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Citation for published version (APA):
Hetem, M. J. J., Haan, de, J. W., Claessens, H. A., Cramers, C. A. M. G., Deege, A., & Schomburg, G. (1991). Characterization and stability of silanized and polymer-coated octadecyl reversed phases. *Journal of Chromatography*, *540*(1-2), 53-76. https://doi.org/10.1016/S0021-9673(01)88798-7

DOI:
10.1016/S0021-9673(01)88798-7

Document status and date:
Published: 01/01/1991

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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Journal of Chromatography, 540 (1991) 53–76
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 867

Characterization and stability of silanized and polymer-coated octadecyl reversed phases

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(First received April 3rd, 1990; revised manuscript received October 1st, 1990)

ABSTRACT

Three different reversed phases for high-performance liquid chromatography obtained with monofunctional octadecylsilane or with cross-linked polymethyloctadecylsiloxane with and without trimethylsilane precapping of the substrate were synthesized on the same batch of Nucleosil silica. After extensive characterization, these phases were subjected to artificial ageing under simulated routine conditions. Changes in properties of the bonded-phase packings were quantified with chromatographic techniques, solid-state ^{29}Si NMR and elemental analysis. These changes are correlated with lipophilic and polar selectivity, accessibility of silanol groups at the surface, loss of silanes/siloxanes, separation performance and silica degradation of the stationary phases.

Improved surface shielding properties and stability towards eluents of high pH are considerable advantages of cross-linked polymer-coated octadecyl stationary phases. Precapping of the substrate with trimethylsilanes prior to polymer coating reduces the concentration of silanol groups to less than 50% and yields stationary phases which exhibit further decreased silanol interactions because the residual silanol groups are shielded in a double manner by the trimethylsilyl groups and by the polymer layer on top of it. This also leads to an increase in stability. The non-precapped polymer-coated stationary phase showed an advanced cross-linking of the polymers after ageing experiments with high pH buffer solutions, resulting in improved silanol shielding and large shifts in selectivity.

After precapping with trimethylsilane-enolate the polymeric methyloctadecylsiloxane coating proved to be more stable than the non-precapped phase. Also, the precapped phase showed hardly any chemical bonding with the silica surface; only Van der Waals and other dispersive interactions with the anchored trimethylsilanes at the surface exist. The high mobility of the polymer coating at the pore surface of the substrate is retained for the precapped stationary phase, also after simulated ageing experiments. This precapped polymer coated stationary phase showed an almost consistent separation performance after ageing experiments with high pH buffer solutions, but a small shift in selectivity was observed.

INTRODUCTION

The inorganic polymer silica is well suited to form small particles of defined geometry and porosity. These are preferred characteristics for support materials in

0021-9673/91/$03.50

packed column systems used in reversed-phase high-performance liquid chromatography (RP-HPLC). The performance and selectivity of chromatographic separations that can be achieved with such packing materials in RP-HPLC depend largely on the microstructure of the organic layer bonded at the silica surface. In spite of the widespread application of RP-HPLC stationary phases, two main problems are still encountered. These concern the reproducible preparation of these phases, having controlled, predictable selectivity, and the relatively poor stability under a number of practical conditions.

During more than two decades, much effort has been put into the synthesis of organic layers to (mainly) silica substrates. These studies are still being continued and include the attachment of mono-, di- and trifunctional ligands to substrates, the last two often resulting in (un)controlled polymeric phases.

It is now recognized that in addition to the properties of the substrate and the density of the ligand surface coverage, the functionality of the bonding agents and the alkyl chain length are important parameters with respect to stability and selectivity. Much attention has been paid to the shielding of the substrate in order to prevent unwanted solute–substrate interactions, *i.e.*, silanophilic interactions.

A minimum surface coverage and chain length, difunctionally attached to the substrate, seem to result in more or less stable stationary phases [1–6]. However, in recent years RP phases have been applied to an increasing extent for the separation of basic compounds and unprotected proteins and peptides, the separations usually being performed at pH < 3 or pH > 8. In these regions many RP phases are not stable over a considerable period of time. This stimulated a further search for selective and stable phases without unwanted substrate–solute interactions.

At present these efforts are concentrated on the preparation of very dense monofunctionally bonded ligands [7] and on the synthesis of polymer phases with controlled structures and properties in order to provide special selectivities [8,9]. The latter phases are synthesized using di- or trifunctional chloro- or alkoxysilanes under strictly controlled reaction conditions, especially with respect to the amount of water during the synthesis.

Also, amongst others, Kirkland *et al.* investigated the properties of bidentate bonded phases and also a number of bulky phases [10]. Both of these types of phases look promising with respect to improved stability. In the latter type, the methyl side groups are replaced with larger groups in order to increase the shielding from unwanted solute–substrate interactions.

Another approach in order to prepare selective and stable stationary phases is the encapsulation of silica or other substrates with a polymeric layer such as polymethylsiloxanes, substituted by long-chain alkyl ligands; polybutadiene [11–14] and copolymerized vinyl-modified silica with acrylic acid [15]. Othsu *et al.* [16] deposited silicone monomer on the substrate, subsequently followed by *in situ* polymerization and attachment of, *e.g.*, octadecyl ligands. There is evidence that a number of these phases exhibit superior stability and shielding properties to conventional ligand-bonded phases. However, the actual structure and bonding of the polymeric layers to the substrate is not clear in all instances and is the subject of further research.

In this study, two reversed stationary phases were prepared on the same batch of silica and extensively investigated, together with one model phase: (i) a conventional dimethyloctadecyl-bonded phase; (ii) a model polymethyloctadecylsiloxane polymer-coated phase, subsequently immobilized by cross-linking and chemical bonding to the silica [11–14]; and (iii) the same as under (ii), but the silica was pretreated by a silanization procedure with trimethylsilane enolate prior to the polymer coating procedure [14].

The last type of RP material is well known for its low concentration of residual silanol groups and a favourable surface shielding by the cross-linked polymer [14]. This polymer is only physically adsorbed at the presilanized silica surface, as will be shown below.

In this paper we lay emphasis on the comparison of shielding effects and stabilities between a conventionally modified ligand phase and siloxane polymer-coated layers, either directly on porous silica or on a hydrophobic precapped silica. These materials were subjected to artificial ageing under simulated routine use conditions [3,17]. After ageing, changes in stationary phase properties were investigated in detail by chromatography, elemental analysis and high-resolution solid-state ^{29}Si NMR. In this way, the influence of the various chemical modifications of the silica substrate on the chromatographic properties and the resistance of the stationary phases towards possible deterioration by the eluents could be determined.

EXPERIMENTAL

Materials

The test components used for chromatographic characterization of the stationary phases before and after the ageing experiments were all of reference grade. The *n*-alkylbenzene homologous series, test mixture 1 (Pierce, Rockford, IL, U.S.A.) and *p*-hydroxybenzoic acid *n*-alkyl ester homologous series, test mixture 2 (Sigma, St. Louis, MO, U.S.A.) were used for the determination of the chromatographic lipophilic and polar selectivity of the reversed phases [7]. A third test mixture containing components with various selectivities was used for comparison with previous papers [11–14]: acetophenone, benzophenone, benzoyl benzoate, ethylbenzene, 2-*n*-octylpyridine, *n*-butylbenzene and *n*-hexylbenzene, all of reference quality. Deionized water was freshly prepared with a Milli-Q system (Millipore, Bedford, MA, U.S.A.). All other solvents were of analytical-reagent grade (E. Merck, Darmstadt, Germany). The eluents used for HPLC were filtered over 0.22-μm membrane filters (Millipore).

