

Effect of acid pretreatment of the silica substrate on the stability of octadecyl modified reversed phases

Citation for published version (APA):

Hetem, M. J. J., Haan, de, J. W., Claessens, H. A., Mussche, P., & Cramers, C. A. M. G. (1990). Effect of acid pretreatment of the silica substrate on the stability of octadecyl modified reversed phases. *Chromatographia*, 29(9-10), 473-481. <https://doi.org/10.1007/BF02261397>

DOI:

[10.1007/BF02261397](https://doi.org/10.1007/BF02261397)

Document status and date:

Published: 01/01/1990

Document Version:

Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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Effect of Acid Pretreatment of the Silica Substrate on the Stability of Octadecyl Modified Reversed Phases

M. Hetem^{1*}/J. de Haan¹/H. Claessens¹/Ph. Mussche²/C. Cramers¹

¹Eindhoven University of Technology, Department of Chemical Technology, Laboratory of Instrumental Analysis, P.O. Box 513, NL-5600 MB Eindhoven, The Netherlands

²Chrompack International B.V., P.O. Box 8033, NL-4330 EA Middelburg, The Netherlands

Key Words

Column liquid chromatography
Stability of reversed phases
Acid pretreatment of silica
Solid state ²⁹Si NMR
Crystalline regions

Summary

Two stationary phases, identically modified and derived from the same experimental Chromspher substrate, but one of which has been pretreated with a hydrofluoric acid solution, are compared after ageing with various eluents under simulated routine conditions. The hydrothermal hydrofluoric acid treatment of the silica substrate prior to modification with dimethyloctadecyl-silane improved both the surface coverage and the stability of the resulting stationary phase compared to those of the original substrate. The stability of the silica-to-silane bonding after modification was characterized by means of chromatographic techniques and elemental analysis. Changes in the rigidity of the substrate were studied by means of solid state ²⁹Si NMR. The effect of the hydrothermal acid treatment on relevant substrate parameters, including formation of crystalline regions at the surface, is also discussed.

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 with relatively aggressive eluents [1–3]. The long term stability of these stationary phases in laboratory practice is limited by hydrolysis of ligands from the surface and hydrolysis of the silica substrate [4–6]. The use of aqueous buffer solutions with pH values below 3 or above 8 and eluents with a relatively high salt content drastically decrease the useful lifetime of RP-HPLC columns [1, 4, 6].

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 proprietary stationary phases. Here, we report on a study into the changes in surface properties brought about by a hydrothermal acidic treatment of the silica substrate before modification. The effect of this treatment on the stability of modified octadecyl silane ligands is studied by ageing these phases under conditions which simulate routine use. Changes in properties after ageing are characterized and partially quantified as reported in earlier publications [4, 6].

The two different octadecylsilane RP-HPLC stationary phases discussed here 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. The second stationary phase was synthesized on the original substrate. The hydrothermal acidic treatment altered some important surface properties, as witnessed by an increase in the silanediol/silanol ratio and ligand density after subsequent modification. We also found inhomogeneous areas of small crystalline micro-structures at the surface of the pretreated substrate. In this work 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, when exposed to ageing, was superior to that of the non-pretreated stationary phase.

Experimental

Materials

The test components used for chromatographic characterization were all of reference grade. The alkylbenzenes used (test mixture 1) were benzene, methylbenzene, ethylbenzene, propylbenzene, and butylbenzene. The alkyl aryl ketones used (test mixture 2) as test compounds were ethanophenone, propanophenone, butanophenone, pentanophenone, hexanophenone and octanophenone (Pierce Chemical

Corp., Rockford, IL, USA). All other solvents and chemicals used, both in simulating routine experiments and in chromatographic characterization, were of analytical grade (E. Merck, Darmstadt, FRG.). The deionized water exhibited an electrical resistance exceeding $10 \text{ Mohm}\cdot\text{cm}^{-1}$, (Milli-Q system, Millipore Corp., Bedford, USA). All eluents were freshly prepared and filtered through $0.22 \mu\text{m}$ membrane filters (Millipore Corp., Bedford, USA) prior to use.

