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CHROMATOGRAPHIC AND SOLID STATE NUCLEAR MAGNETIC RESONANCE STUDY OF THE CHANGES IN REVERSED-PHASE PACKINGS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AT DIFFERENT ELUENT COMPOSITIONS

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SUMMARY

A number of long and short chain reversed-phase packings for high-performance liquid chromatography from two different silica substrates, derivatized with mono- or trifunctional silane reagents, were studied under simulated routine conditions. The changes in the properties of the packings are described in terms of loss of silanes, gain in silanol content and rearrangements of the silica-to-silane bondings. Chromatographic techniques, solid-state NMR spectroscopy and elemental analyses were used to characterize and partially to quantify these changes. As expected, long chain phases are more stable than short chain phases with the same silane-to-silica attachment. More surprisingly, the reversed phases derived from monofunctional silanes are much more stable than phases with the same silane chain length prepared from trifunctional silanes. An explanation of the changes is offered in terms of siloxane hydrolysis and rearrangement plus concomitant polymerization of multifunctional silanes. The impact on the chromatographic behaviour is discussed.

INTRODUCTION

A large variety of bonded phases in high-performance liquid chromatography (HPLC) can be prepared by anchoring different organic moieties to substrates, generally mono-, di- or trifunctional silanes to silica, under several reaction conditions^{1–12}. The derivatization of silica substrates results in a network of structural elements at the surface of the stationary phases, which significantly influence the separation process^{6,10,11,13}. The large group of silica-alkyl bonded phases in combination with more or less polar aqueous-organic mixtures as eluents has led to the development of the highly popular reversed-phase chromatography (RP-HPLC). In spite of the extensive and still increasing application of RP-HPLC phases, a number of fundamental problems have remained unsolved.

(i) Large differences in chromatographic properties between apparently the same phases from different manufacturers, and also between batches of one brand,

can be observed^{14–18}. This might be due to the different properties of the silica substrates applied, the different reagents and reaction conditions. After the derivatization process a number of structural elements at the silica surface should be distinguished like lone, geminal, vicinal residual silanols, siloxane bridges and a number of different bonded alkyl chains. The relative amounts of these structural elements, their properties and topologies determine the final behaviour of the bonded phase^{5–9,11,19,20}.

(ii) In a number of cases the properties of RP-HPLC phases change under practical laboratory conditions^{5,13,21–28}. This is due to the influence of the eluent, which may affect the properties of the stationary phase by solvolysis of ligands, hydrolysis of siloxane bridges to silanols, hydrolysis of alkoxy- or chloro-groups of di- and trifunctional reagents and sorption of additives like ion-pairing agents from the mobile phase¹³. Moreover, in some eluents, especially at higher pH (> 7), dissolution of the silica substrate may occur. Therefore, it is not surprising that retention and selectivity may change during a column lifetime. This may result in several problems especially when RP-HPLC columns are applied routinely. The extensive application of RP-HPLC techniques implies a large variety of eluent compositions, which may affect the stationary phase in different ways.

(iii) In view of the above, it is not surprising that the retention and selectivity models developed for RP-HPLC do not yield satisfactory predictions in a number of cases^{6,29–32}. These models often start from the assumption of an homogeneous stationary phase, which is not in accordance with the facts and might lead to erroneous results^{6,33}. Interactions of the components with the silica substrate, *e.g.*, with residual silanols and siloxane bridges, and, possibly, with solvated ligands also influence the separation^{34–36}. Improvements were made by applying separation models taking into account a multiple retention and selectivity mechanism due to the inhomogeneous and complex structure of the stationary phase. However, for a number of polar components the results of these models are still poor^{2,3,5,37–39}. After the present work was completed, Dill⁴⁰ published a new mechanism in which also solute partitioning into the grafted chains is considered.

(iv) Fundamental insight into the technology of packing of efficient and reproducible columns is still poor and the procedures used at present are mostly based on empirical knowledge⁶. Therefore, in laboratory practice one is often confronted with columns having poor kinetic properties and mechanical stabilities.

To study these phenomena of bonded phases a broad range of characterization techniques has been reported. These characterization of stationary phases and substrates may again be subdivided.

(a) The determination of bulk properties like specific area, size distribution and volume of the pores, particle sizes, etc. These data can be obtained by the well known BET, Coulter counter and other techniques^{41,42}. Moreover, the shape of the substrate particles, the pH, the amount of the adsorbed water and the possible contamination with traces of metals may also influence the final properties of the stationary phase.