Chromatography

All stationary phases were prepared on the same Nucleosil silica (batch 7021; diameters: $d_{\text{particle}} = 5$ μm, $d_{\text{pore}} = 10$ nm; BET surface area, $S_{\text{BET}} = 360$ m^2 g^{-1}) (Macherey, Nagel & Co., Düren, Germany) to eliminate substrate-dependent effects on the stability of the reversed phases [3,7]. This silica was modified with dimethylamine–dimethyloctadecylsilane (DMA–DMODS) [18] to obtain a conventional monodentate bonded *n*-octadecyldimethylsilane phase for the comparison with the polymer-coated phases. The two polymethyloctadecylsilane (0.25 molar ratio *n*-octadecyl groups, carbon coating = 53.8%) coated phases, one precapped with trimethylsilyl–2,4-pentanedione enolate [13], were prepared according to the procedures described previously [12,14], the non-precapped phase being meant as a model (see

Introduction). These three *n*-octadecyl-modified stationary phases will be referred to as a *n*-octadecyldimethylsiloxysilane bonded phase (C_{18}/A), a polymethyloctadecylsiloxane-coated phase ($PMSC_{18}$) and a trimethylsilyl enolate precapped polymethyloctadecylsiloxane-coated phase ($C_1/PMSC_{18}$), respectively. The bulk properties of these modified stationary phases are listed in Table I. The carbon contents of the reversed phases were determined with a Perkin-Elmer Model 240 Analyzer. Trimethylsilyl 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, Germany) 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% variation in column efficiency and a deviation of ±5% in capacity factors and selectivities were allowed for a given series of columns. The equipment and procedure for simulated intensive routine use were described in detail earlier [3]. 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 performed in order to ensure a constant flow through the columns and to avoid the deposition of pump seal material at the top of the column, which could affect the ageing experiments.

The basic and acidic aqueous and aqueous methanol buffers used as eluents for simulated routine use experiments are listed in Table II. In this study the eluent normally used in experiment 4, aqueous sodium hydrogencarbonate buffer (pH 8.4), was replaced with a plain aqueous sodium phosphate buffer (pH 7.5). The plain aqueous high-pH sodium hydrogencarbonate buffer was found to be too destructive for the stationary phases under study. Meaningful chromatographic characterization could not be performed after this specific ageing experiment no. 4.

Two series of homologues, *n*-alkylbenzenes and *p*-hydroxybenzoic acid *n*-alkyl esters, were used for fundamental chromatographic characterization of the phases for

TABLE I

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 phase	Carbon content, P_c (%, w/w)	Ligand surface density, α_1 (see ref. 2, eqn. 1) (μmol m^{-2})	Film thickness of polymer coating, d_f (nm)	^{29}Si MAS NMR relative silanol content
Nucleosil 5-100 #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 content of geminal silanol groups, Q_2, was 0.073 and that of vicinal/"lone" silanol groups, Q_3, was 0.927; see Fig. 5.

[b] These are precapped trimethylsiloxysilane ligands; see C_1/E phase.

TABLE II

ELUENT COMPOSITIONS FOR THE EXPERIMENTS SIMULATING ROUTINE USE

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

Ageing expt. No.	Buffer	pH	Volume fraction of methanol in the eluent	Ion pairing agent (concentration 5 m*M*)
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	Hexanesulphonate
4	0.05 *M* phosphate	7.5	0	–
5	0.05 *M* carbonate	8.4	0.5	–
6	0.05 *M* carbonate	8.4	0.5	Triethylamine

at least four different eluent compositions after each ageing experiment. By multiple linear regression of the logarithm of the capacity factors of a homologous series at a number of suitable eluent compositions, a set of characteristic parameters can be derived, as discussed previously [3,7]. From this set of parameters the values of m_0 and q, denoting mainly the non-specific lipophilic selectivity (interactions) and the polar selectivity (interactions), were calculated, providing the most relevant information on specific stationary phase–solute interactions. These interactions depend largely on the type and actual condition of the stationary phase when identical chromatographic test conditions are applied. With these restrictions, these parameters characterize the contribution of stationary phase–solute interactions to the overall selectivity of a stationary phase.

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 status of the stationary phases was also tested with test mixture 3. With this test mixture the selectivity factor α for the retention of 2-*n*-octylpyridine relative to ethylbenzene was calculated.

The chromatographic characterization was performed with the same equipment as described previously [3,7].

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 [18]. Two columns (150 mm × 4.6 mm I.D.) were packed with these phases. Each column contained *ca.* 1.60 g of stationary phase material. The breakthrough method was employed to determine the modifier coverage at the stationary phase surface as a function of the mobile phase composition. The equipment and experimental design were similar to those published previously [19].

Solid-state ^{29}Si NMR measurements

Solid-state ^{29}Si NMR spectra were obtained on a Bruker CXP 300 Fourier transform NMR spectrometer at 59.63 MHz. Representative samples of 190–230 mg were spun at *ca.* 3.5 kHz using 7 mm O.D. zirconium oxide rotors of the Bruker double-bearing type. ^{29}Si Bloch decay magic angle spinning (MAS) NMR spectra of the original and modified silicas were obtained with a $\pi/2$ pulse and a pulse interval time of 90 s.

^{29}Si MAS NMR spectra were also collected for the C_1/PMSC_{18} phases after the ageing experiments. Typically 620 FIDs (free induction decays) with an acquisition time of 10 ms for the substrate and silanized samples and 100 ms for the polymer coated samples were accumulated in 1K and 2K data points, respectively. Prior to Fourier transformation a line broadening of 10 Hz and 1 Hz, respectively, was applied and subsequently the data files were zero-filled to 8K. ^{1}H–^{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 1 s. Typically, 2000 FIDs with an acquisition time of 10 ms were accumulated in 1K data points and zero-filled to 8K prior to Fourier transformation. The line broadening used was 15 Hz prior to zero-filling. The spectral width for all spectra was 15 kHz.

^{29}Si CP-MAS NMR experiments with variable contacts, between 0.1 and 50 ms, of the C_{18}/A, C_1/PMSC_{18} and 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 with different signals in the ^{29}Si NMR spectrum. 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 [20]. 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 Discussion).

RESULTS AND DISCUSSION

Chromatography

From adsorption isotherms measured with the breakthrough method, the maximum adsorbed ethanol coverage of the C_{18}/A and C_1/PMSC_{18} phase surfaces was obtained. The columns packed with C_{18}/A and C_1/PMSC_{18} contain equal amounts of stationary phase material. The maximum amount of ethanol adsorbed at the C_{18}/A surface was 152 μmol on 1.60 g of material, with a specific surface area, corrected for the ligand content, of 264 m^2 g^{-1}. For the C_1/PMSC_{18} phase the value was 70 μmol on 1.60 g, with a corrected specific surface area of 281 m^2 g^{-1}. From these data, the adsorbed ethanol coverage at the surface could 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 this value was 0.156 μmol m^{-2}. The difference in maximally adsorbed ethanol was partly due to the difference in ligand surface density, *viz.*, 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 I. Ethanol adsorption by accessible residual silanol groups decreases with increasing amount of chemically bonded ligands. However, the large difference in ethanol adsorption, more than twice that for the C_{18}/A stationary phase, illustrates clearly the shielding effect of the polymethyloctadecylsiloxane polymer.

