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STUDY OF THE CHANGES IN MONO-, DI- AND TRIFUNCTIONAL OCTADECYL-MODIFIED PACKINGS FOR REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIFFERENT ELUENT COMPOSITIONS

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SUMMARY

Two different types of silica substrates uniformly modified with reversed-phase high-performance liquid chromatographic (RP-HPLC) phases obtained with mono-, di- and trifunctional octadecylsilanes were subjected to artificial ageing under simulated routine conditions and were subsequently analysed and evaluated. Changes in the properties of the bonded phase packings were characterized and quantified with chromatographic techniques, solid-state NMR and elemental analysis. These changes were correlated with selectivity, loss of silanes, gain in silanol content and rearrangement of the silica to silane bonding. When eluents of extremely high pH were used, the multifunctional octadecylsilane stationary phases showed a higher resistance towards ligand stripping. The substrate shielding of difunctional octadecylsilanes showed superior characteristics due to direct surface attachments. The rigidity of the silica substrate used for modification influences the stability of the RP-HPLC phases, especially for the monofunctionally modified octadecylsilane stationary phase. With the use of multifunctional C_{18} silanes for modification, the influence of the characteristics of the substrate on the stability of the RP-HPLC phases was reduced compared with monofunctional C_{18}. In contrast to monofunctionally modified phases, the polar selectivity of the multifunctionally modified phases remained more or less constant after intensive use with aggressive eluents. A chromatographic characterization method for obtaining information about the stability, capacity, selectivity and silica degradation of different RP-HPLC stationary phases is also presented.

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]. The significance of these studies becomes clear on considering the still increasing popularity of liquid–solid partition reversed-phase chromatography (RP-HPLC). The wide variations 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 such as 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, e.g., chloro and ethoxy groups[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 the synthesis of chemically bonded phases not only determine the selectivity, but also influence their stability towards hydrolysis. Aggressive eluents used as mobile phases 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 slowly, after use for some time the properties of the stationary phase alter, indicated by changes in the capacity and eventually also in the selectivity of the reversed phase.

In this study, 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 altogether, were examined by 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-, di- and even tridentate linkages 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 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 was investigated by subjecting the six modified phases to artificial ageing under simulated routine conditions.

CHARACTERIZATION

In order to describe the qualitative and quantitative changes in the RP-HPLC stationary phases, 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 surface area, particle size, pore size distribution and total volume of the pores of the substrate[19-21]. Possible contamination 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 surface area of the substrate, the average ligand surface density (α_1) can be calculated with an equation derived by Berendsen[14]:

$$\alpha_1 \ (\text{mol m}^{-2}) = \frac{P_c}{S_{BET}[M_c - P_c(M_1 - 1)]} \tag{1}$$

where P_c = the amount of carbon (g/g), S_{BET} = specific BET surface area of the substrate (m² g^{-1}), M_c = the amount of carbon per mole of bonded silane (g mol^{-1}) and M_1 = molecular weight of the silane molecule (g mol^{-1}).

(b) Characterization of surface structure elements should include the nature of various groups present on 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 the 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 on 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 stationary phases should be aimed at in order to relate the results of other stationary phase characterization methods. In this study, the chromatographic experiments were 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 such as phenols, anilines or nitrobenzene, which are indicators of 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 funda-mental chromatographic characterizations 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 relationship between the logarithm of the capacity factors, k', of a homologous series and the eluent composition:

$$\log k' = a - mx \tag{2}$$

and

$$a = a_o + a_1 n_c \tag{3a}$$

$$m = m_o + m_1 n_c \tag{3b}$$

where x is 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. 3a and 3b, Jandera[25,26] derived

$$m = q + pa \tag{4}$$

where

$$p = m_1/a_1 \tag{5a}$$

$$q = m_o - pa_o \tag{5b}$$

As already shown by Jandera, the value of p is independent of the stationary phase. An approximate theoretical calculation of the value of p depends only on the type of eluent, especially the organic modifier[26]:

$$p \approx 2\left(1 - \frac{I_{org}}{I_{H_2O}}\right) \tag{6}$$

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. 6, the theoretical value of p is 0.86 and should be independent of the fraction of organic modifier used.

The constants m_o and q in eqn. 5b describe the selectivity[26]: m_o mainly non-specific, lipophilic selectivity and q specific, polar selectivity. The value of m_o 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 of q should depend more on the nature of the homologous series residue or on the stationary phase involved for characterization. The a_o value equals log k' of the molecular residue (see eqns. 2 and 3a). The a_o value of an essentially non-polar residue such as benzene should give a good indication of the phase ratio of the stationary phase used. A decrease in this a_o value expresses the loss of attached ligands. The a_o value of alkyl aryl ketones includes 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.

EXPERIMENTAL

Materials

The test components used for chromatographic characterization were all of reference grade. Alkylbenzenes (test mixtue 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, IL, U.S.A.). All other solvents and chemicals used for simulating routine treatments and chromatographic characterization were of analytical-reagent grade (E. Merck, Darmstadt, F.R.G.) and demineralized, deionized water (Millipore Milli-Q system, Millipore, Bedford, MA, U.S.A.) was used. 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 methylchlorooctadecylsilane, dimethylchlorooctadecylsilane 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 I.

TABLE I

BULK PROPERTIES OF THE SILICA SUBSTRATES

Property	Hypersil	Nucleosil 100-7
Batch No.	5-192	5061
Mean particle size (μm)	5 ± 1.5	7 ± 1.5
Mean pore size (nm)	12	10
Specific surface area (m^2/g)	170	350
Pore volume (ml/g)	0.7	1.0
Iron content (μg/g)	377 ± 20	76 ± 10
Total silanol content[a] (ratio $\times$ 100%)	15.6	32.8
Relative silanol ratios ($\times$ 100%)[a]		
Dihydroxysiloxane	13.5	11.6
Hydroxysiloxane	86.5	88.4
Silanol density at the surface (contents $\times$ 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 by dividing the total silanol content by the specific surface area.

A 25-g amount of silica substrate was activated at 160°C under reduced pressure for 24 h. A sample of 0.120 mol of dimethylchloro-, methyldichloro- or trichlorooctadecyl silane was melted and dissolved in 100 ml of dry methylene chloride together with 0.15 mol of 2,6-lutidine. The mixture was added to the activated silica still under vacuum and with vigorous stirring to avoid clotting. The reaction mixture was refluxed for 5 h. The silica was then filtered and thoroughly washed with dry methylene chloride, ethanol, ethanol–water (50:50) and finally diethyl ether. The modified silicas were then dried at 50°C for 72 h. 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 was described previously[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 240 h. Basic and acidic aqueous and methanol–aqueous buffers were used (see Table II). The buffer solutions were prepared with equal amounts of buffer salt (calibrated for a total volume of 1 l of 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 II

TREATMENTS FOR SIMULATING ROUTINE-USE EXPERIMENTS

Each column purged with 7000 column volumes of a typical purging eluent; flow-rate 0.5 ml/min; time 240 h; ambient temperature.

