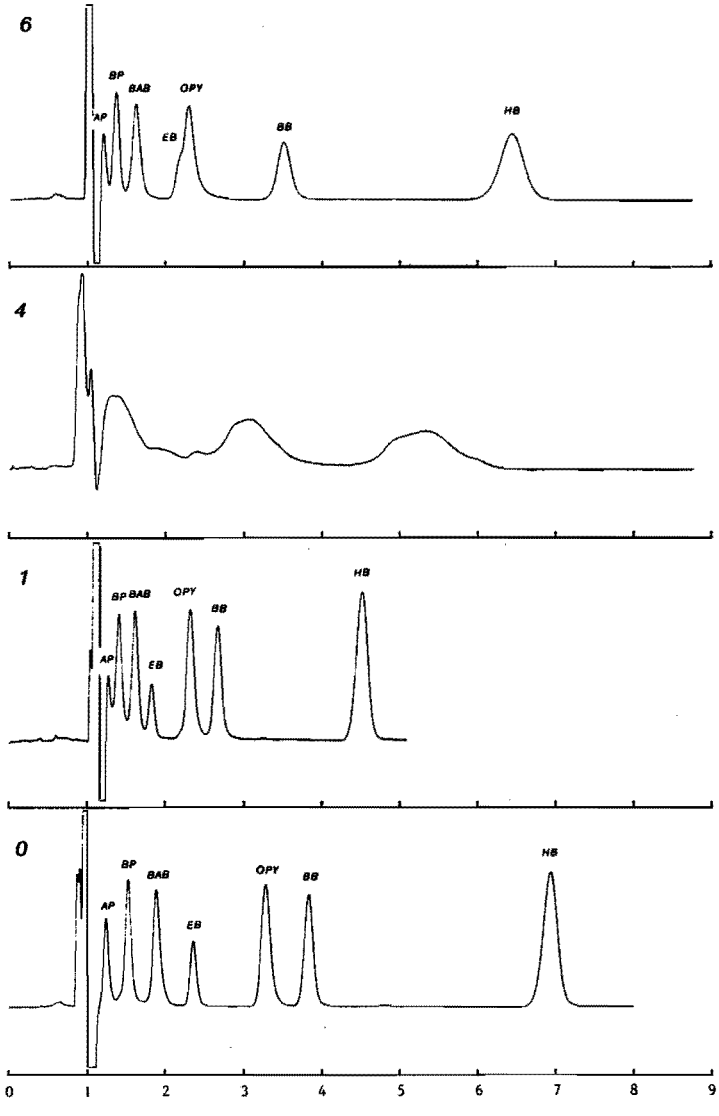

Figure 6.8

Chromatograms of test mixture 3 eluted from the PMSC$_{18}$ stationary phase before ageing (0) and after ageing experiments 1, 4 and 6. Conditions as in Figure 6.7.

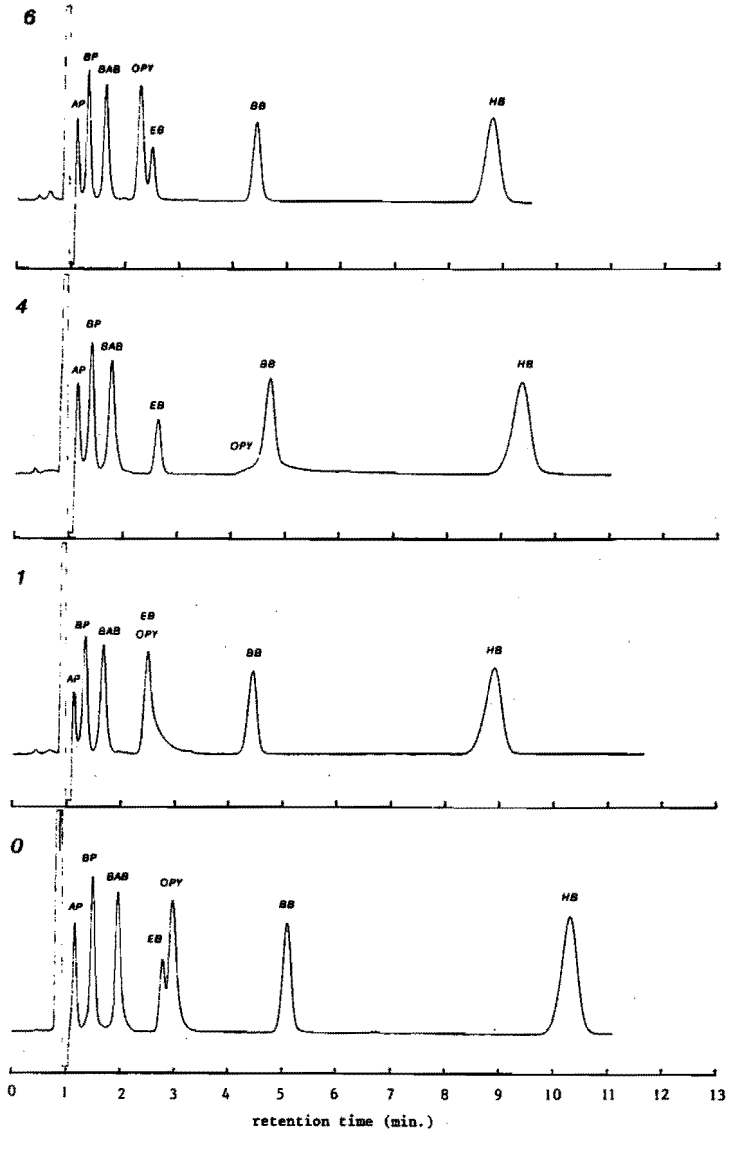

Figure 6.9

Chromatograms of test mixture 3 eluted from the $C_1/PMSC_{18}$ stationary phase before ageing (0) and after ageing experiments 1, 4 and 6. Conditions as in Figure 6.7.

experiments, are depicted in Figs. 6.7, 6.8 and 6.9, respectively. A comparison of these chromatograms already indicates the difference in selectivity of the original phases. Especially, the 2-n-octylpyridine peak is found at different positions in the chromatogram. A small capacity factor for basic 2-n-octylpyridine is supposed to indicate only limited specific interactions with acidic silanol groups [2-4].

This can be translated into a small amount of accessible silanol groups at the surface. Thence, the $C_1/PMSC_{18}$ phase exhibited the smallest amount of accessible silanol groups. This is confirmed by the ethanol adsorption measurements. Furthermore, 2-n-octylpyridine retention seemed to be very sensitive towards changes in stationary phase properties. Shifts in capacity factor values occurred after various ageing experiments. These shifts are summarized in Table 6.XI, where the selectivity factors α(2-n-octylpyridine /ethylbenzene) are listed.

TABLE 6. XI

Selectivity factor α of 2-n-octylpyridine relative to ethylbenzene.

Stationary phases	Ageing experiment no.						
	0	1	2	3	4	5	6
C_{18}/A	2.08	2.02	2.06	2.01	2.00	2.05	2.12
$PMSC_{18}$	1.69	1.74	1.60	1.52	--	1.25	1.06
$C_1/PMSC_{18}$	1.09	1.00	1.00	0.93	2.23	0.83	0.83

With the two polymer coated phases significant changes in this selectivity factor value were noticed after several ageing experiments. After ageing experiments with relatively aggressive eluents the selectivity factors generally decreased for the polymer coated phases. This stands in contrast to the expected decrease in silanol shielding properties due to stationary phase

stripping and disordering of the coating observed in chapters 4 and 5. A possible explanation of this unexpected phenomenon will be discussed in the solid state ^{29}Si NMR section. This decrease in selectivity factor value was remarkably large for the $PMSC_{18}$ phase after ageing experiments with high pH aqueous-methanol buffer solutions.

After similar ageing experiments the $C_1/PMSC_{18}$ phase showed a relatively small decrease in selectivity factor value. Only the high pH plain aqueous buffer increased the selectivity factor value of the $C_1/PMSC_{18}$ phase drastically. The C_{18}/A phase revealed a more or less constant, although high, selectivity factor value for 2-n-octylpyridine relative to ethylbenzene.

TABLE 6. XII

Lipophilic selectivity, m_0(n-alkylbenzenes) values and the polar selectivity, q(p-hydroxybenzoate) values determined by chromatographic characterization for the reversed-phases, before and after the ageing experiments.

Stationary phases	Ageing experiment no.						
	0	1	2	3	4	5	6
m_0(n-alkylbenzenes)							
C_{18}/A	2.91	3.11	3.06	3.04	3.06	3.01	3.00
$PMSC_{18}$	3.12	2.49	3.16	3.13	--	3.19	3.08
$C_1/PMSC_{18}$	2.95	3.14	2.91	3.05	2.94	2.90	3.00
q(p-hydroxybenzoate)							
C_{18}/A	1.55	1.70	1.54	1.71	1.76	1.54	1.78
$PMSC_{18}$	2.09	2.28	1.98	2.19	--	3.11	--
$C_1/PMSC_{18}$	1.75	2.16	1.71	1.46	1.95	1.90	2.20

From the chromatograms of the two homologous test mixtures, obtained with binary methanol-water test eluents with a methanol volume fraction between

0.6 and 0.9, the values of the parameters m_0 and q were determined. Obviously, m_0(n-alkylbenzenes) is to be preferred over m_0(p-hydroxybenzoic acid n-alkyl esters) for the characterization of the lipophilic selectivity, as discussed in detail in chapter 5.

Changes in ligand hydrolysis and/or stationary phase stripping due to ageing will affect the m_0(n-alkylbenzenes) value. These changes will also influence polar interactions with the stationary phase surface, especially for polarizable compounds. The q(p-hydroxybenzoate) value will express the effect of polar changes at the surface of the substrate better than the q(benzene) value. The values for the lipophilic and polar selectivities of the octadecyl modified phases are listed in Table 6.XII. The m_0 value determined for the original $PMSC_{18}$ phase is slightly higher than for the two other phases, due to a thicker polymer coating film (d_f = 1.02 nm) at the surface of the silica pores. The m_0(n-alkylbenzenes) value of all stationary phases did not alter significantly after the ageing experiments, except for the $PMSC_{18}$ phase after ageing experiment 1. It can be concluded that the amount of silanized ligands or coated polymer involved in lipophilic interactions remains more or less constant at the silica surface with the ageing experiments. The drop in m_0 value for the $PMSC_{18}$ phase after ageing experiment 1 with a low pH plain aqueous buffer solution could indicate a severe loss of polymer coating caused by dissolution, see also carbon content analysis. The slightly increased α(2-n-octylpyridine/ ethylbenzene) value indicates also a better accessibility of the surface silanol groups (see above).