The latter polymer is hardly chemically bonded to the silica surface, only physically adsorbed at the trimethylsilanized surface by Van de Waals interactions

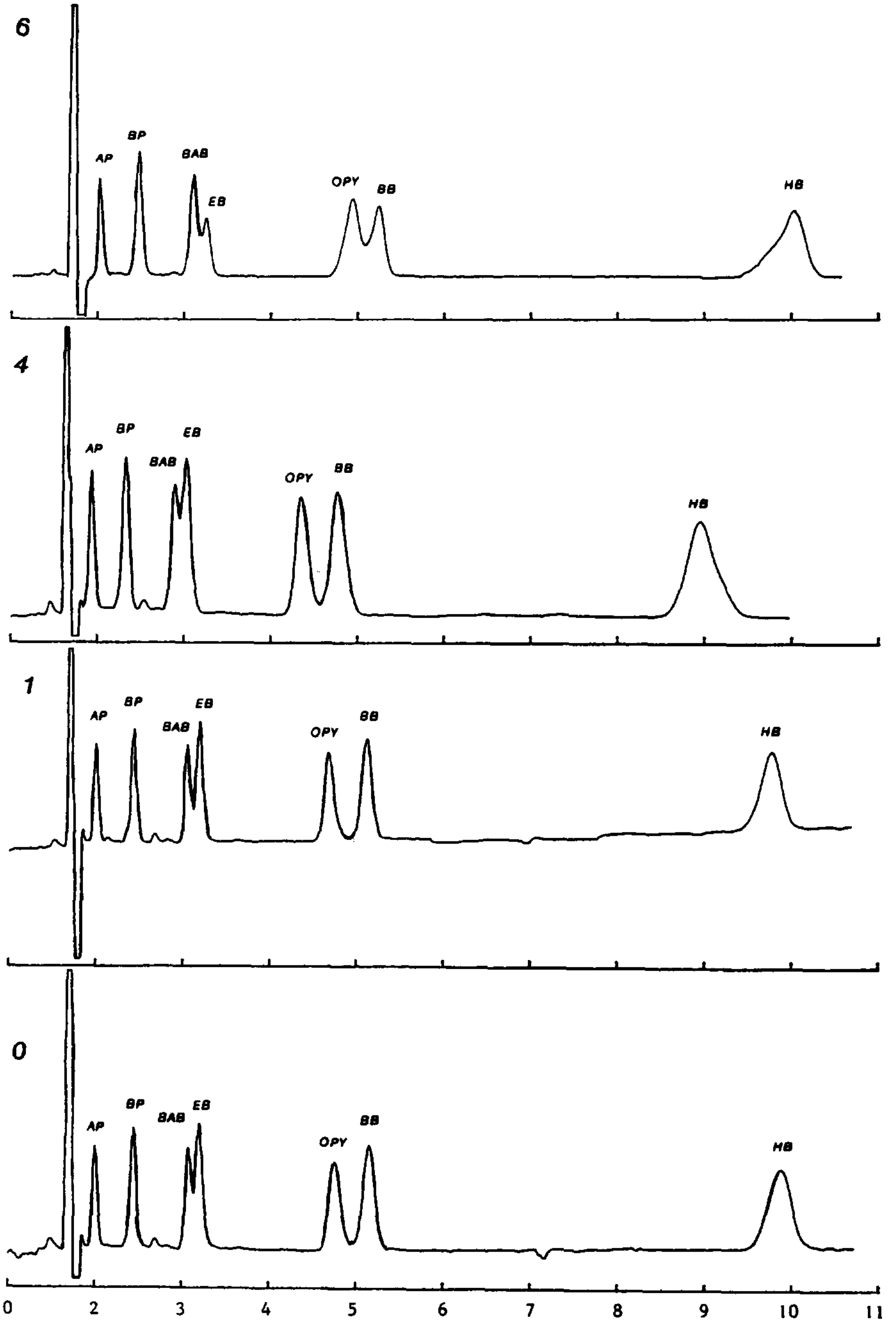

Fig. 1. Chromatograms of test mixture 3 eluted on the C_{18}/A stationary phase before (expt. 0) and after ageing experiments 1, 4 and 6. Chromatographic test conditions: water–methanol (20:80, v/v); UV detection at 254 nm. Compounds: AP, acetophenone; BP, benzophenone; BAB, benzoyl benzoate; EB, ethylbenzene;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

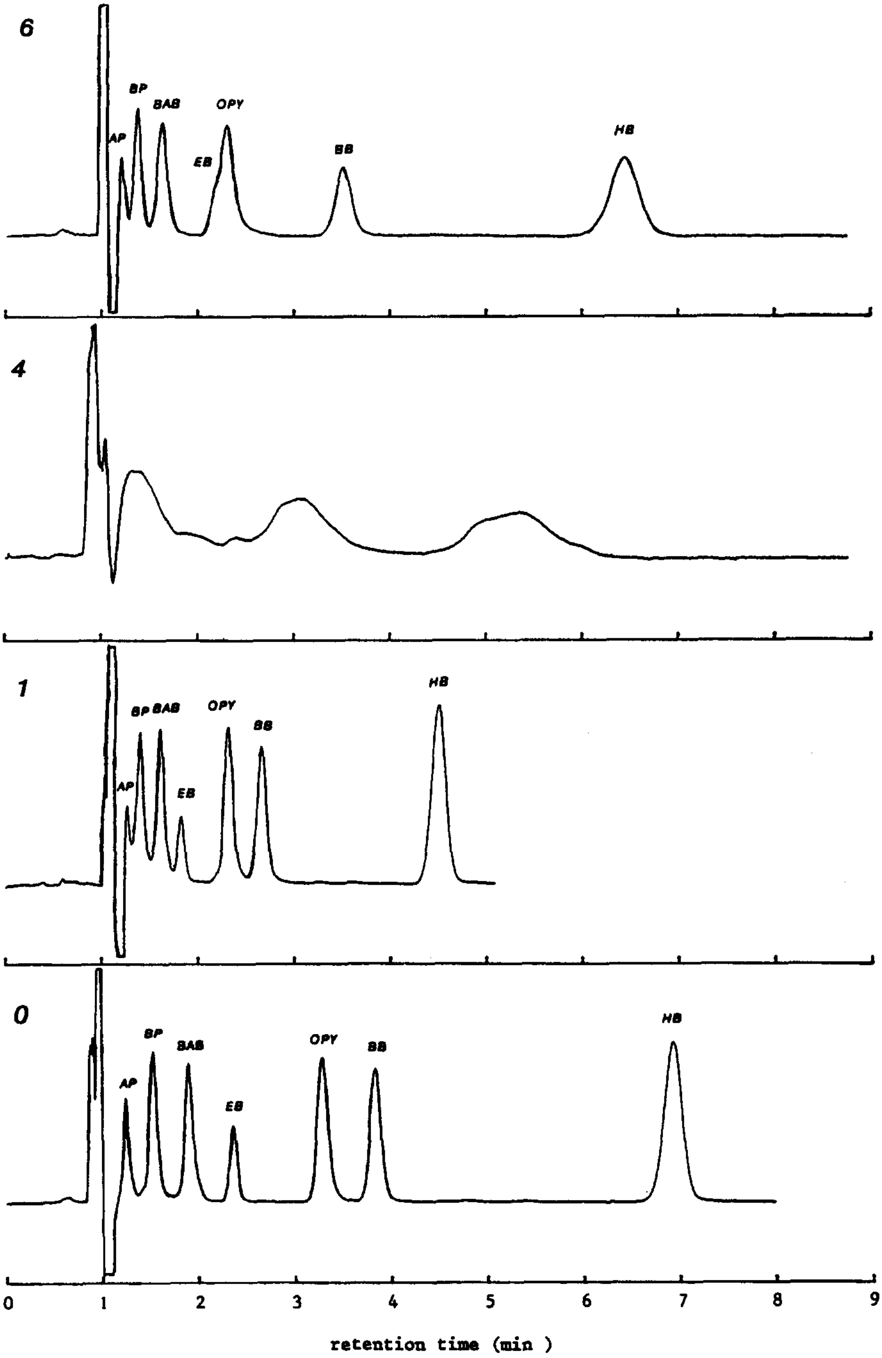

Fig. 2. Chromatograms of test mixture 3 eluted on the PMSC$_{18}$ stationary phase before ageing (expt. 0) and after ageing experiments 1, 4 and 6. Conditions as in Fig. 1.