Chromatography

The two octadecyl stationary phases which form the subject of this study were produced by modification of the same substrate, an experimental Chromspher silica (Chrompack B.V., Middelburg, NL). Part of the silica that had been hydrated according to standard procedures was dehydrated at 450°C . Subsequent hydrothermal treatment with a 0.1 % (w/w) solution of hydrofluoric acid in deionized water at 95°C for 2 hours, increased the amount of the 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 I). The two substrates, untreated and pretreated, were modified with dimethylamine-dimethyloctadecylsilane (DMA-DMODS) [7, 8] 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. The n-pentane was removed by evacuation and, subsequently, the reaction mixture was refluxed at 220°C under a nitrogen atmosphere for 18 hours. The modified silicas were then filtered and thoroughly washed successively with n-pentane, dry acetone and methanol. The two reversed phase silicas thus prepared are referred to in the following discussion as octadecyl Chromspher silica (ODCS) and pretreated octadecyl Chromspher silica (PODCS), respectively. The bulk properties of the modified stationary phases are listed in Table II. The carbon content of the modified C_{18} stationary phases, prior to and after simulated routine use, was obtained with a Perkin Elmer Analyzer, model 240 (Perkin Elmer Corp., Norwalk, CT, USA).

Each stationary phase was used to pack seven identical Swagelock columns, $100 \text{ mm} \times 4.6 \text{ mm}$, i.d. (Crawford Fitting Company, Solon, OH, USA.) 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, while the remaining column was used as a reference column for initial chromatographic characterization. The equipment and procedure for simulated intensive routine use have been formerly described in detail [4]. The basic and acidic aqueous and methanol/aqueous buffers used for ageing were identical as before [6], see Table III. After ageing, the columns were subjected to chromatographic characterization as previously extensively discussed [6], with the two series of homologues, alkylbenzenes and alkyl aryl ketones, at suitable eluent compositions. These chromatographic experiments were performed with a model 100A pump (Beckman Instruments,

Table I Bulk properties of the two silica substrates prior to silane modification; A = untreated Chromspher silica, B = Chromspher silica after the hydrothermal acidic treatment.

	Silica A	Silica B
Mean particle size (μm)	5 ± 1.5	5 ± 1.8
Mean pore size (nm)	7,	7,5
SBET, Specific area (m^2/g)	152,	141,
Pore volume (cm^3/g)	,51	,50
Ion content (ppm) (1)		
- Sodium:	4200,	1500,
- Aluminum:	400,	300,
- Iron:	190,	130,
- Nickel:	20,	50,
relative silanol ratio (2)		
- vicinal/"lone":	1,00	0,88
- geminal:	-	0,12

- (1) determined by atomic absorption spectrometry (AAS); calcium and copper content below 5 ppm.
 (2) determined by solid state ^{29}Si MAS NMR.

Table II 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 ± 1.5	5 ± 1.8
Mean pore size (nm)	6,	6,
SBET, Specific area (m^2/g)	93,	87,
Pore volume (cm^3/g)	0,285	0,286
Ion content (ppm) (1)		
- Sodium:	1500,	200,
- Aluminum:	200,	100,
- Iron:	120,	110,
- Nickel:	50,	100,
Carbon content P_c (w/w) % (2)	10,06	11,59
Ligand surface density		
α_1 (see [6], eq. (1) ($\mu\text{mol} \cdot \text{m}^{-2}$))	3,16	4,03

- (1) determined by AAS; calcium and copper content below 5 ppm.
 (2) determined by elemental analysis.

Berkeley, CA, USA), a model CV-6-VHPa-N60 injection valve equipped with a $20 \mu\text{l}$ loop (Valco, Houston, TX, USA) and a model LC-3 variable wavelength UV-detector (Pye Unicam, Cambridge, UK) operated at 254 nm. Typically volumes of 5–10 μl of the test mixtures were injected. The detector signal was sampled at 10 Hz and integrated with a Nelson 3000 data system (Nelson Analytical, Cupertino, CA, USA).