The polar interactions determined with the homologous series of p-hydroxybenzoic acid n-alkyl esters exhibit a larger value for the polymer coated phases. The overall polarity is larger, probably this is due to interaction with mobile siloxane bonds in the polymethyloctadecylsiloxane coating.

The q(p-hydroxybenzoate) values reveal substantial changes after some ageing experiments, especially for the polymer coated phases. The polar selectivity of both polymer coated phases altered, except when a low pH

methanol-water buffer solution was used as an eluent. For the $PMSC_{18}$ phase the peak shapes in the chromatograms of test mixture 2 after ageing experiments 4 and 6 are so poor that no meaningful value for q can be determined. The $PMSC_{18}$ phase shows the largest changes in polar selectivity. The polar selectivity increased slightly after ageing experiment 1 and 3 and substantially after experiments 5. Here, more polar interactions occur, although the accessibility of the surface silanol is reduced after ageing experiment 5 (see selectivity factor α). We assume that the polymethyl-octadecylsiloxane polymers remain inside the pores caused by immobilization through advanced cross-linking. This explains also the unchanged m_0 values. The physical background of the apparent contradiction between changes in α and q(p-hydroxybenzoate) values will be discussed in the solid state ^{29}Si NMR section. For the $C_1/PMSC_{18}$ phase the q(p-hydroxybenzoate) values increase also, but less than for the $PMSC_{18}$ phase. The C_{18}/A phase shows the most constant selectivity after the different ageing experiments.

Table 6. XIII

Separation efficiency, N_{sys}(n-butylbenzene), calculated from chromatographic data. Experimental conditions: eluent methanol-water, 80:20 (v/v); UV detection: 261 nm.

Stationary phases	Ageing experiment no.					
	1	2	3	4	5	6
C_{18}/A	5432 + 7%	5877 + 6%	5622 + 2%	3098 -44%	5827 + 8%	2267 -58%
$PMSC_{18}$	3648 -32%	4096 -15%	4847 - 2%	--	1697 -64%	1855 -60%
$C_1/PMSC_{18}$	3914 -29%	5133 - 2%	5410 + 5%	3823 -31%	4280 -14%	4345 -15%

The effect of the ageing experiments on the separation performance, N_{sys}, is shown in Table 6.XIII. Because the separation plate number of the

original columns varied by as much as 15 percent, the value of N_{sys} (n-butylbenzene) after the ageing experiments is listed together with the changes in separation plate number due to ageing. The $PMSC_{18}$ phase shows the largest decrease in separation efficiency after several ageing experiments. It can be assumed that the shielding and protection of the silica substrate by the surface attached polymethyloctadecylsiloxanes is less than optimal. The various aggressive ions present in the ageing solvents could cause disorder of the silica surface rather easily. Even the less aggressive solvent with experiment 2 causes a severe drop in column efficiency by stationary phase degradation.

The positive effect of trimethylsilane precapping is quite clear. The stability of the resulting stationary phase improves, although still a considerable deterioration of the silica substrate is observed after ageing experiments with extreme pH plain aqueous buffer solutions. The stability towards aggressive eluents containing organic solvents is improved. This effect is so large, that a superior shielding of the silica surface is observed compared with the octadecyl ligand silanized phase. Presumably, solvation of the polymer coating caused a more effective shielding of the silica surface underneath the polymer.

TABLE 6. XIV

Carbon content P_C (%, w/w) determined by elemental analysis.

Stationary phases	Ageing experiment no.						
	0	1	2	3	4	5	6
C_{18}/A	20.48	20.40	20.35	20.47	20.36	20.33	20.06
$PMSC_{18}$	13.23	10.53	12.87	12.86	14.19	11.79	11.16
$C_1/PMSC_{18}$	16.18	14.92	16.02	16.02	15.58	15.22	15.19

The carbon contents of the stationary phases, determined by elemental

analysis, confirm the superior resistance of the C_{18}/A phase towards ligand stripping with plain aqueous buffer solutions, see Table XIV. Generally, the high pH buffer solvents cause the largest drop in carbon content for the polymer coated phases. Only after ageing experiment 1 the $PMSC_{18}$ phase shows a large drop in carbon content as well.

As discussed before, a substantial amount of polymethyloctadecyl-siloxanes is dissolved in the eluent in this case.
One has to keep in mind though, that changes of the carbon content are of relative value, both the silica and the ligand or polymer coating can dissolve in the eluent.
This explains the apparent gain in polymer content of the $PMSC_{18}$ phase after ageing experiment 4. Here, relatively more silica is dissolved. A large drop in separation efficiency reveals silica dissolution and disordering of the silica surface (see Table 6.XIII). From the carbon content data of the $C_1/PMSC_{18}$ phase it is not clear whether the polymethyloctadecylsiloxane coating or the trimethylsiloxysilane ligands are dissolved to the same extent. However, differences between dissolved amounts of ligands and polymers can be determined by solid state ^{29}Si MAS NMR, see next section.

Solid state ^{29}Si NMR

Table 6.XV lists the siliceous moieties most relevant to this study, with their notations and ^{29}Si NMR chemical shifts. The structures of the species specific for this paragraph are presented in Figure 6.10. The ^{29}Si MAS NMR spectrum of the Nucleosil substrate is depicted in Figure 6.11, together with the deconvoluted signal. The silanol content calculated from this spectrum is listed in Table 6.X. ^{29}Si CP-MAS NMR experiments with variable contact times of all n-octadecyl modified silicas revealed the specific correction factors for the different ^{29}Si NMR adsorptions. With a contact

time of 6 ms for the CP-MAS measurements in this study, the correction factors are calibrated: $I_0/I(6ms)$ and listed in Table 6.XV as well.

TABLE 6. XV

Silane/siloxane moieties, their notations and typical ^{29}Si NMR chemical shifts, see also chapter 2, together with the ^{29}Si CP-MAS NMR correction factors for a contact time of 6ms, $I_0/I(6ms)$.

Modification	Code	Typical ^{29}Si NMR chemical shift (ppm downfield from TMS)	correction factor $I_0/I(6ms)$
n-octadecyldimethyl-siloxysilane	M_1	+ 12.6	1.18
trimethylsiloxysilane	M_1	+ 12.3	1.23
bridged(dimethyldisiloxysilane) or bridged(methyloctadecyldisiloxysilane)	D_2'	- 20	1.18
polydimethyldisiloxysilane or polymethyloctadecyldisiloxysilane	D_2''	- 22.3	1.51
dihydroxysiloxane	Q_2	- 91	--
hydroxysiloxane	Q_3	- 101	1.14
tetrasiloxane	Q_4	- 110	1.23 [a]

[a] Only the tetrasiloxane moieties near the silica surface are detected with the CP-MAS technique. The cross-polarization efficiency depends on R^{-6} for the distance between hydrogen and silicon nuclei.

^{29}Si CP-MAS NMR spectra were obtained for the C_{18}/A stationary phase before and after the ageing experiments, see Figure 6.12.

The calibrated relative contents of siliceous surface moieties are listed in Table XVI. After all ageing experiments the content of n-octadecyldimethyl-siloxysilanes (M_1) increases relative to the hydroxysiloxanes (Q_3) content. Although it seems, that the total amount of both ligands and silanol groups at the surface decreased. One can conclude that relatively more silanol groups have disappeared with the ageing experiments. The high pH buffer

eluents cause also the largest changes in amounts and content ratio, although the changes in ratio are rather small.

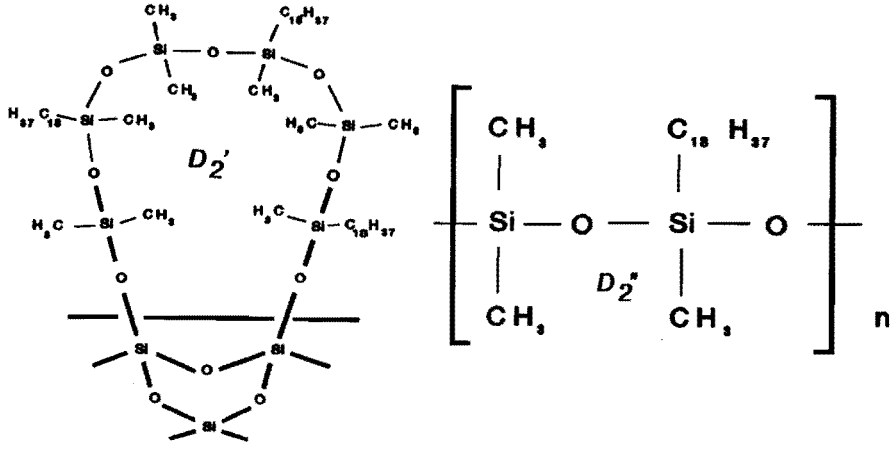

Figure 6.10

Structures of the siliceous surface moieties specific for this paragraph.

TABLE 6. XVI

The relative contents of siliceous moieties of the C_{18}/A phase before and after the ageing experiments, determined by ^{29}Si CP-MAS NMR with the correction factors listed in Table 6.XV.

Ageing exper	Relative content of siliceous surface moieties	
	M_1	Q_3
0	0.53	0.47
1	0.55	0.45
2	0.59	0.41
3	0.57	0.43
4	0.55	0.45
5	0.58	0.42
6	0.62	0.38

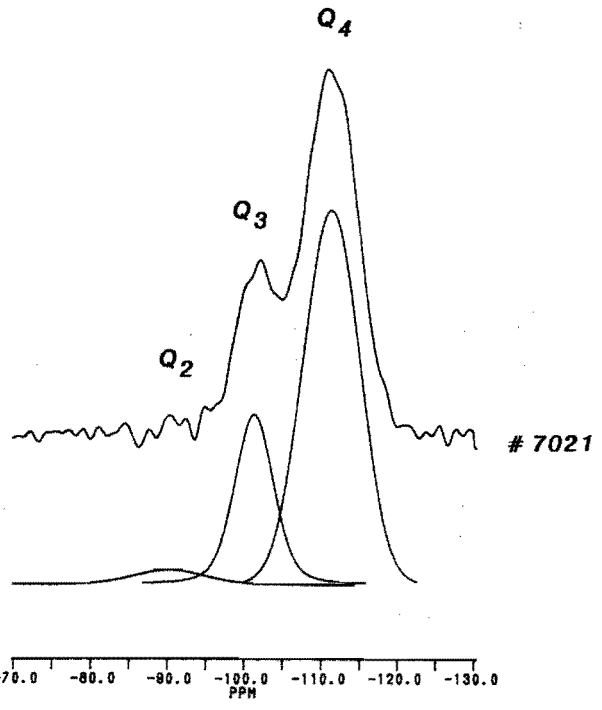

Figure 6.11

A ^{29}Si pulse excited Bloch decay MAS NMR spectrum of the Nucleosil #7021 silica substrate with the deconvoluted signals. N_S = 620; pulse interval time, 90s; acquisition time, 10 ms; line broadening, 10 Hz.

