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IMPROVED SILICA-BASED COLUMN PACKINGS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

J. KÖHLER* and J. J. KIRKLAND*
E. I. du Pont de Nemours and Company, Central Research and Development Department, Wilmington, DE 19898 (U.S.A.)

SUMMARY

Undesired adsorption of basic compounds and the low hydrolytic stability of alkyl bonded-phase ligands can be strongly affected by the existence of highly-acidic, essentially non-hydrogen bonded (or isolated) SiOH groups on silica packings. We have previously proposed that the support for stable silica reversed-phase packings with low adsorptivity for basic compounds should contain the highest, and not the lowest, number of homogeneously distributed, associated SiOH groups. To obtain such desirable supports we fully hydroxylate calcined silica by special techniques involving the dissolution and redeposition of silicic acid. This rehydroxylation process results in no substantial change in the surface area or pore size of the silica support. Subsequently, the properties of these silicas were investigated by chemical, spectroscopic and chromatographic techniques. Fully hydroxylated silicas exhibit: (1) a larger number of associated silanols; (2) higher pH values; (3) markedly lowered adsorptivity for basic compounds; (4) significantly improved hydrolytic stability of bonded-phase ligands; and (5) increased mechanical stability. Large pore-size, reversed-phase packings made from this kind of support produced much better separations of basic peptides and proteins than conventional column packings.

INTRODUCTION

In a previous study[1] we showed that various trimethylsilyl (TMS) modified silicas exhibit different tendencies to adsorb basic organic compounds and different levels of stability during use in reversed-phase chromatography. These characteristics were ascribed to differences in the nature of the silica support making up the packing. In this previous study, chromatographic measurements were correlated with physical and chemical data, such as BET surface areas and pore volume distributions, and the concentration of bonded organic ligand, as determined by elemental analysis. Packings were subjected to thermogravimetric analysis (TGA), ^{29}Si cross-polarization magic-angle-spinning NMR (^{29}Si-CP-MAS NMR), and proton spin-counting

* Permanent address: Max-Planck-Institut für Köhlenforschung, Kaiser-Wilhelm-Platz 1, 4330 Mülheim/Ruhr, F.R.G.

solid-state NMR, to measure the amount of silanol groups on the surface. ^{29}Si NMR and diffuse-reflectance infrared Fourier-transform spectroscopy (DRIFT) revealed important information about the type and/or distribution of SiOH groups in the packing.

In that previous work chromatographic tests showed that most of the TMS-silica materials studied exhibited high adsorptivities for the basic probe, N,N-diethylaniline (N,N-DEA). In addition, these bonded-phase packings were rapidly hydrolyzed when purged with pure water. Curiously, certain TMS-modified silicas (*e.g.*, Nucleosil-TMS and Vydac-TMS) exhibited a much lower order of adsorption for basic solutes, and the TMS bonded phase was more stable than other packing materials. However, these silicas either showed poorer mechanical stability or higher column back-pressures.

The different chromatographic behaviour of the two types of silicas was attributed to the different concentration and type of residual SiOH groups on the surface of the packing. For example, most unmodified, relatively adsorptive silicas (herein called Type A silicas) contain about 5.5–6 μmol SiOH/m^2 after drying at 120°C *in vacuo*, whereas less adsorptive (herein called Type B silicas) showed a surface silanol concentration of at least 7 μmol/m^2. It was determined that the more adsorptive TMS-modified Type A silicas include Adsorbosphere, Hypersil, Spherisorb, RSil and Zorbax®, while less adsorptive TMS-modified Type B silicas include Nucleosil, Chromegabond and Vydac[1]. These results were qualitatively in agreement with another study of the silanophilic contribution of a variety of commercial reversed-phase columns based on the chromatographic behavior of cyclic tetra-aza compounds[2]. It was also determined that Type B silicas can contain some "buried" SiOH groups that confuse analytical data regarding silanol surface concentrations.

Our previous study showed by ^{29}Si-CP-MAS NMR that both Type A and Type B supports contain 32% geminal [Si(OH)$_2$] and 68% single (SiOH) groups. Upon reaction with trimethylchlorosilane, geminal groups totally disappeared on both types of silicas, making it highly unlikely that geminal silanol groups are the source of adsorption and instability problems with silica-based packings. DRIFT revealed the existence of two kinds of silanols —unbonded (or isolated) and bonded (or associated). These groups are different in terms of their hydrogen bonding to their nearest neighbors. We found that the isolated SiOH groups generally react first with silylation agents. We also found that partly TMS-modified Type A materials contained no geminal SiOH groups, but still contained isolated acidic sites which caused strong adsorption for basic samples.

It was concluded that isolated or unbonded, acidic SiOH surface groups on silica-based packings are largely responsible for the adsorption of basic molecules. This conclusion was further supported by the fact that, compared to Type A supports, the less adsorptive Type B materials exhibited higher concentrations of associated or bonded SiOH groups, relative to isolated or unbonded SiOH groups. Macroscopically, the difference in particle acidity, as evidenced by the adsorption of bases, also was correlated to pH measurements; Type B materials exhibited a higher pH value than Type A unmodified silicas. These studies led to a better understanding regarding the desired chemical nature of silicas for use as an optimum high-performance liquid chromatography (HPLC) packing support.

In this paper, we describe procedures whereby the desirable properties, such

as high particle strength and excellent separation efficiency of a Type A support, can be combined with low adsorptivity for basic molecules and increased hydrolytic stability of bonded phase materials. This leads to significantly improved silica-based column packings for HPLC separations.

EXPERIMENTAL

Silylation reagents, silanization of silicas, analytical techniques, column-filling procedures and chromatographic tests were previously described[1]. TMS-modified silicas were subjected to definitive chromatographic testing involving the measurement of retention times (or k' values) and peak shapes for non-polar solutes, 1-phenylhexane and 1-phenylheptane; a polar solute, 5-phenylpentanol; and basic solutes, 2,6-di-*tert.*-butylpyridine and N,N-diethylaniline. After purging columns with known volumes of water at 50°C, k' values were redetermined with the initial aqueous–organic mobile phase system. From these measurements, characteristic hydrolysis curves, relating the k' of test solute *versus* column volumes of water, were used to illustrate column stability.

DRIFT and thermogravimetric analyses (TGA) were carried out as described previously[1]. To remove physically adsorbed water from the silica surface in TGA analyses, 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 calculation of SiOH concentration assumed that two moles of SiOH groups combine on heating to form one mole of water that is lost from the sample during the heating procedure.

Chemicals

Tetrabutylammonium hydroxide was obtained from Aldrich (Milwaukee, WI, U.S.A.). Hydrofluoric acid (49%), ethylenediamine, ammonium hydroxide, nitric acid and dibasic sodium phosphate were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Sodium silicate (SiO_2/Na_2O ratio = 3.25) was supplied by Du Pont.

Particle-strength measurements were performed with an Model 1127 universal testing machine (Instron, Canton, MA, U.S.A.).

Treatment of silicas before silanization

All silica samples to be silanized were first subjected to drying 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.