Solid State ^{29}Si CP-MAS 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 180–

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

Ageing experiment 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 Carbonate	8.4	0	-
5	0.05 M Carbonate	8.4	0.5	-
6	0.05 M Carbonate	8.4	0.5	Triethylamine

220 mg were spun at ca. 3.5 kHz using aluminum oxide rotors of the standard Bruker double bearing type. Solid state ^{29}Si cross-polarization magic angle spinning (CP-MAS) NMR spectra of all the modified C_{18} stationary phases prior to and after simulated routine treatments were obtained with a cross-polarization contact time of 6 ms. For the ^{29}Si CP-MAS NMR spectra of both silica substrates a contact time of 2 ms. was applied. A spin-temperature-alternated CP sequence with quadrature cycling and flip-back of the ^1H nuclei was applied to eliminate experimental artifacts. The pulse interval was 1 s.

Typically 2000 free induction decays (FIDs) with an acquisition time of 10 ms. were accumulated in 1 K data points, zero filled to 8 K prior to Fourier transformation. The line broadening used was 20 Hz prior to zero-filling and Fourier transformation. The spectral width for all spectra was 20 kHz. The different types of surface silane/siloxane groups, with their ^{29}Si NMR chemical shifts, present at the surface have already been reported [6]. Experiments with variable contacts measuring the characteristic CP-behaviour were carried out for both silica substrates and the two octadecyl modified reversed phases. 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 as described above. For a quantitative comparison ^{29}Si Bloch decay magic angle spinning (MAS) NMR spectra of both silica substrates were obtained with a pulse interval of 90 s and typically 1000 FIDs were accumulated.

Results and Discussion

Characterization

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 Figures 1 and 2. The structures of the siliceous moieties most relevant to this paper are depicted in Figure 3.

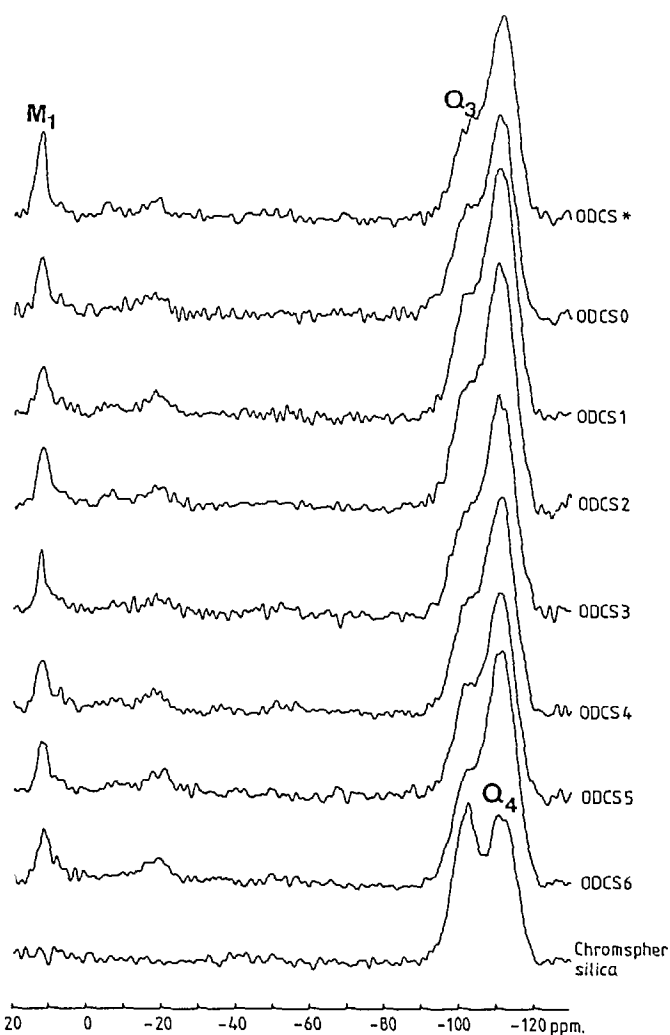


Figure 1

^{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., pulse interval time 1 s., acquisition time 10 ms., line broadening 20 Hz.