The difference between spectra obtained with ^{29}Si MAS NMR and ^{29}Si CP-MAS NMR for siliceous species modified at the silica surface, of interest in this study, is illustrated for the $PMSC_{18}$ stationary phase in Figure 6.13. Especially for mobile polymeric siloxysilanes, loosely attached to the rigid silica surface, the cross-polarization technique can have a very low response. In this sample the polymethyloctadecylsiloxane polymer is either loosely attached at the silica surface or fixed at a few positions only. With the ^{29}Si MAS NMR measurements the loosely attached polymer groups are detected yielding the narrow signal at -22.3 ppm.

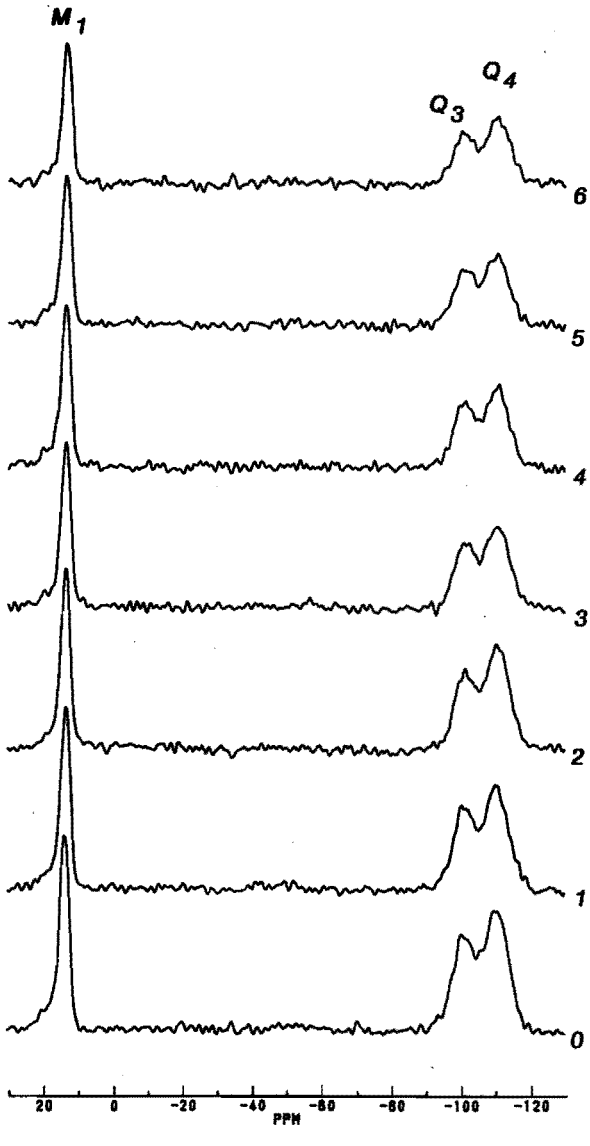

Figure 6.12

^{29}Si CP-MAS NMR spectra of the C_{18}/A phases before and after the ageing experiments. N_S = 2000; contact-time, 6 ms; interval time, 1s; acquisition time, 10 ms; line broadening, 15 Hz.

With the ^{29}Si CP-MAS NMR measurements these groups are hardly detected. The CP-characteristics reveal that with a contact time of 6 ms a small cross-polarization efficiency is obtained in the rather mobile polymer chain. A large correction factor has to be used for the D_2'' species with CP-MAS NMR spectra obtained for this stationary phase, see Table 6.XV.

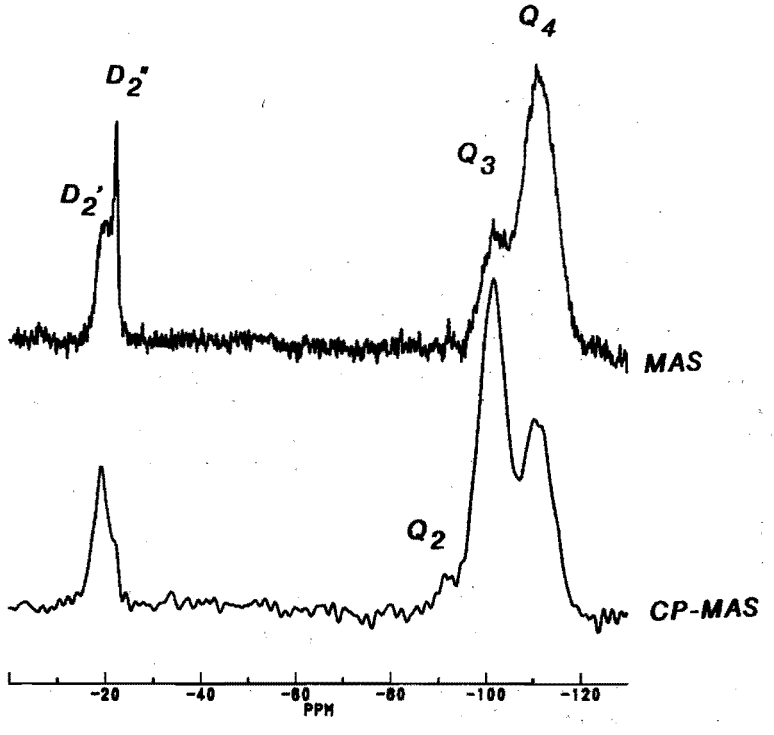

Figure 6.13

The ^{29}Si MAS NMR spectrum and ^{29}Si CP-MAS NMR spectrum of the original $PMSC_{18}$ stationary phase. Conditions as in Figs. 6.11 and 6.12.

As illustrated, part of the polymethyloctadecylsiloxysilane groups are incorporated in loosely attached, mainly physically adsorbed, polymers at the silica surface: the D_2'' groups in the NMR spectrum, while the other part is

chemically bonded forming short loops: the D_2' groups. The formation of these latter siloxysilanes causes a small amount of remaining polymeric hydrocarbonsiloxysilanes, the D_2'' groups. The more mobile polymers are physically adsorbed by the short chemically bonded hydrocarbonsiloxysilane loops at the pore surfaces. This is similar to the methyloctadecylsiloxane polymers which are physically adsorbed at the surface of the trimethylsilane-enolate precapped silica substrate. The ratio between D_2' and D_2'' moieties at the surface of the original $PMSC_{18}$ phase is determined by ^{29}Si MAS NMR, D_2' : D_2'' = 0.63 : 0.37. The amount of unreacted silanol groups, the hydroxysiloxanes, Q_3 is rather high for this stationary phase compared with the other stationary phases, see Table 6.X.

TABLE 6. XVII

The relative contents of siliceous moieties of the $PMSC_{18}$ phase before and after the ageing experiments, determined by ^{29}Si CP-MAS NMR with the correction factors listed in Table XV.

Ageing exper.	Relative content of siliceous surface moieties		
	D_2'	D_2''	Q_3
0	0.16	0.097	0.74
1	0.19	0.088	0.72
2	0.21	--	0.79
3	0.22	--	0.78
4	0.23	0.11	0.66
5	0.26	0.16 *a*	0.58
6	0.24	0.21 *a*	0.55

a The correction factor determined after ageing experiment 5 was, D_2'' : $I_0/I(6ms)$ = 1.22. This value was used here.

Figure 6.14 depicts the spectra of the $PMSC_{18}$ phases before and after the ageing experiments. From these spectra and Table 6.XVII, it is concluded that the amount of silanol groups decreases drastically after the ageing experiments with high pH buffer solutions. Parallel with this decrease in silanol content the amount of polymeric siloxysilanes D_2'' seems to increase.

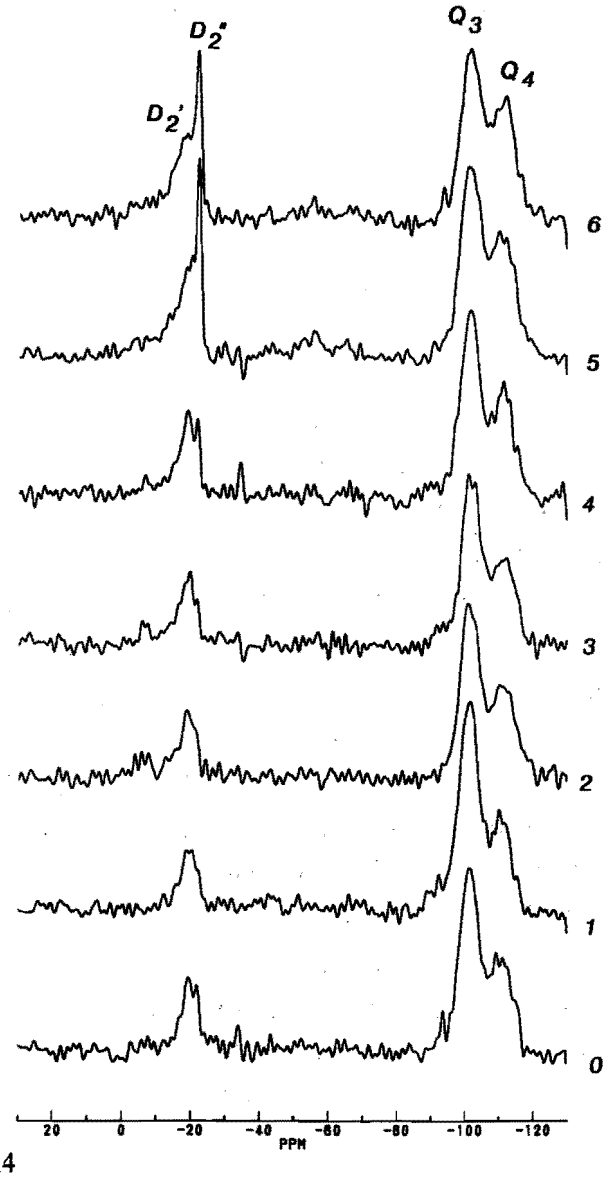

Figure 6.14

^{29}Si CP-MAS NMR spectra of the $PMSC_{18}$ phases before and after the ageing experiments. Conditions as in Figure 6.12.