(b) The qualitative and quantitative determination of structure elements at the silica surface like the various types of silanols, ligands and siloxane bridges. For reversed phases, this type of information can be obtained by a large number of techniques, which in turn may be subdivided into destructive and non-destructive methods. The first group of methods principally interferes with the surfaces of the

phases by solvolysis, pyrolysis and fragmentation reactions. These reaction products can be subsequently analysed by gas chromatography (GC), mass spectrometry (MS) and NMR techniques^{13,43-47}. Non-destructive characterization methods leave the surfaces intact. Infrared and NMR techniques are most important, and in a number of cases complementary, methods to provide qualitative and (semi-)quantitative information about the silanol groups and the bonded ligands at silica surfaces^{13,14,48-58}. For several reasons the qualitative and quantitative determination of the different silanol groups is of great importance. Residual silanols may give rise to unexpected and unwanted interferences with the solvophobic interactions between the stationary phase and solutes^{14,23}.

On the other hand, the amount of the residual silanols gives insight into the extent of the derivatization process. Moreover, further progress in the accurate determination of the several silanol groups, present prior to and after the derivatization process, may contribute to the solution of the controversy about the reactivity of the different silanol types⁵⁹.

The most important methods for the qualitative and quantitative determination of silanols may be subdivided into those principally interfering with the surface of the stationary phase and those which leave these surfaces intact. In the first group of methods, titration or reactions with alkyl-metal compounds are the most important techniques⁵⁹⁻⁶⁶. The latter group of methods includes isotopic proton exchange⁶⁴⁻⁶⁷, infrared and NMR techniques^{13,23,59,68}. Contrary to the isotopic exchange methods, which provide information about the total amount of silanols, infrared and NMR techniques are much more useful in differentiating the various silanol types present at the surfaces investigated. Also a number of chromatographic methods, based on specific retention of polar components by silanols, have been developed⁶⁹⁻⁷². There is, however, one important difference between indirect spectroscopic techniques and direct chromatographic approaches. For the latter, only the accessible silanols, the amount of which may be influenced by the actual eluent composition, can be determined. At present, there is poor agreement between different methods for quantifying the silanols.

(c) The structure of the surfaces is usually expressed in terms of the conformation and mobilities of the ligands. Earlier models of the surfaces of bonded phases are the brush-, fur- and breathing models^{2,73,74}. These models do not satisfactorily explain a number of observations in the chromatography of bonded phases. It has become clear that, besides the types and amounts of bonded ligands at the surface, also their conformations and mobilities may affect the chromatographic properties of RP-HPLC phases. Chain conformations and mobilities are determined by the basic properties of the substrate, type of ligand and the bonding to the surface and, finally, the eluent composition.

Fluorescence, IR and NMR techniques seem to be the methods of choice for studying conformations⁷⁵⁻⁸⁰, whereas NMR and neutron scattering are suitable for determining mobilities in the 10^9 to 10^{11} Hz range, *e.g.*, kink diffusion^{75,81,82}. In order to yield useful results in a reasonable time, ^{13}C NMR measurements of suspensions usually require labelling. Alternatively, results can be obtained without labelling⁸³, but then the information is usually drawn from strong peaks, consisting of overlapping resonances. This information is then necessarily only semi-quantitative in nature. In a number of studies it was found that the mobilities of the alkyl chains

at the substrate's surface (coverage) vary with the type and composition of the mobile phase and, *e.g.*, with its viscosity^{82,84–87}. In one report it was demonstrated by ²H NMR spectroscopy that apolar solvents like hexane and benzene do not significantly solubilize the chains, whereas methanol does⁸⁸. Another paper deals with water association with the stationary phase and its possible chromatographic consequences⁸⁹. Moreover, the mobilities of the methyl group at the end of the alkyl chain increase with the chain lengths and are larger than those of the methylene groups of the chains. Correlations between chain mobilities and chromatographic properties of alkyl bonded phases were studied by Martire and Boehm⁷⁴ and Albert *et al.*⁸⁶.

(d) Chromatographic characterization of stationary phases can be directed towards either the determination of the selectivity or the efficiency properties of these phases. The efficiency properties of stationary phases reflect the kinetic behaviour, *i.e.*, the quality of the packed chromatographic bed. Moreover, also the thermodynamic behaviour, controlled by the surface properties, may play a rôle in the kinetics of bonded phases, *e.g.*, due to secondary interactions between stationary phase and solutes. In the present study the characterization in terms of selectivity of the RP-HPLC phases is emphasized.

In selectivity studies of bonded phases the solvophobic selectivity can be monitored by applying apolar test solutes, showing negligible interactions with the silica substrate^{90,91}. Alternatively, attention can be focused on the determination of the influence of the silica substrates by applying polar test solutes like phenols and anilines, which are indicators of substrate interactions^{14–16}. The chromatographic characterization of the RP-HPLC phases was performed essentially according to the work of Jandera^{90–93} and Smith^{94,95}. It is based on the assumption that the log of the capacity factors, k' , of an homologous series is a linear function of the eluent composition or

$$\log k' = a - mx \quad (1)$$

and

$$a = a_0 + a_1 n_c \quad (2a)$$

$$m = m_0 + m_1 n_c \quad (2b)$$

where x is the volume fraction of the organic part of the eluent, a_0 , a_1 , m_0 and m_1 are constants and n_c is the incremental carbon number of an homologous series.