The roman numerals indicate the typical ageing experiments as outlined in Table III.

From these spectra the relative surface concentration of all siliceous moieties can be calibrated. The results of spectroscopic measurements and elemental analysis are summarized in Tables IV and V for our untreated and treated substrates respectively. Although the

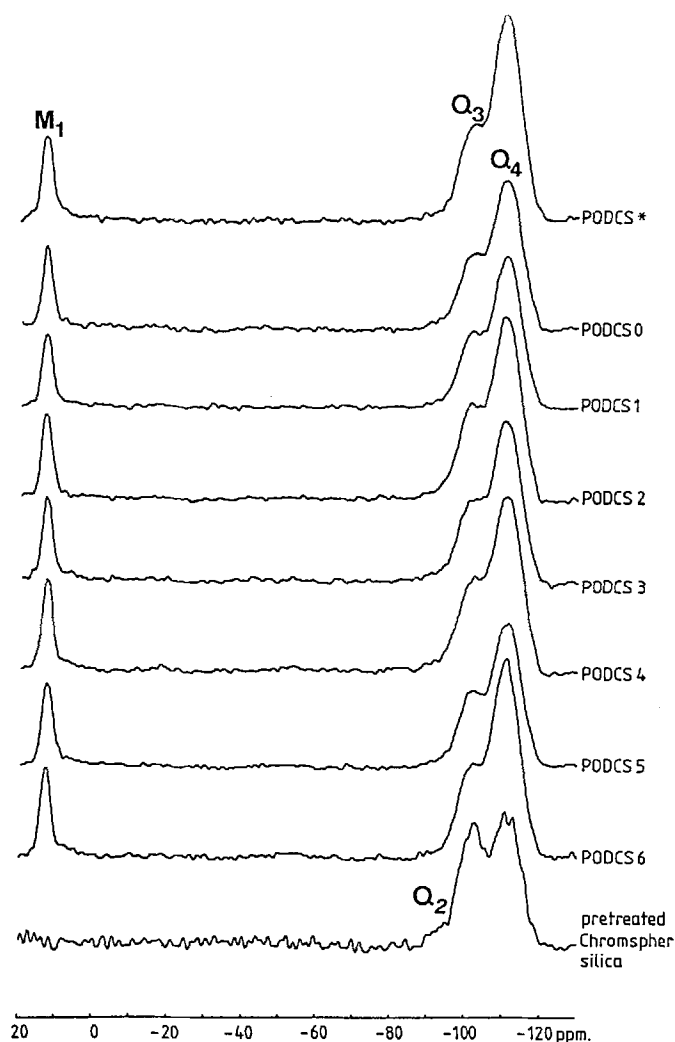


Figure 2
²⁹Si CP-MAS NMR spectra of the pretreated Chromspher silica substrate and PODCS phases before and after each treatment. *N_s* = 2000, contact time 6 ms., pulse interval time 1 s., acquisition time 10 ms., line broadening 20 Hz. The roman numerals indicate the typical ageing experiments as outlined in Table III.

