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COMPREHENSIVE CHARACTERIZATION OF SOME SILICA-BASED STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Comprehensive studies have been carried out on the effect of the silica support in preparing optimized bonded-phase packings for high-performance liquid chromatography. Different covalently bonded reversed-phase silica-based chromatographic materials have been investigated with respect to ligand density, residual silanol group type, and concentration. Results from diffuse-reflectance Fourier-transform infrared spectroscopy, elemental and thermogravimetric analysis, and ^1H solid-state- and ^{29}Si cross-polarization magic-angle spinning nuclear magnetic resonance techniques were combined with liquid chromatographic data to provide quantitative insights regarding the properties of silica-based bonded-phase packings.

We have determined that unfavorable adsorption of basic compounds and low hydrolytic stability of alkyl bonded-phase ligands can be attributed to the existence of isolated, non-hydrogen-bridged, highly acidic SiOH groups. Contrary to common belief, a stable silica support with the lowest adsorptivity for basic compounds must contain the highest possible (and not the lowest) concentration of homogeneously distributed, associated or bonded SiOH groups to ensure a minimum concentration of deleterious highly acidic, isolated silanols. Under these conditions, silica-based bonded-phase packings exhibit higher hydrolytic stability and a substantially lower order of adsorptivity towards basic organic compounds.

INTRODUCTION

Good structural and chemical properties of the chromatographic support are necessary for high-performance liquid chromatographic (HPLC) separations[1]. Structural factors influence the kinetics of the separation process. More importantly, the chemistry of the support surface, together with the nature of the mobile phase, determine the thermodynamic phase equilibrium of a solute and thus its retention time and peak shape. Structurally suitable spherical silica particles of well-defined diam-

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eter, pore size, pore volume, surface area, and rigidity are readily available for HPLC[2]. Also, chemically different silica-based stationary phases, modified with polar and non-polar ligands, can be easily obtained and are widely used.

It has been recognized that the surface of the silica support used for chromatographic packings often is not homogeneous[3]. Besides differences presented by chemically bonded ligands (-CN, -NH$_2$, -diol, -alkyl, etc.), the support also contains unevenly distributed residual SiOH groups as well as trace impurities, all of which interact differently with the mobile phase —and more importantly— with the solute. Deleterious, multiple interactions often become obvious with polar, mainly basic solutes, and the resulting non-linear adsorption isotherms lead to poor chromatographic peak shapes[4–6]. The worst effects of this sort sometimes are observed with multifunctional solutes such as peptides and proteins. Under certain operating conditions, compounds of this type can be denatured, and recoveries are quite poor or variable.

Silica chromatographic supports may be considered as being composed of a "living polymer", which can be slowly dissolved in the presence of water and especially in eluents with basic or acidic buffers. In addition, bonded-phase ligands can be hydrolyzed by such mobile phases, creating new SiOH groups on the packing surface. Thus, it has been repeatedly observed[7,8] that the chromatographic retention properties of such systems are continuously changing with time, making it difficult to gather reproducible chromatographic data. Earlier investigations have clearly shown that SiOH groups on the surface of the silica-based packings play a key role in undesirable retention processes[4,5].

Our goal was to evaluate systematically the concentration and type of SiOH groups on different unmodified and chemically modified silica surfaces by using various analytical techniques such as ^{29}Si cross-polarization magic-angle-spinning nuclear magnetic resonance spectroscopy (CP-MAS NMR), proton magic-angle-spinning (^{1}H-MAS) NMR, thermogravimetric analysis (TGA), diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), and elemental analysis. The purposes of this study were (1) to define the causes for the unwanted adsorption of polar basic solutes, (2) to identify the reason for the rapid deterioration of certain bonded-phase packings, and (3) to develop approaches to improve the characteristics of certain silica-based reversed-phase packings. In this study, different trimethylsilyl (TMS)-modified silicas were investigated as model systems. Chromatographic retention data were correlated with spectroscopic and TGA results to define the nature of the chromatographic packing surface.

EXPERIMENTAL

Chemicals

Chloroalkoxysilanes, 1-phenylheptane, 1-phenylhexane, N,N-diethylaniline, 2,6-di-*tert.*-butylpyridine, 5-phenylpentanol and imidazole were obtained from Aldrich (Milwaukee, WI, U.S.A.). Type 4A Molecular Sieve, disodium ethylenediaminetetraacetate, pyridine, acetylacetone, and HPLC-grade solvents were obtained from Fisher (Pittsburgh, PA, U.S.A.). SAS-Hypersil (C$_1$), Vydac-TP, RSilC$_3$, and Adsorbosphere-TMS were secured from Alltech (Deerfield, IL, U.S.A.). Zorbax-PSM-60, -150, and -300 were supplied by E. I. du Pont de Nemours (Wilmington, DE, U.S.A.).

Chromegabond-TMS and Nucleosil-5-300 (5 μm, 300 Å pores) were a gift of E-S-Industries (Marlton, NJ, U.S.A.) and Nucleosil-5-100 and LiChrospher-Si-200 (a research sample from Merck, Darmstadt, F.R.G.) were a gift of the Max-Planck-Institut für Kohlenforschung, Mülheim/Ruhr, F.R.G. Q_8M_8, TMS-ester of a double four-ring octameric silicic acid, was obtained from Bruker (Billerica, MA, U.S.A.).

All solvents for moisture-sensitive chemical reactions were dried by passage through a 30×5 cm I.D. column, filled with Type 4A Molecular Sieve, which had been activated at 220°C under high vacuum. Chemical reactions were performed under inert conditions, either in an atmosphere of dry argon or in a high vacuum chamber, as indicated.

Preparation of silylation agent

Besides the common chloro- and alkoxysilanes silylating reagents, TMS-enolate $[(CH_3)_3SiO–C(CH_3)=CH–CO–CH_3]$ was used for silanizing the silica supports. This enolate reagent was synthesized using the procedure described in refs. 4 and 10.

Treatment of silicas before silanization

All silica samples to be silanized were dried at 120°C and 0.1 mbar in a vacuum oven for 20 h. Silicas for vacuum-chamber silanizations were dried at the same temperature, but at 10^{-6} mbar.

Selected Zorbax and Nucleosil samples studied during this work were heated in quartz crucibles within a nitrogen-purged furnace to 280, 505, 650 and 850°C. The hot quartz crucible containing the silica was then removed from the furnace and allowed to cool in a vacuum oven to room temperature. All further preparation steps were carried out in an atmosphere of dry nitrogen or argon. The various silicas investigated during this study are listed in Table I, together with heating treatments, effect on surface area and pore structure, and the results of other physical and chemical tests.

Silanization of silicas

TMS-modified silicas were prepared by three different procedures.

Reaction with trimethylchlorosilane

Apparatus. A 250-ml three-neck flask, reflux condenser, argon purge system, heater-stirrer.

Reaction. Dried silica (20 g) was suspended in 100 ml of trimethylchlorosilane and heated to the boiling point for 20 h while the apparatus was purged with dry argon to remove hydrogen chloride formed by the reaction. After it had cooled, the slurry was transferred to a porous frit and washed with 200 ml each of dry toluene, dichloromethane, methanol, methanol–water (1:1) and acetone, successively. The silica then was air-dried and transferred to a 120°C, 0.1-mbar vacuum oven for 20 h of further drying. (Note: this reaction does not lead to the maximum coverage of the silica surface with TMS groups.)

Reaction with trimethylchlorosilane in the presence of pyridine

Apparatus. As described above.

Reaction. Dried silica (20 g) was suspended in 100 ml of toluene, and 50 ml of

TABLE I

INVESTIGATED SILICAS

Sample No.	Silica	Heating temperature§ (°C)	Heating time (h)	Struc-ture*	pH**	Adsorp-tivity***
1	Zorbax PSM-60	–	–	–	4.1	S
2	Zorbax PSM-150	–	–	–	4.3	S
3	Zorbax PSM-300	–	–	–	4.5	S
4	Nucleosil-100	–	–	–	5.2	N
5	Nucleosil-300	–	–	–	6.9	N
6	Vydac-300	–	–	–	5.3	N
7	Zorbax PSM-60	280	24	U	4.1	–
8	Zorbax PSM-60	505	24	U	4.2	–
9	Zorbax PSM-60	650	24	U	4.3	S
10	Zorbax PSM-60	845	19	U	4.3	–
11	Zorbax PSM-60	850	15	U	4.3	–
12	Zorbax PSM-60	1026	5	C	4.7	–
13	Zorbax PSM-60	1100	15	F	–	–
14	Nucleosil-100	1100	15	F	–	–
15	Hypersil-C1	–	–	–	–	S
16	Adsorbosphere-TMS	–	–	–	–	S
17	RSil-C3	–	–	–	–	S
18	Chromegabond-TMS	–	–	–	–	N

　　* Changes in surface area and pore structure after heating: U = unchanged; C = changed; F = fused.
　　** pH of a suspension of 1 g of silica in 50 g of water after 10 min of equilibration.
　　*** Chromatographic adsorptivity of the silanized silica with respect to bases, as judged by retention and peak shape before and after hydrolysis: S = strong adsorption; N = no adsorption.
　　§ Heat treatments in this study; original heat treatments by manufacturers were not considered.

trimethylchlorosilane (large molar excess) and 4.09 g of pyridine (50 mmole) were added. The reaction was carried out at a temperature of 110°C for 65 h. The resulting material was worked up according to the procedure above. This reaction leads to the highest possible coverage of the surface with TMS groups, provided the reaction time is 48 h or longer.

Reaction with trimethylsilylenolate under vacuum

Apparatus. Edwards high vacuum system and the silylation apparatus previously described[4,9,10].

Reaction. Silica (15 g) was dried in the reaction chamber of the silylation apparatus at 200°C and $2 \cdot 10^{-6}$ mbar for 24 h, then allowed to cool to room temperature. Then 30 ml of the trimethylsilylenolate were placed in the dropping funnel under an argon atmosphere, the funnel was evacuated, and the enolate was allowed to contact the dry, gas-free silica. As the reaction proceeded, bubbles of acetylacetone were released. After 1 h, the silica was heated to 60°C for another 4 h. The resulting product was worked up according to the procedure given above. (Note: this reaction is especially useful if high coverage is desired with short reaction times).

Column filling

　　Procedures for the slurry-packing of columns have previously been detailed[11].

Stainless-steel column blanks, 150 mm × 4.6 mm I.D. with mirror-finished walls, were used. Compression fittings with metal screens retained the packing. Prior to chromatographic testing, columns were carefully purged with 2-propanol and methanol.

Chromatographic experiments

Chromatographic experiments were performed with a Du Pont 8800 LC instrument, equipped with column oven, injection valve (Rheodyne, Cotati, CA, U.S.A.) and a Du Pont 860 absorbance detector or Du Pont 862 UV spectrophotometer detector. Solvent containers were stored in well-ventilated areas, and all mobile phases were carefully degassed by helium purge before use. Data were collected on-line and analyzed by the Du Pont Experimental Station DART-computer system[12]. All columns were thermostated at 50°C.