From eqns. 1, 2a and 2b, a_0 equals $\log k'$ of the molecular residue of the homologous series extrapolated to a mobile phase composition of 100% water. The value of a_0 depends predominantly on the type and amount of ligand, and thus allows the direct chromatographic comparison between a number of different alkyl bonded phases. The chromatographic characterization of bonded phases was performed on two test mixtures of homologous series of alkylbenzenes and alkyl aryl ketones. Moreover, a third test mixture of phenol and 2,5-dimethylaniline, to study the more polar interactions of the stationary phase, was used.

In the present paper, we concentrate on the change in properties of RP-HPLC phases under practical laboratory conditions^{5,13,21–28}. Our aim is to investigate the influences of eluent compositions on the qualitative and quantitative changes in the

phases and, subsequently, to study the chemical reactions underlying these processes. Furthermore, we investigated the influences on the chromatographic properties of the phases. Köhler *et al.*^{23,96} described the change in chromatographic properties of a number of bonded phases, but these studies were restricted to the use of water as an eluent. In earlier work we reported the results of a study on the changes in two RP-HPLC phases based on Zorbax silica with a number of different mobile phases¹³. Based on these results, a large number of RP-HPLC phases on different silica substrates have now been subjected to artificial, strictly controlled experiments. This included the continuous, separate subsection of columns, packed with these phases under well defined laboratory conditions, to several eluent compositions, generally applied in RP-HPLC techniques.

Usually, a period of 240 h was needed for a typical experiment. High and low pH values, aqueous and methanol–aqueous buffers were used as eluents. Some basic ion-pairing agents were also included. The eluents were recirculated during these experiments. This might influence the experiments in terms of saturation of the eluent with dissolved silica and/or ligands, so decreasing the extent of the observed phenomena. This would mean that these effects will be more serious in laboratory practice where recirculation of the eluent is not a common practice. The recirculation approach was taken for practical and especially economic reasons. It seems reasonable to consider the results of the present experiments as the minimum changes during normal laboratory use for the different combinations of reversed phases and eluents. Before and after these treatments, the columns were tested chromatographically. Moreover, elemental analyses of the stationary phases, when removed from the columns, were carried out. Finally, solid-state high-resolution NMR spectra of the phases were taken in order to observe the changes in silanol contents and in the types and contents of the ligand-to-surface attachments. In this study, a special problem for the quantitation of the total ligand concentration by elemental analysis is the possible dissolution both of the ligands and of the silica substrate in the eluent. These dissolution processes will generally not occur to the same relative extents to which silanes and substrates occur in the RP phases. Therefore, in some cases, apparently the same or even increasing carbon loads could be “observed” by elemental analysis, while, *e.g.*, NMR data clearly indicate the opposite process.

EXPERIMENTAL AND RESULTS

First, the validity of eqn. 1 was investigated by injecting the two test mixtures of the homologous series of alkylbenzenes and that of the alkyl aryl ketones with three or four different eluent compositions of water and methanol on freshly prepared columns. The capacity factors, k' , of the components were calculated by linearization⁹⁷ of the function

$$\log k' = \log k'_0 + (\log q)n_c \quad (3)$$

where k'_0 is the capacity factor of the molecular residue of the homologous series, $\log q$ the incremental value of the capacity factor of that series and n_c the incremental carbon number.

From these experiments it appeared that the $\log k'$ values are linearly depen-

Fig. 1. Graphical presentation of the relationship between $\log k'$ on Hypersil ODS of the members of two homologous series and the volume fraction methanol in the eluent.

dent on the eluent composition at least in the range 60–90% (v/v) of methanol. A typical example of a graphical plot of the results for Hypersil ODS is presented in Fig. 1. Subsequently, from these k' values the a_0 values were calculated according to eqns. 1, 2a and 2b by multiple regression.

TABLE I
INVESTIGATED SILICAS

Hypersil silica and bonded phases (Shandon Southern Products, Runcorn, U.K.); Zorbax silica and bonded phases (DuPont, Wilmington, DE, U.S.A.).

	<i>Hypersil</i>	<i>Zorbax</i>
Mean particle size (μm)	5	7
Specific area ($\text{m}^2 \text{g}^{-1}$)	170	300
Mean pore size (nm)	12	10
pH (10%, w/w water)	8.2	3.8
Modification	Trimethylchlorosilane (SAS) Octyldimethylchlorosilane (MOS) Octadecyldimethylchlorosilane (ODS) Butyltrichlorosilane (Butyl) Octadecyltrichlorosilane (ODS-t)	Octyldimethylchlorosilane (Z C ₈) Octadecyldimethylchlorosilane (Z C ₁₈)

Seven alkyl bonded phases (Table I), prepared on two different silica substrates, were subjected to simulated routine use. Each stationary phase was packed in columns, 100 mm $\times$ 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, while the remaining column was used for initial chromatographic tests.