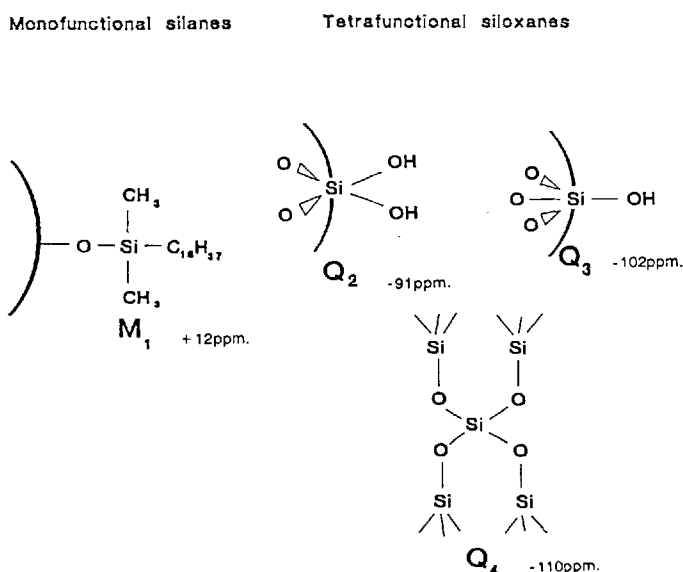


Figure 3
 Structure of siliceous moieties most relevant to this paper, notation and ²⁹Si chemical shift.

results from neither the elemental analysis nor the solid state ²⁹Si CP-MAS NMR show a perfect match the results for both methods before and after the simulation of intensive use 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 of this phenomenon is the formation of more reactive geminal silanol groups (Q₂, 12% more) during this pretreatment. These geminal silanols are formed when siloxane bridges at the surface of the silica substrate are extensively hydrolysed.

As studied by Lork [9] hydrothermal acidic treatments of "soft" silica gels may very well introduce small hexagonal crystalline domains most probably β-tridymite, at the surface. In particular, a high level of

Table IV Results of elemental analysis and solid state ²⁹Si 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 III. The ODCS* and ODSC0 phase denote the original stationary phases before and after packing/chromatographic characterization, respectively.

	relative % Carbon	Ligand density α ₁ (eq. 1 [6]) (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. (eq. 7 [6]):		3.67 %	20.3 %	

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

	relative % Carbon	Ligand density α_1 (eq. 1 [6]) ($\mu\text{mol} \cdot \text{m}^{-2}$)	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. (eq. 7 [6]):		2.46 %	9.10 %	

ionic impurities, as we have in the present case, will enhance the formation and stability of the β -tridymite domains. Feher et al. [10] reported the formation of β -tridymite-like structures from heptameric-trisilanol structures. In our opinion the leaching of silicas with relatively high impurity levels could also lead to heptameric-hexasilanol, and consequently to β -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 4 and Figure 5 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, $T_{1\rho\text{H}}$. Both the 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 respectively, of the Chromspher silica show an approximately single exponential behaviour [11, 12]. The same is true for the dimethyloctadecylsiloxysilane, M_1 , moiety (at $+12$ ppm), and the Q_3 and Q_4 CP curves of the monofunctionally octadecylsilane 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. We assume the coexistence of at least two different regions present at the surface: amorphous silica, with relatively large T_{HSi} , and $T_{1\rho\text{H}}$ values, and crystalline micro-structures, β -tridymite-like, with small T_{HSi} , and $T_{1\rho\text{H}}$ values. This hypothesis