However, this increment of D_2'' moieties is artificial. A ^{29}Si CP-MAS NMR experiment with variable contacts showed a shift in CP-characteristics, the optimal contact time decreased, together with the correction factor $I_0/I(6ms)$. This indicates that the polymethyloctadecyl-siloxysilane groups are more tightly attached to the surface. Probably, hydrolysis of siloxane bonds in the long polymer chains causes fragmentation via hydrolysis, yielding silanols. These polymer silanol groups condense with other polymer silanol groups or surface silanol groups, with formation of a more rigid polymer network with smaller loops. The polymer chains still exist but with a decreased free chain length between the cross-linking knots. The amount of small loops anchored to the surface: D_2' increases likewise. However, a substantial part of the surface silanol groups disappears due to substrate hydrolysis, dissolution and by condensation into siloxane bridges with neighbouring silanol groups.

Both processes, the formation of a more rigid coating layer and the dissolution or condensation of silanol groups explain the changes in chromatographic behaviour of this stationary phase. These are: an increase in polar selectivity q(p-hydroxybenzoate) by the increasing amount of siloxane bonds in the polymer, a more or less constant stationary phase ratio and a decrease in selectivity factor value, α(2-n-octylpyridine/ethylbenzene). These latter changes are particularly evident after ageing with high pH buffer solutions. However, the stability of this stationary phase is rather poor.

The ^{29}Si CP-MAS NMR spectra of the native Nucleosil silica substrate, the trimethylsilane-enolate precapped substrate and the subsequently coated polymethyloctadecylsiloxane stationary phase are depicted in Figure 6.15.

It is calculated that approximately 60 percent of the silanol groups at the native silica surface reacted with the trimethylsilane-enolate. The reaction of the coating polymethyloctadecylsiloxanes with the remaining silanol groups can be neglected, see also Table 6.X.

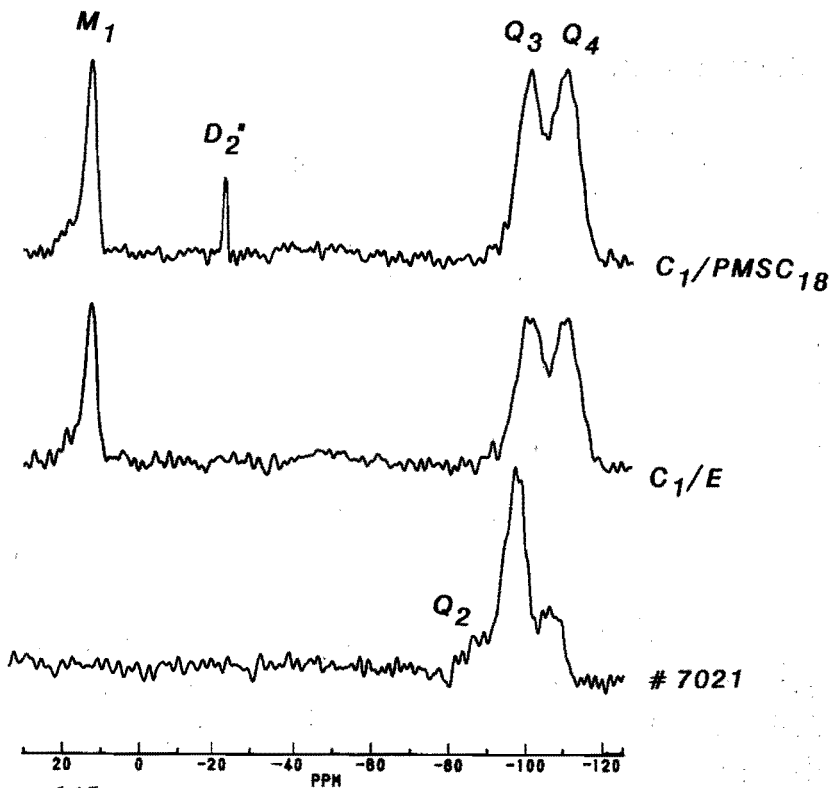

Figure 6.15

^{29}Si CP-MAS NMR spectra of the originate Nucleosil silica substrate (#7021) and the trimethyl modified silica (C_1/E) and the subsequently polymethyloctadecylsiloxane coated stationary phase ($C_1/PMSC_{18}$). Conditions as in Figure 6.12.

Here again, the cross-polarization efficiency of the free polymeric hydrocarbonsiloxanes, D_2'' moieties, is rather poor. Somewhat to our surprise, approximately the same CP-dynamics factors are determined for these moieties at the surfaces of the $PMSC_{18}$ and the $C_1/PMSC_{18}$ phases. One may conclude, that a similar mobility for the methyloctadecylsiloxane polymers on top of the surface anchored short hydrocarbonsiloxysilane loops points to a similar structure of the polymethyloctadecylsiloxane coating.

TABLE 6. XVIII

The relative contents of siliceous moieties of the $C_1/PMSC_{18}$ phase before and after the ageing experiments, determined by ^{29}Si MAS NMR.

Ageing exper.	Relative content of siliceous moieties			
	M_1	D_2^n	Q_3	Q_4
0	0.092	0.19	0.12	0.60
1	0.072	0.18	0.13	0.62
2	0.092	0.19	0.12	0.60
3	0.086	0.20	0.11	0.60
4	0.085	0.19	0.12	0.60
5	0.075	0.20	0.10	0.62
6	0.076	0.20	0.11	0.62

For the $C_1/PMSC_{18}$ phases the CP-behaviour is hardly influenced after ageing experiment no. 5, in contrast with $PMSC_{18}$. The precapped polymer coated stationary phase hardly shows an advanced cross-linking with high pH water-methanol ageing experiments, see also selectivity factors α.
The ^{29}Si MAS NMR spectra will be used for quantification of the siliceous moieties of the $C_1/PMSC_{18}$ after the ageing experiments, see Figure 6.16 and Table 6.XVIII.
Again, a slightly higher silanol content is determined after the ageing experiments with extreme pH plain aqueous buffer solutions. With the low pH plain aqueous buffer the raise in silanol content coincides with a drop in trimethylsiloxysilane ligands and a loss of polymer coating. With the high pH plain aqueous buffer only a small drop in anchored ligands is noticed. The amount of polymer remains constant with high pH buffer solutions. As already observed with chromatography, after ageing experiments with high pH buffer solutions the $C_1/PMSC_{18}$ revealed much better substrate shielding properties than the non-precapped $PMSC_{18}$. The amount of silanol groups decreased slightly, indicating only little silica dissolution.

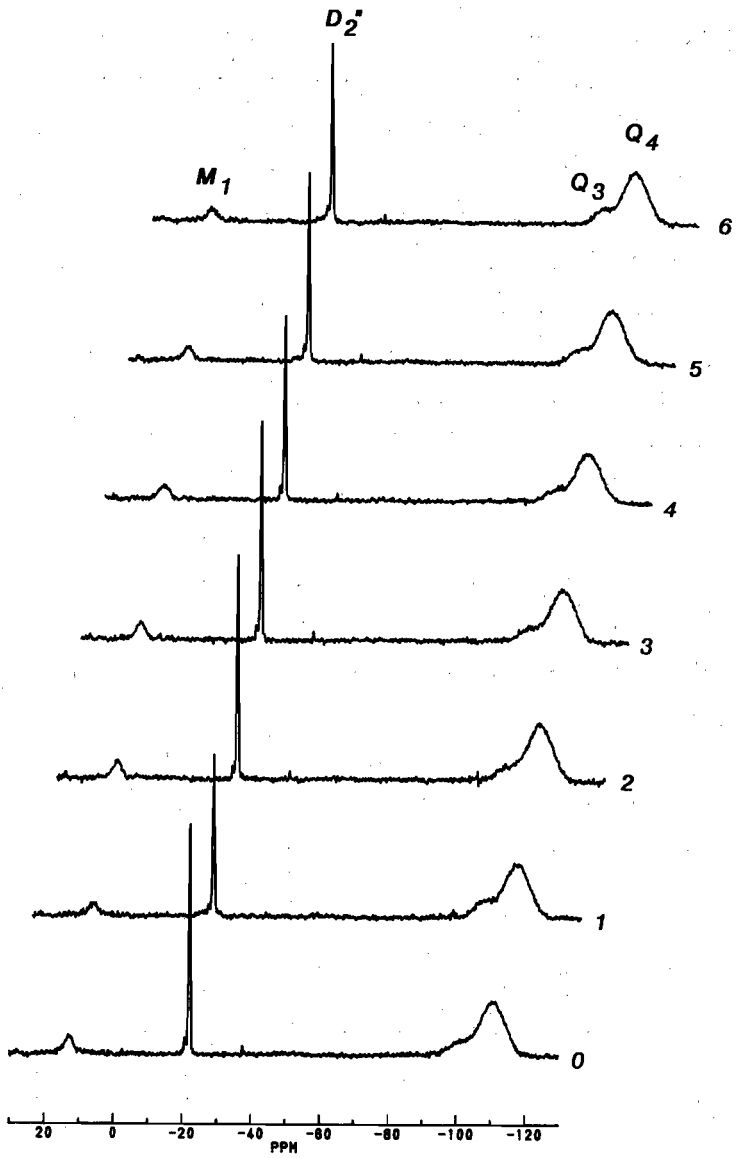

Figure 6.16

^{29}Si Bloch decay NMR spectra of the C_1/PMSC_{18} phases before and after the ageing experiments. Conditions as in Figure 6.10, except acquisition time, 100 ms; line broadening, 1 Hz.