Treat- ment No.	Buffer	pH	Volume fraction of methanol in the eluent	Ion pairing agent and concentration (M)
I	0.05 M phosphate	3.0	0	—
II	0.05 M phosphate	3.0	0.5	—
III	0.05 M phosphate	3.0	0.5	Hexylsulphonate (0.005)
IV	0.05 M hydrogencarbonate	8.4	0	—
V	0.05 M hydrogencarbonate	8.4	0.5	—
VI	0.05 M hydrogeancarbonate	8.4	0.5	Triethylamine (0.005)

In order to include a study of the effect of additional ionic species on bonded phases, at high pH a basic and at low pH an acidic 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. As already discussed by Claessens et al.[1], possible saturation of the eluent with dissolved silica and/or ligands could reduce the ageing effect of the treatment. The influence of multiple sample introduction on 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 phase 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 characterization with the two series of homologues, alkylbenzenes and alkyl aryl ketones, at a suitable eluent composition. These chromatographic experiments were performed with a Model 100 A pump (Beckman, Berkely, 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 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 simulating routine treatment was 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 carbon content, P_c, and the ligand surface density, α_1, of the starting materials are given in Table III.

TABLE III

OCTADECYLSILANE-MODIFIED RP-HPLC STATIONARY PHASES UNDER STUDY, NOTA-
TION, CARBON CONTENTS AND LIGAND SURFACE DENSITIES

Substrate	Modification	Abbre- viation	Carbon content, P_c (%, w/w)	Ligand surface density, α_1 (eqn. 1) ($\mu mol \ m^{-2}$)
Nucleosil 100-7, batch 5061	Dimethylchlorooctadecylsilane	NMO	15.9	2.38
	Methyldichlorooctadecylsilane	NDI	15.6	2.52
	Trichlorooctadecylsilane	NTR	15.6	2.62
Hypersil, batch 5-192	Dimethylchlorooctadecylsilane	HMO	8.5	2.34
	Methyldichlorooctadecylsilane	HDI	8.7	2.55
	Trichlorooctadecylsilane	HTR	8.6	2.63

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 aluminium oxide rotors 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 Bloch
pulse MAS NMR is a time consuming technique but, when the 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. Typically
1000 FIDs (free induction decays) with an acquisition time of 10 ms were accumulated
in 1K data points, 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
simulated routine treatment were obtained with a cross-polarization contact time of
6 ms for the monofunctionally 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 atoms 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 data points, zero-filled to 8K 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.

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 chromato-
graphy itself. Fig. 1 depicts the chromatograms of alkylbenzenes (test mixture 1) eluted
on methyldichlorooctadecylsilane-modified Nucleosil phases (NDI), before and after
treatment III and IV. The characterization of the changes at the surface of the modified
substrates after intensive use, the chemistry of the ageing proces, the impact on
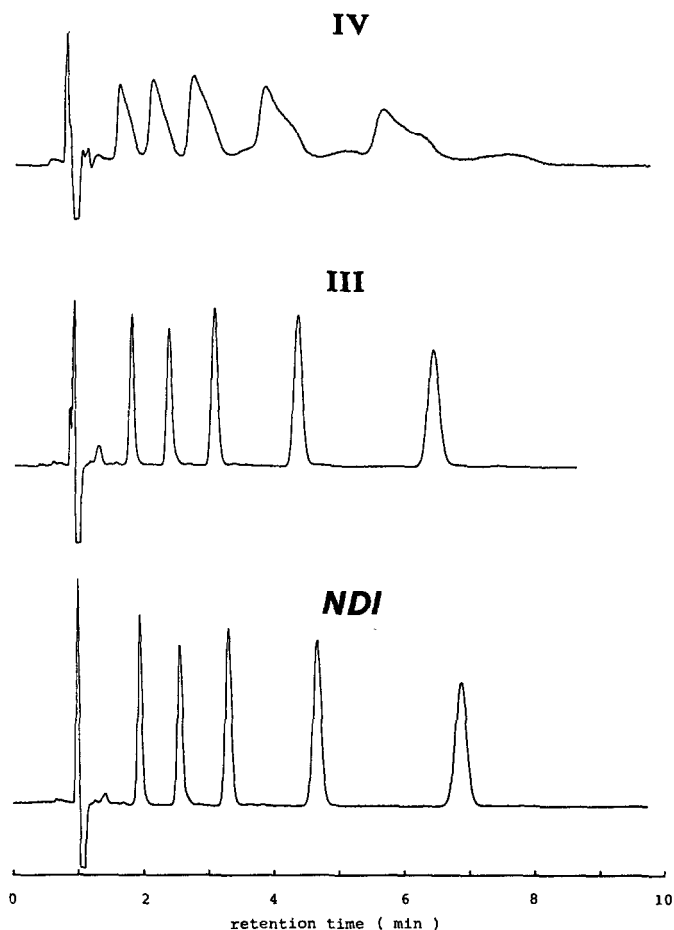chromatography and stationary phase modification are discussed below.

Fig. 1. Chromatograms of the untreated difunctional octadecylsilane-modified Nucleosil silica (NDI) and the identical stationary phase after treatments III and IV according to Table II. Chromatographic test conditions: test mixture 1, alkylbenzenes; water–methanol (30:70, v/v); UV detection at 254 nm.

Condensation of multifunctional silanes

The different types of surface silane modifications most relevant to this paper are collected in Table IV with their ^{29}Si NMR chemical shifts. The corresponding structural elements at the surface of the silica gel are depicted in Fig. 2.

The ^{29}Si MAS NMR spectra of both silica substrates are represented in Fig. 3. The relative amount of silanol groups, mainly hydroxysiloxane (Q_3) groups, determined from these spectra is given in Table I. Owing to the large surface area of the Nucleosil substrate and assuming that 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%. Köhler et al.[3] reported a geminal silanol content of 32% relative to the total amount of silanol for various substrates,

TABLE IV

MOST RELEVANT SURFACE SILANES/SILOXANES, THEIR FUNCTIONALITY, NOTATION AND TYPICAL ^{29}Si CHEMICAL SHIFTS

Modification	Chemical (spectral) function-ality	Topological (network) function-ality	Code	Typical chemical shift (ppm downfield from TMS)
Dimethyloctadecylsiloxysilane	M	1	M_1	+12
Methyloctadecylhydroxysiloxysilane	D	1	D_1	−3
Methyloctadecyldisiloxysilane	D	2	D_2	−11
Paired methyloctadecyldisiloxysilane	D	2	D'_2	−16
Octadecyldihydroxysiloxysilane	T	1	T_1	−48
Octadecylhydroxydisiloxysilane[a]	T	2	T_2	−55
Octadecyltrisiloxysilane[a]	T	3	T_3	−66
Dihydroxysiloxane	Q	2	Q_2	−91
Hydroxysiloxane	Q	3	Q_3	−101
Tetrasiloxysiloxane	Q	4	Q_4	−110

[a] Paired T_2 and T_3 groups and double-paired T_3 groups were not distinguishable.