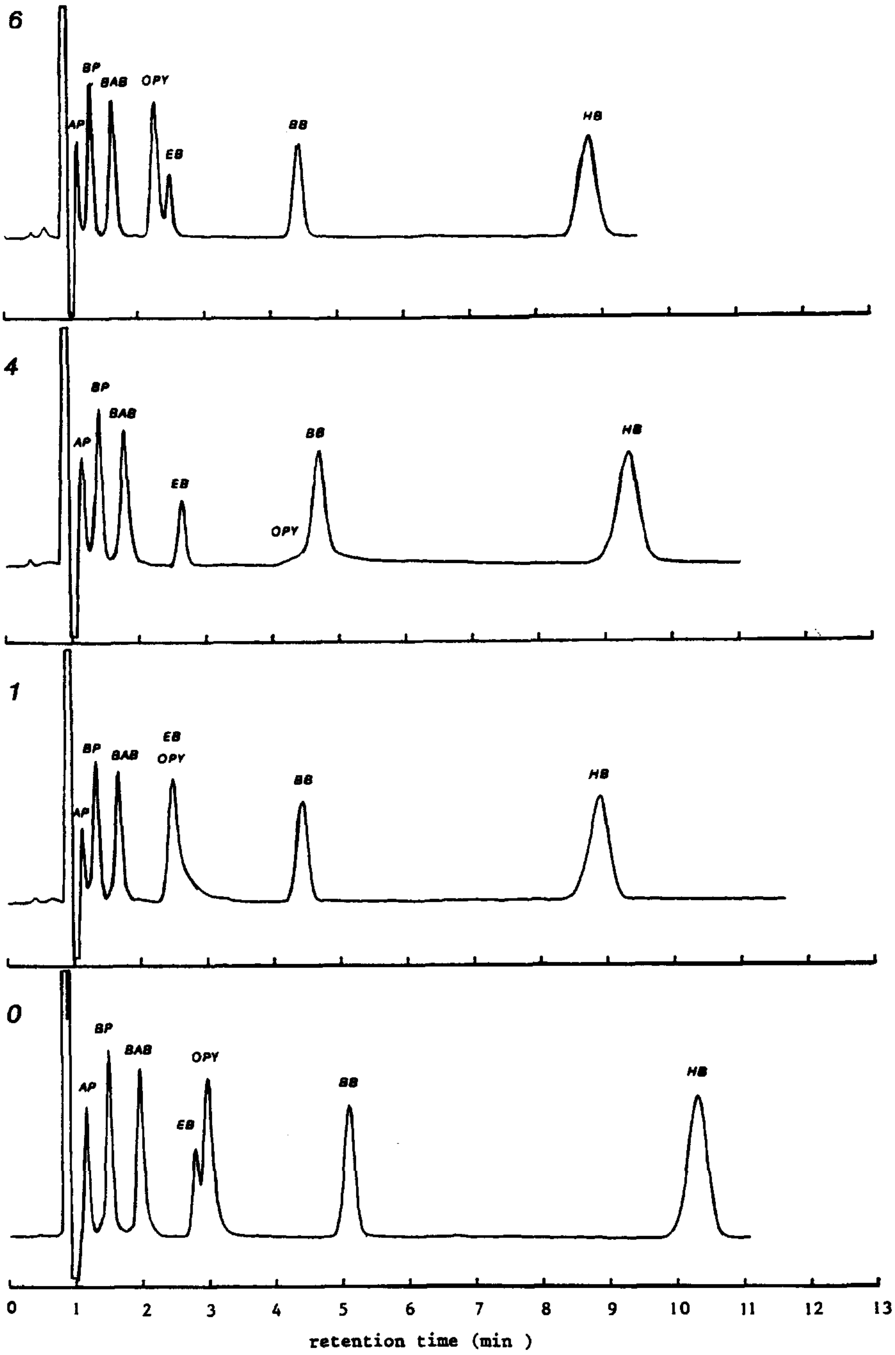

Fig. 3. Chromatograms of test mixture 3 eluted on the $C_1/PMSC_{18}$ stationary phase before ageing (expt. 0) and after ageing experiments 1, 4 and 6. Conditions as in Fig. 1.

between hydrocarbons. A pronounced shielding effect of the residual silanol groups by the polymer, even for small molecules such as ethanol, is thus indicated.

The chromatograms of test mixture 3 eluted on the C_{18}/A, PMSC_{18} and C_1/PMSC_{18} stationary phases before and after some ageing experiments are depicted in Figs. 1, 2 and 3, respectively. A comparison of these chromatograms indicates the difference in selectivity of the original phases. In particular, the 2-*n*-octylpyridine peak is found at different positions in the chromatograms. A small capacity factor for the basic 2-*n*-octylpyridine is assumed to indicate only limited specific interactions with acidic silanol groups [12–14]. This might be interpreted as a small amount of accessible silanol groups at the surface. The C_1/PMSC_{18} phase exhibited the smallest amount of accessible silanol groups, as confirmed by the ethanol adsorption measurements. Further, 2-*n*-octylpyridine retention appeared to be very indicative of changes in stationary phase properties. Shifts in capacity factor values occurred after various ageing experiments. These shifts are summarized in Table III, where the selectivity factors α(2-*n*-octylpyridine/ethylbenzene) are listed.

With the two polymer-coated phases, significant changes in this selectivity factor were observed after several ageing experiments. After ageing experiments with relatively aggressive eluents the selectivity factors generally decreased for the polymer-coated phases. This contrasts with the expected decrease in silanol shielding properties due to stationary phase stripping and disordering of the coating observed in earlier studies [3,7]. 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. 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 for 2-*n*-octylpyridine relative to ethylbenzene.

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. The lipophilic selectivity m_0(*n*-alkylbenzenes) and the hydrophillic selectivity q(*p*-hydroxybenzoic acid *n*-alkyl esters) were calculated as discussed in detail previously [4,7]. Changes in ligand hydrolysis and/or stationary phase stripping due to ageing will affect the m_0(*n*-alkyl-

TABLE III

SELECTIVITY FACTOR α OF 2-*n*-OCTYLPYRIDINE RELATIVE TO ETHYLBENZENE

Stationary phase	Ageing expt. No.						
	0[a]	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

[a] Experiment 0 represents the stationary phase after packing or chromatographic characterization in this and subsequent tables.

benzenes) value. These changes will also influence polar interactions with the stationary phase surface, especially for polarizable compounds. The q(p-hydroxybenzoate) value indicates the effect of polar changes at the surface of the substrate better than the q(benzene) value.

The values of the lipophilic and polar selectivities of the octadecyl-modified phases are listed in Table IV. The m_0 value determined for the original PMSC$_{18}$ phase is slightly higher than those for the other two phases, owing to a thicker polymer coating film (d_f = 1.02 nm) at the surface of the silica pores. The m_0(n-alkylbenzenes) values of all the stationary phases did not alter significantly after the ageing experiments, except for the PMSC$_{18}$ phase after 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 decrease in m_0 value for the PMSC$_{18}$ phase after ageing experiment 1 with a low pH plain aqueous buffer solution might indicate a severe loss of polymer coating caused by dissolution of the substrate; see also the carbon content analysis. The slightly increased α(2-n-octylpyridine/ethylbenzene) value also indicates a better accessibility of the surface silanol groups.

The polar interactions determined with the homologous series of p-hydroxybenzoic acid n-alkyl esters are larger for the polymer-coated phases. The overall polarity is larger, probably owing 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 eluent. For the PMSC$_{18}$ phase the peak shapes in the chromatograms of test mixture 2 after ageing experiments 4 and 6 were so poor that no meaningful value for q could be determined. The PMSC$_{18}$ phase showed the largest changes in polar selectivity. The polar selectivity increased slightly after ageing experiments 1 and 3 and substantially after experiment 5. Here, more polar interactions occur, although the accessibility of the surface silanol was reduced after ageing experiment 5 (see selectivity

TABLE IV

LIPOPHILIC SELECTIVITY, m_0(n-ALKYLBENZENES), VALUES AND THE POLAR SELECTIVITY, q(p-HYDROXYBENZOATES), VALUES DETERMINED BY CHROMATOGRAPHIC CHARACTERIZATION FOR THE REVERSED PHASES BEFORE AND AFTER THE AGEING EXPERIMENTS

Stationary phase	Ageing expt. 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

factor α). We assume that the polymethyloctadecylsiloxane polymers remain inside the pores caused by immobilization through advanced chemical bonding. 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 also increased, but less than for the PMSC$_{18}$ phase. The C_{18}/A phase showed the most constant selectivity after the different ageing experiments.