6.4.3. CONCLUSIONS

The polymethyloctadecylsiloxane coated stationary phases exhibit a selectivity for certain polar compounds, like pyridine containing compounds, that is clearly different from conventionally modified octadecyl phases. Only a small amount of accessible silanol groups was determined with ethanol adsorption measurements and chromatography. However, the overall polarity determined via the homologous series of p-hydroxybenzoic acid n-alkyl esters indicates a higher value after the ageing experiments for these particular coated phases. This is probably due to interactions with mobile siloxane bonds formed in the polymethyloctadecylsiloxane polymer coating.
With high pH water-methanol buffer solutions the polymethyloctadecylsiloxane coating forms a rigid polymer network by advanced cross-linking, more tight to the silica surface. This is observed to a large extent with the $PMSC_{18}$ phase, probably caused by the large amount of remaining silanol groups after polymer modification. Part of the silanol groups react with the hydrolysed polymer chains. However, advanced cross-linking of the polymethyloctadecylsiloxanes does not reduce dissolution of the silica surface underneath. With multifunctionally modified octadecyl ligand phases cross-linking and condensation of the surface attached silanes was also observed after similar high pH ageing experiments described in chapter 4.
The $PMSC_{18}$ phase exhibits the poorest silica shielding properties. Trimethylsilane-enolate precapping improves the stability of the polymethyloctadecylsiloxane coated stationary phase drastically. The more effective interactions between the hydrocarbons of the polymer coating and the trimethylsiloxysilane ligands result in an improved structure and better attachment of the coating layer. Surface shielding by the coated polymers of the $C_1/PMSC_{18}$ phase improves largely in the presence of organic solvents in the eluent. With high pH water-methanol buffer solutions this shielding is superior to the conventionally modified C_{18}/A phase. However,

plain aqueous buffer solutions with high pH values deteriorate the silica surface by minor substrate and ligand hydrolysis and subsequent dissolution. The surface attached octadecyl ligands of the conventional C_{18}/A phase exhibited better shielding properties under these conditions. When RP-HPLC separations with varying, aggressive solvents are necessary, the precapped polymer coated stationary phase should be cross-linked more drastically to minimize shifts in selectivity, caused by advancing cross-linking during use.

Therefore, future research on polymer coated porous phases should also be focused on an enhancement of stability. Better silica shielding and protecting properties would further increase the popularity of these phases. A consistent specific selectivity, incorporated in the polysiloxanes, over a long period of intensive use in LC practice will convince many future users of the convenience of these types of phases. When special care concerning surface shielding and surface wettability for the polysiloxane coating is taken, the polymer coated phases will exhibit an equal or even superior stability compared with conventionally modified phases. A good example is given by the trimethyl precapped polymethyl-octadecylsiloxane phase examined in this study.

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CHAPTER 7

QUANTITATIVE AND QUALITATIVE ASPECTS OF SOLID STATE ^{29}Si NMR

Solid state ^{29}Si CP-MAS NMR provides information regarding the chemistry and individual molecular mobilities of different surface moieties. Furthermore, interactions between deactivating layers and the stationary phases at the silica surface are detected. However, this technique only yields accurate quantitative results provided that adequate precautions are taken.

7.1. SOLID STATE NMR

7.1.1. *General*

Throughout this thesis the structure analyses of silica substrates and of modified silicas rest largely on solid state NMR techniques. It seems therefore appropriate, to stipulate in this chapter some of the salient features. This is all the more of interest since in the samples described in this thesis, a wide variation in mobilities exists. This requires a rather large variation in NMR operating conditions.

For native and modified silicas NMR of ^{13}C and ^{29}Si are of interest. In solids the molecules are generally held rather rigidly. Therefore, neither chemical shift anisotropies nor dipolar interactions will be modulated sufficiently to obtain narrow lines (high-resolution) in NMR spectra of

stationary solid samples. Chemical shift anisotropy influences can be removed from the spectra by the well-documented technique Magic Angle Spinning (MAS) [1,2]. This involves rapid rotation ($\nu_{MAS} \approx$ 1-5 kHz.) of samples around an axis at an angle Θ of 54°44' with the main magnetic field, such that $(3\cos^2\Theta - 1) = 0$. The MAS technique also diminishes dipolar interactions [3], although in most cases not completely. This is the case, e.g. for rare nuclei like ^{13}C and ^{29}Si directly bonded to protons. In these cases, recourse is taken to dipolar decoupling of protons [3]. This technique has been used throughout this study.

7.1.2. *Pulse excitation and relaxation*

Relaxation to thermal equilibrium involves dissipation of energy of the spin system to the lattice in the form of heat and is called "spin-lattice relaxation". Relaxation requires local oscillating magnetic fields with frequency components near ω_0, the nuclear precession frequency or Larmor frequency of the nucleus concerned [3]. The spin-lattice relaxation time (T_1) is defined as:

$$\frac{d\,M_z}{dt} = \frac{-(M_z - M_0)}{T_1} \qquad (7.1)$$

The spin-lattice relaxation time for diluted spins in solids can be rather long. Pfleiderer et al. [4] measured $T_1(Si) \geq 40$ s for tetrasiloxane moieties in silicagel.

For quantitative analyses with pulse excited NMR the appropriate pulse interval times should exceed 5 times the longest T_1 value [5,6]. Thence, for silicagels pulse NMR methods are rather inefficient. For these materials spectra accumulation may take more than 10 h. This problem does not exist for cross-polarization NMR techniques. Only in those cases, where all signals are required with equal response factors, pulse excitation is necessary.

7.1.3. *Principles of cross-polarization NMR*

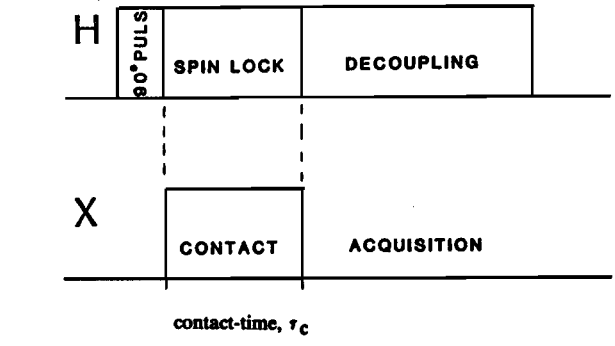
Cross-polarization (CP) is a technique, which derives the magnetization of the diluted rare spins (^{13}C and ^{29}Si) from the more abundant proton spins. This transfer is made to occur in the rotating frame of reference by adjusting the amplitudes of the radio frequency magnetic fields (matching) as described by Hartmann-Hahn [7,8]:

$$\gamma_H B_{1H} = \nu_H = \nu_X = \gamma_X B_{1X} \qquad [Hz] \qquad (7.2)$$

Transfer of magnetization is only the case when both B_{1H} and B_{1X} magnetic fields are applied simultaneously. Both nuclei precess with identical Larmor frequencies in the rotating frame. Energy transfer between the diluted spins and the proton spins is caused by heteronuclear spin flips under CP conditions.

CP occurs only when dipole-dipole interactions are still present in the sample, i.e. the molecular motions should be limited in at least one direction (anisotropic motion). The duration of cross-polarization is called the contact-time, τ_C, see Figure 7.1. Typical cross-polarization times are in the range of 0.5 - 5 ms.

During CP the diluted spins relax through the neighbouring, more abundant protons, which have short spin-lattice relaxation times, typically a few seconds [8,14]. Therefore, fast repetitions can be applied and spectra are obtained within a reasonable time. Moreover, in equilibrium an enhanced magnetization of the diluted spins is achieved following cross-polarization. The enhancement factor is γ_H / γ_X. For ^{13}C and for ^{29}Si these factors amount to approximately 4 and 5, respectively [8,9]. For ^{29}Si nuclei this is only the case for those nuclei which are in close contact with protons (maximally 4 bonds away), owing to the fact that the rate of cross-polarization is proportional with R^{-6} [5,10].



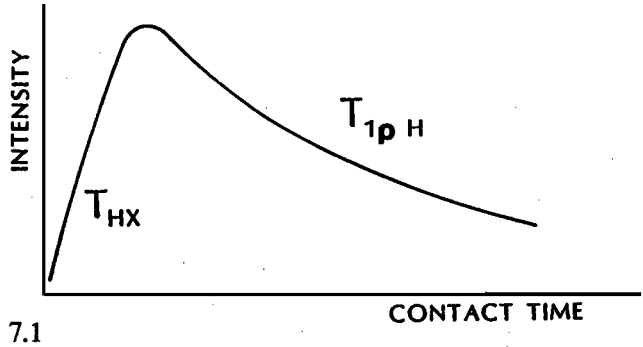


Figure 7.1

The pulse sequence used to obtain cross-polarization from the proton reservoir to silicon-29 spins for solids.

This situation usually prevails for those regions of the silicagel close to the external surfaces, including pore surfaces. For this reason, CP excitation is the method of choice in the investigations of surface modified silicas as described in this thesis.

7.1.4. *Variable contact-time experiments*

The intensity of a resonance line in the CP-MAS NMR spectrum depends on the applied contact-time. A plot of the ^{29}Si NMR signal intensity with increasing contact-time (τ_c) is depicted in Figure 7.2 for a ligand modified silica. This plot shows the signals assigned to the individual siliceous

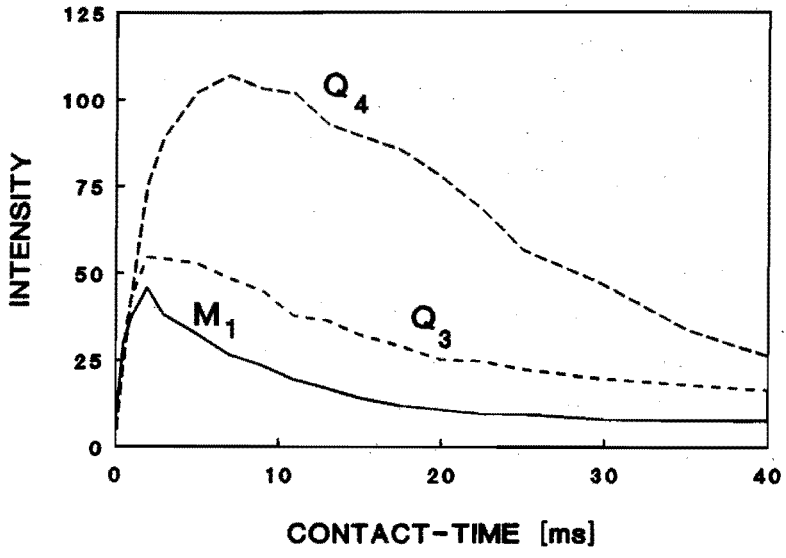


Figure 7.2

Silicon-29 CP-curves of octadecyldimethylsiloxysilane (M_1), hydroxysiloxane (Q_3) and tetrasiloxane (Q_4) moieties in monofunctional Hypersil-C_{18}.

moieties. The signal as a function of the contact-time is described according to [12]:

$$I(\tau) = I_0 \lambda^{-1} * [\, 1 - \exp(-\lambda \tau / T_{HSi})] * \exp(-\tau / T_{1\rho H}) \qquad (7.3)$$

with: $\lambda \equiv 1 + T_{HSi}/T_{1\rho Si} - T_{HSi}/T_{1\rho H}$

Under the assumption that $T_{1\rho Si}$ and $T_{1\rho H}$ are of the same order eqn. 7.3 can be simplified to:

$$I(\tau) = I_0 * [\, 1 - \exp(-\tau / T_{HSi})] * \exp(-\tau / T_{1\rho H}) \qquad (7.4)$$

I_0 is the amplitude of the exponential function, T_{HSi} is the

cross-polarization time constant for signal rise, $T_{1\rho H}$ and $T_{1\rho Si}$ are the spin-lattice relaxation times in the rotating frame (under spin-locked conditions) for signal decay of protons and silicon-29, respectively.
The values of T_{HSi} and $T_{1\rho H}$ can be determined by the contact-time variation experiments. These time constants are sensitive for motions in the 10^2 Hz and 10^5 Hz range, respectively [13]. Therefore, the shape of the CP-curves is an indication for motions in a solid sample in these particular ranges. For comparison, the longitudinal relaxation time T_{1Si} is determined largely by motions in the 10^8 Hz range (Larmor frequency). Taken together, the three time constants yield useful information regarding molecular motions (as viewed through three frequency windows).

