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Ageing Processes of Alkyl Bonded Phases in HPLC; a Chromatographic and Spectroscopic Approach

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Key Words

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FTIR
Chain dynamics

Summary

Laboratory use of HPLC columns packed with C_8 and C_{18} bonded phases leads to changes in selectivities and retention volumes. FTIR, ^1H NMR of hydrolysed bonded phases and solid state ^{13}C- and ^{29}Si NMR were applied to characterize the materials. The results of the various techniques are in fair agreement except solid state NMR. Loss of silane and hydrolysis of surface siloxane groups have been observed for the C_8 bondes phase, while for the C_{18} material the latter process seems to dominate. The solid state NMR results have been tentatively explained in terms of changing chain arrangements and mobilities.

Introduction

Silica alkyl bonded phases (SAP's) are widely used in modern high-performance liquid chromatography (HPLC). The surfaces of these phases are different and depend on the silica source, the type of derivatising reagent and the reaction conditions. The surface can contain the following structural elements [1–3]:

i) differently anchored silane groups, especially if di- and tri-functional reagents are used.
ii) residual silanol and siloxane groups.
iii) short alkyl groups if end-capping is applied.

The groups that are present and their relative amounts will determine the chromatographic character of a stationary phase. Therefore, more than one mechanism will often contribute to the retention. In spite of the dominant role of the mobile phase in reversed-phase chromatography [2, 4], the surface structure is important for the chromatographic behaviour of many solutes [5].

In reversed-phase HPLC the composition of the mobile phase can be varied by a number of parameters: type and percentage of organic modifier, pH, type and concentration of (buffering) salts, of ion-pair reagents and of other additives. In routine analysis with such systems, changes in retention and selectivity are often observed, especially if the mobile phase contains salts and/or ion-pair reagents. Possible causes of these instabilities of the chromatographic systems are:

i) Saponification of silanes by eluents and/or solutes, which results in the formation of more silanols on the surface.
ii) The hydrolysis of unreacted groups (alkoxy, chloro) of di- or tri-functional silanes to silane silanols, if these types of reagent were used for the preparation of the bonded phase. These silanols can then react with the silanol groups of the surface forming bi- or tridentate linkages. This may influence the conformational and/or motional behaviour of the alkyl chains and, so, the interaction with solutes. Moreover, cross polymerization of silane silanols can occur. Surface silanols may then be shielded more effectively which would decrease the hydrophylic interactions.
iii) Sorption of components of the mobile phase, especially when ion-pair reagents are used. This can give a permanent modification of the surface.
iv) Hydrolysis of siloxane groups to silanols which results in a more hydrophylic character.

Up to now these phases have been studied by various techniques, e. g. gas chromatographic and mass spectrometric analysis of pyrolysis products of the phases [6], gas chromatography of saponification products [7, 8] and spectroscopic techniques [9–15]. Solid state NMR experiments

Dedicated to Professor J. F. K. Huber on the occasion of his 60th birthday.

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have been reported for silylated silicagels with particular emphasis on qualitative [16] and quantitative [17, 18] aspects and on the dynamic behaviour of the chains [19]. Specific studies on stationary phases have appeared as well [3, 5]. High-resolution NMR of stationary phases in the presence of solvents has been presented by Gilpin c.s. [20].

The present paper describes a combined investigation of SAP's by liquid chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance Spectroscopy (NMR). Two typical stationary phases, a C_8 and a C_{18} bonded silica, were used for some weeks in ion-pair systems and, subsequently, the materials were compared with fresh materials.

Experimental

HPLC

The Zorbax C_8 and C_{18} bonded silica phases (7μm, Du Pont Company, Wilmington De USA) have been used in chromatographic systems with mobile phases containing water, various organic modifiers, buffers (pH 3–8), sodium alkyl sulphonates, n-decyltrimethylammoniumbromide and/or n-hexylamine. After the systems had been used for about ten weeks, the properties of the columns were studied. The test criteria were the separation factors for the separation of acetanilide, nitrobenzene and benzophenon with acetonitril/water (60:40 v/v) as the mobile phase. For a flow rate of 1.5ml/min, the reduced plate height of nitrobenzene was calculated. Moreover, the retention volume of nitrobenzene in n-hexane as an eluent was determined.

The HPLC pump was a Beckman 110A pump (Beckman, Berkely Ca, USA). Detection was carried out with a Hewlett Packard 1036A fixed wavelength (254nm) UV detector (Hewlett Packard, Waldbronn, GFR).