The equipment for simulating routine use consisted of a six-headed metering pump (Metering Pumps, London, U.K.) provided with laboratory-constructed pulse dampers, allowing each column to be purged separately with a specific eluent (Fig. 2). The pump flow-rates could be controlled independently. All eluents were freshly prepared, preserved with 0.02% sodium azide and filtered over 0.22- μm membrane filters (Millipore, Intertech, Bedford, MA, U.S.A.) prior to use (Table II).

From each of the eluents, 1 l was prepared and purged through the column continuously by recirculating the column effluent, during 240 h at a flow-rate of 0.5 ml/min. So during one cycle of the purging process each column was purged with

Fig. 2. Schematic diagram of the equipment for simulating routine use of columns. a = Pump head; b = pulse damper; c = column; d = eluent bottle. Flow-rate: 0.5 ml/min. Time 240 h (continuous). Ambient temperature.

TABLE II

TREATMENTS FOR SIMULATING ROUTINE USE EXPERIMENTS

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

<i>Treatment No.</i>	<i>Buffer</i>	<i>pH</i>	<i>Volume fraction of methanol in the eluent</i>	<i>Ion-pairing agent and concentration (M)</i>
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	Hexanesulphonate (0.005)
IV	0.05 M Phosphate	3.0	0.5	Hexylamine (0.01)
V	0.05 M Bicarbonate	8.4	0	—
VI	0.05 M Bicarbonate	8.4	0.5	—
VII	0.05 M Bicarbonate	8.4	0.5	Triethylamine (0.005)

about 7000 column volumes of a specific eluent. All purging experiments were performed at ambient temperature. After finishing a typical series of purging experiments, the columns were carefully washed with water, mixtures of water and methanol and finally with pure methanol. Precautions were taken to prohibit the deposition of buffering salts and strong water-methanol gradients in the columns. Subsequently, the columns were subjected to a number of chromatographic experiments with three test mixtures (Table III) at suitable eluent compositions.

These chromatographic test experiments were performed with a Model 6000-A (Waters, Milford, MA, U.S.A.), a Model CV-6-VHPa-N60 injection valve equipped with a 20- μ l loop (Valco, Houston, TX, U.S.A.) and a variable-wavelength UV detector (Type LC-3; Pye Unicam, U.K.) operated at 254 nm. Injections of 1–5 μ l of the test mixtures were performed. The detector output signals were sampled by a DEC μ -PDP-11 microcomputer (sample frequency 10 Hz), and subsequent calculations of the first moments of the peak signals were performed on a VAX-750 computer. Log k'_0 was determined from chromatograms of the test mixtures 1 and 2

TABLE III

TEST MIXTURES FOR THE CHROMATOGRAPHIC CHARACTERIZATION OF THE COLUMNS

Test components dissolved in methanol.

<i>Mixture</i>		
<i>1</i>	<i>2</i>	<i>3</i>
Benzene	—	Phenol
Methylbenzene	Ethanophenone	2,5-Dimethylaniline
Ethylbenzene	Propanophenone	—
Propylbenzene	Butanophenone	—
Butylbenzene	Pentanophenone	—
	Hexanophenone	—

TABLE IV

LOG OF THE CARBON CONTENT, p_c (g/g), OF THE STARTING BONDED PHASES

Type of bonded phase	$\log p_c$
SAS	-1.51
MOS	-1.16
ODS	-0.93
Butyl	-1.57
ODS-t	-1.06
Z C ₈	-0.96
Z C ₁₈	-0.85

according to eqn. 3 by linearization of this function. Subsequently, the a_0 values were calculated from:

$$\log k'_0 = a_0 - m_0 x \quad (2c)$$

The standard deviation of the a_0 measurements was <0.07 . From the k'_0 values of benzene and the data from the chromatograms of test mixture 3, the retentions, r , of 2,5-dimethylaniline and phenol relative to benzene were calculated. Moreover, test mixture 3 was used for qualitative judgement of substrate interactions of silanols. Finally, again after careful washing of the columns with mixtures of water and methanol and pure methanol, the packing was removed from the column and dried at 115°C in vacuum for spectroscopic and elemental analysis. Elemental analysis of the carbon and hydrogen contents was performed on a Model 240 C Analyzer (Perkin-Elmer, Norwalk, CT, U.S.A.). The log of the carbon contents, p_c , of the starting materials is presented in Table IV. The p_c value was used to calculate the ligand surface density according to¹²

$$\alpha_1 = \frac{p_c}{S[m_c - p_c(M_1 - 1)]} \quad (\text{mol/m}^2) \quad (4)$$

where p_c = the amount of carbon (g/g), S = the specific area of the substrate, m_c = the amount of carbon per mol of ligand and M_1 = molecular mass of the ligand.