7.2. QUANTITATIVE ANALYSIS

7.2.1. *Correction factors for CP excitation*

For rigid homogeneous solid samples the CP-curves can generally be analyzed in terms of the two time constants T_{HSi} and $T_{1\rho H}$ (see above). The I_0 values, calculated from the CP-curves, constitute the quantitatively correct NMR responses for the various signals. For fast quantitative analyses with a single contact-time, τ_c, the correction factor $I_0/I(\tau_c)$ of all moieties of concern have to be known for an accurate evaluation [3,5]. Therefore, a fast quantitative analysis of a single sample with unknown CP characteristics is not feasible. On the other hand, most ligand modified silicas described in chapters 4 to 6, can be analyzed within reasonable time with a single contact-time. Accurate quantitative analyses of these materials, which show similar CP characteristics, are performed with a contact-time beyond the optimal τ_c for most CP-curves, see Figure 7.2. For more accurate analyses the characteristic CP time constants have to be determined for each sample, which is rather time consuming. Analysis of samples, which contain both

rigid and more mobile moieties, e.g. the $PMSC_{18}$ stationary phase described in chapter 6 (see also Figure 7.3), can be performed with two different contact-times. Both contacts have to be selected beyond the optima in CP-curves of interest.

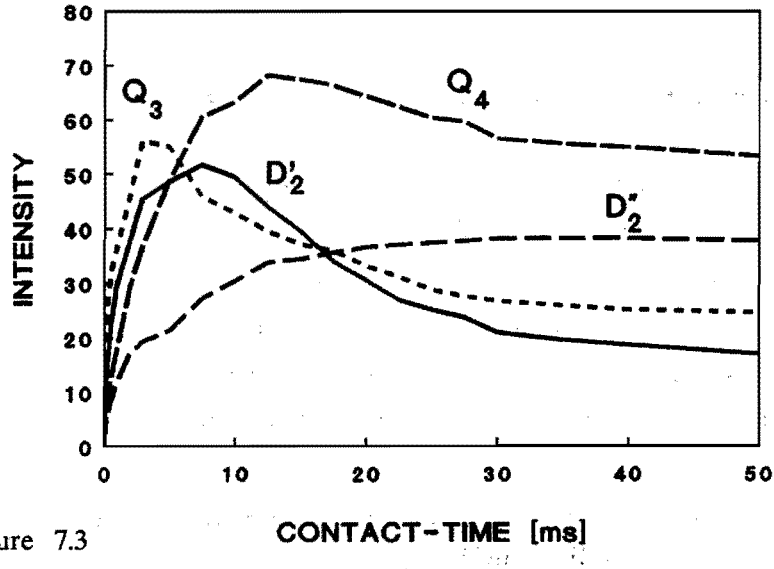


Figure 7.3

Silicon-29 CP-curves of bridged methyloctadecyldisiloxysilane (D_2'), poly methyloctadecyldisiloxysilane (D_2''), hydroxysiloxane (Q_3) and tetrasiloxane (Q_4) moieties of polymethyloctadecylsiloxane modified silica ($PMSC_{18}$).

When sets of two time constants are involved in CP characteristics their relative contribution, i.e. I_0 values, have to be known and accurate quantitative analyses will already become more complicated.

A quantitative evaluation of moieties, that exhibit CP-curves with more than one maximum, e.g. thick-film PMHS deactivated Cab-O-Sil (Figure 3.9), is complicated. The intensities of the maxima give only a rough indication of the amounts of moieties which exhibit a certain molecular motion, see next paragraph. A more accurate quantitative analysis has to be performed with pulse NMR. Even then, only limited results can be obtained. The

CP-curves show that a given signal may represent structures with quite different mobilities. With pulse excitation, given adequate precautions, only the sum of the different structure units can be obtained. Quantitative analyses of very mobile (isotropic) moieties, e.g. the $C_1/PMSC_{18}$ stationary phase in chapter 6, is impossible with CP-MAS NMR.

7.3. SEPARATE DOMAINS IN SOLID SAMPLES

The CP-curves of thick-film PMHS deactivated Cab-O-Sil samples in chapter 3 and of acid pretreated silicagel moieties in chapter 6 clearly showed more than one maximum, see Figures 3.9 and 6.3. An intuitive approach would be to assume overlap of two or more CP-curves, each governed by the normal formula (see eqns. 7.3 and 7.4). In practice, however, it turns out that such an approach can only yield approximate results.

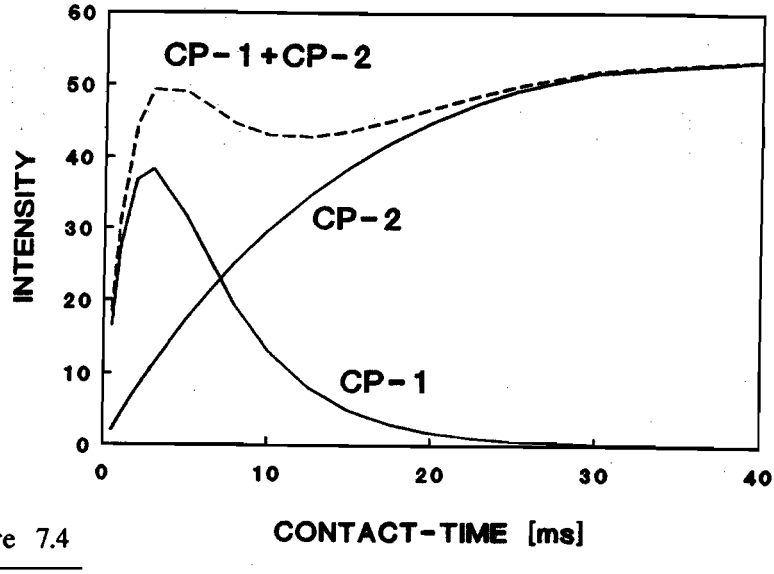


Figure 7.4 Reconstruction of a CP-curve with two sets of time constants.

For reasons yet unknown heteronuclear magnetization transfer, under double resonance conditions, within the mobile parts showed an effect that looks like a delay of approximately 10 ms. This is true, even if the apparent T_{HSi} and $T_{1\rho H}$ values differ by an order of magnitude, see Figure 7.4. Pfleiderer et al. [4,14] circumvented this problem through logarithmic scale linearization of the separate contact-time intervals, thus acknowledging that both magnetization transfer and decay start at the beginning of any CP experiment. Although the existence of two $T_{1\rho H}$ values points at two separate proton reservoirs, CP simulation in this manner fails on certain points. The two proton reservoirs should be considered as mechanically and spatially separated (hardly any ^1H-^1H spin-spin exchange occurs between the two reservoirs).
An example of uncoupled proton reservoirs is discussed by Bronnimann & Maciel for zeolite-HY adsorbed methanol [15]. It can be concluded that with CP excitation, separate carbon and proton reservoirs can be detected. The carbons of the mobile methanol molecules are excited via the protons of the rigid methanol species.

For the acid pretreated silicagel, described in chapter 6, similar mechanically uncoupled domains were detected for the hydroxysiloxane and tetrasiloxane moieties. The explanation could be as follows. In the non-pretreated silicagel there are regions which exhibit different mobilities. Both amorphous and crystalline regions exist in the silicagel. Mechanical coupling between the different regions, however, causes an averaged behavior in the sense of mobilities. During acid pretreatment a number of siloxane bonds will be broken. Afterwards, the rigid crystalline regions and the more mobile amorphous regions become uncoupled and separate CP-curves begin to show up. It seems that this process is enhanced in the presence of ionic impurities in the silica matrix. However, the uncoupling is not completed, otherwise the resulting separate CP-curves should each be analysable in terms of two time constants. The more mobile regions could be reviewed as having fractal

dimensions of the order of 2.0 - 2.2 [16], i.e. their behaviour is becoming more analogous to that of e.g. the one side anchored alkyl chains.
A useful example of this kind of behaviour of an ensemble of spins can be found for octyl modified silicas, see Figure 7.5. The ^{13}C CP-curves of methyl and methylene moieties reveal distinct differences in CP characteristics at different locations in the anchored octyl chain caused by different mobilities. Each carbon position in the chain corresponds with an individual set of transfer and relaxation parameters. The form of the CP-curves of carbon nuclei relatively remote from the surface, is similar to that of mobile silicagel parts. This can be construed as support of the silicagel model presented above.

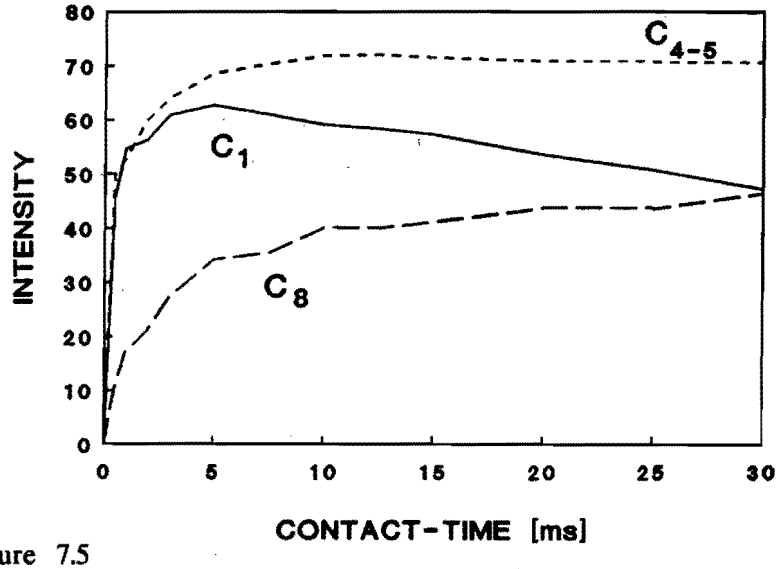


Figure 7.5

Carbon-13 CP-curves of several methyl and methylene moieties in the anchored octyl chain; carbon positions according to figure 3.8.