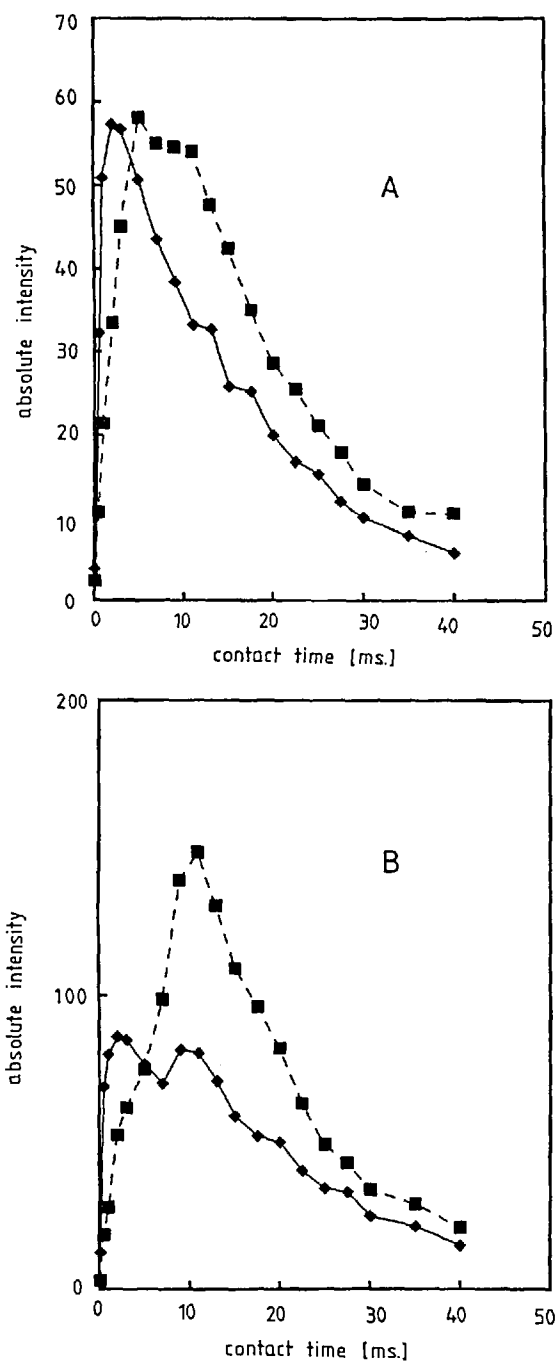


Figure 4 Characteristic CP-behaviour of ^{29}Si atoms in Chromspher silica [A] and hydrothermally acidic treated Chromspher silica [B].
 $-\diamond-$ Q_3 : -102 ppm; $-\square-$ Q_4 : -110 ppm.