Two different test mixtures were used during this study: (1) "alkylbenzene mixture": 10 μl of 1-phenylheptane and 10 μl of 1-phenylhexane, dissolved in 4 ml of methanol; (2) "polarity mixture": 25 μl of a solution composed of 250 μl of 5-phenylpentanol, 10 μl of N,N-diethylaniline, 50 μl of 2,6-di-*tert.*-butylpyridine, and 1000 μl of 1-phenylheptane, dissolved in 4 ml of methanol.

Injections of 5–10 μl of these mixtures produced satisfactory chromatographic peak sizes on a 1-mV recorder at 254 nm detection wavelength and an absorbance of 0.05 of full-scale (a.u.f.s.).

Freshly prepared columns were initially tested with both the "alkylbenzene" and "polarity" mixtures, using methanol–water in 80:20, 70:30 or 60:40 ratio, depending on the solvent strength needed to produce satisfactory k' values for the test solutes. Retention times, k' values, and column plate counts for the different peaks were determined for each chromatogram. The retention time of the basic probe, N,N-diethylaniline, relative to the neutral compound, 1-phenylheptane, was used to designate the adsorptivity of column packings. Table II contains chromatographic results for the different bonded silica-based packings investigated during this study.

To determine the chemical stability of the bonded-phase packings, columns were purged at 50°C with several hundred column volumes of deionized water, normally at a flow-rate of 1 ml/min. The columns then were purged with 20 column volumes of pure methanol, and the test compounds were rechromatographed using the original eluent composition (normally methanol–water, 70:30 or 60:40). By repeating the purging process and the retention measurement, characteristic hydrolysis curves (k' *versus* column volumes of water) were established for every packing material.

Elemental analysis

Elemental analysis data on bonded-phase packings were obtained from Micro-Analysis (Wilmington, DE, U.S.A.) or from Galbraith (Knoxville, TN, U.S.A.). Reliable standards of similar samples crosschecked by other laboratories were submitted with every set of samples. Duplicate analyses for carbon, hydrogen, and nitrogen were normally performed. Some of the pertinent data on these packings are reported in Table II.

TABLE II

RETENTION DATA FOR DIFFERENT TMS-MODIFIED SILICAS

Sample No.	Silica	Method of silylation*	%C**	Coverage*** ($\mu mol/m^2$)	k_1'[†]	k_2'[††]	α[†††]
1	Zorbax PSM-60	CA	2.36	1.55	1.14	ads	§
1	Zorbax PSM-60	CP	4.50	3.11	15.89	3.69	4.31
1	Zorbax PSM-60	EV	4.66	3.23	21.00	ads	§
1[§§]	Zorbax PSM-60	CP	4.38	3.01	20.36	15.38	1.32
2	Zorbax PSM-150	CP	1.73	3.17	4.27	15.04	0.28
3	Zorbax PSM-300	CP	0.81	4.01	1.74	3.27	0.53
4	Nucleosil-100	EV	5.35	3.66	16.18	2.39	6.77
4	Nucleosil-100	CP	5.10	3.47	14.68	2.25	6.52
5	Nucleosil-300	EV	1.27	3.94	2.09	0.77	2.71
6	Vydac-300	EV	1.41	5.43[§§§]	2.64	0.59	4.47
7	Zorbax PSM-60	CP	4.32	3.02	–	–	–
8	Zorbax PSM-60	CP	3.89	2.72	–	–	–
9	Zorbax PSM-60	CP	3.50	2.42	7.50	3.88	1.93
15	Hypersil Cl	–	2.61	–	6.51	1.91	3.41
16	Adsorbosphere-TMS	–	1.99	–	6.07	1.77	3.43
17	RSil-C3	–	5.59	–	5.73	ads	–
18	Chromegabond-TMS	–	4.98	–	16.68	2.87	5.81

* CA = with trimethylchlorosilane, argon-purge; CP = with trimethylchlorosilane, pyridine; EV = with trimethylsilylenolate, vacuum.

** Carbon percentage as determined by elemental analysis.

*** Coverage of the silica surface with alkyl ligands.

[†] k' value for 1-phenylheptane; V_0 for all columns 1.68 ± 0.02 cm³; mobile phase methanol–water (60:40).

[††] k' value for N,N-DEA; ads = adsorption.

[†††] Selectivity: k_1'/k_2'.

§ Not calculated — very small.

[§§] After wash with EDTA.

[§§§] Value higher than the maximum previously reported for TMS-modified silica (4.75 $\mu mol/m^2$). This and other unreported results suggest that "buried" SiOH groups can be accessed and reacted during silanization. TGA measurements have shown that Vydac-300 has a total SiOH concentration of ca. 14 $\mu mol/m^2$, indicating a relatively high population of "buried" SiOH groups for this material.

BET data

Surface areas and pore size distributions were measured by the conventional Brunauer–Emmett–Teller (BET) method[15]. Instrumentation consisted of a PDP-8M computer-controlled Digisorb 2500, and a manual 2100 Surface Area Analyzer (Micromeritics, Norcross, GA, U.S.A.). Samples were normally outgassed at 200°C and 10^{-5} mbar. Samples submitted for analysis were periodically compared with authentic surface area standards. The precision of the surface area measurements was usually within the 4–5% range, but infrequently varied as much as 10–15%. Results of the BET measurements are summarized in Table III.

pH measurements

All pH measurements of solutions and silica suspensions were carried out with a φ 43 pH-METER (Beckman, Fullerton, CA, U.S.A.), equipped with automatic temperature compensation and a Beckman refillable combination electrode. The elec-

TABLE III

BET DATA ON SILICAS

Values in parentheses determined by mercury intrusion. Silica numbers as in Table I.

Silica No.	Surface area (m^2/g)	Volume at saturation (ml/g)	Pore volume (ml/g)	Average pore diameter ($\overset{\circ}{A}$)
1	443 (440)	547	0.85 (0.63)	77
2	157	408	0.63	162
3	56 (89)	399	0.62 (0.51)	442 (338)
4	456 (413)	826	1.28 (0.92)	113
5	92 (144)	614	0.95 (0.82)	415 (400)
6	74 (116)	429	0.67 (0.55)	359 (285)
7	436	540	0.84	77
8	432	522	0.81	75
9	433	528	0.83	76
10	463	575	0.89	77
11	436	534	0.83	76
12	184	193	0.30	65
15*	162	389	0.60	150
16*	182	321	0.50	111
17*	415	556	0.87	83
18*	401	490	0.76	76

* TMS-modified silicas.

trode was calibrated with pH 4, 7 and 10 standard solutions, depending on the range investigated.

TGA measurements

TGA measurements were conducted with a Du Pont Model 990 TGA-Ana-lyzer. In these tests, 20 to 100 mg of silica was loaded in a small quartz crucible and placed in the TGA-analyzer. To remove physically adsorbed water from the silica surface, the sample was heated to 120°C at a rate of 10°C/min while dry nitrogen gas was passed through the heating chamber at a flow-rate of 50 ml/min. The sample was maintained at this temperature until no further weight loss could be observed. At this point, the temperature was increased to 300°C at the same heating rate as before and held at this temperature until a constant weight was reached. The same process was repeated at 500, 700, 900, 1050, and 1200°C. At each temperature, a characteristic weight loss could be observed for every sample.

The silica samples in this study usually began to soften above 1000°C. Sig-nificant weight losses could be detected after 1 h of heating at above 1000°C with certain samples. To ensure that this observation was not due to artifacts (*e.g.*, for-mation of silicon nitride), experiments were repeated with argon as the purging gas. No differences in the TGA curves could be detected.

A control experiment (no sample) also showed no apparent weight loss at temperatures above 1000°C, indicating no significant response due to buoyancy effects at the high temperature. Also, observed weight loss above 1000°C cannot be due to desorption of gas during the melting process, since BET measurements re-

vealed that only extremely small amounts of gases are adsorbed on the silica surface at higher temperatures (*e.g.*, 380°C).

ICP-AES data

To define the level of impurities in the silica samples, inductively coupled plasma–atomic emission spectroscopy (ICP–AES) was carried out on a Model 34000 B ICP–AES instrument (Applied Research, Sunland, CA, U.S.A.). Results obtained by this technique are summarized in Table IV.

TABLE IV

ICP–AES DATA

Values in parts per million. N.O. = not observed.

Element	LiChrospher Si200	Nucleosil-5-100	Zorbax PSM-60	Zorbax PSM-60 EDTA
Copper	N.O.	9	245	N.O.
Manganese	31	N.O.	N.O.	N.O.
Iron	445	76	68	N.O.
Zirconium	N.O.	N.O.	<25	N.O.
Palladium	N.O.	N.O.	N.O.	N.O.
Antimony	625	N.O.	N.O.	N.O.
Barium	81	6	N.O.	N.O.
Calcium	N.O.	130	N.O.	N.O.
Chromium	N.O.	N.O.	115	N.O.
Lithium	N.O.	<5	<5	N.O.
Aluminium	1100	N.O.	N.O.	N.O.
Sodium	2900	56	105	N.O.
Titanium	235	57	41	N.O.

^{29}Si *CP-MAS NMR data*

Selected silica samples were analyzed by ^{29}Si CP-MAS NMR to define the type and concentration of SiOH groups associated with the packings. Spectra were obtained on a Bruker CXP-300 NMR spectrometer at 59.6 MHz. A proton cross-polarization time of 13 ms was used, followed by decoupling, both with γ-H_2 = 40 kHz. The Hartmann–Hahn matching condition for optimum cross-polarization was carefully calibrated with Q_8M_8[13]. The magic angle was adjusted to minimize the line-width of the first-order quadrupole transitions of ^{127}I (60.0 MHz) in potassium iodide or of ^{79}Br (75.2 MHz) in potassium bromide[14]. MAS rates of *ca.* 4 kHz produced spectra with no observable spinning sidebands. Typically, 10^3 to 10^4 scans were obtained with a recycle time of 10 s.

Chemical shifts were referenced with an external sample of hexamethylcyclo-trisiloxane (−9.2 ppm) in chloroform. Spectra were transferred to a VAX computer (Digital, Maynard, MA, U.S.A.) for analysis. After performing a non-linear least-squares fit of the spectra, the areas of the resulting Gaussian lines were calculated separately.

DRIFT experiments

DRIFT was performed on selected silica samples to characterize the state of SiOH groups on the packings. Spectra were obtained on a Model 7199 spectrometer (Nicolet, Madison, WI, U.S.A.) equipped with a wide-range mercury–cadmium–telluride detector (specific detectivity $D^* = 2.0E10$), operating at 2 cm^{-1} spectral resolution. The Fourier transform was zero-filled twice to provide interpolated data points every 1 cm^{-1}. A weak apodization function F3[16] was used, and 256 scans were co-added to improve the signal-to-noise ratio. All data were obtained by collecting diffusely scattered radiation with an in-line parabolic collection system, similar to that of Fuller and Griffiths[17].

Approximately 30 mg of the silica samples were loaded into an evacuable cell with minimal packing. Thus, the materials were examined as loosely packed powders. The physically adsorbed surface water was removed by evacuating for 1 h; estimated pressures at the samples under vacuum were 10^{-4} to 10^{-5} Torr. An extended study with over 24 h of pumping established that 1 h of pumping was sufficient to remove surface water. In several cases the temperature of samples was increased under vacuum to observe the dehydroxylation process. Temperatures were measured with a chromel alumel thermocouple, immersed in the powder. In some cases externally preheated or calcined samples were investigated.