The results of the spectroscopic, chromatographic measurements and the elemental analysis are summarized in Tables V–XI, where p_c = the amount of carbon (g/g); α_1 = surface coverage of ligands (mol/m²), eqn. 4; NMR, ligand = degree of silylation as defined by [ligand]/([ligand] + [silanol]); a_0 = capacity factor of benzene at an eluent composition of 100% water, eqns. 1 and 2; NMR, SiOH = relative concentration of silanol groups (= 1 minus the degree of silylation); r_{phenol} = retention of phenol relative to benzene and r_{dma} = retention of 2,5-dimethylaniline relative to benzene.

As pointed out earlier in this paper, some of the p_c and consequently of the α_1 data do not seem to agree with the corresponding NMR data, but this should be ascribed to dissolution of the silica substrate. Typical chromatograms of an untreated Hypersil butyl-modified silica and the same stationary phase after purging, experiments V and VI, are presented in Fig. 3.

Fig. 3. Chromatograms of an untreated Hypersil Butyl-modified silica (A) and the same stationary phase after purging experiment V (B) and experiment VI (C), according to Table II. Purging conditions: 7000 column volumes; flow-rate 0.5 ml/min; ambient temperature. Chromatographic test conditions: test mixture I, alkylbenzenes; eluent, water-methanol (50:50, v/v); detection, UV at 254 nm.

TABLE V

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE SAS MODIFICATION OF HYPERSIL SILICA

Treatment	Ligand				Silanol		
	Elem. analysis		NMR ligand	Chrom. α_0	NMR SiOH	Chromatographic	
	$\log p_c$	α_1				r_{phenol}	r_{dma}
0	-1.51	5.44	0.41	1.14	0.59	0.37	1.17
I	-2.05	1.48	0.08	0.34	0.92	0.46	5.56
II	-1.63	4.07	0.30	1.07	0.70	0.37	0.93
III	—	—	—	—	—	—	—
IV	-1.61	4.19	0.34	1.10	0.66	0.37	1.09
V	-1.74	3.12	0.16	0.68	0.84	0.23	1.09
VI	-1.76	2.96	0.22	0.80	0.78	0.39	1.11
VII	-1.89	2.15	0.12	0.47	0.88	0.13	1.50

TABLE VI

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE MOS MODIFICATION OF HYPERSIL SILICA

Treatment	Ligands		Silanol				
	Elem. analysis		NMR ligand	Chrom. a_0	NMR SiOH	Chromatographic	
	$\log p_c$	α_1				r_{phenol}	r_{dma}
0	-1.16	3.78	0.49	2.03	0.51	0.38	0.89
I	-1.15	3.81	0.46	2.05	0.54	0.43	1.05
II	-1.13	4.03	0.46	2.03	0.54	0.38	0.99
III	—	—	—	—	—	—	—
IV	-1.16	3.76	0.46	2.03	0.54	0.37	1.02
V	-1.16	3.79	0.50	2.03	0.50	0.40	0.94
VI	-1.16	3.78	0.50	2.01	0.50	0.36	0.73
VII	-1.18	3.57	0.46	1.96	0.54	0.34	0.67

²⁹Si cross polarization magic angle spinning (CP MAS) NMR

²⁹Si NMR spectra were obtained at 59.63 MHz on a Bruker CXP 300 spectrometer using a Beams-Andrew type probe and rotor or a double air-bearing probe of more recent design. MAS rotation speeds were 3500–3800 Hz. Usually, 4000–6000 transients were accumulated with the Beams-Andrew probe while 2000 transients were sufficient with the double air-bearing probe. The pulse interval was 1 s. Contact times were 2 ms or, for checking purposes, 6 ms. A value of 2 ms is close to the optimum for silanol groups and multidentate surface-linked groups^{50,53}. A larger value has been recommended in cases where trimethylsiloxy groups are to be cross-polarized⁵³. It is known that longer contact times lead to difficulties in maintaining a stable Hartmann-Hahn match^{50,53}. This will happen more easily upon trying to

TABLE VII

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE ODS MODIFICATION OF HYPERSIL SILICA