determined by ^{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. For relative comparison, representative ^{29}Si CP-MAS NMR spectra of the monofunctional octadecylsilane-modified Nucleosil substrate, the difunctional
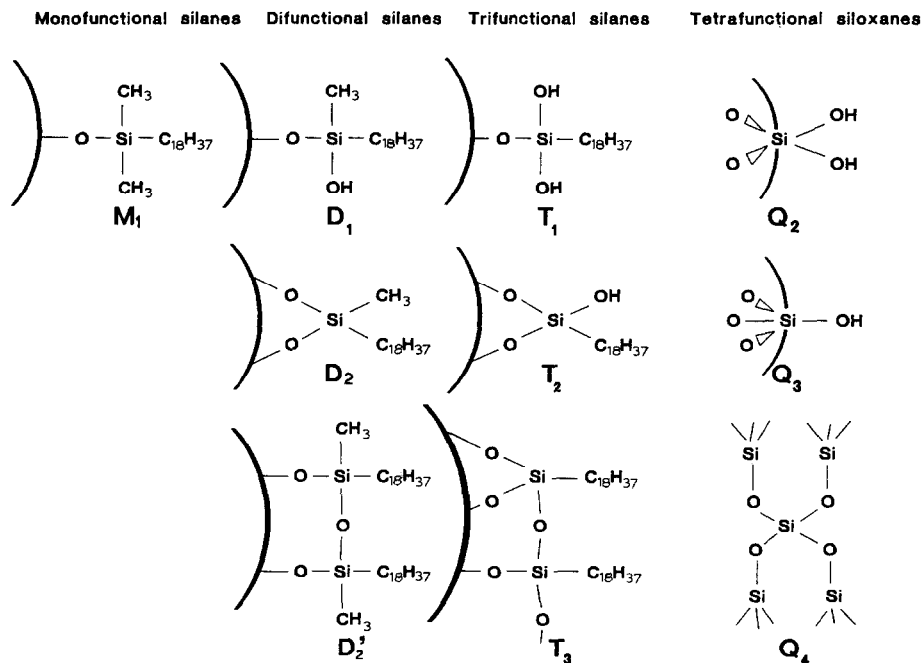

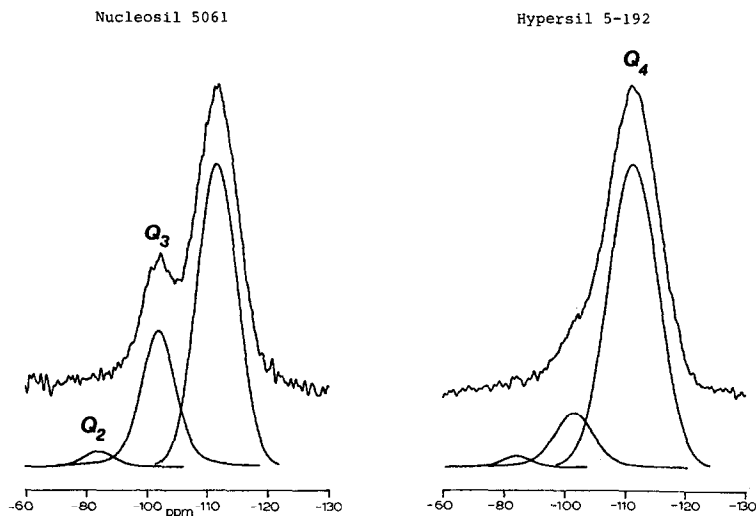Fig. 2. Structure of the siliceous surface moieties most relevant to this paper with their notations.

Nucleosil 5061   Hypersil 5-192

Fig. 3. ^{29}Si Bloch pulse MAS NMR spectra of the original Hypersil and Nucleosil silica substrates with the deconvoluted signals. $N_s = 1000$; pulse interval time, 90 s; acquisition time, 10 ms; line broadening, 10 Hz.

octadecylsilane-modified Nucleosil substrate, the trifunctional octadecylsilane-modified Nucleosil and the trifunctional octadecylsilane-modified Hypersil substrate before and after treatments I, III and IV are shown in Figs. 4, 5, 6 and 7, respectively. The spectra of mono- and difunctional octadecylsilane-modified Hypersil were identical with those of 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 the Q_3 and Q_4 signals, which generally show different optimum contact times. To prevent the spectral difference, 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 ligand, when used intensively with 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 Fig. 5). A similar condensation reaction occurred with the trifunctional octadecylsilane-modified stationary phases. Octadecyldihydroxysiloxysilane (T_1) and octadecylhydroxydisiloxysilane (T_2) moieties formed on modification were dehydrated to form octadecylhydroxydisiloxysilane (T_2) and octadecyltrisiloxysilane (T_3) moieties, respectively (see
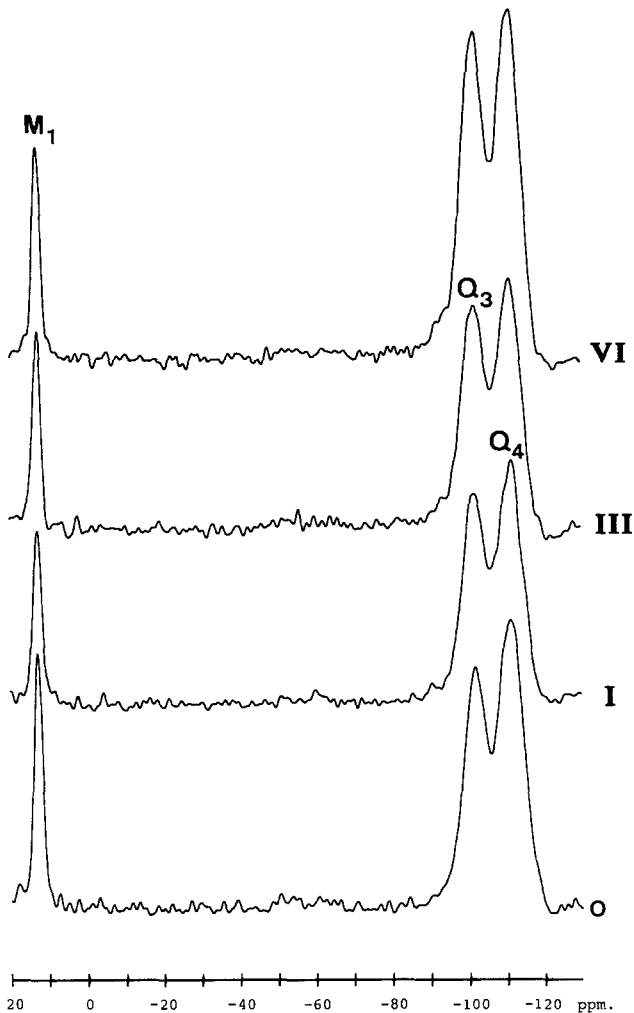
Fig. 4. ^{29}Si CP-MAS NMR spectra of monofunctional octadecylsilane-modified Nucleosil silica, before (0) and after treatments I, III and VI. $N_s = 2000$; contact time, 6 ms; interval time, 1 s; acquisition time, 10 ms; line broadening, 20 Hz.