Frequencies were assigned to the peak position of the sharp hydroxyl peak by examining the spectra with a high level of scale expansion and using an interactive graphics cursor.

Solid-state proton NMR data

Solid-state proton spin-counting and ^{1}H-MAS NMR were carried out to define the concentration and structure of SiOH groups and alkyl ligands on various silicas. Three different types of experiment were carried out: (a) proton spin-counting NMR on silica samples heated to different temperatures, (b)[1] ^{1}H-MAS NMR on dry samples and samples after reaction with [^{2}H$_4$]methanol, (c) non-spinning ^{1}H NMR on dry samples and samples after reaction with [^{2}H$_4$]methanol.

To make these measurements, 200-mg samples were placed in 10-mm O.D. NMR tubes, equipped with stopcocks and ground-glass fittings for connection to a vacuum system. The samples were heated overnight at a temperature between 120 and 150°C at 10^{-4} Torr to remove physically adsorbed water. Selected samples also were heated to temperatures up to 500°C. The tubes were removed from the vacuum system, and the nonspinning proton spectra were obtained with the samples in the original evacuated tubes.

For MAS spectra, the samples were transferred to rotors under anhydrous conditions in a drybox. After the spectra were taken, an excess of [^{2}H$_4$]methanol was added to certain samples while they remained in the same containers and spectra were retaken. The addition of perdeuterated methanol was performed under anhydrous conditions; typically, 220 mg of [^{2}H$_4$]methanol per 160 mg of dry silica was used.

All NMR experiments were performed on a Bruker CXP-300 spectrometer. The nonspinning proton spectra were obtained at 90 MHz, employing a 21-kGauss iron-core magnet. This arrangement has the geometric advantage that sample tubes with stopcocks can be inserted in the NMR probe and subsequently reconnected to

the vacuum system for further treatment. The spectra were obtained with single 90° (3-μs) pulse excitation. Proton contents were quantitatively determined by comparison of the initial free-induction decay (FID) intensity with that of a standard water sample. All spectra were taken on resonance, and a magnitude calculation of the FID was performed before making intensity measurements and plotting of FID.

^{1}H-MAS spectra were obtained at 300 MHz in a 71-kGauss superconducting magnet, using a MAS probe with sapphire rotors and Kel-F caps (Doty Scientific, Columbia, SC, U.S.A.). The spinning rate was 2.0 kHz. This procedure gave rise to a pattern of strong sidebands separated at 6.7 ppm. Since the MAS spectra are exclusively used for site identification rather than for intensity measurements, only the center band region of the MAS spectra is plotted for display in this paper. The delay time between pulses was 20 s for the dry samples and 100 s for samples with [^{2}H$_4$]methanol. All spectra were obtained at room temperature.

RESULTS AND DISCUSSION

Chromatographic data on commercial reversed-phase silica supports

Chromatographic experiments were performed with different commercially available silicas to gather definitive data on what has been called, sometimes quite inaccurately, "adsorptive" silicas or silica supports with "poor stability". These terms generally have expressed the user's dissatisfaction with the performance of a particular silica-based column. "Adsorptive" supports cause low recoveries and poor peak shapes for a variety of solutes. Some of these by their very nature simply cannot be chromatographed with reversed-phase HPLC systems without modifying the mobile phase with deactivating additives.

The phrase "poor stability" is generally attributed to several phenomena within the column, such as the development of voids during use, change in k' values for standard compounds, etc. The appearance of voids in columns can be due to low mechanical stability of the silica particle or a faulty packing process. Voids also can result when the column is exposed to drastic mechanical or hydraulic shocks. In many cases of column instability, highly basic eluents have been used. These mobile phases are so aggressive that they simply dissolve the silica backbone of the packing.

But even in cases where operator errors could be excluded, some silicas seem to adsorb organic bases more strongly than others. With some silicas, the retention times of standard compounds do not change during intensive use of the column. On the other hand, chromatographic data cannot be reproduced with other silica packings.

In this study "adsorptivity" was not determined by measuring adsorption isotherms, but more practically by using sensitive chromatographic experiments that compare the retention times and peak shapes of special test solutes on different silica-based reversed-phase packings. Test solutes were carefully chosen not only to cover a wide range of polarity and basicity, but also to insure elution under reversed-phase conditions without mobile-phase additives. Solutes were also selected to facilitate UV detection at very low concentrations at a convenient wavelength. 5-Phenylpentanol was chosen as a polar, non-basic test-solute molecule, and 1-phenylhexane and 1-phenylheptane were selected as compounds that are retained mainly by lipophilic interactions and dispersion forces. 2,6-Di-*tert.*-butylpyridine was used as a

lipophilic, sterically hindered, basic solute, and finally, N,N-diethylaniline was employed as a polar, basic probe.

The relative retention of the basic molecules with respect to the non-polar molecules was used to judge the adsorptivity of packings with different alkyl ligand coverages in various solvent systems. Relative retention values were utilized to compensate for small changes in chromatographic conditions between different runs.

The chemical stability of the packing was judged by measuring the retention times of the non-polar compounds before and after purging the column with pure water. This treatment results in cleavage of alkyl ligands from the silica surface by hydrolysis. To hasten hydrolysis, the column was heated to 50°C. When the column loses alkyl ligands by hydrolysis, retention times of the non-polar test solutes decrease. Thus, the k' values of these non-polar compounds *versus* the amount of water purged through the system (determined as column volumes of water) is a quantitative measure for the chemical stability of the bonded stationary phase on the silica support.

TMS-coated silicas were chosen as a model for the tests, since reversed-phases with small alkyl ligands are hydrolyzed much faster than phases with long alkyl chains. Presumably, the longer alkyl chains inhibit attack of the surface by water. Among the commercially available stationary phases, trimethylsilane-modified packings exhibit the poorest stability, and thus represent the most sensitive test system for observing changes.

Fig. 1 summarizes a typical hydrolysis experiment for Zorbax PSM-60 after reaction with trimethylchlorosilane in the presence of pyridine (sample 1-CP, cf. Table II). The k' value for 1-phenylheptane decreased from *ca.* 15.5 to *ca.* 4 after purging the column with 2500 column volumes of water. Decreased k' values were also observed for 1-phenylhexane, 5-phenylpentanol, and 2,6-di-*tert.*-butylpyridine. The peak for the substituted pyridine coincided with that for 1-phenylheptane during the first two measurements. However, after the second hydrolysis step, the basic 2,6-di-*tert.*-butylpyridine probe was eluted after the non-polar compound. This pattern indicates that polar and basic functionalities begin to interact with the increased number of active surface SiOH groups that are formed during hydrolysis of the bonded phase. The result is increasing retention of the polar and basic solutes compared with 1-phenylheptane, which is mainly retained by its hydrophobic interaction with non-polar alkyl ligands.

The formation of acidic SiOH groups as a result of the hydrolysis of the alkyl ligand also leads to a drastic increase in retention time for the basic polar compound, N,N-diethylaniline (N,N-DEA). Even prior to hydrolysis of the bonded phase, N,N-DEA exhibited a very broad and asymmetric peak; its shape became worse with each hydrolysis step. However, the N,N-DEA peak was altered by injecting this compound into the column successively several times. With every injection the N,N-DEA retention time was decreased and peak shape was improved.

These effects suggest that N,N-DEA is adsorbed on a relatively small concentration of high-energy sites that cause a non-linear isotherm, until these sites are blocked by strong adsorption of the basic amine. Prior to hydrolysis of the TMS-modified surface, only a few injections of N,N-DEA are required to achieve this effect. Based on this observation, it can be calculated that only *ca.* 10 nmole of N,N-DEA are necessary to decrease its retention time markedly. The amount of
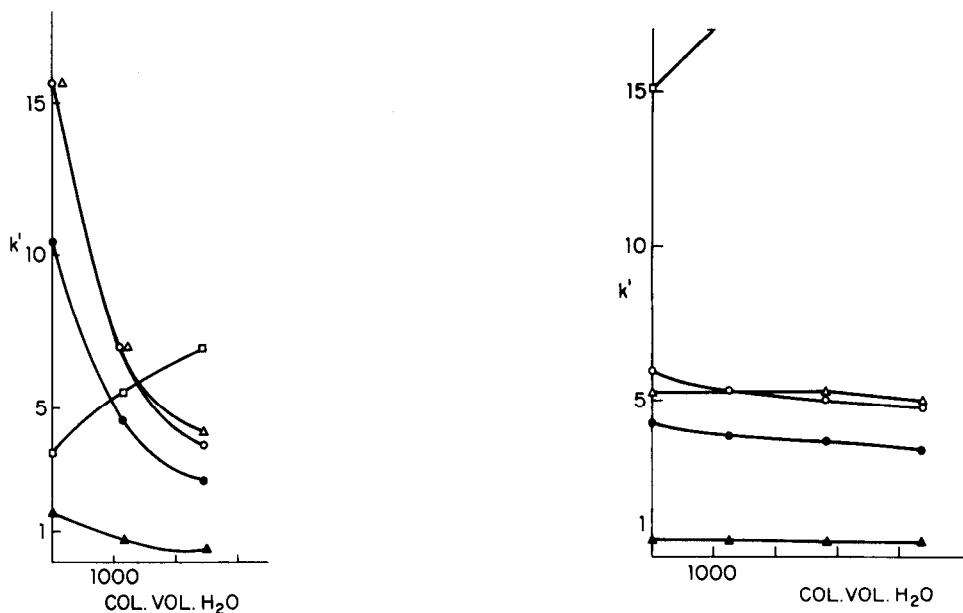
Fig. 1. Hydrolysis of fully TMS-modified Zorbax PSM-60. Starting Zorbax PSM-60: surface area = 443 m^2/g; pore volume = 0.84 ml/g; average pore diameter = 77 Å. Silanized silica: sample 1-CP, Table II; %C = 4.50; surface area = 372 m^2/g; pore volume = 0.71 ml/g; average pore diameter = 77 Å; water purge for hydrolysis, 1.0 ml/min at 50°C; chromatographic test, 60% methanol in water at 1.0 ml/min, 50°C. Key: $\bigcirc$ = 1-phenylheptane; $\triangle$ = 2,6-di-*tert.*-butylpyridine; $\bullet$ = 1-phenylhexane; $\square$ = N,N-diethylaniline; $\blacktriangle$ = 5-phenylpentanol. COL. VOL. = column volumes.

Fig. 2. Hydrolysis of C_8-modified Zorbax PSM-60. %C = 10.71; surface area = 307 m^2/g; pore volume = 0.54 ml/g; average pore diameter = 70 Å; water purge for hydrolysis, 2.0 ml/min at 50°C; chromatographic test, 80% methanol in water at 1.0 ml/min, 50°C. Key as in Fig. 1.

N,N-DEA adsorbed on the surface decreased with consecutive injections, as evidenced by the continuing increase of peak area.