The effect of the ageing experiments on the separation performance, N_{sys}, is shown in Table V. Because the plate numbers of the original columns varied by as much as 15%, the value of N_{sys}(n-butylbenzene) after the ageing experiments is listed together with the changes in the plate numbers after the ageing experiments. The PMSC$_{18}$ phase showed the largest decrease in 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 optimum. The various aggressive ions present in the ageing solvents could cause disorders of the silica surface fairly easily. Even the less aggressive solvent in experiment 2 causes a substantial decrease in column efficiency owing to degradation of the stationary phase.

The positive effect of trimethylsilyl precapping is clear. The stability of the resulting stationary phase improves, although considerable deterioration of the silica substrate was still observed after ageing experiments with plain aqueous buffer solutions of high or low pH. The stability towards organic solvents containing aggressive eluents was improved. This effect is so strong that superior shielding of the silica surface was 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.

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 VI). Generally, the high pH buffer solvents cause the largest decrease in carbon content for the polymer-coated phases. Only after ageing

TABLE V

EFFICIENCIES, N_{sys}(n-BUTYLBENZENE), CALCULATED FROM CHROMATOGRAPHIC DATA

Experimental conditions: eluent, methanol–water, 80:20 (v/v); UV detection, 261 nm.

Stationary phase	Ageing expt. No.[a]					
	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%

[a] Percentages indicate increase (positive numbers) or decrease (negative numbers) of efficiencies after the ageing experiments.

TABLE VI

CARBON CONTENT, P_c (%, w/w), DETERMINED BY ELEMENTAL ANALYSIS

Stationary phase	Ageing expt. 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

experiment 1 did the $PMSC_{18}$ phase show a large decrease in carbon content also. As discussed before, a substantial amount of polymethyloctadecylsiloxanes is dissolved in the eluent in this case.

One has to bear in mind, however, that changes in the carbon content are relative, and 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 decrease in efficiency reveals silica dissolution and disordering of the silica surface (see Table V). From the carbon content data for the $C_1/PMSC_{18}$ phase, it is not clear whether the polymethyloctadecylsiloxane coating and 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 the next section).

Solid-state ^{29}Si NMR

Table VII lists the siliceous moieties most relevant to this study, with their notations and ^{29}Si NMR chemical shifts. The structures of these species are presented in Fig. 4. The ^{29}Si MAS NMR spectrum of the Nucleosil substrate is depicted in Fig. 5,

TABLE VII

SILANE/SILOXANE MOIETIES, THEIR NOTATIONS AND TYPICAL ^{29}Si NMR CHEMICAL SHIFTS [12], TOGETHER WITH THE ^{29}Si CP-MAS NMR CORRECTION FACTORS FOR A CONTACT TIME OF 6 ms, $I(0)/I(6\text{ ms})$

Modification	Code	Typical ^{29}Si NMR chemical shift (ppm downfield from TMS)	Correction factor, $I(0)/I(6\text{ ms})$
n-Octadecyldimethylsiloxysilane	M_1	+ 12.6	1.18
Trimethylsiloxysilane	M_1	+ 12.3	1.23
Bridged (dimethyldisiloxysilane) or bridged (methyloctadecyldisiloxysilane)	D'_2	− 20	1.18
Poly(dimethyldisiloxysilane) or poly(methyloctadecyldisiloxysilane)	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}, where R is the distance between hydrogen and silicon nuclei.

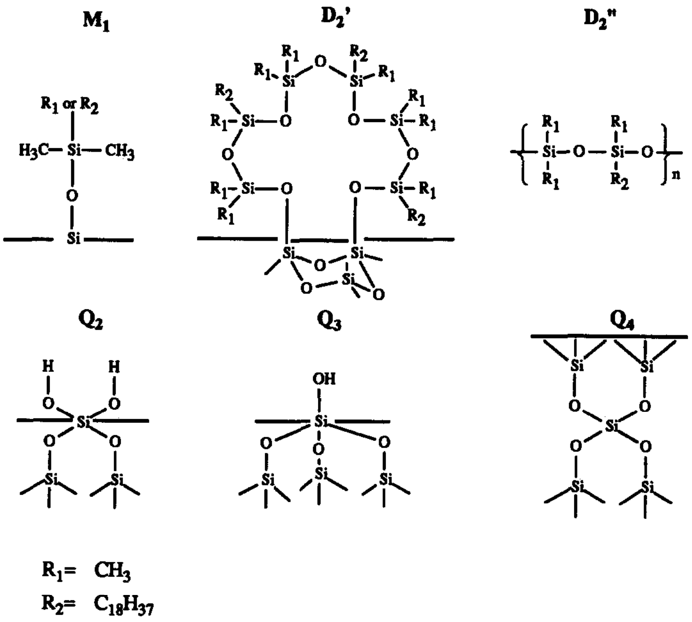

Fig. 4. Structures of the siliceous surface moieties most relevant to this study.

together with the deconvoluted signals. The silanol content calculated from this spectrum is listed in Table I.

^{29}Si CP-MAS NMR experiments with variable contact times ("CP characteristics") of all the *n*-octadecyl-modified silicas revealed the specific correction factors for the different ^{29}Si NMR resonances. With a contact time of 6 ms for the CP-MAS measurements in this study, the correction factors were calibrated, $I(0)/I(6\ \text{ms})$, and are also listed in Table VII.

^{29}Si CP-MAS NMR spectra were obtained for the C_{18}/A stationary phase before and after the ageing experiments (Fig. 6). The spectra in Fig. 6 are all scaled in the same way. The measured contents of siliceous surface moieties, relative to the untreated sample, are given in Table VIII. It is obvious that the contents of both *n*-octadecyldimethylsiloxysilanes (M_1) and the hydroxysiloxanes (Q_3) decrease. During the measurements, the usual precautions were taken to ensure maximum compatibility between the different samples (see above). The accuracy of our approach can be estimated to be *ca.* 5% relative.

A second conclusion from Fig. 6 is that the amount of silanol groups is reduced relatively more than that of the ligands, especially after treatments 3–6. The high pH buffer eluents also cause the largest changes in amounts and content ratio, although the changes in ratio are small.

There is an important difference between pulse and cross-polarization (CP) excitation of MAS NMR spectra. The former method, simply referred to as MAS NMR, can be made largely non-selective by using pulse interval times at least three times as long as the largest longitudinal relaxation time (T_1) in the sample [21]. Typical

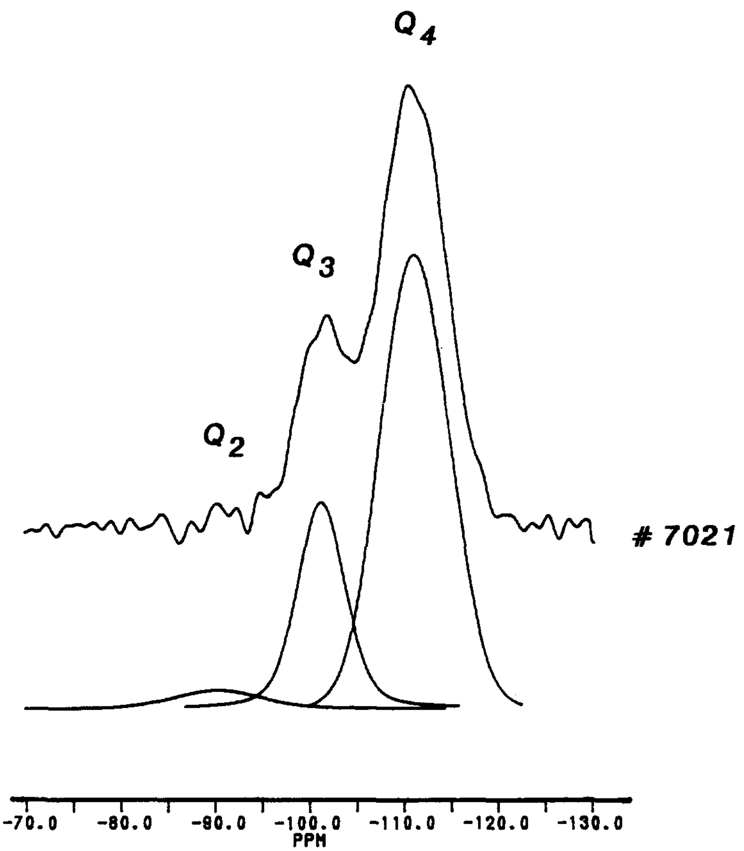

Fig. 5. ^{29}Si Bloch decay MAS NMR spectrum of the Nucleosil 7021 silica substrate with the deconvoluted signals. Number of scans (N_s) = 620; pulse interval time, 90 s; acquisition time, 10 ms; line broadening, 10 Hz.