For dehydroxylation, silica samples were heated in quartz crucibles in a nitrogen-purged furnace to remove all water and most of the silanol groups from the silica surface. In all cases the temperature was increased in three steps (100, 200 and 450°C) to 850°C. Samples were allowed to equilibrate at each of these temperatures for 8 h, and finally held at 850°C for at least 3 days. The hot quartz crucible containing the silica then was removed from the furnace and placed in a vacuum oven to cool to

TABLE I

EXPERIMENTS WITH SILICAS

Data for silicas 1–10 and 13 are taken from ref. 1. Silica: Z = Zorbax, N = Nucleosil, V = Vydac, (XXX) = pore size in Å. Temperature: temperature in °C at which silica has been tempered (3 days). Add.: rehydration agents: A = water–HF, B = water–tetrabutylammonium hydroxide, C = steam, D = water–ethylenediamine, E = water–calcium carbonate, F = water–sodium silicate, G = water–ammonium hydroxide, H = silicon tetrachloride + water hydrolysis, I = water, + = treatment at room temperature. Conc.: Concentration of additive in water in ppm (100 ml water/10 g silica). pH 1: pH of silica suspended in the rehydration liquid. Time: hydrolysis time in h. Treatment: additional treatment of the rehydrated silica: * = filtration of the hot suspension, A = wash with water to neutrality, B = wash with ammonium hydroxide–water (pH 9), C = wash with nitric acid (1 ml conc. HNO_3 in 200 ml water), D = boil in water for 2 days, E = wash with acetone, F = dry *in vacuo* at 120°C, G = wash with acetic acid. pH 2: pH of a suspension of 1 g of silica in 50 g of water after at least 10 min of equilibration. Structure: changes in (compared to starting material): A = surface area, B = pore size, C = pore volume as determined by BET, D = destruction of particle, E = no change in structure. SiOH: changes in SiOH concentration per surface area unit (compared to starting silica); < = decrease, > = increase, = = constant, relative to starting material. Adsorptivity: chromatographic adsorptivity of the silanized silica with respect to bases, as judged by retention and peak shape before and after hydrolysis: S = strong adsorption; M = moderate adsorption, N = no adsorption.

No.	*Silica*	*Temperature (°C)*	*Add.*	*Conc.*	*pH 1*	*Time*	*Treatment*	*pH 2*	*Structure*	*SiOH*	*Adsorptivity*
1	Z (60)	–	–	–	–	–	–	4.08	–	–	S
2	Z (150)	–	–	–	–	–	–	4.27	–	–	S
3	Z (300)	–	–	–	–	–	–	4.53	–	–	S
4	N (100)	–	–	–	–	–	–	5.15	–	–	N
5	N (300)	–	–	–	–	–	–	6.89	–	–	N
6	V (300)	–	–	–	–	–	–	5.34	–	–	N
7	Z (60)	1100	–	–	–	–	–	–	A, B, C	<	–
8	N (100)	1100	–	–	–	–	–	–	A, B, C	<	–
9	Z (60)	1026	–	–	–	–	–	4.74	A, B, C	<	–
10	Z (60)	850	–	–	–	–	–	4.30	E	<	–
11	Z (60)	650	G	3500	9.00	4	A, E, F	4.86	A, B	>	S
12	Z (60)	650	I	–	–	60	A, E, F	4.92	E	=	M
13	Z (60)	245	–	–	–	–	–	4.34	E	<	–

14	Z (60)	845	I	–	–	60	A, E, F	4.55	E	=	S
15	Z (60)	850	E	20 000	6.63	19	G, A, F	5.89	A, B, C	>	S
16	Z (60)	–	I	–	–	24	A, E, F	4.68	–	–	–
17	Z (60)	–	I	–	–	48	A, E, F	4.87	–	=	–
18	Z (60)	850	I	–	–	45	A, E, F	–	E	=	M
19	Z (60)	–	I	–	–	144	A, E, F	5.47	A	>	N
20	Z (60)	850	A	100	3.32	24	A, E, F	5.26	A	>	N
21	N (100)	800	–	–	–	–	–	–	A, B, C	<	–
22	N (100)	800	I	–	–	72	A, E, F	7.12	A, B, C	>	M
23	Z (60)	850	I	–	–	100	A, E, F, *	–	A, B, C	>	S
24	Z (60)	–	C	–	–	7	A, E, F	4.04	E	>	S
25	Z (60)	850	G	–	9.30	17	A, E, F	5.38	A, B, C	>	S
26	Z (60)	850	F	–	9.99	2	A, C, E, F	–	B, C	>	M
27	Z (300)	–	I	–	–	144	A, E, F	5.19	D	>	–
28	Z (300)	850	A	75	–	72	A, D, E, F	–	B, C	>	N
29	Z (60)	–	A	100	–	20	A, E, F	4.17	A, C	=	M
30	Z (60)	–	A	100	–	20	A, D, F	–	A, B, C	>	–
31	Z (60)	–	A	100	–	20	A, D, B, F	–	A, B	=	M
32	Z (60)	850	A	50	–	72	A, D, F	4.11	A, B	>	S
33	Z (60)	–	H	–	–	15	A, E, F	5.07	A, B, C	>	S
34	Z (60)	850	A	200	3.11	72	A, B, E, F	5.31	A, C	>	N
35	Z (60)	850	B	–	8.00	25	A, C, E, F	5.29	A, B, C	>	M
36	Z (60)	850	B	–	9.00	26	A, C, E, F	5.59	A, B, C	>	N
37	Z (60)	850	A	400	2.27	120	A, B, D, E, F	5.21	A, C	>	N
38	Z (300)	850	A	400	2.27	120	A, B, D, E, F	5.24	B, C	>	N
39	Z (60)	850	D	–	9.00	24	A, C, E, F	5.27	A, C	>	N
40	Z (60)	–	G+	–	9.99	18	A, C, E, F	4.80	A, C	>	N

room temperature. All further preparation steps were carried out in an atmosphere of dry nitrogen or argon.

Rehydroxylation of silicas

Dehydroxylated silicas were rehydrated or rehydroxylated by a variety of methods. Typically, samples were placed in water containing small amounts of different activators such as hydrofluoric acid, ammonium hydroxide, tetrabutylammonium hydroxide or ethylenediamine[3]. The pH values of silica–water suspensions (typically 10 g of silica in 100 ml of water) ranged from 2.7 to 10, depending on the

TABLE II

BET DATA ON SILICAS

Data for silicas 1–10 and 13 taken from ref. 1. Values in parentheses were determined by mercury intrusion.

*Silica No.**	*Surface area* (m^2/g)	*Volume at saturation* (*ml/g*)	*Pore volume* (*ml/g*)	*Average pore diameter* (*Ångstrom*)
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)
9	184	193	0.30	65
10	436	534	0.83	76
11	355	549	0.85	96
13	463	575	0.89	77
15	182	489	0.76	167
18	430	519	0.81	75
19	419	542	0.84	80
20	417	536	0.83	80
21	430	760	1.18	110
22	237	721	1.12	189
23	373	605	0.94	101
24	438	557	0.87	79
25	195	386	0.60	123
26	436	490	0.76	70
27	58	258	0.40	279
28	57 (91)	264	0.41 (0.50)	289 (333)
29	389	498	0.77	80
30	380	604	0.94	99
32	356	561	0.87	98
33	402	451	0.70	70
34	347	447	0.69	80
35	390	593	0.92	95
36	356	501	0.78	87
37	272	523	0.81	120
38	57 (87)	338	0.53 (0.50)	372 (345)
39	224	511	0.80	142
40	387	472	0.73	76

* Compare Table I.

additive used, and reaction times varied from 4 to 144 h. If the rehydration was carried out at elevated temperature, the suspension normally was allowed to cool to room temperature before particles were removed from the reaction mixture by filtration.