A different TMS material (Table II, sample 1-CA) was also measured under the same conditions. This product was prepared by reaction of Zorbax PSM-60 with an excess of trimethylchlorosilane and purging of the suspension with argon to remove hydrogen chloride byproduct. Whereas silica 1-CP exhibited a coverage with alkyl ligands of 3.11 $\mu mol/m^2$, silica 1-CA was modified with only 1.55 μmol trimethylsilane per m^2. Therefore, the 1-CA sample was only partially modified with TMS groups. The retention time for the non-polar compounds on silica 1-CA was found to be relatively short, and N,N-DEA was totally adsorbed, even after injection of more than 100 nmole. The sterically hindered weak base, 2,6-di-*tert.*-butylpyridine, also was strongly adsorbed and exhibited three times the k' value of the non-polar 1-phenylheptane. Thus, the tendency for adsorption is significantly greater for partially *versus* fully silanized Zorbax.

Hydrolysis of longer-chain alkyl ligands on the surface of certain silica supports takes place in highly aqueous mobile phases, but generally proceeds more slowly than for short-chain bonded phase packings. Fig. 2 shows the hydrolysis curve for a Zorbax PSM-60 silica which had been allowed to react with dimethyloctylchloro-
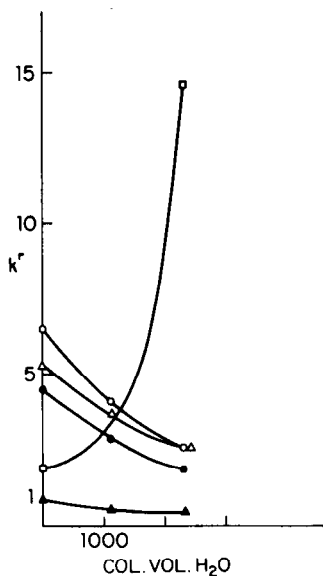
Fig. 3. Hydrolysis of SAS-Hypersil (Cl). Sample 15, Table II; %C = 2.61; surface area = 162 m²/g; pore volume = 0.60 ml/g; average pore diameter = 150 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.
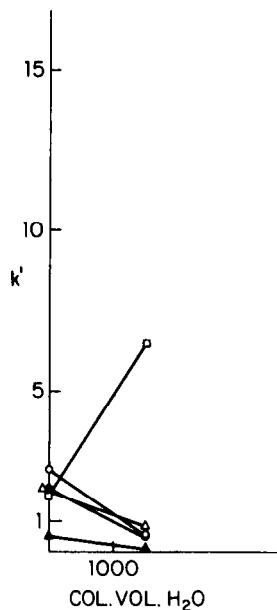
Fig. 4. Hydrolysis of Adsorbosphere-TMS. Sample 16, Table II; %C = 2.00; surface area = 182 m²/g; pore volume = 0.50 ml/g; average pore diameter = 111 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.

silane (without endcapping with chlorotrimethylsilane). As expected, elemental analysis revealed that this material contains much more carbon than the TMS samples. BET data show that surface area, pore volume, and pore size are decreased according to the increased size and the concentration of the alkyl ligand on the surface (cf. caption for Fig. 2). The data in Fig. 2 also indicate that PSM-60-C8 is not hydrolyzed as rapidly as TMS-coated silicas; only a small decrease of the k' value for 1-phenylheptane, 2,6-di-tert.-butylpyridine, 1-phenylhexane and 5-phenylpentanol was observed during the hydrolysis test. However, the substituted pyridine peak was observed to change its position with 1-phenylheptane as a result of the slight ligand hydrolysis. Even prior to purging the Zorbax PSM-60-C8 column with water, N,N-DEA was strongly adsorbed; its peak shape deteriorated, and retention increased with every hydrolysis step. We speculate that the slower hydrolysis pattern for Zorbax PSM-60-C8 relative to the TMS-modified material is based on the greater difficulty of hydrated hydroxide ions to approach the silica surface for hydrolysis. As the carbon chain length of the ligand is increased, increasing surface tension or steric factors become more important in inhibiting access of hydroxide ions to the base silica.

Based on these results, we concluded that certain silica packing materials contain adsorptive sites, the strength and/or concentration of which depends on the coverage of the silica with alkyl ligands. However, adsorption of the organic-base
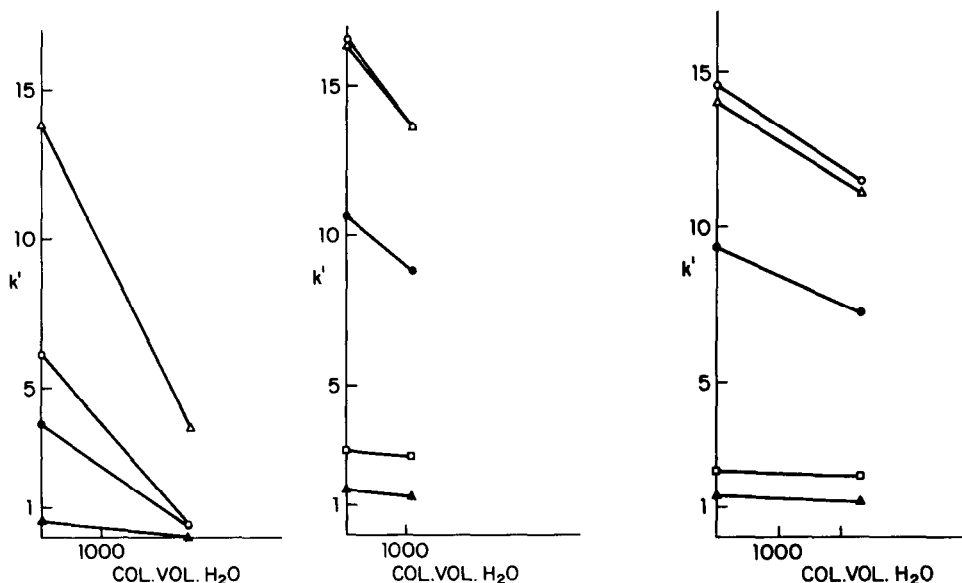
Fig. 5. Hydrolysis of RSil-C3. Sample 17, Table II; %C = 5.60; surface area = 415 m²/g; pore volume = 0.87 ml/g; average pore diameter = 83 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1. N,N-Diethylaniline is totally adsorbed.

Fig. 6. Hydrolysis of Chromegabond-TMS. Sample 18, Table II; %C = 4.98; surface area = 401 m²/g; pore volume = 0.76 ml/g; average pore diameter = 76 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.

Fig. 7. Hydrolysis of Nucleosil-TMS (trimethylchlorosilane-modified). Sample 4-CP, Table II; %C = 5.10; starting silica surface area = 456 m²/g; pore volume = 1.28 ml/g; average pore diameter = 113 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.

probe occurs even with different types of alkyl ligand on this silica. Poorest stability of the bonded-phase coating was most obvious for packings with the smallest organic TMS ligand.

Various commercially available TMS silicas exhibited different hydrolysis characteristics, as indicated by the plots in Figs. 3–6. The captions for these figures also contain data on the amount of carbon for the particular stationary phase, as determined by elemental analysis, as well as BET data on the silanized materials. All phases, with the exception of Chromegabond-TMS, exhibit low chemical stability of the alkyl ligand and strong adsorptivity for basic compounds. This pattern is very similar to that found for packings made from Zorbax. Our studies have shown that most of the commercially available silica supports for HPLC exhibit relatively strong adsorptivity for basic compounds and poor stability of alkyl bonded-phase ligands. Because of availability, Zorbax was arbitrarily used in further studies as a model for this form of silica in our work ("Zorbax-type").

The hydrolysis of Nucleosil-based packings proceeds differently, as illustrated in Figs. 7 and 8. In this case, Nucleosil-5-100 silica (5 μm, 100 Å pores) was made to react in our laboratory with trimethylchlorosilane in the presence of pyridine, and
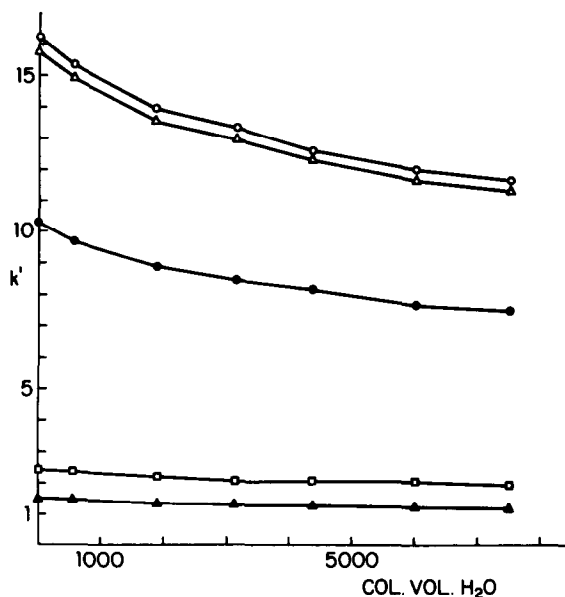
Fig. 8. Hydrolysis of Nucleosil-TMS (enolate-modified). Sample 4, Table II; %C = 5.35; rest, same as for Fig. 7, except first hydrolysis step (*ca.* 500 column volumes), flow-rate 1.0 ml/min, then 2.0 ml/min for rest of water purge. Key as in Fig. 1.

in a second experiment with TMS-enolate by using the vacuum method (cf. Table II). Both reversed-phases showed excellent chemical stability and low adsorptivity with respect to basic test probes.

On the other hand, both of the Nucleosil columns exhibited about twice the back-pressure compared to columns filled with the other TMS-silicas. We believe that this effect was caused by "fines" formed from these weaker Nucleosil particles during the column-filling process. Scanning electron microscopy was not able to detect any fines prior to column packing. However, after removing the column matrix after testing, we found that a slurry of the silica in methanol exhibited fines that settled much slower than the original silica spheres. These fines were seen as a "mist" in the clear slurrying liquid, and light microscopy showed smaller particles than the original spheres.

Vydac-TP-TMS produced hydrolysis and adsorption data similar to that for Nucleosil-TMS, and thus can be considered a relatively non-adsorptive packing with good chemical stability of the alkyl ligands (cf. Table II). The hydrolysis curves for Chromegabond-TMS show a pattern very similar to that for Nucleosil-TMS, presumably because this material is based on Nucleosil silica.