Treatment	Ligands		Silanol				
	Elem. analysis		NMR ligand	Chrom. a_0	NMR SiOH	Chromatographic	
	$\log p_c$	α_1				r_{phenol}	r_{dma}
0	-0.93	3.41	0.45	2.25	0.55	0.24	2.42
I	-0.95	3.18	0.55	2.30	0.45	0.28	0.62
II	-0.93	3.35	0.46	2.31	0.54	0.25	1.29
III	-0.95	3.23	0.43	2.31	0.57	0.25	0.77
IV	-0.95	3.24	0.42	2.31	0.58	0.25	0.62
V	-0.95	3.26	0.60	2.31	0.40	0.25	0.70
VI	-0.95	3.23	0.45	2.29	0.55	0.24	0.61
VII	—	—	—	—	—	—	—

TABLE VIII

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE BUTYL MODIFICATION OF HYPERSIL SILICA

Treatment	Ligands		Silanol				
	Elem. analysis		NMR ligand	Chrom. a_0	NMR SiOH	Chromatographic	
	$\log p_c$	α_1				r_{phenol}	r_{dma}
0	-1.57	3.52	0.47	0.74	0.53	0.32	2.42
I	-1.55	3.70	0.47	0.77	0.53	0.55	1.47
II	-1.53	3.83	0.50	0.81	0.50	0.33	0.91
III	-1.53	3.84	0.54	0.78	0.46	0.36	0.98
IV	-1.52	3.91	0.41	0.72	0.59	0.36	0.99
V	-1.84	1.83	0.26	0.19	0.74	0.32	1.36
VI	-1.69	2.61	0.36	0.56	0.64	0.39	1.09
VII	—	—	—	—	—	—	—

reach “full cross-polarization efficiency” as was advocated recently by Köhler *et al.*²³. We found no larger discrepancies between mono- and trifunctionally derivatized silicas upon using contact times of 2 ms than with 6 ms. The longer contact times are not required for monodentate surface-linked silanes with longer alkyl chains, such as in octyl and octadecyl phases. Therefore, the only RP phase in this study where a contact time of 2 ms is relatively short is Hypersil SAS. Experiments in duplicate indicated that the total estimated uncertainty amounts to *ca.* 10%, a value slightly better than that claimed recently by us⁹⁸.

The data in Tables V–XI summarize the relative areas of the silane and silanol signals in the NMR spectra, as defined in the previous paragraph. Thus only the degree of silylation follows directly from these data, and not the changes in magni-

TABLE IX

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE ODS-t MODIFICATION OF HYPERSIL SILICA

Treatment	Ligands		Silanol				
	Elem. analysis		NMR ligand	Chrom. a_0	NMR SiOH	Chromatographic	
	$\log p_c$	α_1				r_{phenol}	r_{dma}
0	-1.06	2.68	0.32	1.91	0.68	0.29	0.80
I	-1.01	3.08	0.34	1.90	0.66	0.31	1.18
II	-1.05	2.75	0.20	1.92	0.80	0.30	1.26
III	-1.04	2.86	0.36	1.94	0.64	0.30	0.74
IV	-1.05	2.73	0.31	1.84	0.69	0.25	0.79
V	-1.04	2.87	0.15	1.45	0.85	0.22	0.85
VI	-1.08	2.56	0.29	1.72	0.71	0.29	0.92
VII	—	—	—	—	—	—	—

TABLE X

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE Z C₈ MODIFICATION OF ZORBAX SILICA

Treatment	Ligand		Silanol				
	Elem. analysis		NMR ligand	Chrom. a ₀	NMR SiOH	Chromatographic	
	log p _c	α ₁				r _{phenol}	r _{dma}
0	-0.96	4.2	0.70	2.28	0.30	0.34	0.83
I	-0.95	4.3	0.67	2.29	0.33	0.34	0.82
II	-0.96	4.2	0.61	2.29	0.39	0.34	0.86
III	—	—	—	—	—	—	—
IV	-0.97	4.1	0.54	2.27	0.46	0.33	0.85
V	-0.95	4.3	0.69	2.31	0.31	0.36	0.75
VI	-0.98	4.1	0.54	2.27	0.46	0.37	0.76
VII	-0.99	3.9	0.48	2.21	0.52	0.38	0.70

tudes of silane and silanol signals separately. In order to provide these latter data, the NMR signal areas can also be compared in an "absolute" way. This is illustrated for the untreated materials in comparison with materials subjected to treatment V for the following reversed phases: Hypersil SAS (Fig. 4), Hypersil ODS (Fig. 5), Hypersil Butyl (Fig. 6) and Hypersil ODS-t (Fig. 7).

For one given reversed phase material, the CP MAS NMR spectra were recorded for one consecutive series of experiments. In those cases, the uncertainties are as stated above. In principle, we could also compare ²⁹Si CP MAS NMR spectra of different reversed phases in the same absolute manner. In such a case, however, the uncertainty would be larger, because the NMR measurements were performed at different periods, *i.e.*, after different adjustments of the cross-polarization Hartmann-Hahn match.