Steps were taken to avoid adsorption of the rehydroxylation agent on the final silica support. Silicas rehydroxylated with basic additives were washed with nitric acid and then with water to neutrality. Hydrofluoric acid-treated samples were rinsed with ammonium hydroxide (pH 9) and then washed with water until the filtrate exhibited a pH of 7. Most of the silicas were finally washed with acetone and dried *in vacuo*. A summary of the different experiments performed during this part of the study is given in Table I. BET and mercury intrusion data on these samples are summarized in Table II.

RESULTS AND DISCUSSION

We have previously concluded that low SiOH concentrations on a silica support surface significantly affect bonded-phase chemical stability and the adsorption of organic bases[1]. In this study, we also found that the concentration of SiOH on relatively adsorptive Type A silicas is significantly lower than on the less adsorptive Type B silicas. In the preparation of most commercially available silicas, a sintering process normally is incorporated to strengthen the particle. For use as a chromatographic support, this dehydroxylated silica must then be hydroxylated so that silanization reactions can be carried out to prepare reversed-phase packings.

Rehydration of dehydroxylated silicas is accomplished by various methods, such as heating in aqueous acid, extensive boiling in water, etc. However, such processes are not usually sufficiently rigorous to create total rehydroxylation with a surface SiOH concentration of about 8 μmol/m^2; with Type A silicas the concentration of SiOH groups often is about 5–6 μmol/m^2 (ref. 1). (See Tables I–IV for comparison; some data from ref. 1 are included in these tables.) Therefore, an important aspect of the present work was to develop procedures for the complete hydroxylation of the surface and obtain the chromatographically desirable support surfaces described in ref. 1.

De- and re-hydroxylation of Nucleosil

As previously indicated, Type B packings exhibit higher silanol surface concentrations than Type A materials. This fact suggests that Type B particles are produced without heating above about 150°C, or, alternatively, that this material is sintered for strengthening and then completely rehydroxylated. To elucidate this point, a Nucleosil 5-100 silica sample was heated to 800°C for 24 h to produce a silica that had been heat-treated similarly to Type A silicas. As expected, BET data (Table II, silicas 4 and 21) revealed that this heating process did not significantly affect surface area, pore size and pore volume. Next, this dehydrated silica was boiled in water for 72 h, as suggested by Gobet and Kováts[4], dried, and silanized with TMS. This sintered and rehydrated material (Nucleosil, silica 22 in Table II) showed a drastic decrease in surface area (237 m^2/g compared to 430 m^2/g for starting silica 21, Table II) a small decrease in pore volume, and the formation of much larger pores. This effect is in strong contrast to the same treatment on the particular Type

TABLE III

RETENTION DATA FOR DIFFERENT TMS-MODIFIED SILICAS

Silica: type of silica, see Table I. Hyp = Hypersil C_1, Ads = Adsorbosphere-TMS, Ros = RSil-C3, Chr = Chromegabond-TMS, * = after wash with EDTA. Silylation: method of silylation: CA = with trimethylchlorosilane, argon-purge; CP = with trimethylchlorosilane, pyridine; EV = with trimethylsilylenolate, vacuum. %C: carbon percentage as determined by elemental analysis. Coverage: coverage of the silica surface with alkyl ligands in μmol/m^2. Solvent: A = methanol–water (80:20), B = methanol–water (70:30), C = methanol–water (60:40), k'_1: k' value for 1-phenylheptane, k'_2: k' value for N,N-diethylaniline; Ads = adsorption. α: selectivity: k'_1/k'_2. +, Data taken from ref. 1.

*Silica**	*Silylation*	*%C*	*Coverage*	*Solvent*	k'_1	k'_2	α
1 +	CA	2.36	1.55	C	1.14	Ads.	**
1 +	CP	4.50	3.11	C	15.89	3.69	4.31
1 +	EV	4.66	3.23	C	21.00	Ads.	**
1* +	CP	4.38	3.01	C	20.36	15.38	1.32
2 +	CP	1.73	3.17	C	4.27	15.04	0.28
3 +	CP	0.81	4.01	C	1.74	3.27	0.53
4 +	EV	5.35	3.66	C	16.18	2.39	6.77
4 +	CP	5.10	3.47	C	14.68	2.25	6.52
5 +	EV	1.27	3.94	C	2.09	0.77	2.71
6 +	EV	1.41	5.43	C	2.64	0.59	4.47
11	CP	4.55	3.92	C	12.92	12.51	1.03
12	CP	5.15	3.61	C	22.06	10.73	2.06
14	CP	4.78	3.94	C	23.67	27.57	0.86
15	CP	2.57	4.14	C	7.28	9.15	0.80
18	EV	5.22	3.77	C	28.26	11.51	2.46
19	EV	5.65	4.23	C	19.85	4.47	4.44
20	CP	4.91	3.63	C	9.31	2.94	3.17
22	CP	2.91	3.62	C	4.80	2.01	2.39
23	EV	4.98	4.13	C	22.25	Ads.	**
24	EV	5.00	3.53	B	6.62	Ads.	**
25	EV	2.98	4.52	C	9.55	5.28	1.81
26	EV	5.29	3.78	A	2.36	6.14	2.60
28	CP	0.80	3.96	C	2.27	0.54	4.20
29	EV	5.13	4.09	B	6.09	1.61	3.78
31	EV	5.44	–	B	5.86	1.67	3.51
32	CP	5.00	4.34	B	5.53	11.14	0.50
33	CP	5.11	3.94	B	5.46	Ads.	**
34	EV	4.55	4.01	B	5.38	1.30	4.14
34	EV	4.55	4.01	C	19.96	2.82	7.08
35	EV	5.05	4.01	B	5.92	2.20	2.69
36	EV	5.08	4.42	B	7.35	1.80	4.08
37	EV	3.44	3.78	B	3.29	1.10	3.00
38	EV	0.76	3.76	C	2.15	0.89	2.42
39	EV	2.87	3.78	C	2.50	0.82	3.05
40	EV	–	–	B	5.55	1.50	3.70
Hyp +	–	2.61	–	C	6.51	1.91	3.41
Ads +	–	1.99	–	C	6.07	1.77	3.43
Ros +	–	5.59	–	C	5.73	Ads.	**
Chr +	–	4.98	–	C	16.68	2.87	5.81

* Compare Table I.

** Not calculatable, very small.

TABLE IV

TGA DATA: TOTAL WEIGHT LOSS (%) UP TO 1200°C

Heated at 120°C to constant weight prior to analysis. Judgment about increase or decrease of SiOH concentration can only be made by taking BET data into consideration.

*Silica**	*Weight loss (%)*	*Comment*
1	2.1	Starting silica
2	0.5	Starting silica
3	0.3	Starting silica
4	3.7	Starting silica
11	3.2	Increase of SiOH conc.
12	2.2	No change of SiOH conc.
15	1.7	Increase of SiOH conc.
17	2.1	No change of SiOH conc.
18	2.1	No change of SiOH conc.
19	2.7	Increase of SiOH conc.
20	2.6	Increase of SiOH conc.
22	2.7	Increase of SiOH conc.
23	2.1	Increase of SiOH conc.
24	3.2	Increase in SiOH conc.
25	2.1	Increase of SiOH conc.
27	1.0	Increase of SiOH conc.
28	0.8	Increase of SiOH conc.
30	2.4	Increase of SiOH conc.
32	2.1	Increase of SiOH conc.
34	3.0	Increase of SiOH conc.
36	2.9	Increase of SiOH conc.
38	0.5	Increase of SiOH conc.