Chromatographic differences between silicas

To insure that differences between the TMS-coated silicas (*e.g.*, silica 1-CA and 1-CP, Table II) are not caused by different silylation procedures, a Zorbax PSM-60 sample was silanized by several methods, including the "enolate" method. Although slightly higher TMS coverage was obtained on Zorbax by the enolate
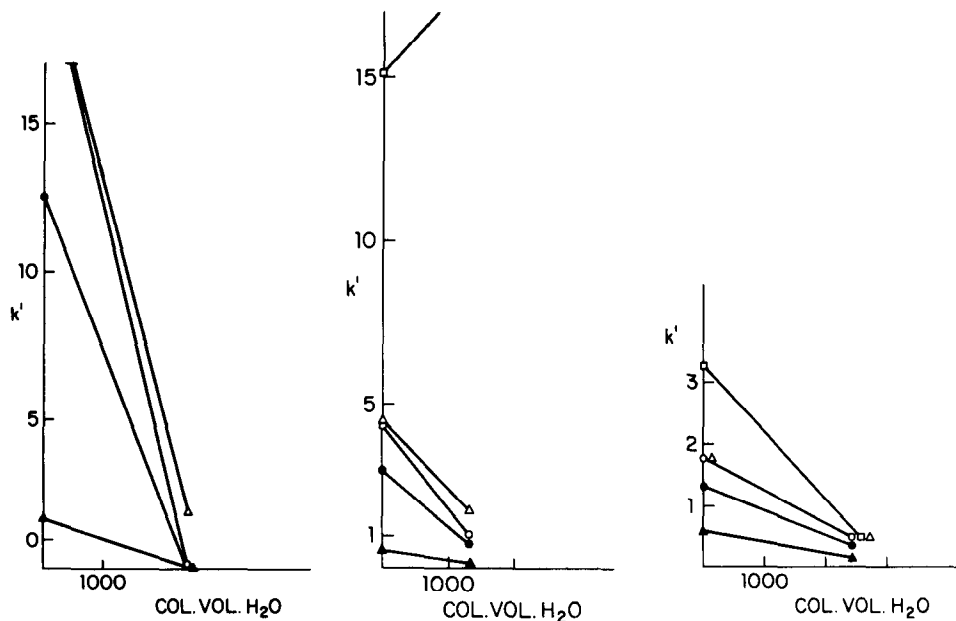
Fig. 9. Hydrolysis of Zorbax-TMS (enolate-modified). Sample 1-EV, Table II; %C = 4.66; surface area = 370 m²/g; pore volume = 0.71 ml/g; average pore diameter = 77 Å; water purge, 1.0 ml/min at 50°C; chromatographic test as in Fig. 1. Key as in Fig. 1. N,N-Diethylaniline is totally adsorbed.

Fig. 10. Hydrolysis of TMS-modified Zorbax PSM-150. Sample 2-CP, Table II; %C = 1.74; starting silica surface area = 143 m²/g; pore volume = 0.59 ml/g; average pore diameter = 165 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.

Fig. 11. Hydrolysis of TMS-modified Zorbax PSM-300. Sample 3-CP, Table II; %C = 0.81; starting silica surface area = 56 m²/g; pore volume = 0.62 ml/g; average pore diameter = 338 Å; water purge and chromatographic test as in Fig. 1. Key as in Fig. 1.

method than for trimethylchlorosilane (3.23 *vs.* 3.11 μmol/m², respectively), the chemical stability of the resultant alkyl ligand actually was decreased, relative to the trimethylchlorosilane-modified Zorbax silica 1-CP (see Fig. 2). In addition, N,N-DEA was totally adsorbed, as documented by the hydrolysis data in Fig. 9.

The BET data in Table III reveal that Nucleosil-5-100 silica supports contain larger pores than Zorbax PSM-60 (average pore diameter, 11.3 nm for Nucleosil and 7.7 nm for Zorbax). To exclude the possibility of a pore-size difference being responsible for the different adsorption properties, Zorbax PSM-150 (162 Å average pore diameter) and Zorbax PSM-300 (338 Å average pore diameter by mercury intrusion; BET data are less reliable for larger pores) were silanized with trimethylchlorosilane and tested according to the procedure described above. Results are shown in Figs. 10 and 11. In summary, both phases lose their alkyl ligands very rapidly, and N,N-DEA is strongly adsorbed on both packings prior to hydrolysis. However, on the Zorbax PSM-300-TMS packing, adsorption of the aniline derivative is somewhat decreased, relative to PSM-60 TMS material. We speculate that this effect may be due to the fact that the surface of Zorbax PSM-300 is more highly hydrolyzed (higher μmol/m² of SiOH groups) than Zorbax PSM-60. Alternatively,

Zorbax PSM-300 is an aggregate of much larger colloidal silica microparticles than Zorbax PSM-60 so that surface SiOH groups on the Zorbax PSM-300 are closer together and can form more stable hydrogen-bonded pairs[25]. This feature could leave fewer unbonded (isolated), highly acidic SiOH for interaction with basic solutes.

It was concluded, therefore, that neither the silylation agent nor the pore size of the silica cause the significant differences between Nucleosil- and Zorbax-type silicas.

pH measurements and ICP–AES data

The pH values for the suspensions of different "pure" silicas in water provide insight regarding the acidity of the bulk particles and, therefore, their tendency to adsorb organic bases. LiChrospher exhibited a pH value of 9.2; Nucleosil, 5.2; and Zorbax, 4.1. It can be argued that silica supports with higher pH-values might be more favorable for separating basic compounds. However, it was found that LiChrospher Si 200 totally adsorbs organic bases, such as N,N-DEA or 2-*n*-octylpyridine.

The very high pH value of LiChrospher Si-200 cannot be caused by the silica itself, but is apparently due to impurities. Table IV gives the results of ICP–AES on the three samples described above. These data strongly suggest that the high pH of LiChrospher is caused by the extensive contamination of this sample with sodium.
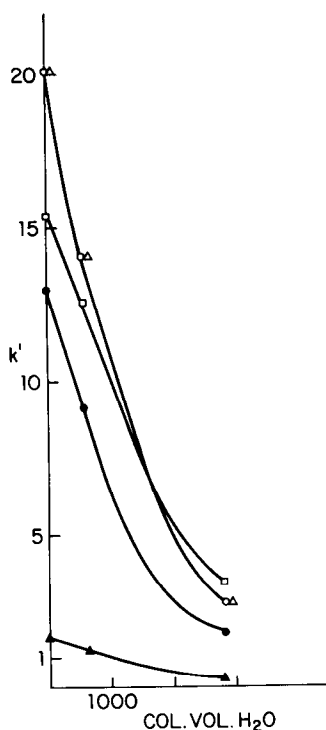
Fig. 12. Hydrolysis of EDTA-treated Zorbax PSM-60-TMS. Sample 1-CP (see 8th footnote to Table II); %C = 4.38; water purge, 4.0 ml/min for first step, then 1.0 ml/min; chromatographic test as in Fig. 1. Key as in Fig. 1.

On the other hand, the data in Table IV indicate that Nucleosil and Zorbax silicas are quite pure. Therefore, differences in pH for these materials cannot be explained by impurities. The data in Table IV show that the particular Zorbax sample studied contained 245 ppm of copper, which is known to adsorb nitrogen bases by complexation. To exclude the influence of heavy metals on the observed adsorption phenomena, 15 g of Zorbax PSM-60 was suspended in 100 ml of a 1% Na_2EDTA solution for 1 h, washed with 50 ml of fresh EDTA solution, and then with water to neutrality. After filtering, washing with acetone and drying, the silica was silanized with trimethylchlorosilane and tested. The results of the hydrolysis experiment are given in Fig. 12. Stability of the TMS ligand was not improved by the EDTA treatment. On the other hand, adsorptivity for bases was decreased, possibly due to the deactivation of the silica by adsorption of the basic EDTA on acidic surface groups of the silica. Contrary to earlier experiments, the retention time for N,N-DEA was increased with every injection. ICP–AES (cf. Table IV) gave no evidence for any heavy metal impurities in this EDTA-washed silica sample.

We conclude that for a pure silica, such as Nucleosil and Zorbax, only acidic SiOH groups can be responsible for different pH values. It appears that Zorbax contains more acidic SiOH groups than Nucleosil, and this explains their different bonded-phase stability and tendency to adsorb bases.

^{29}Si CP-MAS NMR data

Previous studies have shown that ^{29}Si CP-MAS NMR can provide information about the type and number of silanol sites on a silica[18]. This technique measures bulk as well as surface properties and can distinguish geminal SiOH groups, single SiOH groups, as well as silicon atoms with no silanols. We have determined that this method can also be used to determine the concentration of various types of SiOH
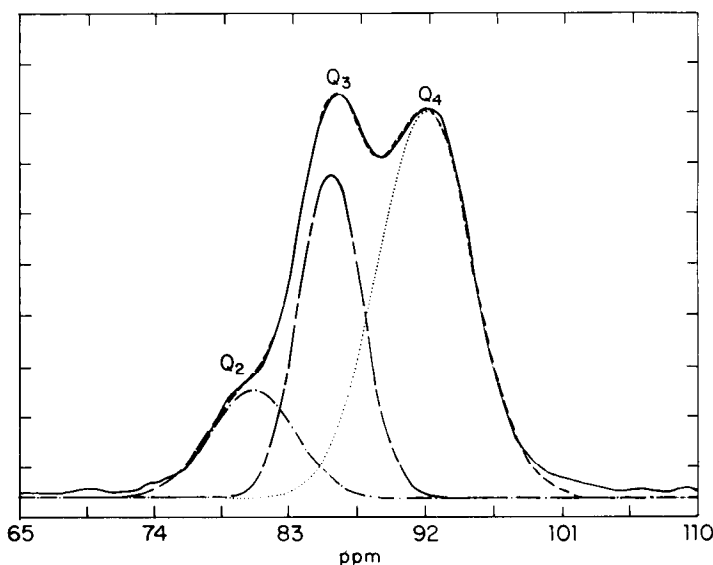

Fig. 13. ^{29}Si CP-MAS NMR of Zorbax PSM-60. See text for description.

groups on a silica surface. Untreated and silanized Zorbax and Nucleosil samples were characterized by this techniques. Fig. 13 shows a spectrum of Zorbax PSM-60 in the range from -65 to -110 ppm. In this presentation Q_2 is the signal for geminal SiOH groups $[=Si(OH)_2]$, Q_3 is the signal for single SiOH groups $[\equiv Si(OH)]$, and the Q_4 peak describes silicon atoms bearing no silanols $[Si(OSi)_4]$. This spectrum was deconvoluted mathematically into individual peaks for the various components under the assumption that the three different peaks are strictly Gaussian. The dashed line in Fig. 13 is the sum of the three deconvoluted peaks. Since this summation is virtually identical with the original signal, it can be concluded that an accurate deconvolution was performed.

To ensure the accuracy of these data, a fundamental question had to be answered: Are these peak areas directly proportional to the number of surface sites? It is well known that differences in the cross-polarization rates (T_{IS}^{-1}) are reflected in differences in the relative intensities of the observed resonances. Such differences would introduce an unknown, systematic error into the calculated surface coverage, unless an experimental cross-polarization time is chosen which is long compared with all of the inverse-rate constants. The inverse cross-polarization rates of the surface sites on a Fisher silica gel, as determined by Sindorf and Maciel[18] were 2.3, 2.9, and 13 ms for the Q_2, Q_3, and Q_4 sites, respectively, and the proton rotating-frame relaxation time $(T_{1}\rho_{H})$ was ca. 21 ms. Thus, if a cross-polarization time of 10 ms or longer is used for the measurement, the systematic error in the relative numbers of Q_2 and Q_3 sites is negligible. It has been suggested previously that this effect also holds for the silane silicon site, $M[19]$. To determine the desired conditions for the materials studied here, a variable cross-polarization time experiment was performed on silica-1CA (Table II). Results are summarized in Fig. 14.