Figs. 6 and 7). The amount of surface silanol groups did not alter accordingly. On the contrary, a slight increase in 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 polymerization across the surface. This is in agreement with the results of methyltrichlorosilane modifications of silica gels with different water contents published by Sindorf and Maciel[7]. Our results indicate that multidentate linkage with other silanes at the surface prevented hydrolysis of the anchored ligands, in contrast to the monofunctionally modified octadecyl phases. In the latter instance, a substantial increase in silanol content is found during ageing. Condensation of multifunctional silanes was more
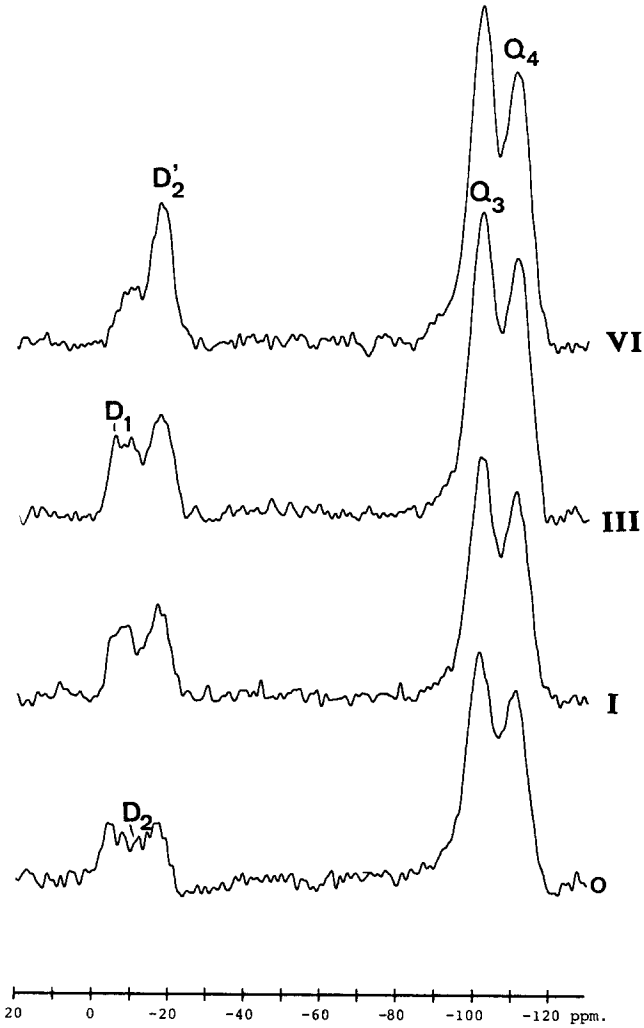
Fig. 5. ^{29}Si CP-MAS NMR spectra of difunctional octadecylsilane-modified Nucleosil silica before (0) and after treatments I, III and VI. Conditions as in Fig. 4, except contact time, 2 ms.

pronounced when buffers of high pH were used as eluents for simulation of intensive use.

The effect of ion pair agents added to aqueous–methanol buffers of low and high pH on the stationary phases studied here is not completely clear at this stage. All multifunctionally modified stationary phases except trifunctionally modified Nucleosil showed an enhanced condensation of surface silanes on addition. For trifunctional octadecylsilane Nucleosil condensation was reduced by the methanol content of the aqueous–methanol buffer.

It should be noted that the trifunctionally modified Nucleosil used for the simulation experiments already showed progress of condensation of octadecyldi-
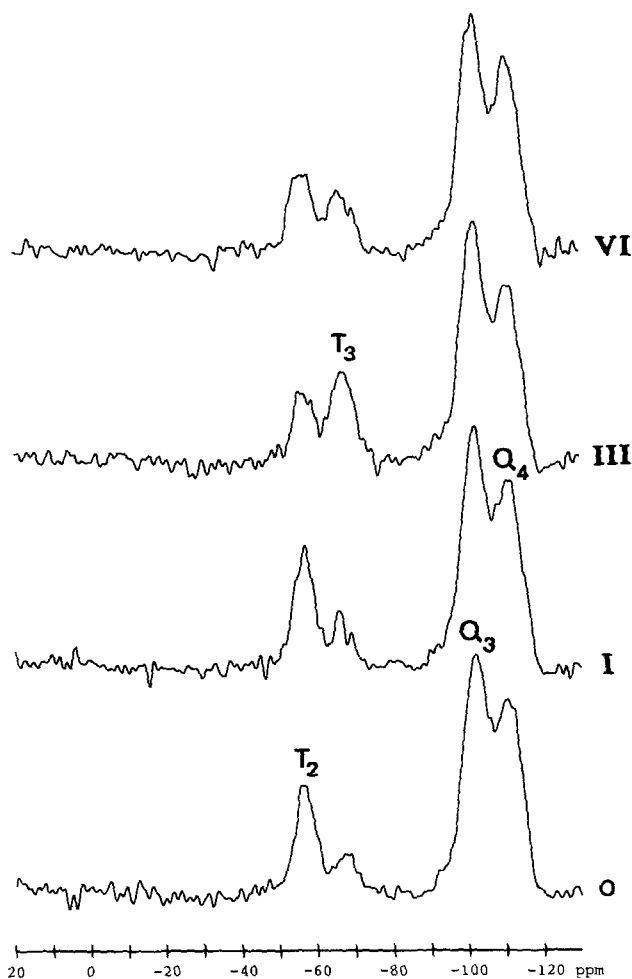
Fig. 6. ^{29}Si CP-MAS NMR spectra of trifunctional octadecylsilane-modified Nucleosil silica before (0) and after treatments I, III and VI. Conditions as in Fig. 5.

hydroxysiloxysilane (T_1) and octadecylhydroxydisiloxysilane (T_2) groups in the time elapsed between synthesis and the start of the chromatographic experiments. The concentration of T_1 groups was low and that of T_3 groups was much higher for the NTR phase than for the HTR phase used (see also Figs. 6 and 7). After a longer period no further changes at the surface of the trifunctionally modified Nucleosil were noticed.

The results of the spectroscopic and chromatographic measurements and the elemental analysis are summarized in Tables V–VII for modified Nucleosil silica and in Tables VIII–X for the modified Hypersil silica.
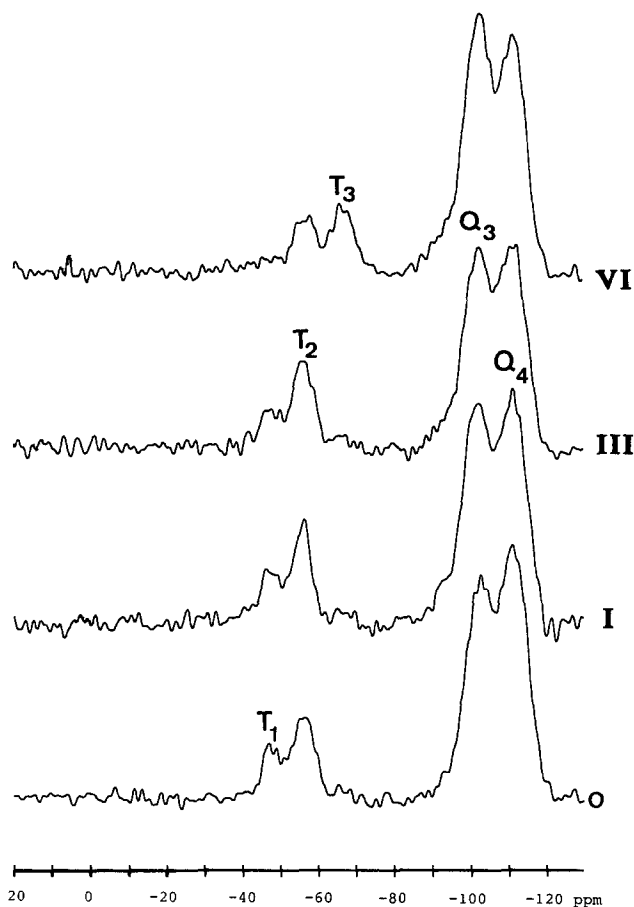
Fig. 7. ^{29}Si CP-MAS NMR spectra of trifunctional octadecylsilane-modified Hypersil silica before (0) and after treatments I, III and VI. Conditions as in Fig. 5.