FTIR

FTIR spectra were obtained on a Nicolet 60-SX FTIR spectrometer equipped with a MCT detector. Approximately 2mg of sample was ground together with 300mg KBr under a nitrogen atmosphere and pressed into a pellet. 30,000 scans were averaged for each spectrum. The intensities of the bands for the alkyl groups were measured in first derivative spectra. The spectrum of underivatized silicagel was subtracted before calculating the derivative spectrum. The band intensities were normalized with respect to the amount of silicagel in the sample which was estimated from the intensities of the Si-O vibrations, measured at 1160, 1100, 1000 and 465cm^{-1}.

HRNMR

High-resulution NMR spectra were recorded on a Bruker WP 200 spectrometer, using a pulse angle of 20° and an acquisition time of 5.4s. For high-resolution NMR studies a known amount (40–50mg) of stationary phase was saponified at room temperature with a mixture of 0.7ml 3N NaOH in D_2O and 0.1ml deuteromethanol until the solid phase was dissolved. The organic material was extracted with deuterochloroform. The solution was dried and NMR spectra were recorded, using 20.0μl 1,2,4-trichlorobenzene as an internal quantitative standard.

CPMASNMR

CPMASNMR spectra were recorded on a Bruker CXP 300 spectrometer using ca. 200mg of stationary phase in an Andrew type rotor of Deldrin (^{29}Si NMR) or of boron nitride (^{13}C NMR). The rotors were spun at ca. 3.8kHz at the magic angle. Typically, 1800 to 3600 spectra were accumulated using pulse delay times of 1s and contact times of 4ms (^{29}Si) or 5ms (^{13}C). The spectra were recorded with flip-back and spin temperature alternation in order to speed up accumulation and to suppress artefacts, respectively. In addition, ^{13}C cross-polarization characteristics were measured for the C_8 and the C_{18} phases to ensure that a contact time of 5ms is a useful standard value in our experiments.

Results

HPLC

The HPLC data in Table I show that the separation factors decrease considerably during the use of the columns. The retention volume of nitrobenzene in a chromatographic system with n-hexane as a mobile phase increases, when the separation factors decrease. No significant change in the reduced plate height of the columns except one was observed after use.

FTIR

The results of the FTIR spectra are summarized in Table II. The data demonstrate that silane groups are lost from the reversed-phase material during HPLC experiments. The amount of silane groups is decreasing in the octyl silane samples in the order I (unused), II, III, IV. The decrease is reflected by the $-CH_2-$ (2930, 2860cm^{-1}), CH_3 (2970 cm^{-1}) as well as the Si-CH_3 (845cm^{-1}) vibrations. In sample IV less than 50% of the original amount of silane groups is retained. For the octadecyl silanes a much smaller decrease is observed in the order V (unused), VI, VII.

Table I HPLC results

I–IV initially packed with C_8 phase; V–VI initially packed with C_{18} phase.

Test components 1, 2 and 3 are acetanilide, nitrobenzene and benzophenon, respectively.

α = separation factor; h = reduced plate height; V_R = retention volume in ml of nitrobenzene in n-hexane as an eluent.

Column	$\alpha_{1,2}$	$\alpha_{2,3}$	h_2	$V_{R,2}$
I	1.88	1.59	4.1	10.0
II	1.67	1.47	7.0	11.6
III	1.52	1.42	4.3	13.0
IV	1.35	1.33	3.0	20.0
V	1.78	1.74	2.6	5.3
VI	1.69	1.67	4.3	9.8
VII	1.56	1.57	4.0	13.5

Table II IR and NMR results

a Relative intensities from the derivatives of difference spectra *versus* silicagel. For ν-OH direct from the spectrum, the value for silicagel is 12.1. Estimated inaccuracies are ± 0.5. An absorption at 790 cm^{-1} points to $Si(CH_3)_2$.

b mmol/g.

c Relative intensities with respect to I and V, respectively.