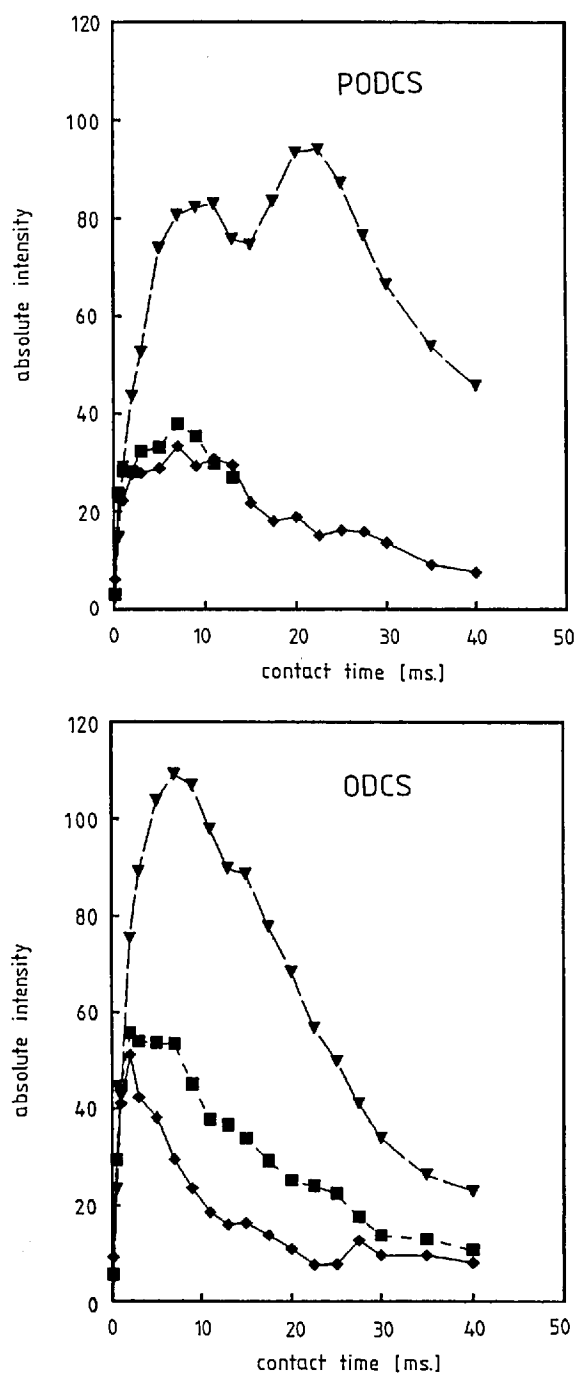


Figure 5
Characteristic CP-behaviour of ^{29}Si nuclei in ODCS and PODCS stationary phases.
-♦- +12 ppm; -■- -102 ppm; -▼- -110 ppm.

is in agreement with the considerable wash out of foreign ions from the silica substrate (mainly sodium ions) by the hydrothermal acidic treatment.

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 had already lost a considerable amount of alkyl chains during 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 physically adsorbed 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 the reference for further comparison.

The relative deviation (RD) of the reported values, see Tables IV and V, were calibrated by comparing the values determined before and after ageing treatments relative to the value before, as calibrated by Eq. (7) [6]. The RD 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 RD 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, as discussed before [6]. 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, inevitably influences the ratio of M_1 to Q_3 given in Tables IV and V. In this case the RD value will be relatively large.

Chromatography

From the chromatograms of alkylbenzenes (test mixture 1) and alkyl aryl ketones (test mixture 2) the capacity factors were determined before and after the ageing experiments. Also for each column the separation efficiency, $N(\text{sys})$, 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 a more heterogeneous size distribution of the PODCS particles the separation efficiency of all packed columns with the PODCS phase was 3 to 5 % lower than that of the identically packed ODCS columns. The chromatograms of the alkylbenzene test mixture before and after the simulated ageing experiments 1, 5 and 6 of the PODCS reversed phase are depicted in Figure 6. 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 7. From this type of diagram 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 Eq. (2) and (3), previously published by our group [6], 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). By the use of Eq. (5a) and (5b) [6] the derived values for p and q were calculated.

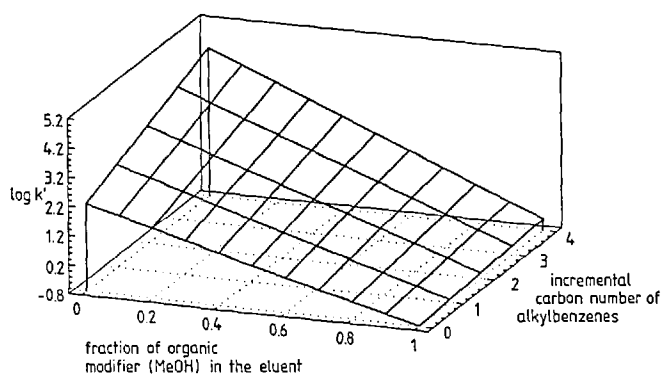
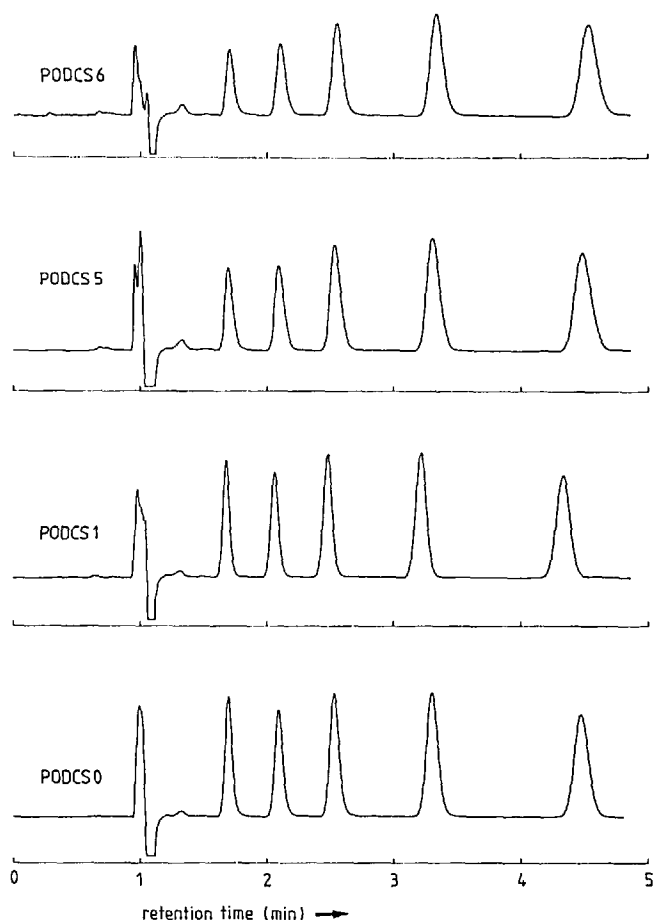