TABLE V

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC MEASUREMENTS OF THE MONOFUNCTIONAL OCTADECYLSILANE-MODIFIED NUCLEO-SIL SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) $= 0.80$.

Stationary phase NMO treatment	Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$)	NMR		Chromatography: a_o	
		Ligand M_1	Silanol Q_3	Alkyl-benzenes	Alkyl aryl ketones
–	2.38	0.34	0.66	2.22	0.81
I	2.32	0.24	0.76	2.31	1.01
II	2.31	0.32	0.68	2.34	0.41
III	2.26	0.23	0.77	1.80	0.32
IV	2.06	0.23	0.77	1.73	0.25
V	2.13	0.32	0.68	1.76	0.32
VI	2.11	0.28	0.72	1.91	0.21

TABLE VI

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC MEASUREMENTS OF THE DIFUNCTIONAL OCTADECYLSILANE-MODIFIED NUCLEOSIL SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) = 0.65.

Stationary phase NDI treatment	Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$)	NMR				Chromatography: a_0	
		Ligand			Silanol Q_3	Alkyl-benzenes	Alkyl aryl ketones
		D_1	D_2	D'_2			
–	2.52	0.16	0.08	0.18	0.58	1.96	0.31
I	2.45	0.19	0.03	0.16	0.62	1.76	0.15
II	2.47	0.17	0.02	0.17	0.64	1.87	0.08
III	2.50	0.15	–	0.21	0.64	1.45	−0.05
IV	2.32	0.12	–	0.22	0.66	1.36	−0.07
V	2.44	0.15	–	0.20	0.65	1.53	−0.06
VI	2.37	0.08	–	0.24	0.68	1.46	−0.12

Stationary phase hydrolysis

The influences of treatments I–VI on the capacity factors of the methyldichloro-octadecylsilane-modified Nucleosil (NDI) stationary phase for test mixture I are shown in Fig. 8 with the eluent methanol–water (80:20, v/v). The decreased capacity factors after all treatments 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 as the a_0 values for the alkylbenzene test mixture (see below). A typical diagram of log k', determined by regression analysis from chromatographic results, *versus* the volume

TABLE VII

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC MEASUREMENTS OF THE TRIFUNCTIONAL OCTADECYLSILANE-MODIFIED NUCLEOSIL SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) = 0.85.

Stationary phase NTR treatment	Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$)	NMR				Chromatography: a_0	
		Ligand			Silanol Q_3	Alkyl-benzenes	Alkyl aryl ketones
		T_1	T_2	T_3			
–	2.62	0.02	0.24	0.10	0.64	2.06	2.44
I	2.57	–	0.22	0.11	0.67	2.38	−0.06
II	2.56	0.03	0.21	0.05	0.71	2.11	1.34
III	2.57	–	0.25	0.09	0.66	1.95	0.86
IV	2.95	–	0.09	0.20	0.71	1.35	−1.25
V	2.58	–	0.11	0.15	0.74	1.72	−0.04
VI	2.55	–	0.16	0.13	0.71	1.97	−0.83

TABLE VIII

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC MEASUREMENTS OF THE MONOFUNCTIONAL OCTADECYLSILANE-MODIFIED HYPER-SIL SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) = 0.84.

Stationary phase HMO treatment	Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$)	NMR		Chromatography: a_o	
		Ligand M_1	Silanol Q_3	Alkyl-benzenes	Alkyl aryl ketones
–	2.34	0.29	0.71	2.37	0.99
I	1.45	0.11	0.89	1.89	0.71
II	1.81	0.22	0.78	2.28	0.34
III	1.82	0.18	0.82	1.97	0.47
IV	1.55	0.16	0.84	2.01	−0.19
V	1.79	0.18	0.82	1.73	0.86
VI	1.63	0.17	0.83	1.98	0.06

fraction methanol (x) and the incremental carbon number of the alkylbenzene (n_c) for the methyldichlorooctadecylsilane-modified Hypersil (HDI) stationary phase after treatment V is shown in Fig. 9, where the linear dependence of the log k' values on both the eluent composition and the incremental carbon number is shown. According to eqns. 2 and 3, the a_o, m_o, 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 and 0.999, indicating reliable values for all constants estimated by the regression model proposed by Jandera[25]. With eqn. 5a the matching values for p were calculated.

TABLE IX

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC MEASUREMENTS OF THE DIFUNCTIONAL OCTADECYLSILANE-MODIFIED HYPERSIL SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) = 0.88.

Stationary phase HDI treatment	Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$)	NMR				Chromatography: a_o	
		Ligand			Silanol Q_3	Alkyl-benzenes	Alkyl aryl ketones
		D_1	D_2	D_2'			
–	2.55	0.07	0.20	0.05	0.68	2.09	0.49
I	2.39	0.05	0.15	0.12	0.68	1.91	−0.29
II	2.44	0.05	0.18	0.17	0.62	1.91	0.57
III	2.45	–	0.17	0.16	0.67	2.01	0.49
IV	2.29	0.04	0.14	0.11	0.71	1.90	−0.36
V	2.36	0.04	0.12	0.14	0.70	2.01	0.14
VI	2.21	0.04	0.10	0.17	0.69	1.60	0.32

TABLE X

RESULTS OF ELEMENTAL ANALYSIS, NMR EXPERIMENTS AND CHROMATOGRAPHIC
MEASUREMENTS OF THE TRIFUNCTIONAL OCTADECYLSILANE-MODIFIED HYPERSIL
SILICA BEFORE AND AFTER TREATMENTS I–VI

Mean value of p (eqn. 5a) $= 0.84$.

| Stationary phase HTR treatment | Ligand α_1 (eqn. 1) ($\mu mol\ m^{-2}$) | NMR | | | Silanol Q_3 | Chromatography: a_0 | |
| | | Ligand | | | | Alkyl-benzenes | Alkyl aryl ketones |
		T_1	T_2	T_3			
–	2.63	0.18	0.18	–	0.64	1.97	0.51
I	2.51	0.07	0.22	–	0.71	1.76	0.31
II	2.54	0.10	0.22	–	0.68	1.82	0.48
III	2.57	0.10	0.23	–	0.67	1.80	0.41
IV	2.69	–	0.12	0.15	0.73	1.23	−0.86
V	2.40	0.02	0.15	0.11	0.72	1.54	−0.06
VI	2.38	–	0.12	0.14	0.76	1.37	−0.70