* Compare Table I.

A silica studied (Zorbax®), which showed no chromatographically significant change in surface area or pore structure (Table II, silicas 18 and 23). These data suggest that some of the walls between the pores of the dehydrated Type B Nucleosil dissolve and redeposit during the boiling-water rehydration process, resulting in larger pore diameters, but only small changes in pore volume.

Our previous study showed that purging a reversed-phase column with water at 50°C constitutes a stringent test of bonded-phase ligand stability, and that the trimethylsilyl group is especially useful as a model phase because of its susceptibility to hydrolysis under these conditions[1]. In hydrolysis–chromatographic experiments with dehydroxylated and rehydrated Type B Nucleosil, several differences were observed, compared to original material. The k' values for the test compounds were smaller, due to a lower surface area of the support after treatment. While N,N-DEA was not strongly adsorbed, the peak was unsymmetrical after initial injection, and its retention time with respect to the non-polar compounds was larger. This rehydrated packing lost its alkyl ligand very rapidly, relative to the original Type B-TMS sample, even though the initial coverage of the surface (3.62 μmol/m^2) was not significantly different.

Thus, it appears that an original Type B silica can be converted to a Type A material by sintering (dehydroxylation) and rehydration. However, it is important to note, that the pore structure of the Type B silica, Nucleosil, was strongly affected by a boiling-water rehydration process, suggestive of relatively poor mechanical stability of these particles.

Rehydration of Type A silica with boiling water

As noted in Tables I and II, the sintering of a Type A silica, Zorbax®-PSM-60, at 850°C for 3 days did not affect the surface area, the pore volume or the pore size of the silica (*cf.*, silicas 1 and 10). Attempts to rehydroxylate such heated samples completely by boiling in water as long as 100 h (silicas 12, 14, 18 and 23), similar to procedures proposed by Gobet and Kováts[4] for fumed silica, were unsuccessful (see Tables I–IV). TGA data indicated that a SiOH concentration level of about 6.5 μmol/m^2 could not be exceeded by this technique. In addition, stability of the bonded-phase alkyl ligand and the adsorptivity of the packing were not improved by this simple long-term boiling in water. For example, although silica 23 (Table I) had been rehydroxylated for a longer time than silica 18 (100 *vs.* 45 h), the latter actually exhibited less adsorption for N,N-DEA. This difference may have been due to the fact that silica 23 was filtered hot, while silica 18 was allowed to cool slowly to room temperature before filtering. This latter procedure may have led to a more homogeneous distribution of SiOH groups on the surface by a more even redeposition of silica.

In another experiment, Type A Zorbax® PSM-60 was dehydroxylated, rehydrated with nitric acid to a silanol concentration of *ca.* 5 μmol/m^2, and then boiled in water (15 g of silica in 1.5 l of deionized water) for 144 h. After cooling the suspension slowly to room temperature, the silica was filtered and dried (Table I, silica 19). The pH value of the rehydrated silica was increased to 5.47 from the original value of about 4.1. A negligible (*ca.* 5%) decrease in surface area was the only observable change in structural and mechanical properties. The TGA curve in Fig. 1 shows that the SiOH concentration for this rehydroxylated silica was increased to 7.0 μmol/m^2, compared to the original material that contained *ca.* 5 μmol/m^2 (*cf.* Table IV). No "buried" silanols could be detected in the silica by TGA.

The higher level of SiOH groups on this material was also confirmed by a silanization reaction. Table III shows that the coverage of the silica with TMS ligand (vacuum silanization with trimethylsilylenolate[1]) was increased to 4.23 μmol/m^2, compared to the 3.23 μmol/m^2 coverage on the original Type A Zorbax®. For this rehydroxylated Type A silica, 61% of the silanols were reacted with TMS, according to ^{29}Si-CP-MAS NMR. Fig. 2 shows a ^{29}Si NMR spectrum of this rehydroxylated material (Table I, silica 19) prior to silanization. The concentration of geminal groups [Si(OH)$_2$, peak 3, Fig. 2] on the rehydroxylated silica was slightly decreased, compared to the original silica.

Resolution of the three signals for silicon by ^{29}Si-CP-MAS NMR was significantly improved by saturating the sample with water vapor. Accuracy of the data is not affected by water vapor, because only silicon atoms are monitored. This is in contrast to most other techniques used in this study, where contamination with moisture leads to inaccurate results.

Different analytical methods showed the same level of SiOH groups on the

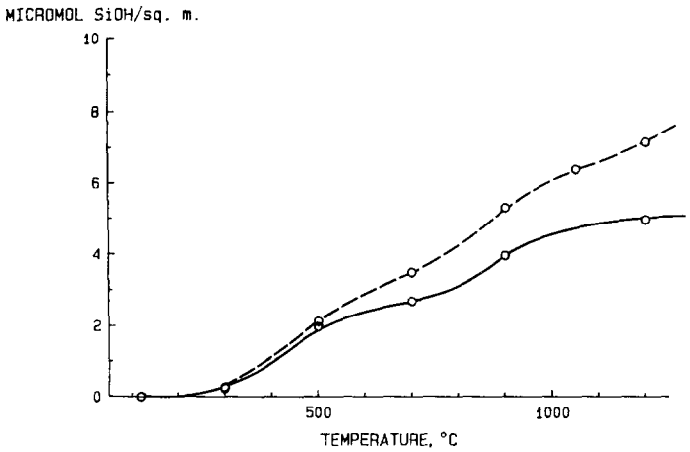

Fig. 1. Thermogravimetric analysis of silicas. Solid line: Type A silica (silica 1, Table I). Dashed line: Type A silica, rehydroxylated in boiling water for 6 days (silica 19, Table I).

100-h boiling-water hydrolyzed Type A sample. The overall concentration of SiOH groups on the surface of the boiling-water rehydrated silica (silica 19), was 6.9 μmol/m^2, calculated by using the surface coverage with TMS, determined by elemental analysis, and the percentage of reacted silanols, as measured by ^{29}Si NMR. This value checks well with the 7.0 μmol/m^2 value determined by TGA, and the 6.5 μmol/m^2 value determined by proton spin-counting NMR.

Fig. 3 shows the hydrolysis data for the 144-h boiling-water-hydrolyzed, TMS-modified Type A support, compared to data for the original TMS-modified material. The chemical stability of the TMS-ligand is improved, as judged by retention of the neutral test compound, 1-phenylhexane. More importantly, the retention time of the basic solute N,N-DEA does not increase with hydrolysis of the ligand,

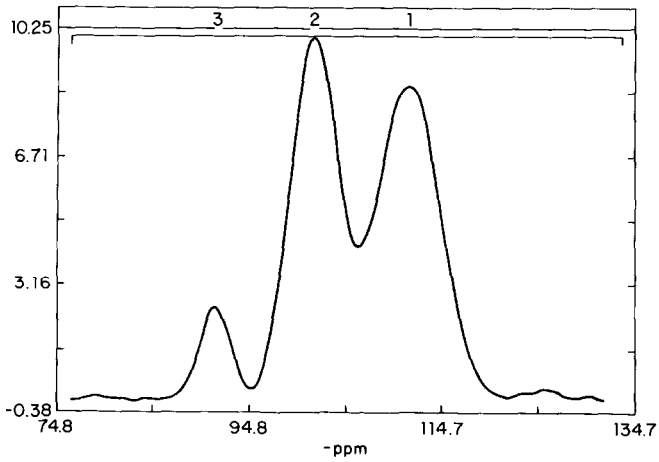

Fig. 2. ^{29} Si NMR spectrum of partly hydroxylated Type A silica, rehydrated in boiling water. Sample 19, Table I. Peaks: 1 = $Si(OSi)_4$; 2 = SiOH; 3 = $Si(OH)_2$.