Column packing		I	II	III	IV	V	VI	VII
FTIR[a]								
CH_3	2970 cm^{-1}	4.0	3.2	2.4	1.5	2.3	1.9	1.9
CH_2	2930 cm^{-1}	8.0	6.3	4.1	2.7	15.8	14.0	13.5
CH_2	2860 cm^{-1}	4.6	3.5	2.2	1.5	15.3	13.1	12.6
$SiCH_3$	845 cm^{-1}	8.3	6.0	6.2	4.0	5.1	4.3	3.7
OH	3420 cm^{-1}	5.1	8.2	4.1	13.1	3.1	4.6	6.3
^{1}H HRNMR[b]								
$C-CH_2-C$	δ=*ca* 1.25 ppm	5.2	4.5	4.4	3.4	9.4	9.0	8.6
$C-CH_3$	δ=*ca* 0.9 ppm	0.85	0.71	0.68	0.47	0.59	0.57	0.50
$Si-CH_2-C$	δ=*ca* 0.5 ppm	0.84	0.73	0.69	0.47	0.52	0.54	0.54
$Si-CH_3$	δ=*ca* 0.1 ppm	1.96	1.52	1.59	1.12	1.11	1.07	1.02
^{13}C CPMASNMR[c]								
$Si-CH_3$	δ=*ca* 0 ppm	1.00	0.60	0.63	0.57	1.00	0.85	1.48
C_1	δ=*ca* 18 ppm	1.00	0.87	0.70	0.65	1.00	1.02	1.53
C2, C7	δ=*ca* 23 ppm	1.00	0.80	0.71	0.64	–	–	–
C2, C17	δ=*ca* 23 ppm	–	–	–	–	1.00	0.88	1.70
C3	δ=*ca* 34 ppm	1.00	0.84	0.78	0.72	1.00	0.87	1.58
C4, C5	δ=*ca* 30 ppm	1.00	0.74	0.61	0.63	–	–	–
C4–C15	δ=*ca* 30 ppm	–	–	–	–	1.00	0.89	2.01
C6	δ=*ca* 32 ppm	1.00	0.73	0.67	0.66	1.00	–	1.89
C8	δ=*ca* 12 ppm	1.00	0.82	0.58	0.54	–	–	–
C18	δ=*ca* 13 ppm	–	–	–	–	1.00	–	–
^{29}Si CPMASNMR[c]								
Silane–Si	δ=*ca* 13 ppm	1.00	1.08	0.71	0.71	1.00	0.90	0.93
=SiOH	δ=*ca* −102 ppm	1.00	1.36	1.16	1.53	1.00	1.24	1.20
=Si=	δ=*ca* −110 ppm	1.00	0.79	0.91	0.83	1.00	1.18	0.99

The intensity of the OH stretching at 3420 cm^{-1} is increasing with the ageing of the material. However, the intensity of this band may also be influenced by some absorbed water, in the first derivative spectra it appears that this band has two components (Fig. 1).

HRNMR

The results of the high-resolution NMR spectra are summarized in Table II. The data demonstrate that octyldimethyl- and octadecyldimethyl silanes are present. The results show a decrease in silane content for the C_8 phase after use, but no significant differences between the C_{18} samples. The results for the different groups are consistent with each other.

CPMASNMR

Quantitative results, corrected for the differences in sample amounts are presented in Table II. Some representative spectra are shown in Figs. 2 and 3. The ^{29}Si CPMASNMR spectra of the C_8 phases show clearly that the silane content

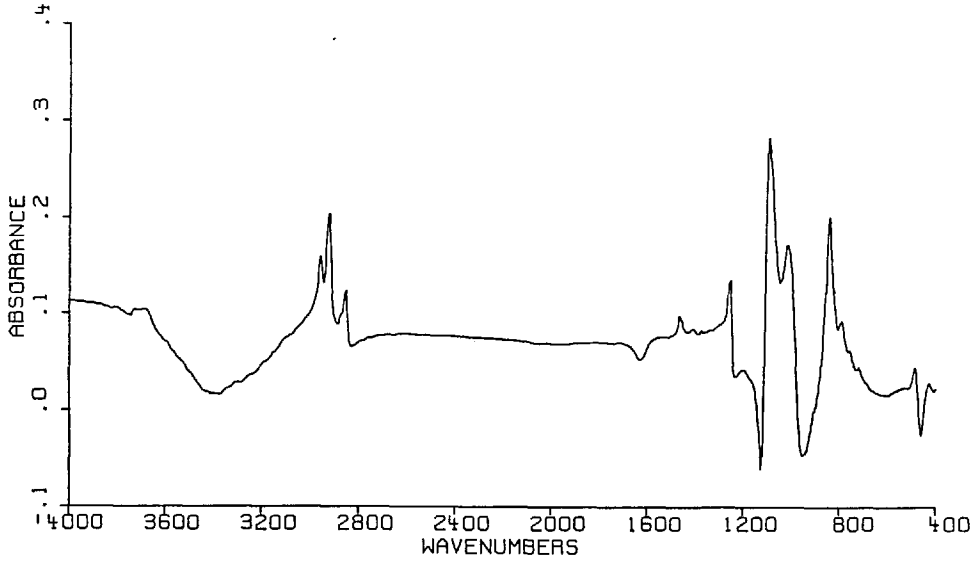

Fig 1
FTIR difference spectrum of sample I *versus* underivatized silica.