T_1 values for derivatized silica gels are *ca.* 30 s [22,23]. The CP method, on the other hand, is intrinsically selective [24]. Quantification is sometimes problematic (see the beginning of this section), but the determination of the several time constants involved in the CP process can be used to determine (differences in) motional behaviour of the sample molecules. In this study, molecular moieties with widely differing motional behaviours are involved, often within one sample. This renders the choice between the two excitation methods often non-trivial.

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 Fig. 7. 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. The CP characteristics

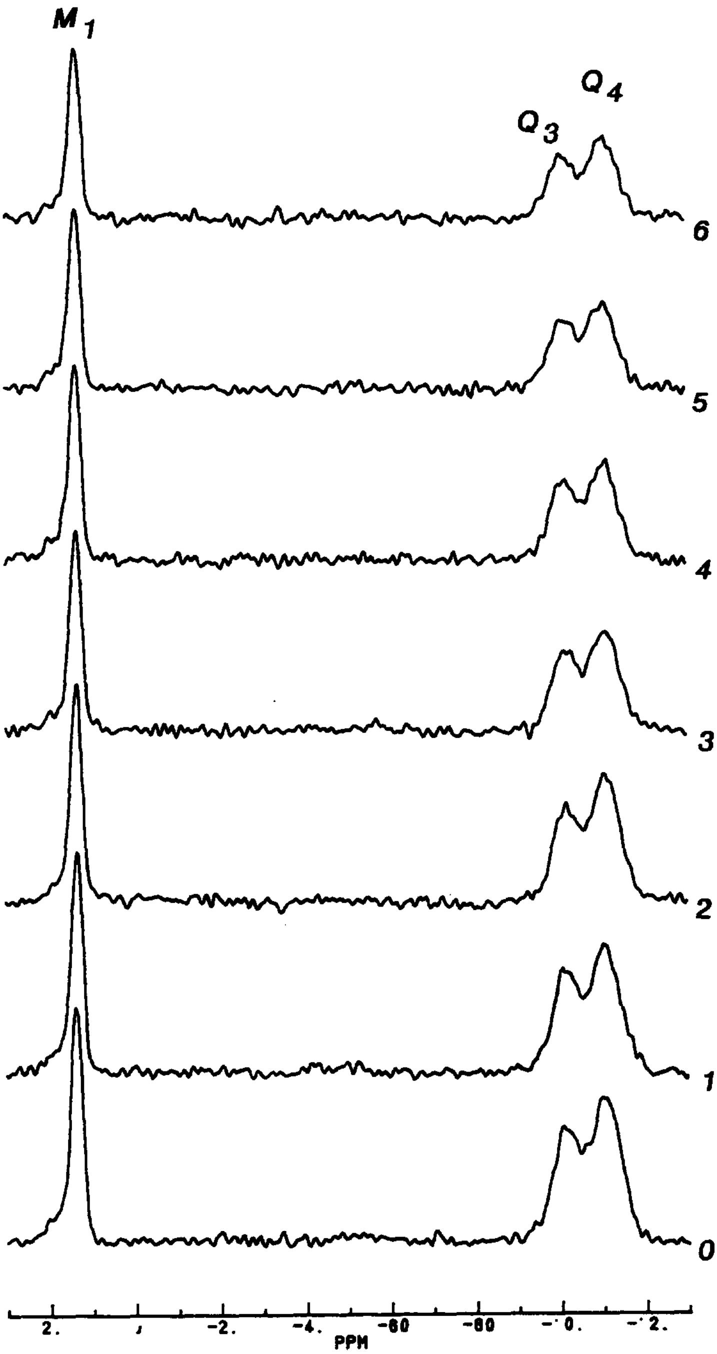

Fig. 6. ^{29}Si CP-MAS NMR spectra of the C_{18}/A phases before (expt. 0) and after the ageing experiments $N_s = 2000$; contact time, 6 ms; interval time; 1 s; acquisition time, 10 ms; line broadening, 15 Hz.

TABLE VIII

CONTENTS OF SILICEOUS MOIETIES OF THE C_{18}/A PHASE AFTER THE AGEING EXPERIMENTS RELATIVE TO THE UNTREATED MATERIAL, DETERMINED BY ^{29}Si CP-MAS NMR WITH THE CORRECTION FACTORS LISTED IN TABLE VII

Ageing expt. No.	Relative content of siliceous surface moieties	
	M_1	Q_3
0	1.00	1.00
1	0.88	0.88
2	0.84	0.81
3	0.79	0.67
4	0.74	0.62
5	0.72	0.57
6	0.67	0.52

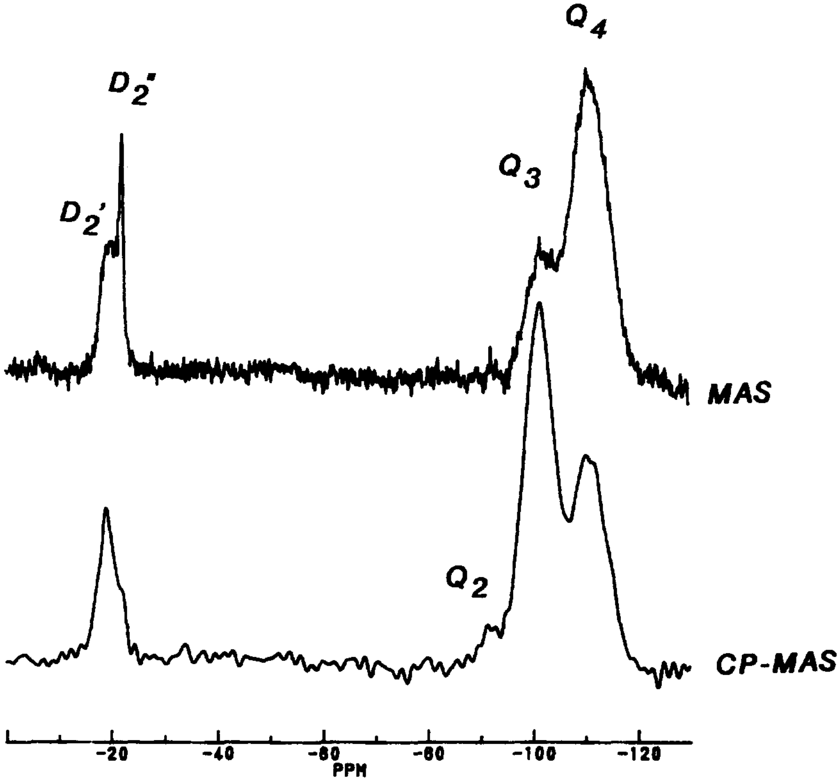

Fig. 7. ^{29}Si MAS NMR spectrum and ^{29}Si CP-MAS NMR spectrum of the original PMSC$_{18}$ stationary phase. Conditions as in Figs. 5 and 6.