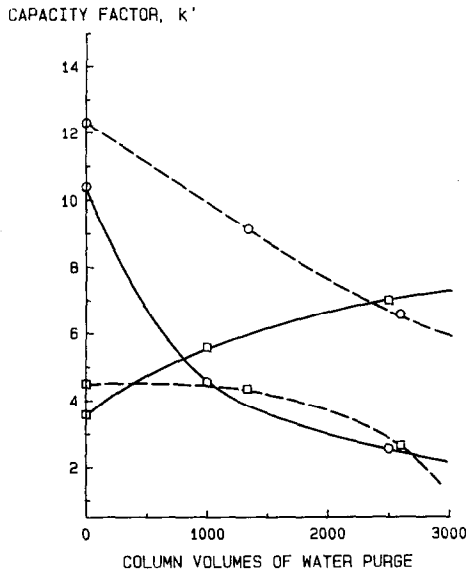

Fig. 3. Degradation of TMS-modified Type A silica rehydroxylated in boiling water. Silicas 1 and 19, Tables I–III. Conditions: column purge with water, 1.0 ml/min at 50°C; chromatographic test, 60% aq. methanol at 1.0 ml/min., 50°C; columns, 150 × 4.6 mm. Solid lines: (○) untreated Type A, 1-phenylhexane; (□) untreated Type A, N,N-diethylaniline. Dashed lines: (○) hydrolyzed type A, 1-phenylhexane; (□) hydrolyzed Type A, N,N-diethylaniline.

indicating that the surface of the base silica consisted mostly of associated or bonded SiOH groups, with few unbonded or isolated acidic SiOH groups. N,N-DEA also was eluted with a much better peak shape than from the packing prepared from the starting silica. With the packings used in this study the sterically-hindered basic 2,6-di-*tert.*-butylpyridine sample showed retention behavior essentially equivalent to a neutral compound.

Rehydration with aqueous base

Studies on the rehydroxylation of silica in boiling water suggested that dissolution and redeposition of silica is involved in the desired rehydration process. As indicated in the previous section, rehydration by simple boiling in water is very slow and often incomplete. Therefore, to increase the rehydroxylation rate, water was modified with certain basic activators. Rehydroxylation was attempted with (a) ammonia at a pH 9 at 100°C and atmospheric pressure (Table I, silica 11); (b) ammonia at a pH 9.3 in a closed glass vial at 100°C (silica 25); (c) ammonia at pH 10 and room temperature with a partly rehydroxylated Type A (silica 40); (d) calcium carbonate (silica 15); (e) ethylenediamine at pH 9 and 100°C (silica 39); and (f) tetrabutylammonium hydroxide with pH values of 8 and 9 (silicas 35 and 36). Results of these rehydration reactions are summarized in Tables I–IV.

All experiments in which bases were used for rehydroxylation led to an increase in SiOH concentration but also to at least modest changes in the physical properties of the silicas. Packings produced by vigorous treatment with ammonia or calcium carbonate also exhibited unfavorable chromatographic properties. Conversely, a

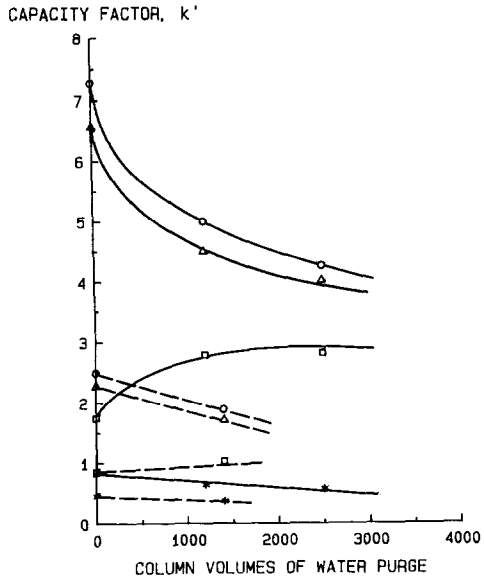

Fig. 4. Degradation of TMS-modified, rehydroxylated Type A silica. Solid lines: rehydroxylation with tetrabutylammonium hydroxide, silica 36, Tables I–III. Conditions same as Fig. 3, except water purge rate at 2.0 ml/min. Dashed lines: rehydroxylated with ethylenediamine, silica 39, Tables I–III. Conditions same as Fig. 3, except chromatographic testing with 70% aq. methanol. (○) 1-Phenylheptane; (△) 2,6-di-*tert.*-butylpyridine; (□) N,N-diethylaniline; (✳) 5-phenylpentanol.

gentle ammonia treatment at room temperature led to a desirable silica (silica 40, Table I). We postulate that although the SiOH concentration of the silica surface was increased by hydrolysis with certain basic activators, the resulting supports that exhibited poor chromatographic behavior also had a heterogeneous surface of SiOH groups.

In addition to the room-temperature ammonia reaction, tetrabutylammonium hydroxide (TBAH) and ethylenediamine (ED) treatments produced significantly improved packing materials with regard to level of SiOH groups and adsorption of organic bases. TBAH at pH 8 was insufficient to rehydrate the silica completely; however, this activator worked quite well at 100°C and a pH of 9. Fig. 4 describes the results of hydrolysis experiments with Silicas 36 (TBAH treatment) and 39 (ED treatment). In both cases the chemical stability of the bonded-phase packing was significantly improved and the unfavorable adsorption of N,N-DEA was measurably decreased, relative to starting silica (see Figs. 3 or 10).

Although quaternary alkylammonium bases aggressively dissolve silica, these compounds can be effective for surface rehydroxylation. This is the case even though tetramethylammonium, tetrapropylammonium and tetrabutylammonium hydroxide show equal or an even greater tendency than sodium hydroxide to attack the silica surface. Apparently, the reason for tetraalkylammonium hydroxides being effective activators is that, when used at the proper pH, very little of the free base remains in solution; most of it is adsorbed as a monolayer on the silica surface, making the silica somewhat hydrophobic[6]. In this case, there are ample hydroxyl ions to catalyze the making and breaking of siloxane groups, while the monolayer of activator on the

surface retards the dissolution and deposition of silica. Therefore, the process can be conveniently interrupted before the degree of hydroxylation of the surface passes beyond the optimum and the structure of the silica pores is seriously affected.

The ethylenediamine-treated silica 39 exhibited smaller k' values than silica 1 due to nearly 50% loss in surface area (Fig. 4); however, the former silica also showed excellent peak shapes for the basic test solutes. The significant surface area decrease suggests that the conditions of the treatment may have been somewhat too vigorous in this instance. In all cases the basic rehydroxylation conditions used did not adversely affect the strength of the particles. Columns were packed at 10 000 p.s.i. without particle fracture and the production of fines that would increase column back-pressure.