Similar cross-polarization rates were observed for each of the sites. However,
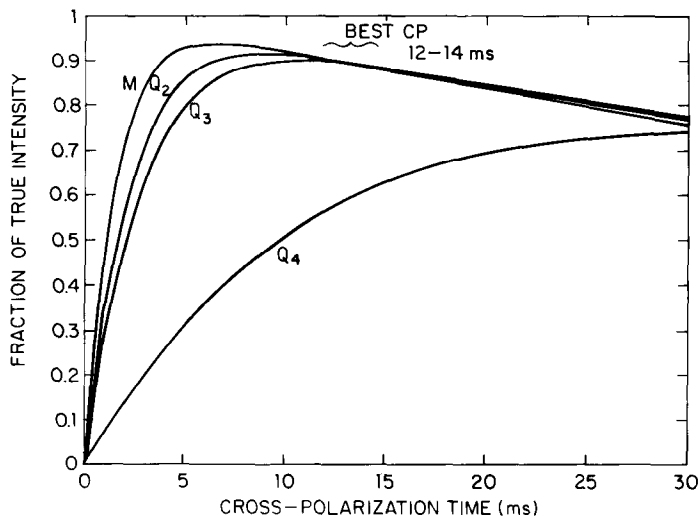

Fig. 14. ^{29}Si MAS NMR: CP time experiment. Sample 1-CA, partially TMS-modified Zorbax PSM-60. Curves: M = CH$_3$Si; Q$_2$ = Si(OH)$_2$; Q$_3$ = Si(OH); Q$_4$ = Si(OSi)$_4$.
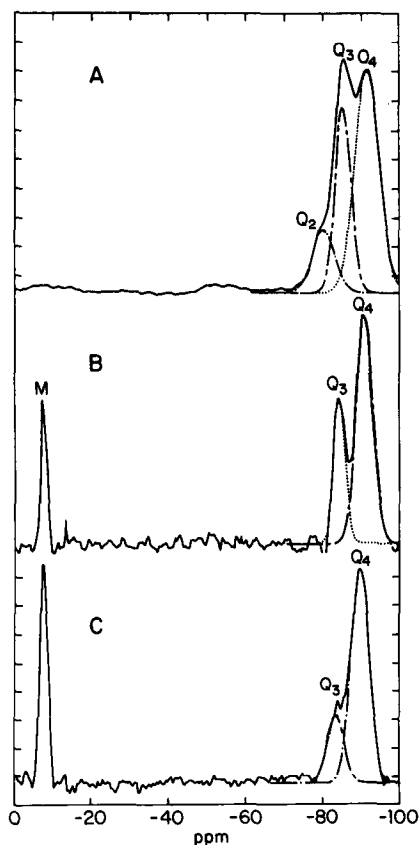
Fig. 15. ^{29}Si CP-MAS NMR spectra of silicas. (A) Zorbax PSM-60 (starting material); (B) partially TMS-modified Zorbax PSM-60 (sample 1-CA, Table II); (C) fully TMS-modified Zorbax PSM-60 (sample 1-CP, Table II). Cross-polarization time, 13 ms.

proton $T_1\rho_H$ was much longer; a lower limit of 14 ms was determined. Fig. 14 shows that accurate relative intensities of Q_2, Q_3 and M are obtained for a cross-polarization time of 13 ± 1 ms.

With the optimum NMR conditions described above, it was possible to deduce several important conclusions regarding bonded-phase silicas prepared by different procedures. Fig. 16 shows ^{29}Si spectra for untreated Zorbax PSM-60, partly-modified Zorbax 1-CA, and fully modified Zorbax 1-CP. (M is the signal for the silicon atoms with an alkyl ligand.) The relative integrated intensities (areas) of Q_2, Q_3, Q_4, and M in Fig. 15 are a direct measure of the relative numbers of the sites.

According to Sindorf[20], the fraction of silicons in the surface layer bearing geminal pairs of hydroxyl groups is

$$f_g = Q_2/(Q_2 + Q_3) \tag{1}$$
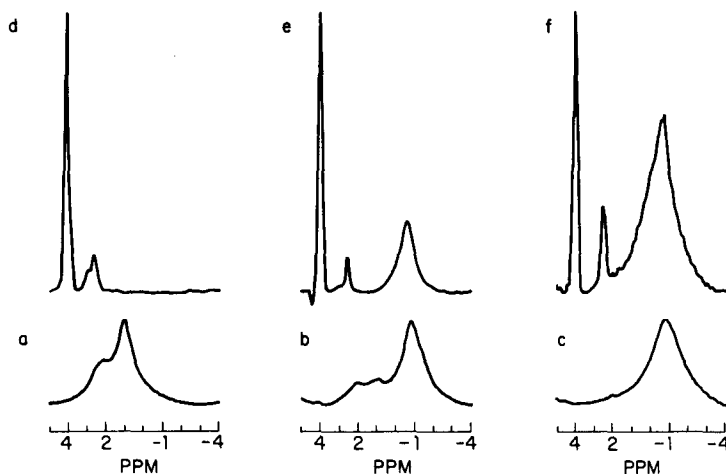
Fig. 16. ^{1}H-MAS NMR spectra of silicas. (a) Zorbax PSM-60; (b) partially TMS-reacted Zorbax PSM-60 (sample 1-CA, Table II); (c) fully TMS-reacted Zorbax PSM-60 (sample 1-CP, Table II); (d)–(f) same samples exchanged with added [²H₄]methanol.

The relative number of modified and unmodified surface sites is

$$f = M/(Q_2 + Q_3) \tag{2}$$

The fraction θ_T of the total number of hydroxyl groups that are modified with TMS is then given by:

$$\theta_T = M/(M + Q_3 + 2Q_2) = f/(1 + f + f_g) \tag{3}$$

(Note that θ_T, as defined here, is unity only if both hydroxyl groups of each geminal pair and all lone hydroxyl groups have reacted. This value differs from θ, as defined by Sindorf[20], which is unity if only one hydroxyl group of each geminal pair and all lone hydroxyls have reacted.)

Using eqn. 1, it was determined that both Zorbax and Nucleosil contain 32% geminal [Si(OH)₂] and 68% single [SiOH] silanol groups.

The NMR results in Fig. 16 show that for the partially TMS-modified Zorbax 1-CA sample (Table II), 33% of the SiOH groups present on the starting silica have reacted. However, 60% of the SiOH groups had been eliminated by silanization for the fully TMS-reacted Zorbax 1-CP sample. (The same starting silica for both samples had been dried *in vacuo* prior to silanization.) These measurements indicate that geminal silanol groups react first during silanization; geminal SiOH groups are missing even for the partly modified silica 1-CA. It is believed that only one hydroxyl group on the geminal groups can react with the silane because of steric factors.

For the fully modified Nucleosil sample (4-CP), the fraction of reaction silanols was determined by NMR as 66%. On the other hand, Nucleosil that had been heated to 200°C at atmospheric pressure and silanized by the enolate method was observed by NMR to contain a larger number of isolated SiOH groups than TMS-modified

Zorbax. Also, some residual geminal SiOH groups were noted. NMR measurements indicated that only 39% of the total silanols on Nucleosil prepared in this fashion were converted by the enolate reaction. Since no clear difference in coverage with alkyl ligands and in the chromatographic properties could be observed between the chlorosilane and enolate reaction (4-CP and 4-EV, Table II), it was concluded that Nucleosil-5-100, when *not* heated *in vacuo*, has some trapped SiOH groups beneath the surface which chromatographically are not relevant.

It is apparent that, while ^{29}Si CP-MAS results cannot explain differences in adsorptivity and stability between silicas, this method is valuable for measuring the fraction of reacted silanol groups after a silanization.

^{1}H-MAS NMR and reaction with [^{2}H$_4$]methanol

Useful characterizations of silica surfaces also may be carried out with ^{1}H-MAS NMR by which fairly well resolved spectra are obtained in samples with diluted and/or mobile proton species. The objective of experiments with Zorbax PSM-60, silica 1-CA, and silica 1-CP (Table II) was to determine the concentration of surface hydroxyls and alkyl ligands on the bonded-phase packings. However, Fig. 16 a–c clearly show that the signals for the SiOH groups at 1.0 and 2.2 ppm are not sufficiently separated from the peak for the methyl groups at -0.8 ppm. Thus, the direct ^{1}H-NMR technique does not provide the desired information.
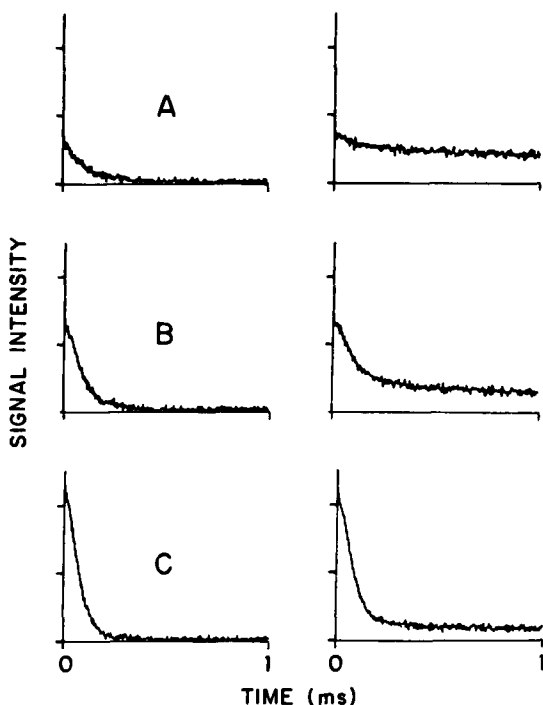

Fig. 17. Non-spinning proton FID of silicas by proton NMR. (A) Zorbax PSM-60; (B) partially TMS-modified Zorbax PSM-60 (sample 1-CA, Table II); (C) fully TMS-modified Zorbax PSM-60 (sample 1-CP, Table II) before (left) and after (right) addition of [^{2}H$_4$]methanol.

On the other hand, a substantial increase in information can be obtained by the addition of [²H₄]methanol to silica samples. With this approach the protons in the SiOH groups are completely exchanged with deuterium, and the peak for the resulting C²H₃OH at 4.1 ppm is well resolved from the methyl silane peak. Fig. 16d–f show the ¹H-MAS NMR spectra of three silica samples after addition of an excess of deuterated methanol. (The additional peak at 2.6 ppm is due to residual methyl protons in the [²H₄]methanol.) Unfortunately, experimental complications prohibited the quantitative evaluation of this convincing qualitative experiment.

A more informative experiment was conducted with deuterated methanol and a much simpler proton NMR technique. In this approach, the proton FIDs of non-spinning samples were monitored before and after the addition of [²H₄]methanol. The technique is based on the fact that solids have fast FIDs (steep slopes), and liquids have slow FIDs (shallow slopes). Fig. 17 shows the FIDs for the three silicas with and without [²H₄]methanol. The FID signals for the samples with [²H₄]methanol have changed to a combination of a solid component (fast decay) and a liquid component (slow decay). Extrapolation of the liquid FID to time zero allows the estimation of the fraction of the protons that have moved from surface hydroxyl positions to the methanol. According to these measurements, 25% of the hydroxyls have been substituted by TMS groups in the silica 1-CA sample, and 50% in silica 1-CP. These data agree reasonably well with the 33% and 60%, measured by ^{29}Si CP-MAS, as described above.