Figure 7

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

Figure 6

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

Table VI Chromatographic characterization parameters, determined with regression and the correlation coefficient for regression for the **alkylbenzene** homologous series on the **ODCS** phase before and after each ageing experiment. Experimental conditions as outlined in Table III.

	a_0	m_0	p	q	r	$N(\text{Sys}) (1)$
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

(1) Determined for butylbenzene eluted with an eluent containing 80 (v/v) % of methanol. Before the ageing experiments columns 1 to 6 showed separation efficiencies of $N(\text{sys}) = 6800 \pm 200$.

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

	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

Table VIII Chromatographic characterization parameters, determined by regression and the correlation coefficient for regression for the **alkylbenzene** homologous series on the **PODCS** phase before and after each ageing experiment. Experimental conditions as outlined in Table III.

	a_0	m_0	p	q	r	N (Sys) (1)
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

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

Table IX Chromatographic characterization parameters, determined by regression and the correlation coefficient for regression for the **alkyl aryl ketone** homologous series on the **PODCS** phase before and after each ageing experiment. Experimental conditions as outlined in Table III.

	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

Tables VI and 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 VIII and 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 by use of the regression model used in this study. An exception are the stationary phases after ageing experiment 4. The eluent used with this experiment apparently caused 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 VI and VIII for the ODCS and PODCS phases respectively, represents the capacity factor of these stationary phases.

As expected, the separation efficiency of both stationary phases decreased after prolonged 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 the 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 by the ageing. The typical CP-curves with shoulders or double maxima of M_1 , Q_3 and Q_4 ^{29}Si nuclei changed back to an almost normal CP-behaviour as given by the untreated Chromospher 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 $T_{1\rho\text{H}}$ values were still larger than for ODCS phases.

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 Table VI to 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 true for the most apolar residue of a homologous series of test solutes used in this study, the alkyl benzene 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 (alkyl benzenes) increased after all ageing experiments at high pH, indicating increased lipophilic selectivity. It may be concluded that the a_0 and m_0 values of the 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 in a previous study [6], where α_1 was about $2.55 \mu\text{mol} \cdot \text{m}^{-2}$. In our opinion, only part of the densely stacked C_{18} ligands of the stationary phase before ageing were 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 alkyl aryl ketones, the value of q did not change significantly after prolonged exposure to relatively aggressive eluents.

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, β -tridymite-like, will be present at the silica surface. This study shows that after this acid treated silica gel is subjected to monofunctional modification with octadecyl silanes it 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 due to substrate hydrolysis. It can be concluded that the crystalline

regions were formed close to the substrate surface and were destroyed by hydrolysis.

After the hydrothermal treatment with a 0.1 (w/w) % hydrofluoric acid solution the silica surface also contained about 12 % 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 % higher octadecylsilane ligand density, which is presumed to be due to 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 in 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 the characterization procedures used in this study.

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Received: Jan. 15, 1990

Accepted: Jan. 31, 1990

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