Ageing of pretreated silicagel under HPLC conditions and exposure to humid air apparently destroyed the boundaries between the two domains

and a more homogeneous, averaged magnetization transfer and decay was observed again.

The thick-film PMHS deactivated Cab-O-Sil samples, described in chapter 3, showed an extremely weak coupling of domains for the methylhydrodisiloxysilane moieties at the surface. With increasing silylation temperatures of thick-film PMHS coated Cab-O-Sil the magnetically uncoupled domains remain, although the separation between the two domains becomes less clear. The domain that contains more rigid moieties increases at the expense of the more mobile moieties with increasing silylation temperatures. Subsequent coating, however, transformed the two uncoupled domains to an almost homogeneous reservoir for all methylhydrodisiloxysilane moieties, see Figure 3.15.

7.4. CONCLUSIONS

^{29}Si CP-MAS NMR with contact-time variation of surface modified silicas yields additional information regarding the structure in the silica itself and the (mono)layers at the silica surface. CP-curves with more than one maximum indicate the presence of magnetically uncoupled proton reservoirs and thus the existence of mechanically and spatial separated domains. This information can not be obtained in a simple way by other methods.

Fast quantitative analyses with single contact CP excitation is feasible with certain restrictions for CP dynamics. Classes of samples which reveal similar CP characteristics can be analysed in this way within a reasonable time. Quantitative analyses of solid samples which contain very mobile moieties, with isotropic motions, are only feasible with pulse NMR.

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SUMMARY

In chromatography usually silica is applied as substrate for the attachment of stationary phases. In this thesis, several studies dealing with modified silica substrates are described.

The first part of this thesis discusses several investigations to optimize deactivation and coating of fused-silica capillary columns for GC and of a non-porous silica model substrate.

In the second part of this thesis, surface characterization studies of a number of selected chemically modified silicagels are described. When modified silicagels are used in RP-HPLC practice, unfavorable changes in chromatographic performance occur. The observed changes in surface structure are discussed in detail.

CHAPTER 2 of this thesis describes a solid state ^{29}Si CP-MAS NMR study after the deactivation of a model silica substrate with diphenyltetramethyl-disilazane (DPTMDS) and polydimethylsiloxane (PDMS). The formation of polymeric chains attached at the silica surface after silylation at high temperatures (T > 350°C) is discussed. The polymeric chains present after silylation shield those silanol groups, which are not removed by the silylation reaction. Upon silylation with DPTMDS no polymer chains are detected, even if the conditions selected favor polymeric growth. On the other hand, the amount and mobility of polymeric chains formed upon PDMS silylation at the silica surface depends largely on the silylation temperature.

CHAPTER 3 discusses deactivation of fused-silica capillaries and a non-porous silica model substrate with polymethylhydrosiloxane (PMHS) at moderate silylation temperatures. The inner-wall of fused-silica capillaries still contains active sites like silanol and siloxane groups, which are unfavorable for the chromatographic separation process. The effect of

subsequent coating with polymethyloctadecylsiloxane (PMODS) on the deactivating film is discussed as well.
These studies are performed with CGC and with solid state ^{29}Si CP-MAS NMR. Optimal deactivation of the fused-silica surfaces is obtained by a thick-film (film thickness, 5-10 nm) at silylation temperatures around 290 °C in the presence of a small amount of physisorbed water.
The nature and the structure of the deactivating film depends largely on the film thickness. With thick-film (*ca.* 5 nm) PMHS silylation a thin, rigid, cross-linked, chemically bonded layer is formed at the silica surface, with on top of that anchored polymeric PMHS chains. Here, the deactivating film exists of two distinct layers. The more mobile polymeric chains, which still contain reactive sites, offer adequate anchoring sites for subsequently coated and immobilized stationary phases. With thin-film (*ca.* 1 nm) PMHS deactivation only the more rigid, densely cross-linked layer is formed.
The application of short narrow-bore capillary colomns, optimally deactivated with PMHS and coated with a thick-film PMODS coating in fast GC analysis is demonstrated.

CHAPTER 4 describes the changes appearing with a number of chemically modified silicas for RP-HPLC, brought about by use in laboratory practice. The stationary phases discussed are mono-, di- en trifunctional octadecyl modified silicas silylated with equal coverages at two different silicas. The unfavorable changes are also studied by physico-chemical analysis methods like solid state ^{29}Si CP-MAS NMR and elemental analysis.
The octadecylsilane ligands are slowly stripped from the surface by aggressive mobile phases and more silanol groups appear. This ligand dissolution process causes considerable changes in chromatographic selectivity, especially with monofunctionally modified silica. With the difunctionally modified silica a severe condensation of the remaining octadecylmethyl-hydroxysilane moieties is observed after ageing experiments with high pH (pH $\approx$ 8.4) buffer solutions. Bidentates are mainly formed with

neighbouring silanes at the surface. These bidentates reduce the stripping of ligands and a better shielding of the silica underneath is obtained. With RP-HPLC characterization only small changes in selectivity are determined. Condensation of ligand silanes is also observed with trifunctionally modified silicagel. The rigid, densely cross-linked silanes prevent dissolution of the anchored ligands, but not to the same extent as for bifunctionally modified silicagels.

The RP-HPLC phases described in **CHAPTER 5** differ in *n*-alkyl chain length. The silica shielding properties by monofunctionally modified alkylsilanes with chain lengths between C_1 en C_{18} are studied with chromatography and physico-chemical analysis methods. Similar techniques as described in chapter 4 are used, together with BET-measurements. In the second part of chapter 5 dissolution of the different stationary phases in a MAS rotor is studied with ^{29}Si MAS NMR .
All measurements reveal that alkyl ligands with a C_8 or longer *n*-alkyl group show a superior shielding of the silica surface. The direct dissolution studies of the modified silicas show that short *n*-alkyl ligands dissolve also by oligomeric dissolution of the silicagel underneath. The longer alkyl ligands show efficient shielding and the stripping of *n*-alkylsilanes progresses mainly by monomer dissolution.

In **CHAPTER 6** two improved octadecyl RP-HPLC phases are discussed, which show a better resistance towards stationary phase deterioration by aggressive solvents. These phases are compared with C_{18} phases, obtained by classical modification on the same batch of silicagel.
In the first part of this chapter, an acid pretreatment of silicagel is applied to increase the amount of reactive silanol groups at the silica surface. This pretreatment involves hydrothermal washing with an 0.1% (w/w) HF solution. The amount of silanediol groups increases drastically and a 30 percent higher octadecylsilane surface concentration is obtained upon

modification. The resulting stationary phase shows a better resistance towards stationary phase stripping with high pH buffer solutions.
A second phenomenon is detected upon the HF pretreatment. By this pretreatment the small crystalline areas, originally incorporated in the silicagel, are "mechanically separated" from the amorphous part of the silica. The nuclei of these two domains experience different mobilities, which can be concluded from solid state ^{29}Si CP-MAS NMR.

In the second part of this chapter two polysiloxane coated stationary phases are characterized. The polysiloxane contains methyl groups and *n*-octadecyl side chains. Immobilisation of the polymer coating proceeds through cross-linking. One of the polymer coated phases is precapped with trimethylsilane-enolate. Solid state ^{29}Si CP-MAS NMR reveals that the polymer chains of this latter stationary phase are probably only physisorbed at the trimethylsilane modified surface. Precapping improved the resistance of the polymer coated phase against deterioration. However, a shift in selectivity, caused by high pH buffer solutions, is noticed.

The various solid state NMR techniques used in this thesis are discussed in CHAPTER 7. Solid state ^{29}Si CP-MAS NMR provides information regarding the chemistry and individual molecular mobilities of different surface moieties. Furthermore, interactions between deactivating layers and the stationary phases at the silica surface are detected.
Quantitative solid state ^{29}Si CP-MAS NMR measurements, however, are beset with difficulties. Measurements with a single contact-time are only accurate when the time constants involved in cross-polarization (T_{HSi}, $T_{1\rho H}$ and $T_{1\rho Si}$) are known. With these time constants the correction factor for the applied contact-time can be calculated.

SAMENVATTING

In de chromatografie worden veelal silica substraten gebruikt voor de aanhechting van stationaire fasen. Het onderzoek, dat in dit proefschrift beschreven wordt, omvat een aantal studies aan gemodificeerde drager-materialen voor chromatografie.
Het eerste deel van dit proefschrift behandelt een studie naar het optimalizeren van de deactivering en coating van fused-silica capillairen voor CGC.
Het tweede deel van dit proefschrift behandelt een studie naar de oppervlakte struktuur van een aantal geselekteerde chemisch gemodificeerde silicagelen. Bij gebruik van gemodificeerde silicagelen als stationaire fasen in de vloeistofchromatografie blijken ongewenste veranderingen in chromatografische eigenschappen op te treden. De veranderingen in oppervlaktestruktuur, die hierbij optreden, worden uitgebreid beschreven.

In **HOOFDSTUK 2** van dit proefschrift wordt een vaste stof ^{29}Si CP-MAS NMR studie beschreven naar de deactivering van een model silica substraat met difenyltetramethyldisilazane (DPTMDS) en polydimethylsiloxaan (PDMS). Indien er bij deactiveren een netwerk van polymere ketens aan het silicagel oppervlak gevormd wordt, worden ook de silanol groepen, die niet weggenomen zijn door silylering afgeschermd. De vorming en de eigenschappen van polymere ketens, gebonden aan het silica oppervlak bij silylering met een hoge temperatuur (T > 350°C) wordt uitgebreid geëvalueerd.
Bij silylering met DPTMDS worden geen polymere ketens gevormd, ook niet als reactiecondities gekozen worden welke de ketengroei stimuleren. Bij PDMS silylering is de beweeglijkheid en de hoeveelheid van de langere polymeer ketens, die aan het oppervlak gebonden worden afhankelijk van

de silyleringstemperatuur.