TABLE XI

RESULTS OF THE NMR, CHROMATOGRAPHIC MEASUREMENTS AND ELEMENTAL ANALYSIS OF THE Z C₁₈ MODIFICATION OF ZORBAX SILICA

Treatment	Ligand		Silanol				
	Elem. analysis		NMR ligand	Chrom. a ₀	NMR SiOH	Chromatographic	
	log p _c	α ₁				r _{phenol}	r _{dma}
0	-0.84	2.43	0.42	2.34	0.58	0.25	0.63
I	-0.86	2.34	0.37	2.33	0.63	0.25	0.66
II	-0.85	2.42	0.50	2.34	0.50	0.23	0.65
III	—	—	—	—	—	—	—
IV	-0.86	2.36	0.52	2.40	0.48	0.27	0.70
V	-0.86	2.34	0.40	2.37	0.60	0.26	0.65
VI	-0.86	2.31	0.31	2.36	0.69	0.24	0.58
VII	-0.87	2.25	0.34	2.33	0.66	0.27	0.64

Fig. 4. ^{29}Si CP MAS NMR spectra of Hypersil-SAS: (A) before treatment; (B) after treatment V.

DISCUSSION AND CONCLUSIONS

In the present investigation, basically two different types of RP-HPLC phases, obtained with either mono- or trifunctional silanes, have been subjected to artificial ageing and subsequent analyses (see Table I). For both types, “long” (C_{18}) and “short” (monofunctional C_1 *versus* trifunctional C_4 !) phases were investigated. For monofunctional phases, a comparison was also made between octyl and octadecyl chains on two different types of silica: Hypersil and Zorbax. The surface concentration of alkyl ligands of Zorbax ODS was lower than that on its Hypersil counterpart; the two octyl phases were comparable in this respect (difference *ca.* 10%). This can be concluded from the elemental analyses of the starting materials (not influenced by side-effects of treatment with basic eluents, see Experimental) together with the different specific areas.

Fig. 5. ^{29}Si CP MAS NMR spectra of Hypersil-ODS: (A) before treatment; (B) after treatment V.

The specific areas of Hypersil and Zorbax silica are *ca.* 170 and 300 m^2/g , respectively. ^{29}Si CP MAS NMR spectra indicate that the degree of silylation of Zorbax Z C₈ is clearly higher than that of Hypersil MOS, while those of the two octadecyl phases are comparable (see headings "NMR ligand" in Tables V–XI) for the untreated materials. Both octyl phases exhibit an higher degree of silylation than the comparable octadecyl phases, especially for the two Zorbax phases. This finding is in accordance with common practice. The degree of silylation of the Hypersil-Butyl phase was higher than that of Hypersil ODS-t, according to ^{29}Si CP MAS NMR spectroscopy. This result was also obtained from elemental analysis (different carbon contents corrected for differences in chain lengths, same silica substrates).

The k' values from the chromatographic tests and, especially, ^{29}Si CP MAS NMR spectroscopy showed that monofunctionally derivatized Hypersils are much more resistant towards exposure to HPLC eluents than their trifunctionally derivatized analogues. This is best demonstrated by comparing Hypersil ODS with Hypersil ODS-t with the same chain lengths, see, *e.g.*, Figs. 5 and 7. This was most evident

Fig. 6. ^{29}Si CP MAS NMR spectra of Hypersil Butyl: (A) before treatment; (B) after treatment V.

when subjecting the phases to 0.05 M bicarbonate buffer (pH 8.4), with or without 50% (v/v) methanol (treatments VI and V, respectively, in Table II).

Rather drastic effects were also found for Hypersil Butyl (Fig. 6). The ^{29}Si CP MAS NMR spectra show extensive hydrolysis of the monodentate, trifunctional silane-surface linkage with subsequent formation of bi-/and tridentate linkages (cross-polymerization, see below). The latter process is particularly important upon treatment with eluents containing 50% (v/v) methanol and, to a lesser extent, with a basic eluent. On the other hand, addition of ion pairs did not clearly influence the process (found by NMR spectroscopy, results not shown). The changes in the ratios between mono-, bi- and tridentate silane-surface linkages, brought about for Hypersil Butyl and Hypersil ODS-t, are remarkably similar, given the large difference in chain lengths, *cf.*, the spectral regions -45 to -65 ppm in Figs. 5 and 7 for untreated (A) and treated (B) reversed phases. Moreover, silane chains are being removed from the surface, presumably by hydrolysis, by basic eluents. This effect is stronger for the ODS-t phase than for the butyl phase, *cf.*, the growth of the SiOH signals at *ca.* -102 ppm, in Figs. 5B and 7B with respect to Figs. 5A and 7A, respectively. We assume that this difference should be attributed to the lower degree of silylation of the ODS-t phase because longer alkyl rests should, for a given degree of silylation, protect the surfaces more efficiently than shorter chains. Different influences of chains of various lengths can be observed for the monofunctionally derivatized Hypersils

Fig. 7. ^{29}Si CP MAS NMR spectra of Hypersil ODS-t: (A) before treatment; (B) after treatment V.

described in this study. Here the results are according to expectations: the very short chains of the Hypersil SAS phase do not protect the reversed phase from hydrolysis under most conditions. Extensive hydrolysis of silane ligands is observed together with some hydrolysis of siloxane bridges: on an absolute basis, the increase in silanol content is larger than the loss in ligands, *cf.*, the signal areas in Fig. 4 at *ca.* 12 ppm (silane) and at *ca.* -102 ppm (shoulder, silanol) before and after treatment.