Characterization of silicas by DRIFT

Previous discussions have indicated that IR spectroscopy is a valuable technique for characterizing silica surfaces. For example, Kondo *et al.*[21] have shown that
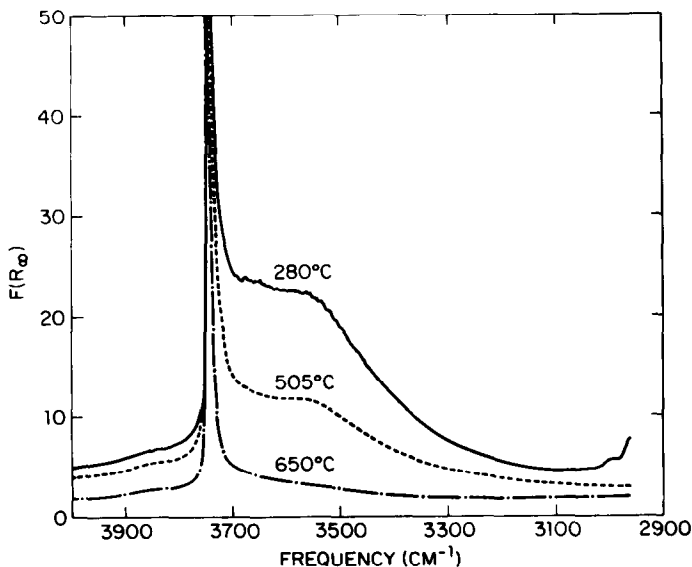

Fig. 18. DRIFT spectra of heated silica. Sample, Zorbax PSM-60. F (R∞) = Kubeka–Munk units.

absorption IR spectroscopy can identify inactive surface hydrogen-bonded SiOH groups produced from different amorphous silicas by means of hydrothermal reaction at high pH and temperature. In this study, we used the versatile DRIFT technique to identify the type and concentration of different silanol groups on silicas. We believe that the DRIFT method provides information on both surface and interior SiOH groups on the silica, since the particle dimension studied (*ca.* 5 μm) is roughly that of the incident IR wavelength.

It is well known that the heating or calcining of silicas decreases the concentration of SiOH groups within the structure[22]. In our study, samples of Zorbax PSM-60 were heated in a furnace to 280, 505, and 650°C, and investigated by DRIFT. Fig. 18 shows three spectra in the SiOH region from 4000 to 2900 cm^{-1}. A relatively sharp peak for "isolated" SiOH groups at *ca.* 3750 cm^{-1} and a broad signal for "associated" or hydrogen bridge-bonded silanols at about 3550 cm^{-1} can be observed. Thus, DRIFT provides a convenient means of distinguishing unbonded (or "isolated") from bonded or associated SiOH groups. It does not, however, provide separate identification of geminal and vicinal SiOH groups.

With increasing temperature, the integral of the overall SiOH peak area becomes smaller. This effect is due to a loss of SiOH groups in the form of water when new siloxane (–Si–O–Si–) linkages are formed. In addition, peak intensity is shifted from bonded to unbonded silanols as the degree of bonding is decreased and the number of unbonded SiOH groups is increased. This effect can clearly be seen in Fig. 19, which shows the expanded frequency region from 3800 to 3700 wavenumbers. Frequencies shift for the unbonded silanols to higher values with higher temperatures. The shift can be interpreted as a result of decreased interaction of the remaining SiOH groups with their neighbors.

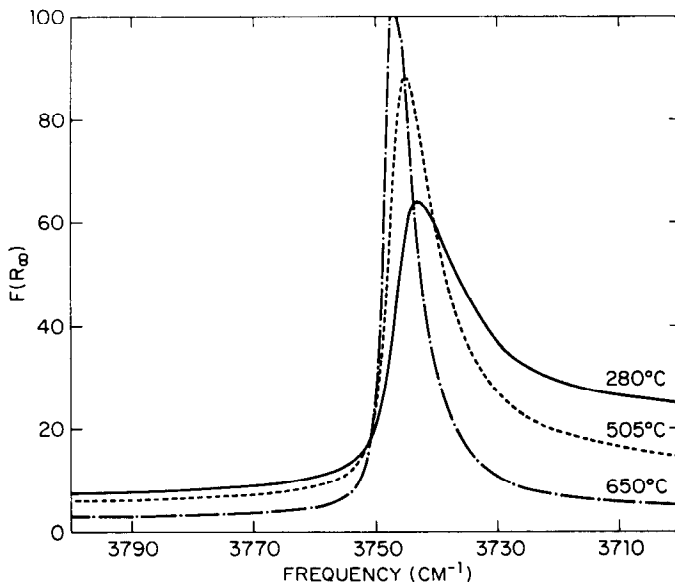Although the number of silanols decreased as a result of heating, the pH value

Fig. 19. DRIFT frequency shift on heating silica. Sample, Zorbax PSM-60.
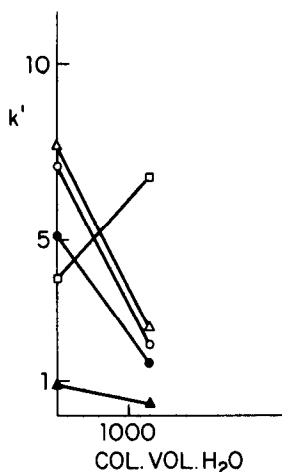
Fig. 20. Hydrolysis of heat-treated fully TMS-modified Zorbax PSM-60. Sample 9-CP, Table II; %C = 3.45; water purge and chromatographic tests as in Fig. 1. Key as in Fig. 1.

of these calcined samples did not increase drastically. For example, a Zorbax PSM-60 which had been heated to 850°C for 3 days, still exhibited a pH-value of 4.30 (cf. Table I). It is believed that unbonded or isolated SiOH groups must be primarily responsible for this low pH-value, since they are the last to disappear during heating. It also is likely that the acidity of these isolated SiOH groups increased somewhat as the concentration (and possibility for intermolecular bonding) decreases.

Not all of the surface silanols on silicas react with alkylsilanes, because of steric factors. Based on chemical, spectroscopic, and elemental analysis data, it can be concluded that only ca. 70% of SiOH groups disappear in the most rigorous silanization reaction; a significant number remain. An attempt was made to decrease this number further by heat treatment of the silica prior to silanization. In this approach, a Zorbax PSM-60 sample was heated to 650°C and silanized with TMS under dry conditions by the enolate method. However, it was evident on the basis of DRIFT results that the resulting bonded-phase support still contained SiOH groups. These groups remained unmodified even though it can be assumed that the reduced concentration of SiOH groups should have eliminated the possibility for hindering or steric effects.

From this result it can be deduced that either the very weak proton donor reaction byproduct (acetylacetone) cleaved –Si–O–Si– bonds to form new silanols, or more likely, that SiOH groups were formed during the work-up procedure in aqueous medium. Fig. 20 shows that, during hydrolysis of this packing with water, the retention time for N,N-DEA is markedly increased. It can be concluded, therefore, that partly dehydrating a silica surface and treating the residual silanols with alkylsilanes does *not* lead to a stable, non-absorptive surface.

The difference between SiOH groups on Zorbax PSM-60 and Nucleosil-5-100 is clearly defined by DRIFT, as shown in Fig. 21. Although Zorbax has a higher particle density that leads to a somewhat higher column-packing density, Nucleosil still exhibits a significantly higher concentration of silanols. Associated or bonded
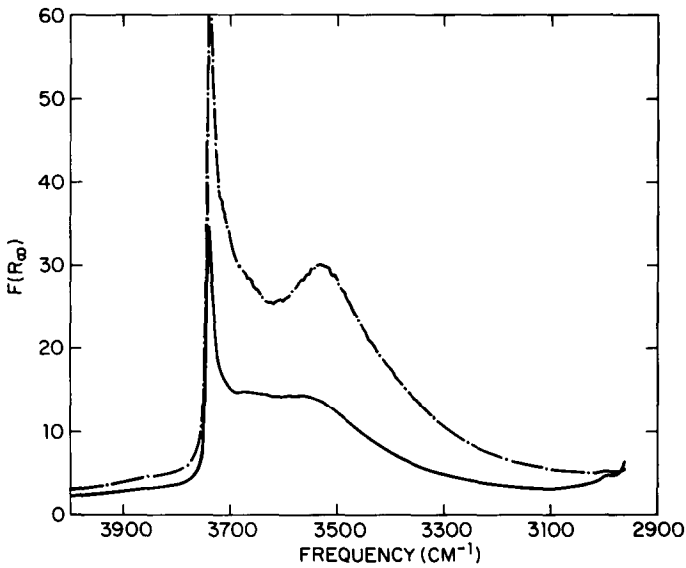
Fig. 21. DRIFT comparison of SiOH groups for silicas. - · - · -, Nucleosil 5-100; ———, Zorbax PSM-60.

silanol groups (3600–3500 cm^{-1}) are also much more prominent for Nucleosil than Zorbax. An expansion of this frequency region (Fig. 22) reveals that the sharp peak for the isolated or unbonded SiOH groups on the Nucleosil is at a lower frequency than the same peak for Zorbax. The maximum for the absorption of the unbonded silanol groups on Zorbax is 3741 cm^{-1}, compared with 3738 cm^{-1} on Nucleosil. This
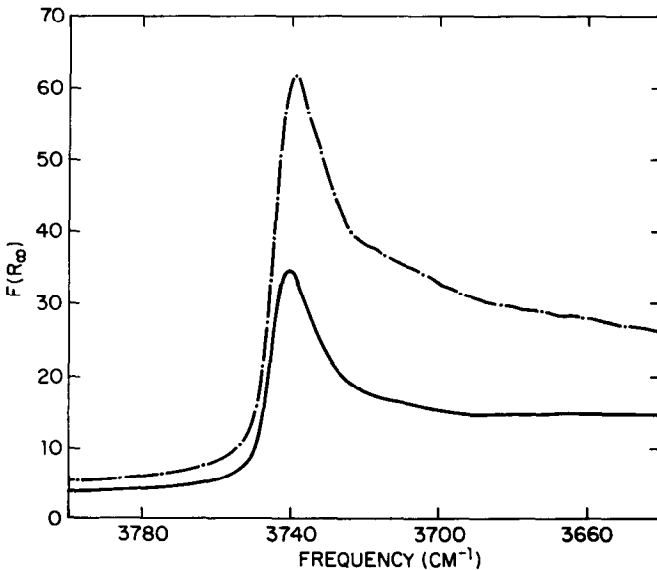

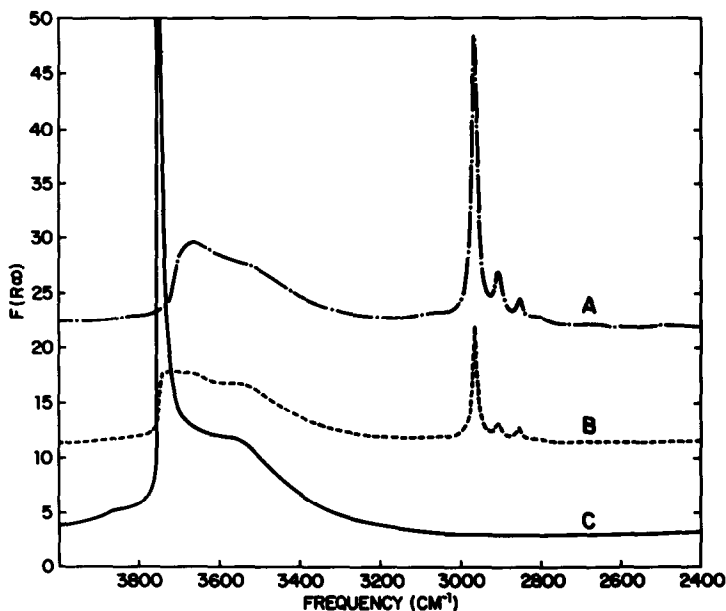Fig. 22. Comparison of isolated SiOH by expanded-scale DRIFT. Curves as in Fig. 21.