These studies suggest that rehydroxylation with certain basic activators creates a homogeneous, high concentration of highly bonded (associated) SiOH groups that result in superior chromatographic performance for basic compounds and significantly improved stability of bonded-phase column packings. It is important to note that, prior to silanization, all silicas were washed with nitric acid to insure that residual base was not simply deactivating acidic SiOH groups by surface adsorption of the basic agent.

Unsuccessful silica rehydroxylations

Rehydroxylation of calcined silicas were also attempted by other chemical reactions, and by steam treatment of a dehydroxylated Type A silica. It was apparent from DRIFT results that in the latter case considerable associated SiOH groups were transformed into isolated silanols. Based on a low pH value of the silica, DRIFT data, and strong chromatographic adsorptivity, we conclude that the homogeneity of the SiOH distribution actually was degraded (silica 24, Table I). Reactions with silicon tetrachloride followed by water hydrolysis (silica 33), and deposition of silicic acid from sodium silicate on the surface (silica 26), were also unsatisfactory. Although the concentration of surface SiOH groups was increased by these treatments, chromatographic properties were poor, probably also because of the relative inhomogeneity of the SiOH groups on the surface that left a relatively large population of unbonded (isolated) highly acidic sites.

We conclude that optimum chromatographic properties cannot be achieved by a simple increase in silanol concentration alone. It appears that homogeneous distribution of the SiOH groups is also necessary so that these groups can associate to produce the desired, less active surface.

Rehydroxylation with hydrofluoric acid

We have determined that hydrofluoric acid is also an effective agent for producing the desired surface on silica supports. We speculate that the fluoride ion at low pH values acts as a catalyst to dissolve silica as SiF_4. This species is immediately hydrolyzed to form silicic acid with regeneration of fluoride ion. The silicic acid then homogeneously precipitates on the support surface in a fully hydrated state with a maximum of homogeneous, associated SiOH groups.

In a series of reactions with hydrofluoric acid, 15 g of Type A silica was rehydroxylated in 150 ml of boiling water which contained 50, 100, 200, or 400 ppm hydrofluoric acid. Depending on the amount of hydrofluoric acid the pH value of

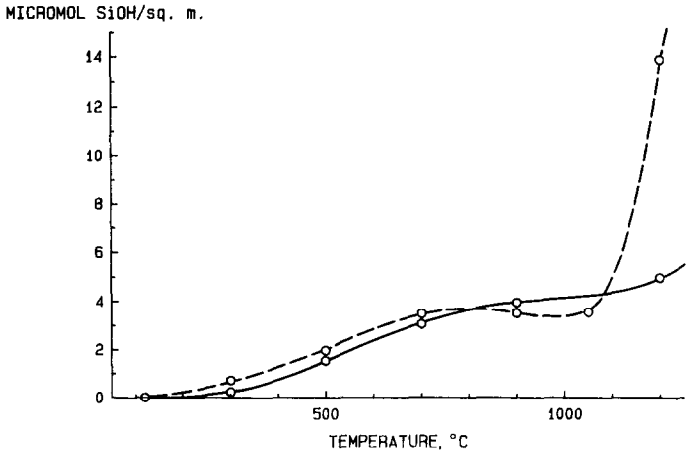

Fig. 5. Thermogravimetric analysis of silicas. Solid line: Type A silica (silica 1, Table I). Dashed line: Type A silica, hydroxylated with 100 ppm of hydrofluoric acid (silica 20, Table I).

the suspension varied between 2 and 4. Reaction times were 1–5 days. The results of these experiments are summarized in Tables I–IV. These data show that rehydroxylation with 50 ppm hydrofluoric acid (silica 32) was not completely effective. There was a slight increase in SiOH concentration on the silica surface, but chromatographic properties were poor, and the pH value of the silica remained relatively low (4.11). However, using 100 ppm hydrofluoric acid as activator in a 24-h reaction led to a support with a pH value of 5.26 (no washing of the product with ammonium hy-

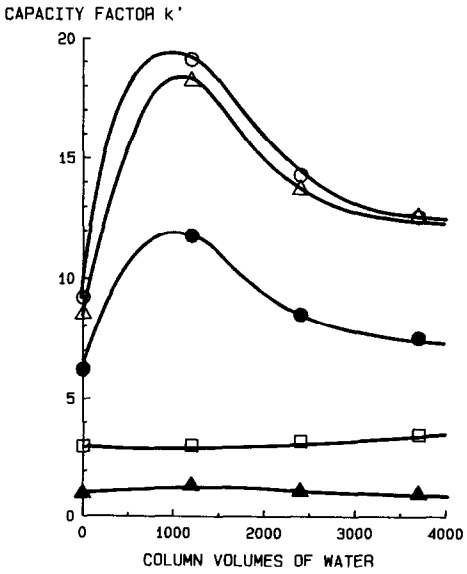

Fig. 6. Degradation of TMS-modified dehydroxylated Type A silica rehydroxylated with 100 ppm hydrofluoric acid. Silica 20, Tables I–III. Conditions same as in Fig. 3. (○) 1-Phenylheptane; (△) 2,6-di-*tert.*-butylpyridine; (●) 1-phenylhexane; (□) N,N-diethylaniline; (▲) 5-phenylpentanol.

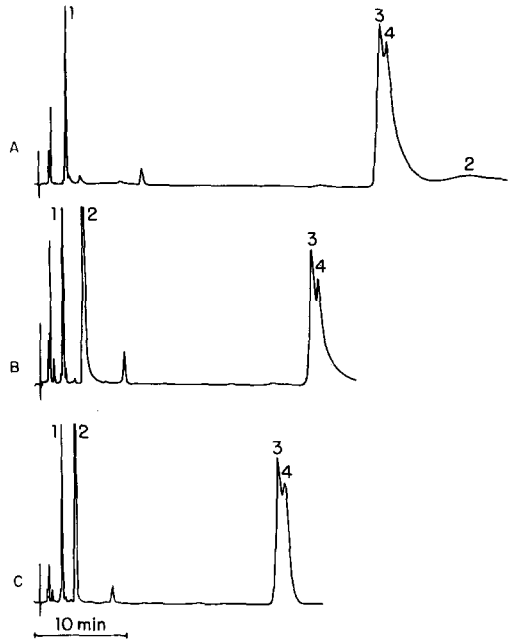

Fig. 7. Chromatographic testing of TMS-modified silicas. (A) Incompletely rehydroxylated Type A silica (silica 14, Tables I–III); (B) hydrofluoric acid-partially rehydroxylated Type A silica (silica 20); (C) Type B silica (silica 4). Conditions: columns, (A and B) 150 × 4.6 mm, (C) 150 × 4.4 mm; mobile phase, 60% aq. methanol; flow-rate, 1.0 ml/min; 50°C; pressure, (A and B) 60 bars, (C) 120 bars. Peaks: 1 = 5-phenylpentanol; 2 = N,N-diethylaniline; 3 = 2,6-di-*tert.*-butylpyridine; 4 = 1-phenylheptane.

droxide, silica 20). As Table II shows, the surface area of this sample actually was slightly decreased from 443 to 417 m^2/g by this treatment. The TGA results in Fig. 5 reveal that the SiOH concentration on the support surface was significantly increased by this rehydroxylation process, relative to the starting silica. The drastic weight loss above 1050°C in TGA suggests the formation of "buried" SiOH groups (Fig. 5). Interestingly, after several days of aging the silica at room temperature, a similar TGA showed a larger weight loss during the drying process at 120°C and smaller weight loss during melting. This result can be explained by assuming that the crosslinking or polymerization of initially present, low-molecular-weight silicic acid forms higher-molecular-weight silica gel with an appropriate release of water. Thus, such "buried" SiOH groups beneath the surface are apparently eliminated by this aging process.