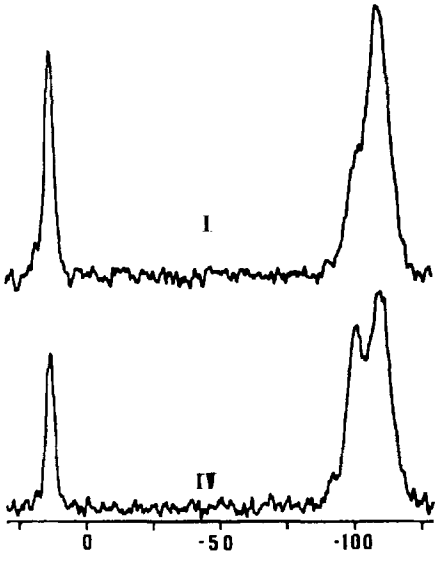

Fig. 2
^{29}Si CPMAS NMR spectra of samples I and IV.

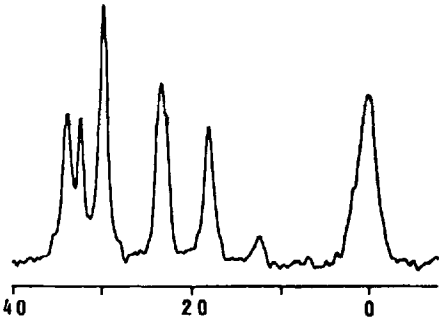

Fig. 3
Typical ^{13}C CPMAS NMR spectrum of sample I.

decreases after usage, although the decrease, as judged from the ^{29}Si NMR spectra alone was significantly smaller than the values indicated by the other spectrocopic methods. However, the signal intensity in CP MAS NMR does not only depend on the amount of material, but also on the motional properties of the nuclei measured.

Another feature, clearly evident from a comparison of the spectra in Fig. 2 is the enhanced concentration of silanol groups (signal at −101 ppm). Even geminal silanediol moieties are detected as a small signal near −92 ppm. For the C_{18} material the silane signals are virtually equal before and after use. The silanol signal with respect to the silane signal is stronger for the unused C_{18} phase than for the unused C_8 phase, indicating a lower surface concentration of silanes. The relative increase of the silanol signal after using the C_{18} phase is lower than for the C_8 material.

The ^{13}C CP MAS NMR spectra demonstrate a considerable decrease in silane signals reflected by CH_3 as well as CH_2 resonances for the used C_8 phase. On the other hand, comparison of samples V and VII show an increase in silane signal after use of the C_{18} material. An interpretation of these observations, based on molecular dynamics is given in the discussion.

Discussion and Conclusions

The HPLC data in Table I show that the separation factors decrease considerably during use of the columns. As these solutes can interact with the hydrophobic part of the stationary phase and with the residual silanol groups, the cause of this decrease is not clear from these data. However, the increase of the retention volume for nitrobenzene in the chromatographic system with n-hexane as a mobile phase demonstrates that the number of exposed silanol groups increases.

Surprisingly, we did not observe a change in the reduced plate height, which can be correlated with the decrease in the separation factors. It is remarkable that the C_8 column (IV in Table I) with the lowest separation factors has a relatively high efficiency. Apparently, the kinetic properties of the stationary phase are not influenced by the change of the surface. The data suggest that the silica particles do not dissolve in the mobile phase.

From the proton NMR data conclusions can be drawn about the chemical nature of the silanes. The ratios of the molar concentrations of methylene and methyl groups in a carbon chain are close to 6 and 16 for the C_8 and C_{18} materials respectively, as expected.

Further, the concentrations for Si-CH_2 and Si-CH_3 groups are consistent with the assumed presence of octyldimethyl- and octadecyldimethyl silanes. There are only monofunctional silane groups attached to the surface which is confirmed by the presence of the 12.3 ppm resonance in the ^{29}Si CPMASNMR spectra and the absence of resonances which have been implicated for bi- and tri-functional reagents [3, 16].

There is hardly any evidence for the presence of groups resulting from end-capping. There appears to be some excess of Si-CH_3 groups from the proton NMR data for the C_8 materials which could be the result of the introduction of additional trimethylsilyl groups. On the other hand, trimethylsilyl groups bonded to a stationary phase show a characteristic vibration in the infrared spectrum at 760 cm^{-1} which was not detected in the samples investigated for this study.