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 should be concluded that there is
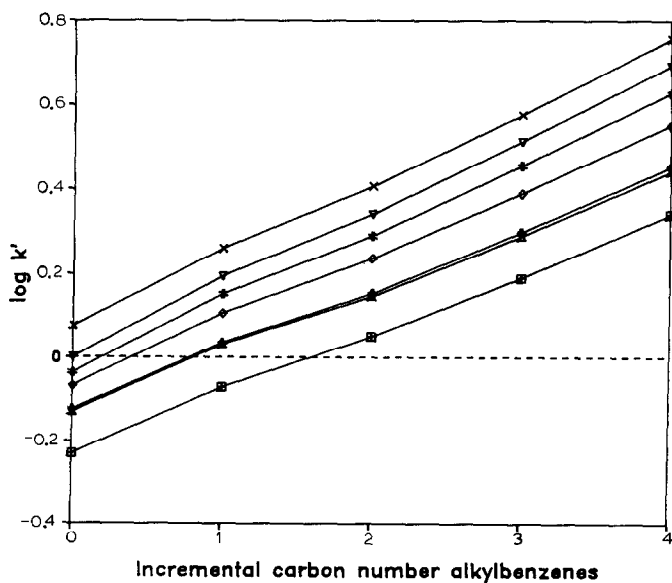

Fig. 8. Influence of treatments I–VI on the capacity factor k' determined by chromatography for
alkylbenzenes (text mixture 1). Eluent: methanol–water (80:20, v/v). ×, NDI, no treatment; ∇, NDI, II; ≠,
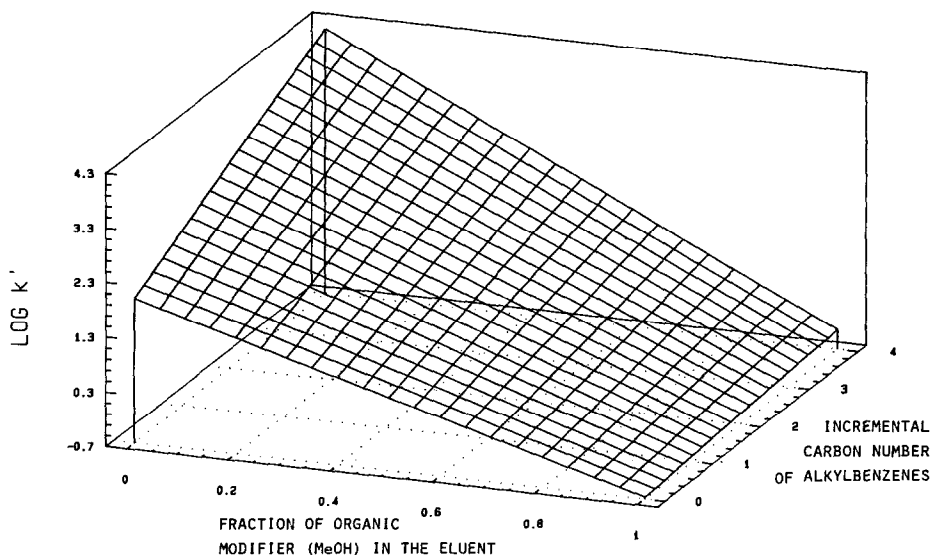NDI, I; ◇, NDI, V; +, NDI, III; △, NDI, VI; □, NDI, IV.

Fig. 9. 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 0.9 and extrapolated for x between 0 and 1, and the incremental carbon number of the alkylbenzene homologous series for the difunctional octadecylsilane-modified Hypersil silica (HDI) after treatment V.

a correlation between especially the a_o values determined for alkylbenzenes and the ligand density α_1 calculated with eqn. 1 after elemental analysis, both indicating eventual stripping of ligands from the surface of the silica. However, the observed correlation between α_1 and a_o (alkylbenzene residue) for both stationary phases was poor, with correlation coefficients of r (Nucleosil) $= 0.70$–0.86 and r (Hypersil) $= 0.56$–0.85. Plots of the α_1 values as a function of the a_o (alkylbenzene residue) values for both the Nucleosil and the Hypersil stationary phases are depicted in Figs. 10 and 11, respectively.

The a_o values fluctuated to a larger extent than the α_1 values, especially for multifunctionally modified phases. The observed deviation between α_1 and a_o values is due to the fact that α_1 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, such as 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_1 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
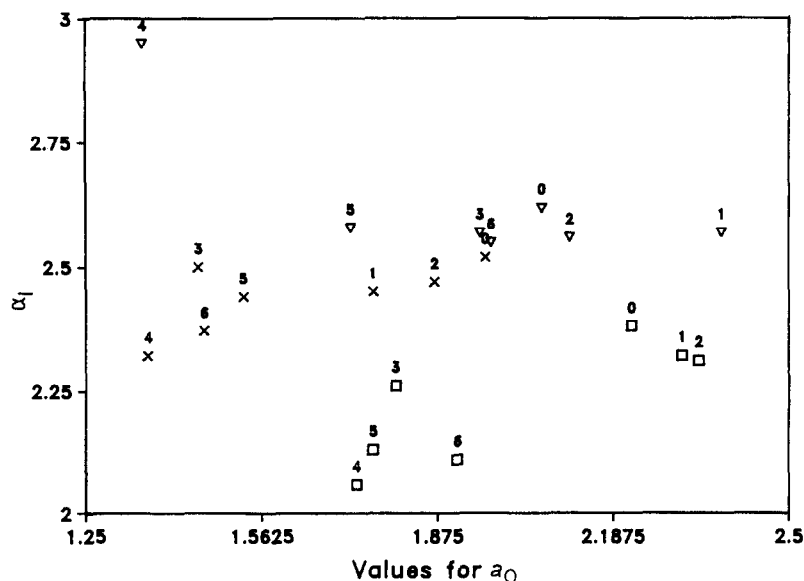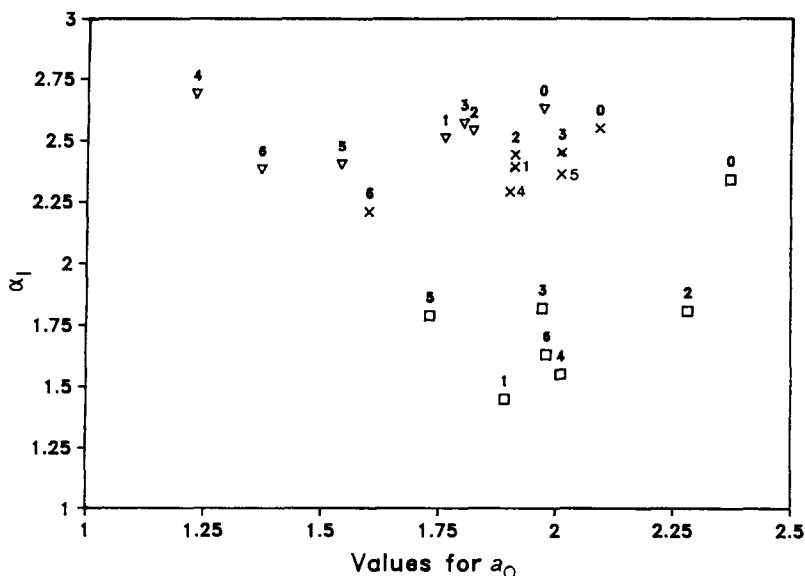
Fig. 10. Octadecyl ligand surface density α_1 (μmol m^{-2}) *versus* a_0 (alkylbenzenes) for modified Nucleosil stationary phases before (0) and after each treatment (numbers 1–6 represent treatments I–VI). $\square$, NMO; $\times$, NDI; ∇, NTR.