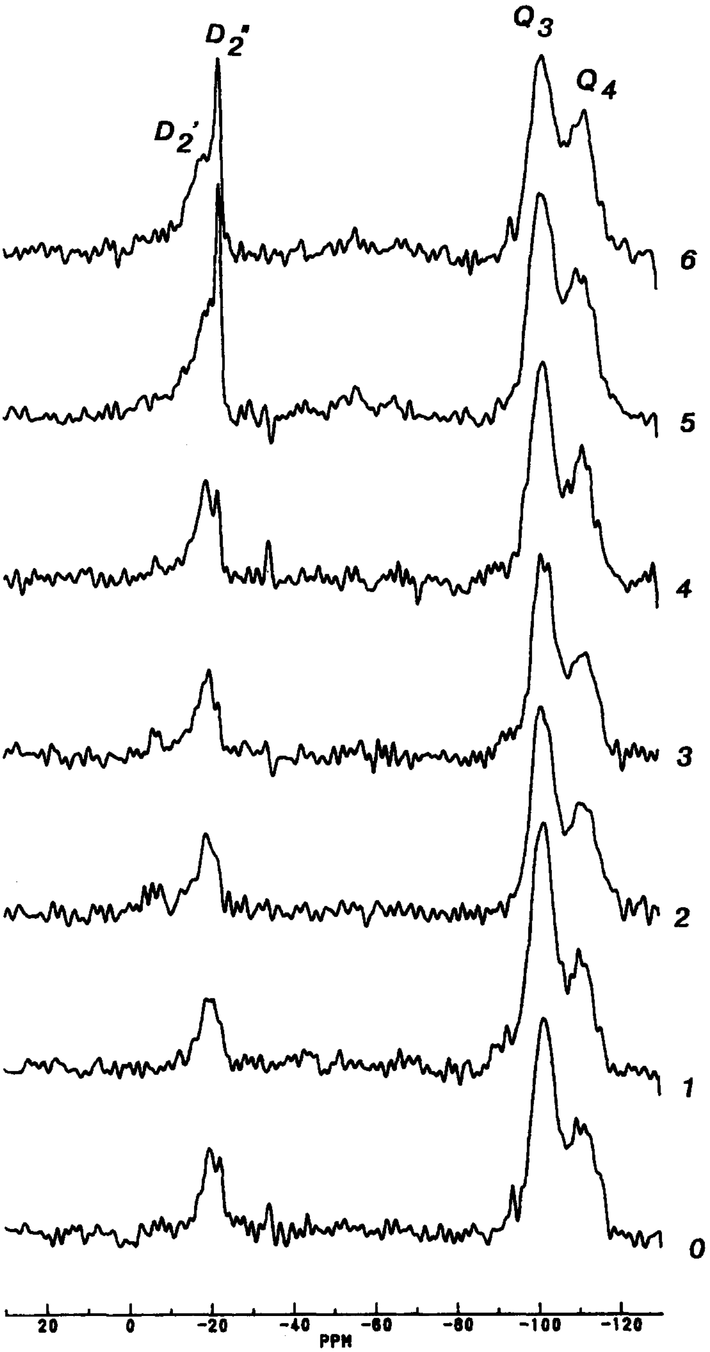

Fig. 8. ^{29}Si CP-MAS NMR spectra of the PMSC$_{18}$ phases before (expt. 0) and after the ageing experiments Conditions as in Fig. 6.

reveal that with a contact time of 6 ms a very small cross-polarization efficiency is obtained for the rather mobile polymer chain. A large correction factor must therefore be used for the D''_2 species with CP-MAS NMR spectra obtained for this stationary phase with a contact time of 6 ms (see Table VII).

Some of the polymethyloctadecylsiloxysilane groups are incorporated in loosely attached, mainly physically adsorbed, polymers at the silica surface, *i.e.*, the D''_2 groups in the NMR spectrum, while the other part is chemically bonded, forming short loops, *i.e.*, the D'_2 groups. The formation of these latter siloxysilanes causes the amount of remaining polymeric hydrocarbonsiloxysilanes (D''_2) to be small. The more mobile polymers are physisorbed by the short chemically bonded hydrocarbon siloxysilane loops at the pore surfaces. This is similar to the methyloctadecylsiloxane polymers being physically adsorbed at the surface of the trimethylsilyl enolate precapped silica substrate. The ratio between D'_2 and D''_2 moieties at the surface of the original $PMSC_{18}$ phase was determined by ^{29}Si MAS NMR to be D'_2:D''_2 = 0.63:0.37. The amount of unreacted silanol groups (Q_3, determined with CP MAS NMR) is high for this stationary phase compared with the other stationary phases (see Table I).

Fig. 8 depicts the CP-MAS NMR spectra of the $PMSC_{18}$ phases before and after the ageing experiments. From these spectra and Table IX, it was concluded that the amount of silanol groups decreases drastically after the ageing experiments with high pH buffer solutions. In parallel with this decrease in silanol content, the amount of polymeric siloxysilanes changes; a first glance at the spectrum would indicate an increase in D''_2. However, this increment in D''_2 moieties is partly artificial. A ^{29}Si CP-MAS NMR experiment with variable contacts showed a shift in CP characteristics with the treatments, and the optimum contact time decreased, together with the correction factor $I(0)/I(6\text{ ms})$. This indicates that the polymethyloctadecylsiloxysilane groups are more tightly attached to the surface. Probably, hydrolysis of siloxane bonds in the long polymer chains causes fragmentation. These polymer silanol groups condense with other silanol groups (polymer or surface) with the formation of a more rigid polymer network with smaller loops. The polymer chains still exist but with

TABLE IX

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 VII

Ageing expt. No.	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: $I(0)/I(6\text{ ms})$ = 1.22. This value was used here.

a decreased free chain length between the cross-linking knots. The amount of small loops anchored to the surface, D'_2, increases accordingly. However, a substantial number of the surface silanol groups disappear owing to substrate hydrolysis, dissolution and condensation with neighbouring silanol groups to form siloxane bridges.

Both processes, the formation of a more rigid coating layer and the dissolution or condensation of silanol groups, explain the changes in the chromatographic behaviour of this stationary phase. These are an increase in polar selectivity *q(p*-hydroxybenzoate) by the increasing number 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 poor.

The ^{29}Si CP-MAS NMR spectra of the native Nucleosil silica substrate, the trimethylsilyl enolate precapped substrate and the subsequently coated polymethyloctadecylsiloxane stationary phase are depicted in Fig. 9. It is calculated from the

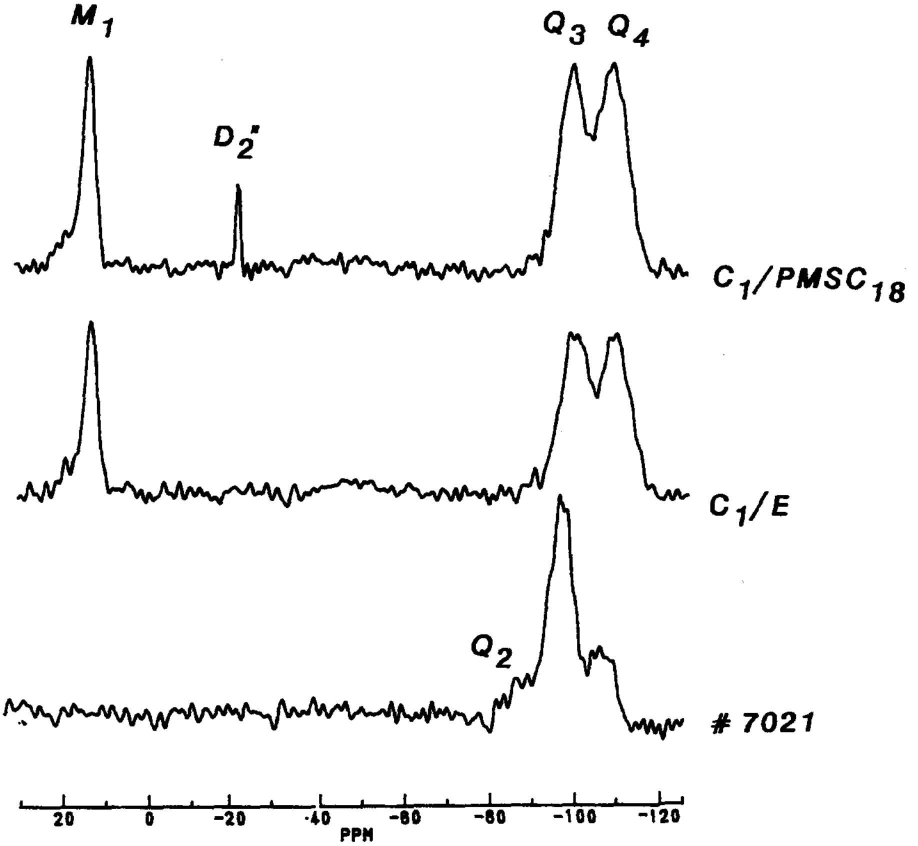

Fig. 9. ^{29}Si CP-MAS NMR spectra of the originate Nucleosil silica substrate (#7021) and the trimethyl-modified silica (C_1/E) and the subsequently polymethyloctadecylsiloxane-coated stationary phase ($C_1/PMSC_{18}$). Conditions as in Fig. 6.