Chromatographic testing confirmed the deactivation of silica by the hydrofluoric acid treatment, as indicated in Fig. 6 for the silanized silica 20 sample. This reversed-phase packing exhibited very low adsorptivity for organic bases. Curiously, we noted that the retention time for the non-polar test compounds actually initially *increased* during purging of this column. This result may be explained by the presence of "excess" unreacted silicic acid on the support surface that shows decreased retention for compounds mainly retained by hydrophobic interaction. Apparently, this "excess" silica acid is dissolved by the water purge; dissolution did not increase the

adsorption of bases. In addition, the chemical stability of the alkyl ligand on this packing was improved, relative to starting silica.

Thus, rehydration of the surface of Type A silicas with aqueous HF results in substantial improvement of chromatographic properties. Fig. 7 compares the chromatographic behavior of an incompletely rehydroxylated TMS-modified Type A silica (silica 14, Tables I–III) (Fig. 7A) and TMS-modified Type B silica ("enolate" reaction, silica 4) (Fig. 7C), with a hydrofluoric acid hydroxylated, TMS-Modified Type A silica from a preliminary study (silica 20) (Fig. 7B). Note that peak 2 for N,N-DEA in Fig. 7A is shifted from last in the chromatogram to a second position in Fig. 7B because of a greatly reduced interaction of this organic base with acidic silanol groups on the packing surface. The peak shape is markedly improved, and the chromatogram looks similar to that produced with silanized Type B silica (Fig. 7C).

Excess silicic acid should either not be initially deposited on the particles or be appropriately removed before column use, since this excess material may result in retention time increase after purging the column with water. To illustrate chromatographic effect of excess silica on the sample just discussed (silica 20, Table I), a heated silica sample of the same starting material was exposed to water containing 100 ppm of hydrofluoric acid in the same manner. However, in this case, prior to silanization, the sample was extensively washed with ammonium hydroxide at a pH of 9. Fig. 8 shows the results of subsequent hydrolysis experiments with this TMS-modified silica column. As expected, the chemical stability of the TMS ligand was measurably improved, compared to silica that had not been especially rehydroxylated (silica 1-CP,

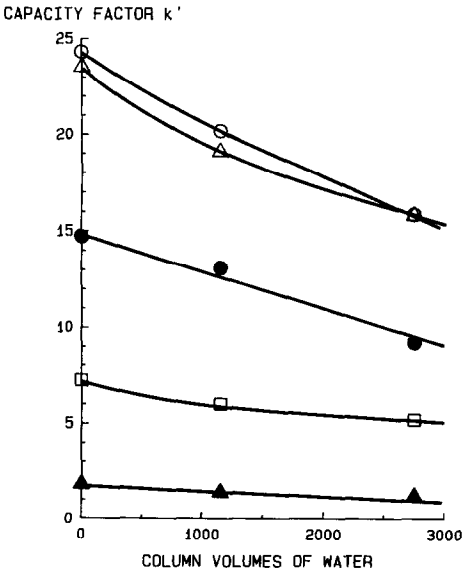

Fig. 8. Degradation of TMS-modified dehydroxylated Type A silica, rehydroxylated with 100 ppm hydrofluoric acid; ammonia wash of silica 20 (Table I) prior to bonding. Conditions same as in Fig. 3. (○) 1-Phenylheptane; (△) 2,6-di-*tert.*-butylpyridine; (●) 1-phenylhexane; (□) N,N-diethylaniline; (▲) 5-phenylpentanol.

Tables I–III). However, in addition, no increase in retention times for the non-polar solutes could be observed during the initial purging process. Also, the retention of N,N-DEA was not increased as a result of hydrolysis with the purge water. On the other hand, the adsorption properties of this silica sample were stronger than those of the sample without ammonium hydroxide washing (silica 20, Tables I–III), as documented by the higher k' values for N,N-DEA and by poorer peak symmetry.

Rehydroxylation reactions with an insufficient amount of hydrofluoric acid do not produce silica surfaces with the desired properties when incompletely hydroxylated silicas are used as starting materials. In experiments with silicas 29–31, a Type A silica which had been rehydrated to a silanol concentration of about 5 μmol/m^2 by aqueous nitric acid treatment was used as a starting material. Hydrolysis of this silica with 100 ppm hydrofluoric acid led to an increase in surface SiOH concentration; adsorption of organic bases on the TMS-modified surface also was significantly decreased. However, the homogeneity of the surface was not sufficient to produce small k' values and good peak shapes for N,N-DEA. An increase in hydrofluoric acid concentration to 200 ppm and reaction at 100°C for 3 days (followed by a rinse of the silica with ammonium hydroxide at a pH of 9) produced the best results, even when completely dehydroxylated (heated at 850°C) Type A silica was used (silica 34).

Optimum rehydration experiments produced silica with definitive characteristics. Rehydrations of a 850°C-heated, Type A silica (Zorbax® PSM-60), carried out with 200 ppm hydrofluoric acid, led to a *ca.* 25% decrease in surface area and a 15% decrease in pore volume (final pH 5.3). Fig. 9 shows chromatograms on this hydrofluoric acid-hydrolyzed material (silica 34) after silanization with TMS by the "enolate" method. The resulting packing exhibited very low column back-pressure, combined with low adsorptivity for bases. N,N-diethylaniline is eluted as an extremely sharp peak with a small k' value, indicating a very low level of interaction with acidic SiOH groups on the packing.

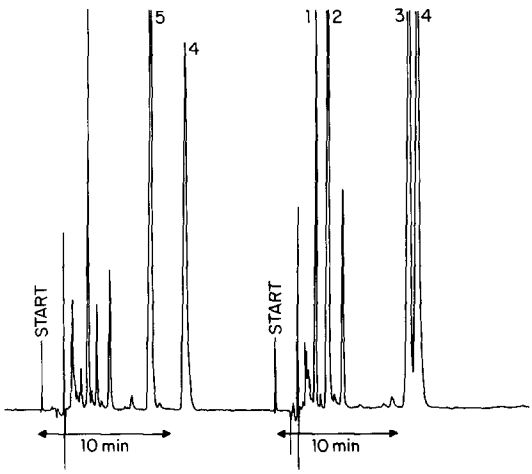

Fig. 9. Chromatogram with TMS-modified dehydroxylated Type A silica, rehydroxylated with 200 ppm of hydrofluoric acid. Silica 34, Tables I–III. Conditions: column, 150 × 4.6 mm; mobile phase, 70% aq. methanol; 50°C; flow-rate, 1.0 ml/min; 50 bars; 254 nm. Peaks: 1 = 5-phenylpentanol; 2 = N,N-diethylaniline; 3 = 2,6-di-*tert.*-butylpyridine; 4 = 1-phenylheptane; 5 = 1-phenylhexane.