HOOFDSTUK 3 behandelt de deactivering van fused-silica capillairen en een fumed silica, als model substraat, met polymethylhydrosiloxaan (PMHS) bij lagere silyleringstemperaturen. Voor CGC geldt dat de binnenwand van de fused-silica capillairen te veel, voor chromatografie ongunstige, actieve silanol en siloxaangroepen bevat.
Tevens is de wederzijdse invloed van een specifieke coating, polymethyl-octadecylsiloxaan (PMODS), op de struktuur van de deactiverende film en vice versa onderzocht. Deze studies zijn uitgevoerd met CGC en vaste stof ^{29}Si CP-MAS NMR.
Een optimale deactivering van het silica oppervlak wordt verkregen bij dikke films (laagdikte 5-10 nm) en een silyleringstemperatuur van 290 °C, waarbij alleen aan het oppervlak gefysisorbeerd water wordt toegelaten. De struktuur van de deactiverende film blijkt sterk afhankelijk te zijn van de filmdikte. Bij dikke films (*ca.* 5 nm) is na silylering direkt aan het silica-oppervlak een chemisch gebonden dunne laag van sterk vernet polymeer aanwezig, met daarop meer beweeglijke, langere maar verankerde PMHS ketens. De totale deactiverende film bestaat dus uit twee lagen. De beweeglijke polymeerketens met de nog aanwezige reactieve groepen vormen goede aanhechtingsmogelijkheden voor stationaire fasen die op de gedeactiveerde silica gecoat worden. Bij dunne films (*ca.* 1 nm) is alleen het chemisch gebonden sterk vernette polymeer aanwezig.
Toepassingen van optimaal met PMHS gedeactiveerde en met een dikke PMODS film bedekte kolommen met een kleine diameter worden gedemonstreerd.

HOOFDSTUK 4 behandelt de veranderingen van een aantal chemische gemodificeerde silicagelen door gebruik in de praktijk. De onderzochte fasen zijn mono-, di- en trifunctioneel chemisch gebonden octadecylsilaan silicas afgeleid van twee verschillende silicagelen. De oorzaken van ongewenste

veranderingen, die optreden in RP-HPLC, worden ook bestudeerd met fysisch-chemische technieken als vaste stof ^{29}Si CP-MAS NMR en element analyse.
De octadecylsilaanliganden worden bij gebruik met agressieve mobiele fasen langzaam van het oppervlak los gemaakt, waarbij silanol groepen gevormd worden. Dit proces veroorzaakt vooral bij monofunctioneel gemodificeerde silicagelen de selektiviteitsveranderingen die waargenomen worden in RP-HPLC. Bij difunctioneel gemodificeerde silicagelen treedt condensatie van de overgebleven silaanhydroxyl groepen op, in het bijzonder indien mobiele fasen met een hoge pH (pH ≈ 8.4) gebruikt worden. De bidentaten die gevormd worden (hoofdzakelijk met nabuur silanen) voorkomen dat meer liganden van het oppervlak verdwijnen. De stationaire fase is dus meer resistent, ook doordat de onderliggende silicagel beter afgeschermd wordt. In de RP-HPLC wordt slechts een kleine verschuiving van selektiviteiten waargenomen. Bij trifunctioneel gemodificeerde silicagelen treedt eveneens condensatie van ligand-silanen op. Het onderliggende silica wordt echter niet zo goed afgeschermd als bij de difunctionele bidentaten het geval is.

De RP-HPLC fasen, die in **HOOFDSTUK 5** onderzocht zijn verschillen in *n*-alkyl ketenlengten van de liganden. De afscherming van silicagel door monofunctioneel gemodificeerde alkylsilanen met ketenlengten tussen C_1 en C_{18} is zowel met chromatografie als met fysisch-chemische analyse-technieken onderzocht. De in hoofdstuk 4 toegepaste analyse-technieken zijn aangevuld met een uitgebreide BET-analyse. In het tweede deel van hoofdstuk 5 wordt het oplossen van de verschillende stationaire fasen direkt in de MAS rotor met ^{29}Si MAS NMR gevolgd.
Uit alle metingen blijkt dat alkylliganden met een *n*-alkyl ketenlengte van C_8 of langer een betere afscherming van het silicageloppervlak geven. Uit de direkte metingen aan het oplosproces van de stationaire fasen wordt duidelijk dat korte *n*-alkyl liganden mede verdwijnen doordat de

onderliggende silicagel als kleine oligomere deeltjes oplost. Bij langere liganden treedt een veel sterkere monolaag afscherming op en lossen de *n*-alkylsilanen voornamelijk op als monomeer.

In **HOOFDSTUK 6** worden twee "verbeterde" typen octadecyl RP-HPLC fasen geëvalueerd op hun resistentie tegen agressieve mobiele fasen. Deze fasen worden vergeleken met op klassieke wijze gemodificeerde C_{18} tegenhangers op identieke silicagel. In het eerste deel van dit hoofdstuk wordt een voorbehandeling met zuur van het silicagel toegepast om meer reactieve silanolgroepen aan het silicaoppervlak te krijgen. Deze voorbehandeling met een 0.1% (w/w) HF oplossing vergroot ook het aantal silaandiol groepen, waardoor een 30 procent hogere belading met octadecylsilanen mogelijk blijkt. De resulterende fase vertoont mede hierdoor een betere resistentie tegen mobiele fasen met een hoge pH.
Er treedt nog een tweede effect op met deze HF voorbehandeling. Door de etsende werking van HF worden kleine kristallijne gebieden, die aanwezig zijn aan het silicageloppervlak, "mechanisch losgekoppeld" van het amorfe deel van de silica. De siliciumkernen in deze twee domeinen ondervinden verschillende beweeglijkheden, die nu met vaste stof ^{29}Si CP-MAS NMR te detecteren zijn.

In het tweede deel van dit hoofdstuk worden een tweetal met polysiloxaan gecoate stationaire fasen bestudeerd. Het polysiloxaan dat gebruikt wordt als coating bevat methylgroepen en *n*-octadecyl zijketens. De immobilisatie van de polymere coating geschiedt door vernetting. Eén van de twee met polymeer gecoate fasen is van te voren gesilyleerd met enolaattrimethylsilaan, waardoor de polymere ketens gefysisorbeerd op de trimethylsilanen komen te liggen, zonder chemische binding met het oppervlak. De trimethylsilaan voorbehandeling verbetert de resistentie van de polymeer gecoate fase.

In **HOOFDSTUK 7** worden de diverse vaste stof kernspinresonantie-spectrometrie technieken, die in dit proefschrift gebruikt zijn, geëvalueerd. Uit dit proefschrift blijkt dat het gebruik van de ^{1}H-^{29}Si kruis-polarizatie de voorkeur verdient voor de analyse van silicaoppervlakken. Met deze methode wordt bruikbare informatie verkregen betreffende de chemie van de aanhechting en de interacties tussen de deactiverende film en de stationaire fasen aan het silicaoppervlak. Verder is het mogelijk met vaste stof ^{29}Si CP-MAS NMR de individuele moleculaire beweeglijkheden van de groepen in het monster te detecteren.
Kwantitatieve vaste stof ^{29}Si CP-MAS NMR metingen zijn inherent moeilijk. Bepalingen bij één contact-tijd kunnen betrouwbaar uitgevoerd worden, indien de tijdskonstanten die het kruis-polarisatie proces bepalen (T_{HSi}, $T_{1\rho H}$ en $T_{1\rho Si}$) bekend zijn, zodat de correctiefactor voor de gebruikte contact-tijd berekend kan worden.

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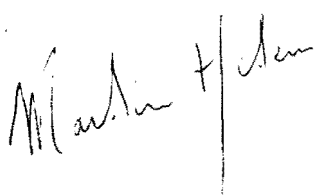

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STELLINGEN

behorende bij het proefschrift

A FUNDAMENTAL STUDY OF CHEMICALLY MODIFIED SILICA SURFACES IN CHROMATOGRAPHY

van

Martin J.J. Hetem

1 **Het gebruik van diphenyltetramethyldisilazaan voor de deactivering van kapillaire scheidingskolommen veroorzaakt niet de door Reiher beschreven polymerisatie van dimethylsiloxaanketens aan het oppervlak.**

T. Reiher, J. HRC & CC, 10 (1987) 159-161.

2 **De toepassing van silicagelen gemodificeerd met multifunctionele ligandsilanen leidt tot stabielere stationaire fasen.**

Dit proefschrift hoofdstuk 4.

3 **Het opnemen van kruispolarisatie-kurven bij de toepassing van ^{29}Si CP-MAS NMR aan gemodificeerde silicagelen is noodzakelijk voor de optimalisering van meetkondities voor kwantitatieve analyse.**

J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, J. Chromatogr. 352 (1986) 275-305.
Dit proefschrift hoofdstuk 7.

4 **Het gebruik van één nomenclatuur voor silicium-structuren in silicaat oplossingen en in silicagelen is nodig voor grensvlakverleggend onderzoek.**

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5 **De molekuulgewichtsbepaling van styreen oligomeren met behulp van dichtheid geprogrammeerde SFC gekombineerd met UV-absorptie detectie levert foutieve waarden.**

Y. Hirata, F. Nakata, Chromatographia, 21 (1986) 627-630.

6 **Korte alkylketens gemodificeerde silicagelen verouderen versneld onder chromatografische kondities door het oplossen van de bulksilica via silicaat-oligomeren.**

Dit proefschrift hoofdstuk 5.

7 **De invoering van een uniforme test voor de karakterisering van de kwaliteit, de selektiviteit en de aktiviteit van kapillaire scheidingskolommen in GC zal de verstandhouding verbeteren tussen enerzijds de kolommakers en anderzijds de kolomgebruikers.**

K. Grob jr., G. Grob, K. Grob, J. Chromatogr., 156 (1978) 1-20.

8 **Onder de auteurs van de navolgende publikatie is kennelijk een expert verantwoordelijk voor de coordinatie van de bijdragen van de mede-auteurs.**

"Expert Systems for Chemical Analysis (ESPRIT Project 1570)" by D. Goulder, T. Blaffert, A. Blokland, L. Buydens, A. Chhabra, A. Cleland, N. Dunand, H. Hindriks, G. Kateman, H. van Leeuwen, D. Massart, M. Mulholland, G. Musch, P. Naish, A. Peeters, G. Postma, P. Schoenmakers, M. de Smet, B. Vanderginste, J. Vink, Chromatographia, 26 (1988) 237-243.

9 **Klaas Vaak was de eerste die het belang van gemodificeerde silica naar waarde schatte.**

Martin J.J. Hetem Eindhoven, 19 juni 1990.