For the longer monofunctional silanes, see, *e.g.*, Fig. 5, the ligand hydrolysis is generally very much slower and here the siloxane hydrolysis constitutes the main change in the reversed phase, in accordance with results obtained earlier in this laboratory¹³. These latter results of hydrolyses are derived from ^{29}Si NMR data.

There are no significant differences in stability between the octyl or the octadecyl reversed phases on Hypersil and those on Zorbax. Our results indicate that reversed phases with long-chain trifunctional silanes seem to be less "resistant" than comparable phases synthesized with monofunctional silanes. The conformational equilibria of the silane chains in the HPLC eluents may be influenced to a certain extent by the way in which the silane is attached to the surface (mono-, bi- or tri-dentate). Therefore, the "hydrophobic shielding" of the surface by the chains might vary but this variation is probably too small to cause large differences in stabilities as seen in the present study. The smaller degree of silylation of Hypersil ODS-t with respect to Hypersil ODS-m (*ca.* 25% by elemental analyses and ^{29}Si NMR spectroscopy) might play a minor rôle, see also above.

Intrinsic differences in the chemical stabilities of surface-to-silane bonds (in fact: siloxane bridges) as a consequence of the different substitution patterns at the silane silicon atom are conceivable. In our view, however, the following explanation seems most plausible. Monodentate surface linkages of trifunctional silanes are known to undergo polymerization rather easily^{99,100} under influence of water. Furthermore, the mode of attachment of trifunctional silanes to silica surfaces (direct reaction with silica silanols followed by cross-polymerization *versus* physisorption of silanes to silica, polymerized in solution) has been a matter of controversy¹⁰⁰. We now conjecture that from a surface largely occupied with monodentate, trifunctional silanes in water at high pH, especially in the presence of an organic modifier, a number of silanes are hydrolyzed and solvated. A certain percentage of those silanes will remain in solution and this accounts for the decreasing surface concentration of ligands. The balance will polymerize both along the surface and perpendicular to it. The resulting reversed phase will consequently exhibit an higher degree of vertical polymerization. The vertical polymers probably do not contain more than four or five monomer units, because otherwise the ²⁹Si CP MAS NMR signals could not be generated with contact times of 2–6 ms. A slight tendency towards longer optimum contact times was, in fact, observed. A more or less analogous situation has already been mentioned by Sindorf and Maciel⁹⁹ in their description of the synthesis of silylated silicas starting with bifunctional (chloro-)silane, but they did not consider it a major process. The process of vertical polymerization at the silica surface may be facilitated by partial dissolution of the silica material in the eluent, particularly in view of the rather high pH value⁸⁶. It is also quite understandable that, once the vertical polymerization has taken place, the chromatographic properties of the phase have changed drastically. Especially when methanol is present, the phase is probably much more solvated⁸⁹. Any ordering effects in and among the chains anchored to the silica surface⁴⁰ will be strongly diminished. Hence, also the partitioning of organic solutes into the phase⁴⁰ will become increasingly difficult with concomitant decreases in *k'* values. On the other hand, these effects will be similar for members of homologous series, causing relative retentions to remain more or less constant. Finally, silanol groups at the surface will be more abundant and much easier to reach.

The number of reversed phases presented in this paper is limited to seven. This means that some conclusions may be tentative and it is to be anticipated that other reversed phases might yield (slightly) different results. With these limitations in mind we would like to present the following final conclusions regarding the phases and eluents discussed.

Tables V–XI indicate that, as expected, long chain phases are more resistant than short chain phases with the notable exception of Hypersil *tert*.-butyl *versus* Hypersil ODS-t. Furthermore, a previous conclusion that monofunctional phases are more stable than their trifunctional counterparts is sustained.

Of the eluents used in the present study, those containing 0.05 M bicarbonate buffer (pH $\approx$ 8) are most aggressive for those reversed phases where we found significant changes. The influence of the silica substrate is confined to only two types in this paper: Hypersil and Zorbax.

Current research comprises a continuation of the simulated routine use experiments with subsequent analyses as described above and extension of other types of silicas and silane-to-silica attachments including bidentate moieties.

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