more stable stationary phases, 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

Fig. 11. Octadecyl ligand surface density α_1 (μmol m^{-2}) *versus* a_0 (alkylbenzenes) for modified Hypersil stationary phases before (0) and after each treatment (numbers 1–6 represent treatments I–VI). $\square$, HMO; $\times$, HDI; ∇, HTR.

high pH buffer eluents, especially with treatment IV. Elemental analysis sometimes showed an "increase" in the ligand density at the surface after treatment. This, however, is caused by rapid dissolution of the silica substrate rather than by extra hydrolysis of the silane ligands during ageing. The relatively higher hydrolysis of substrate material was only observed in this study 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–C_4) instead of the difunctional alkylsilanes used for modification of the silica substrate in most other studies. They also noticed that these "bidentates" showed a higher resistance towards hydrolysis of the anchored silanes. We have noticed before that the anchoring of longer alkylsilane modifications was more stable owing to the shielding effect of these longer alkyl chains[1]. In this study, with difunctionally modified C_{18} phases the loss of ligands at the surface after the exposure treatments 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 alkylsilanes 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 treatment IV in Fig. 1. 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 substrate shielding in the latter case.

The relative deviations of the results determined by the characterization methods used in this study are listed in Table XI. The relative deviations (RD) of the reported values were calibrated by comparing the determined values before and after treatment with the value before treatment (see eqn. 7):

$$RD = \frac{\sum_{i=1}^{n} \left| X_{after} - X_{before} \right|}{n} \cdot \frac{1}{X_{before}} \tag{7}$$

where X is the value determined by a specific characterization method and n is the number of experiments or treatments performed.

These relative deviations give a summary of the results listed in Tables V–X for comparison of the stabilities of the stationary phases examined. From the relative deviations for elemental analysis and solid-state ^{29}Si NMR experiments it can be

TABLE 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: α_1	NMR		Chromatography: a_0	
		Total ligand	Silanol	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

concluded that on the Nucleosil substrate the di- and trifunctional octadecysilane stationary phases were more stable than the monofunctional octadecylsilane phase for the same surface coverage. The relative deviations of a_0 (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 chromatographic treatments. 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. Compared with commercially available reversed-phase monofunctional octadecyl Hypersil phases, the ligand density in this study was relatively low [α_1 = 2.34 compared with α_1 (commercial Hypersil C_{18}) = 3.41]. This probably explains the poor stability of the HMO stationary phase compared with a previous study[1].

Selectivity

Selectivity in RP-HPLC depends on both the mobile and the stationary phase used. To judge the selectivity of the mobile phase, the mean values of p are listed in Tables V–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 of p = 0.65. This deviation was found after regression for both homologous test mixtures and was double checked. No reasonable explanation could be found for this deviation from the theoretical value.

More specific stationary phase–solute interactions were obtained from the values of m_0 for lipophilic and q for polar selectivity. The plots of m_0 versus a_0 for both homologue residues on the Nucleosil and Hypersil stationary phases are depicted in Figs. 12 and 13, respectively. As already pointed out by Jandera[26,27], there is a good correlation between the non-polar contribution to the selectivity m_0 and the capacity factor of the stationary phase represented by a_0 values. The correlation coefficients were r (alkylbenzenes) = 0.92–0.98 and r (alkyl aryl ketones) = 0.97–0.996. As
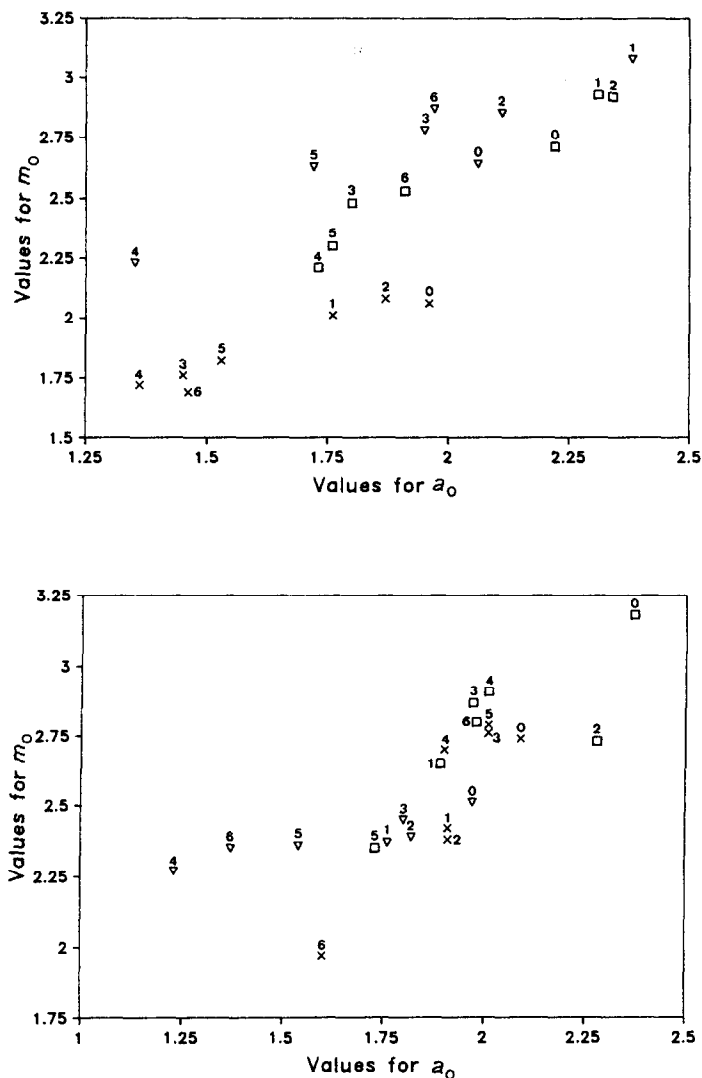
Fig. 12. Chromatographically determined m_o values (lipophilic selectivity) *versus* a_o for the alkylbenzene homologous series on modified Nucleosil (top) and Hypersil (bottom) stationary phases before (0) and after each treatment (numbers 1–6 represent treatments I–VI). Top: □, NMO; ×, NDI; ▽, NTR. Bottom: □, HMO; ×, HDI; ▽, HTR.