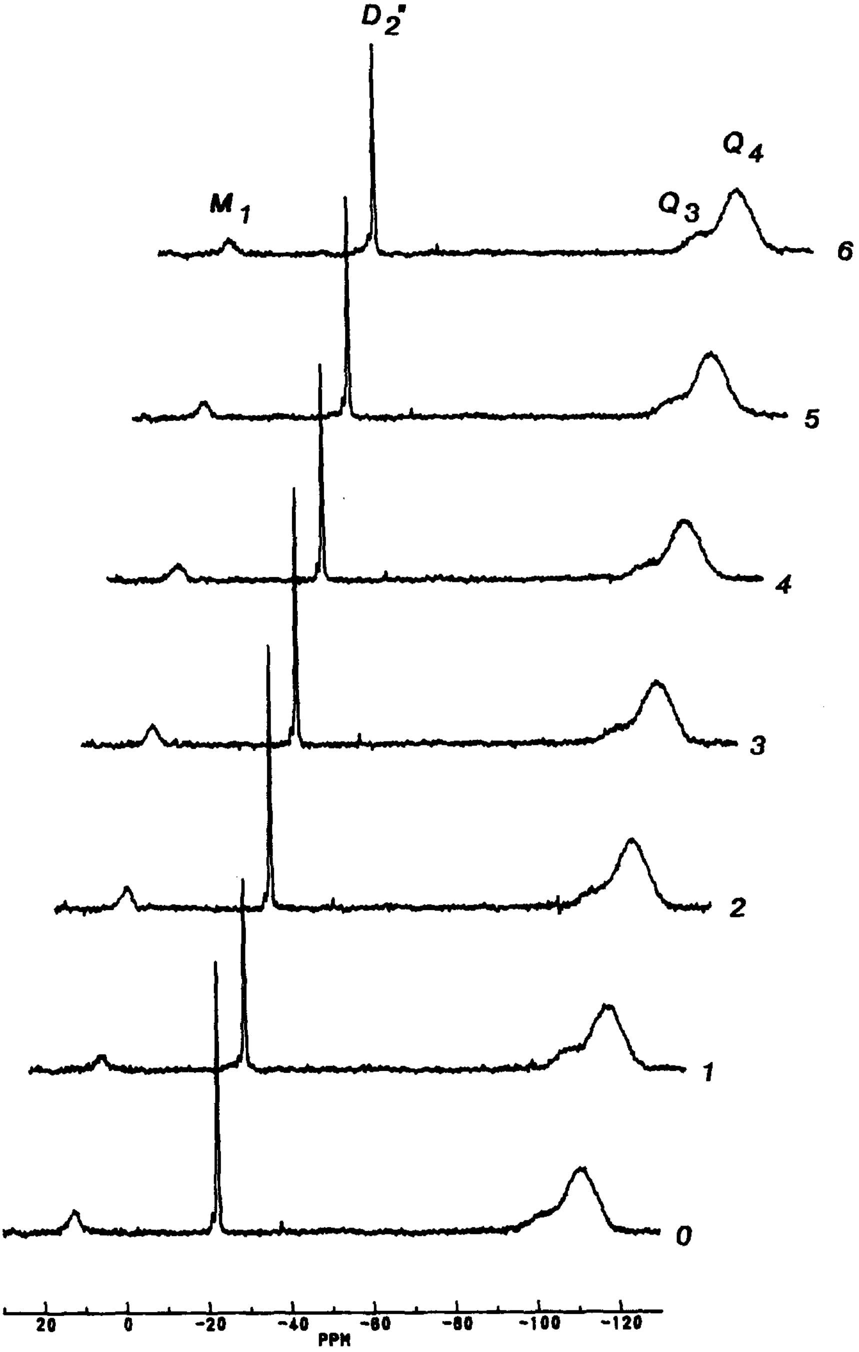

Fig. 10. ^{29}Si Bloch decay NMR spectra of the C_1/PMSC_{18} phases before (expt. 0) and after the ageing experiments. Conditions as in Fig. 5 except acquisition time, 100 ms; line broadening, 1 Hz.

TABLE X

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 expt. No.	Relative content of siliceous moieties			
	M_1	D''_2	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

results that *ca.* 60% of the silanol groups at the native silica surface reacted with the trimethylsilyl enolate. The reaction of the coating polymethyloctadecylsiloxanes with the remaining silanol groups can be neglected (see also Table I). Here again, the CP efficiency of the polymeric hydrocarbon siloxanes, D''_2 moieties, is poor. To our surprise, approximately the same CP characteristics were determined for these moieties at the surfaces of the PMSC_{18} and the C_1/PMSC_{18} phases. One may conclude that similar mobilities for the methyloctadecylsiloxane polymers on top of the surface-anchored short hydrocarbon siloxysilane loops point to similar structures of the polymethyloctadecylsiloxane coating. However, for the C_1/PMSC_{18} phases the CP behaviour was hardly influenced after ageing experiments with high pH water–methanol, in contrast to PMSC_{18}. The precapped polymer-coated stationary phase thus hardly shows an advanced cross-linking under these conditions; see also the selectivity factors α. The non-precapped phase forms cross-links.

For reasons already mentioned, 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 Fig. 10 and Table X).

Again, a slightly higher silanol content was determined after the ageing experiments with extreme pH plain aqueous buffer solutions. With the low pH plain aqueous buffer the increase in silanol content coincides with a decrease in trimethylsiloxysilane ligands and a loss of polymer coating. With the high pH plain aqueous buffer, only a small decrease in anchored ligands is observed. 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 limited silica dissolution.

CONCLUSIONS

The polymethyloctadecylsiloxane-coated stationary phases exhibit a selectivity for polar compounds like those incorporating pyridine, which clearly differs from conventionally modified octadecyl phases. Only a small amount of accessible silanol groups could be determined with ethanol adsorption measurements and chromatography. However, the overall polarity determined by the homologous series of *p*-hydroxybenzoic acid *n*-alkyl esters reveals a larger value for these particular coated phases than for the conventional C_{18}/A phase. This is probably due to interactions with mobile siloxane bonds 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 tightly connected to the silica surface. This is observed to a large extent with the $PMSC_{18}$ phase, probably owing to 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 were also observed after similar high pH ageing experiments in a previous study [3].

The $PMSC_{18}$ phase exhibits the poorest silica shielding properties. Trimethylsilane enolate precapping improves the stability of the polymethyloctadecylsiloxane-coated stationary phase substantially. 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 considerably 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 cause the silica surface to deteriorate as a result of 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 various 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 of the polysiloxane coating is taken, the polymer-coated phases should exhibit a stability equal or even superior to that of conventionally modified phases. A good example is the trimethyl precapped polymethyloctadecylsiloxane phase examined in this study.

ACKNOWLEDGEMENTS

The authors gratefully thank J. G. M. Janssen for fruitful discussions and the characterization of adsorption sites at the silica surface of the modified phases. They also thank M. van Straten and B. Vermeer for technical assistance.

These investigations were supported by The Netherlands Foundation for

Chemical Research (SON) with financial aid from The Netherlands Organization for Scientific Research (NWO) under grant No. 700-344-009.

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