The results of the direct FTIR and indirect ^{1}H NMR analysis show a gradual decrease of the silane content for the four C_8 samples in the order I, II, III and IV. The agreement between different signals in either one technique and between the two techniques is, in general, reasonable. It seems justified to conclude that tha most deteriorated C_8 phase lost approximately 50% of its silane groups.

The FTIR spectra further indicate an increase of hydroxyl groups by a factor of 2.5 between I and IV. A similar increase is indicated by the ^{29}Si CP MAS NMR spectra which show the development of both silanol and geminal silanediol signals. In fact, the FTIR hydroxyl signal in sample IV is about as strong as in the silicagel from which the reversed phase was synthesized. Because only 50 percent of the silane chains have been removed by the use of the column, these results indicate that part of the silanol groups originate from hydrolysis of siloxane bonds at the surface. The results of FTIR and ^{1}H HRNMR indicate at most

only a minor loss in silane groups for the C_{18} phases, which conclusion is sustained by the ^{29}Si CPMASNMR spectra. The FTIR and the ^{29}Si CPMASNMR spectra indicate an increase in hydroxyl groups or silanol groups, respectively. These results are fully consistent with the decrease in hydrophobicity of the surface which was demonstrated by the increase of the retention volume of nitrobenzene.

Combining the results for the C_8 and C_{18} phases it appears justified to conclude that two processes are the main contributors to column ageing:

i) saponification of silanes with formation of surface silanols, and
ii) hydrolysis of surface siloxane bridges with formation of silanols.

For the C_{18} phase it is clear that siloxane hydrolysis is the most important process. For the C_8 phase silane saponification is very significant, but it is hard to separate the relative contributions of the two processes to the ageing effect.

Since there is no reason to believe that the strength of the surface to silane linkages is different for the two phases studied, it appears that in the C_{18} phase the linkage is less accessible for eluents and/or solutes. On one hand this might seem strange because the actual molar coverage is lower for the C_{18} phase, on the other hand, the longer C_{18} chains may be more effective in shielding the surface linkages when they are folded back to the surface.

Probably, the octadecyl chains are more prone to build up fur-like combinations, the octyl chains are likely to prefer brush type arrangements. In the fur model one could then envisage a preference for the hydrocarbon parts for the regions of the surface to silane bonds. However, we should remark that the C_8 and C_{18} phases have not been treated identically, and definite conclusions on the different behaviour of the two phases should await further systematic studies. Such studies are underway.

Finally, we discuss the results of the CP MAS NMR spectra, which, at first sight, are not fully consistent with the other spectroscopic results. While other techniques show a substantial decrease in silane content in the used C_8 material, this was not accuretely reflected by the ^{29}Si NMR spectra. Further, the ^{13}C NMR spectra seemingly indicate an increase in silane content for used C_{18} material. The lack of quantitative correlation of the sample amount with the signal intensity is related to the method of signal excitation by cross polarization. A simple description is given by Hays [21]. According to the theory, the signal intensity depends on the contact time and the optimal contact time depends on the local mobility at the site of the excited nucleus. The local mobility will vary along the chain and will also be influenced by the arrangements of the silane chains on the surface. The deviations of NMR signal intensity can, therefore, be explained by changing chain mobilities in the ageing process. A tentative interpretation is as follows:

The ^{29}Si NMR spectra were recorded with a contact time optimal for rather mobile chains [17]. Lowering the surface coverage might increase the mobility and thereby increase the signal intensity, partially compensating for the lower quantity. Surface silanol groups are not expected to change much in mobility, and therefore their NMR signal would provide a reliable estimate for their concentration.

In a similar way, the increase in ^{13}C NMR signal in the used C_{18} phase could be the result of an increased mobility. In this case, the surface coverage is hardly changing, but the replacement of surface siloxanes by less hydrophobic silanol groups could increase the mobility of the chains by disrupting a compact structure in which the chains are folded back to the surface (fur-type arrangement).

Summarizing, we conclude that a variety of instrumental techniques give a consistent picture of the changes in reversed phase column materials after use. Two processes, silane- and surface siloxane hydrolysis occur. The combined techniques do not only give quantitative information on the various chemical groups at the surface, but also about the dynamics and arrangements of the silane chains. Further work to systematically investigate the effects of specific treatments on various reversed phase materials is underway.

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