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_o values for the alkyl aryl ketone residue both on Nucleosil and Hypersil stationary phases are shown in Fig. 14. The influence of the exposure treatments 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
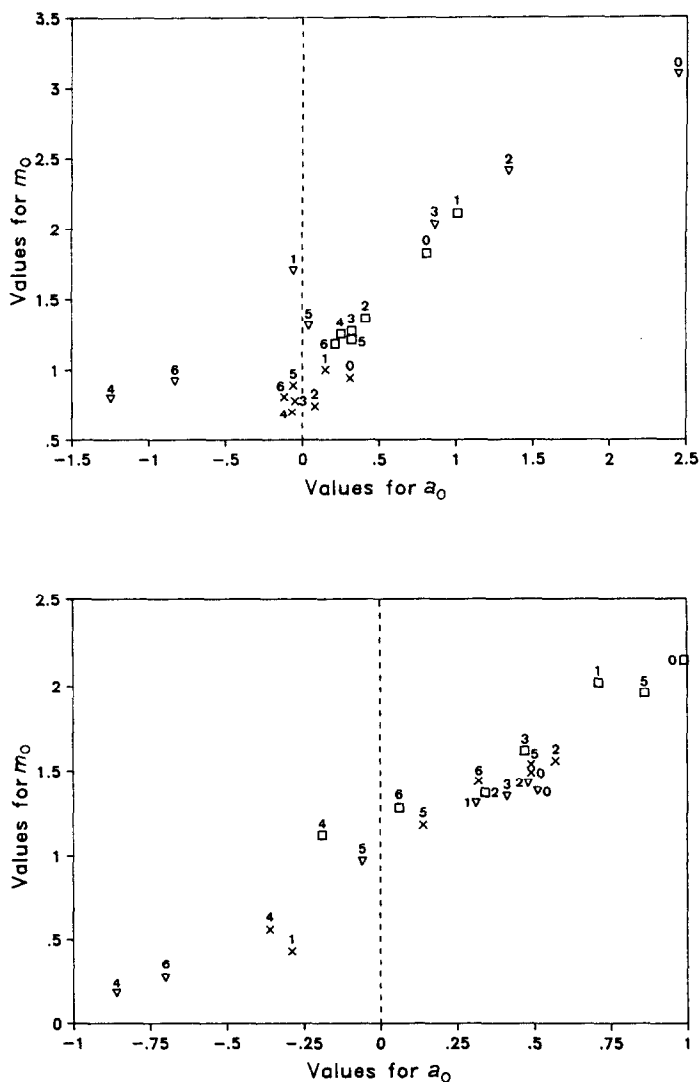
Fig. 13. Chromatographically determined m_0 values (lipophilic selectivity) *versus* a_0 for the alkyl aryl ketone homologous series on modified Nucleosil (top) and Hypersil (bottom) stationary phases before (0) and after each treatment (numbers 1–6 represent treatments I–VI). Top: □, NMO; ×, NDI; ∇, NTR. Bottom: □, HMO; ×, HDI; ∇, HTR

polar selectivity for alkyl aryl ketones, and especially for the HMO stationary phase an extreme decrease in 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 of 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-
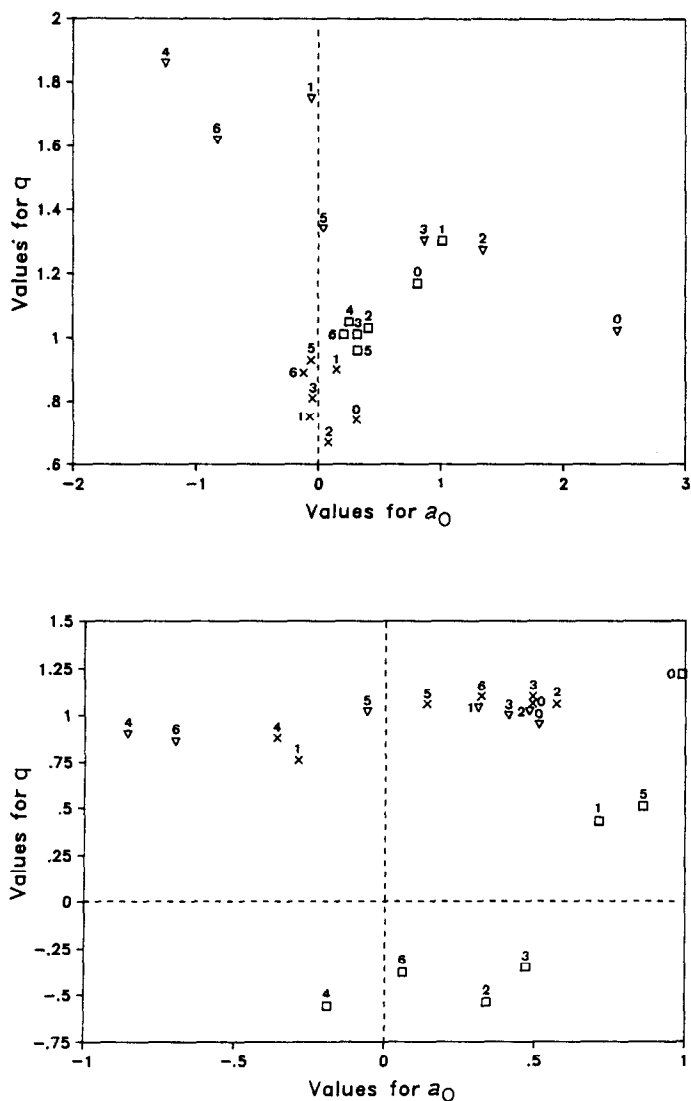
Fig. 14. Chromatographically determined q values (polar selectivity) and a_o values for the alkyl aryl ketone homologous series on modified Nucleosil (top) and Hypersil (bottom) stationary phases before (0) and after each treatment (numbers 1–6 repesent treatments I–VI). Top: □, NMO; ×, NDI; ▽, NTR. Bottom: □, HMO; ×, HDI; ▽, HTR.

modified Nucleosil phase. The substrate shielding of this stationary phase seems poor, indicated also by higher q values after several treatments. Substrate shielding by siloxane bridges directly at the surface, which occurs especially with bidentate, difunctional octadecyl-modified phases, D_2 and D_2' groups, prevented ligand and substrate hydrolysis and showed a remaining selectivity after the exposure treatments. Difunctionally modified stationary phases with a bidentate linkage are to be preferred 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 found that the value of q (alkyl aryl ketones) did not indicate more specific polar interactions between the solute and the increasing amount of silanol groups measured by solid-state ^{29}Si NMR. Probably the "polarity" of the alkyl aryl ketone residue (steric hindrance of 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 exposure treatments, 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, owing to the rather apolar residue of the alkylbenzene homologous series.

Exposure treatments, a practical guide
        A comparison between the effects of different exposure treatments can be used to evaluate the choice of the eluents listed in Table II. From the results of the different characterization techniques, it is obvious that eluents of both low and high pH should be included in the series of exposure treatments. For the aqueous buffer with pH 8.4, used in treatment IV, severe degradation of the silica substrate was always observed. However, for more discriminating information about the stability of modified phases, the pH of the aqueous buffer should be selected at *ca.* 7.5, especially for less-stable RP-HPLC phases. Mixed eluents containing organic modifiers with aqueous buffers can be used with higher aqueous pH values, because the presence of the modifier decreases 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 exposure treatments will deprive the experiments of additional, useful information. The present study involved a wide variety of eluents for exposure treatments.
        For routine laboratory interest, a comparison of different stationary phases should be carried out with a more aggressive eluent than those used in practice to decrease the exposure time. Especially when aqueous eluents with abnormal pH values (below 4 and above 7) are to be used, one should consider "ageing" of the stationary phases. Information about the stability, selectivity, capacity and silica degradation can be obtainad by the chromatographic characterization method described above combined with information on the column efficiency.

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 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 decrease in polar selectivity was determined.

For Nucleosil and Hypersil silica, differences in 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 the subject of further research in these laboratories.

ACKNOWLEDGEMENTS

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