Fig. 23. DRIFT spectra of silicas. (A) Fully TMS-modified Zorbax PSM-60 (sample 1-CP, Table II); (B) partially TMS-modified Zorbax PSM-60 (sample 1-CA, Table II); (C) Zorbax PSM-60.

difference is significant within the experimental error of the method (± 1 cm^{-1}). Based on such DRIFT measurements, it is concluded that Zorbax contains a higher concentration of non-hydrogen-bridge-bonded silanol groups than Nucleosil.

The higher acidity of the Zorbax surface may be caused by the fact that unbonded SiOH groups are essentially surrounded by siloxanes. These siloxane groups withdraw electrons that enhance the acidity of unbonded SiOH groups. On the other hand, fully hydroxylated surfaces such as Nucleosil, have fewer siloxane groups; therefore, surface SiOH groups are less acidic[24].

DRIFT also can be used to characterize residual SiOH groups on silanized silicas. Fig. 23 shows IR spectra for unmodified Zorbax PSM-60, partially modified silica 1-CA, and fully modified silica 1-CP in the range 4000–2400 wavenumbers. Isolated and associated SiOH groups can be observed between 3400 and 3800 cm^{-1}, as well as alkyl ligands near 3000 cm^{-1}. It is apparent that unbonded SiOH groups disappear first during the silanization reaction with trimethylchlorosilane. The partly modified sample (1-CA) still contains a substantial concentration of unbonded silanols, whereas the concentration of these groups is very small for the fully modified silica 1-CP. Isolated, non-hydrogen-bridge-bonded silanols, as determined by DRIFT, can be single or geminal SiOH groups.

We have determined that DRIFT results on unbonded SiOH groups closely correlate with chromatographic data. It may be recalled that we previously concluded from heating experiments and pH measurements that unbonded SiOH groups are believed to be responsible for the high surface acidity, and that highly acidic groups are most likely to interact strongly with bases such as N,N-DEA. As indicated above, DRIFT data show that unmodified Zorbax contains a large number of these un-
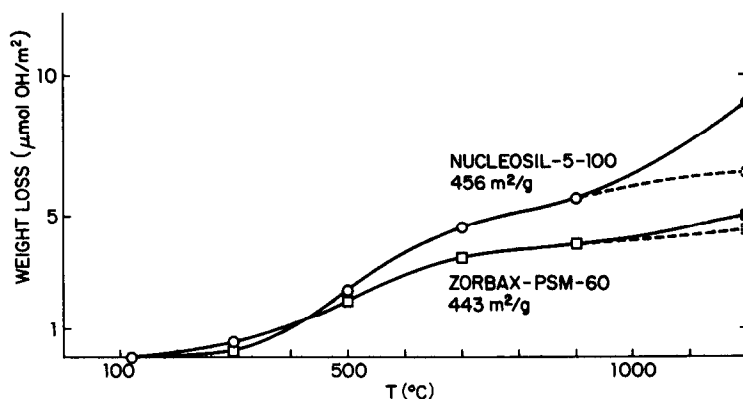
Fig. 24. TGA analysis of silicas. Weight loss after drying at 120°C: 10.9% for Nucleosil (O); 5.6% for Zorbax (□). Solid line, weight loss after equilibrium; broken line, weight loss immediately after reaching 1200°C.

bonded, acidic SiOH groups. As a result, unmodified Zorbax PSM-60 not only totally adsorbs N,N-DEA in a chromatographic experiment with a 60:40 methanol–water mobile phase, but also exhibits very long retention times for 2,6-di-*tert.*-butylpyridine, the weak base in the polarity test mixture.

Partially TMS-modified Zorbax PSM-60 (silica 1-CA) behaves very similarly. However, because the concentration of acidic SiOH groups in a 150 × 4.6 mm I.D. column of this packing is very large, relative to the few nanomoles of bases contained in 10 μl of polarity mixture, the surface of this packing cannot be deactivated with a few injections.

Even though the fully TMS-modified Zorbax PSM-60 (silica 1-CP) contains a very low concentration of acidic SiOH groups that cannot be detected by DRIFT, the presence of these groups is quite obvious chromatographically. These acidic groups on the silica surface are deactivated by injecting a few nanomoles of base as described above.

As a result of the experiments in this part of the study, it now can be concluded that *isolated acidic silanols* are responsible for the adsorption of bases on both silanized and non-silanized silica supports.

TGA and proton spin-counting NMR

The difference between unmodified Zorbax PSM-60 and Nucleosil-5-100 silica is clearly revealed by TGA. Fig. 24 shows a plot of μmol OH/m² weight loss *versus* heating temperature for these two silicas. These results are based on the assumption that the silicas only lose water, and that one mole of water is released as a product of the siloxane bridge formation from two SiOH groups. The dashed lines in this plot represent the weight loss immediately after reaching 1200°C. The solid lines between 900 and 1200°C describe the *total* weight loss of the silica during sintering when the surface area is decreasing very rapidly. (Other experiments indicated that the actual melting process for both samples began above 1050°C.)

From these TGA data we conclude that Nucleosil-5-100 exhibits a significantly higher total SiOH concentration than Zorbax PSM-60. The total weight loss of Nu-

TABLE V

SILANOL CONCENTRATION ON SILICA (ZORBAX PSM-60)

Values in μmol/m^2. Chemical determination with propyldiborane at 150°C, 4.5 μmol/m^2.

$T(°C)$	^1H NMR	TGA
120	5.9 and higher	5.1, 5.2, 5.5
150	5.4	5.0
200	5.1	4.9
300	4.2	4.5
400	3.8	3.9
500	3.1 ($\pm$10%)	3.1 ($\pm$5%)

cleosil is equivalent to *ca.* 9 μmol SiOH/m^2, whereas Zorbax appears to contain only *ca.* 5.5 μmol SiOH/m^2.

Nucleosil also loses more weight during heating at above 1000°C than Zorbax. This observation is in accordance with ^{29}Si NMR data, which suggested incorporated or "buried" SiOH groups for Nucleosil-5-100 heated at atmospheric pressure. Thus, both TGA and ^{29}Si NMR measurements include "buried" as well as surface SiOH in the determination.

Chemical reaction with propyldiborane (previously carried out at the Max-Planck-Institut für Kohlenforschung in Mülheim/Ruhr, F.R.G.) showed 6.5 μmol SiOH/m^2 for Nucleosil-5-100, and 4.5 μmol SiOH/m^2 for Zorbax PSM-60. Steric factors apparently do not allow a complete reaction of propyldiborane with all SiOH groups.

Tables V and VI compare TGA with proton spin-counting NMR results for the SiOH concentration of Nucleosil and Zorbax at different calcination temperatures. For Zorbax, the data of the two characterization methods check very well. (At 120°C the NMR data were not reproducible, probably owing to drying problems.) However, NMR and TGA data for Nucleosil are totally different. This apparent inconsistency is caused by different sample pretreatment. In proton spin-counting NMR experiments, samples were dried *in vacuo*, which removed buried SiOH groups.

TABLE VI

SILANOL CONCENTRATION ON SILICA (NUCLEOSIL-5-100)

Values in μmol/m^2. Chemical determination with propyldiborane at 150°C, 6.5 μmol/m^2.

$T(°C)$	^1H NMR	TGA
120	8.33	9.00, 10.00
150	7.06	9.00
200	6.25	9.00
300	5.11	8.80
400	3.64	8.00
500	2.94 ($\pm$10%)	6.60 ($\pm$5%)

TABLE VII

FRACTION OF REACTED SILANOLS ON SILICAS

θ_T = fraction of modified silanol groups; EA = elemental analysis.

Material	Sample (Table II)	$\theta_T(EA, TGA, {}^1H\text{-}Count)$	$\theta_T({}^{29}Si\ MAS)$
Nucleosil-5-100-TMS	4-CP	0.56	0.66
Nucleosil-5-100-TMS-E	4-EV	0.41	0.39
Partially TMS-Modified Zorbax PSM-60	1-CA	0.32	0.33
Fully TMS-Modified Zorbax PSM-60	1-CP	0.62	0.60
		($\pm 7\%$)	($\pm 10\%$)

In the TGA experiment, analyses were carried out in an inert gas at atmospheric pressure. Under these conditions SiOH groups apparently are trapped within closed pores and can only be released at very high temperatures (above 1000°C).

Taking account of the "buried" silanols, we have determined that the *surface* SiOH concentration for Nucleosil-5-100 is 7.0 ± 0.7 μmol/m^2, and for Zorbax PSM-60 (CW-47) 5.0 ± 0.5 μmol/m^2. For these results, samples have to be dried at 150°C and 10^{-4} mbar.

It is important to note that the higher SiOH concentration on Nucleosil causes lower adsorptivity with respect to bases, a higher pH value of the silica, and a higher hydrolytic stability of the alkyl ligand, compared with Zorbax-type silicas.

Comparison of different analytical techniques

Data produced by different analytical techniques were rigorously compared to evaluate the accuracy of analytical data regarding initial SiOH surface concentration, alkyl ligand density resulting from silanization, and the fraction of modified silanols. Although ^{29}Si CP-MAS NMR directly measures the fraction of modified silanols on a silica surface, this value can also be calculated by using data from the other characterization techniques. For example, the total silanol concentration on a non-silanized silica can be determined by TGA. By contrast, proton NMR using [^{2}H$_4$]methanol exchange provides information on SiOH groups only on the surface of the silica. Since elemental analysis describes the carbon content of a reacted silica, the coverage of the support with organic groups can be calculated with the knowledge of the molecular weight of the ligand and the surface area. The fraction of reacted silanols is given by the ratio of this coverage and the overall number of initially present silanols, determined by various methods. Table VII compares two independently measured values for the fraction of modified silanol groups on different silicas. The data agree within the experimental limits indicated, clearly verifying the accuracy of the different analytical results.

CONCLUSION

Contrary to common belief, a stable silica support with low adsorptivity for basic compounds should contain the highest, and not the lowest, number of homogeneously distributed, associated SiOH groups to ensure a minimum concentration

of highly acidic, isolated silanols. Such silica-based packing exhibit significantly higher hydrolytic stability and a substantially lower order of adsorptivity towards basic compounds. We have developed techniques for preparing this type of support; results of these studies will be published soon.

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