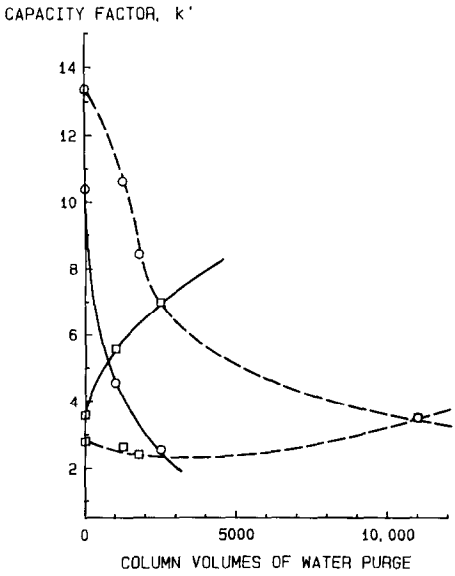

Fig. 10. Comparative degradation of TMS-modified silicas. Silicas 1-CP and 34, Table III; silica rehydroxylated with 200 ppm of hydrofluoric acid. Conditions and key same as in Fig. 3.

Proper rehydroxylation of the silica surface with HF also results in a markedly improved stability of bonded TMS groups. Fig. 10 shows the results of the hydrolysis experiments with a 200 ppm hydrofluoric acid-rehydroxylated Type A silica, silanized by the enolate reaction. This hydrofluoric acid-rehydrolyzed material exhibited a much greater stability than the TMS-modified starting material. Very small k' values for N,N-DEA were exhibited (no adsorption), and good peak shape was maintained, even after the column was purged with 11 000 column volumes of water.

More rigorous hydrolysis of Type A silicas with hydrofluoric acid produced a chromatographically desirable support, but significant changes in the silica pore structure resulted. Complete rehydroxylation of a Type A silica was achieved with 400 ppm hydrofluoric acid and a reaction time of 5 days; however, the surface area decreased from 443 to 272 m^2/g, and the average pore diameter was increased from 7.7 to 14.2 nm. TMS-modified packing from such a support exhibited short retention times for N,N-DEA with good peak shape, indicating no adsorption of this basic compound. Also, the resulting alkyl ligand was relatively stable. Changing the purge from water to 0.1 *M* disodium hydrogen phosphate buffer did not increase the hydrolysis rate of this packing.

Relation of DRIFT to chromatographic adsorption

Our data suggest that it is possible to predict with DRIFT results whether a non-silanized silica will show desirable chromatographic properties, based solely on information obtained from SiOH absorption. Fig. 11 shows IR spectra in the SiOH fundamental absorption region for a commercial Type A silica (Zorbax® PSM-60; starting silica 1), the same silica, rehydroxylated by boiling in water for 6 days (silica 19) and a Type B silica (Nucleosil 5-100). With reasonable assumptions of similar

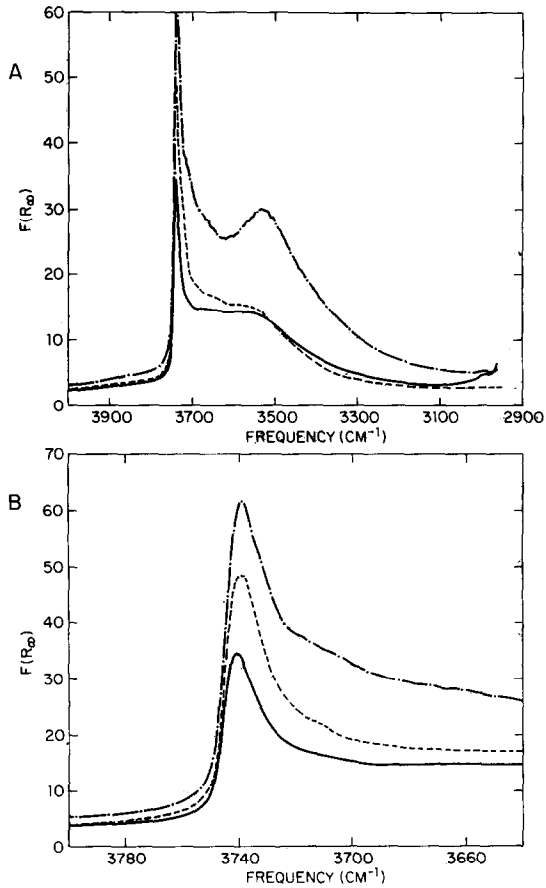

Fig. 11. DRIFT spectra of silicas. Samples: (——) Zorbax® PSM-60, starting silica 1; (– – –) Zorbax® PSM-60 rehydroxylated by boiling in water for 6 days, silica 19; (–.–) Nucleosil 5-100, silica 4 (A) SiOH fundamental absorption region; (B) expanded frequency range for isolated silanols.

packing densities and response factors for this DRIFT technique, these spectra strongly suggest that the Type A silica has the lowest and the Type B silica has the highest silanol concentration. (Note that any "buried" SiOH groups may be included in the DRIFT measurement.) Boiling in water clearly increased the SiOH concentration on the Type A silica (dashed line, Fig. 11A). Fig. 11B shows an expanded plot of the peak for the isolated (unbonded) silanols on these silicas. With increasing association of the SiOH groups (increased hydrogen bonding), the absorption peak for isolated SiOH groups is shifted to lower frequencies and is significantly broadened. Fig. 12 demonstrates the same effect for a Type A silica (silica 1, Table I), and the same sample rehydroxylated with 200 ppm hydrofluoric acid (silica 34).

However, an increase in SiOH concentration does not always lead to a lower frequency shift of the peak for isolated silanols. Fig. 13 demonstrates this effect for a highly adsorptive silica (silica 33, Table I) that was produced by reaction of a Type A silica with silicon tetrachloride, followed by subsequent hydrolysis with water. In

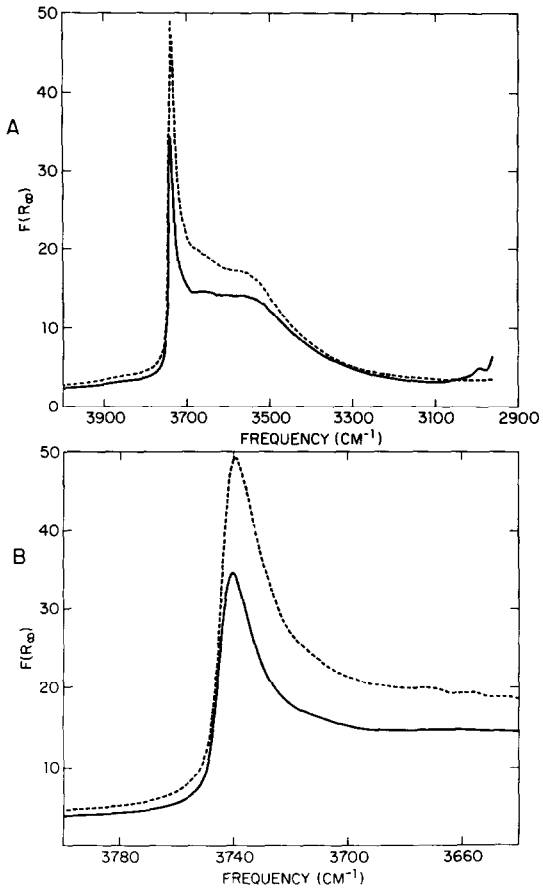

Fig. 12. DRIFT spectra of silicas. Samples: (——) Zorbax® PSM-60, silica 1; (---) Zorbax® PSM-60 rehydroxylated with 200 ppm of hydrofluoric acid, silica 34 (A) SiOH fundamental absorption region; (B) expanded frequency range for isolated silanols.

this sample, the number of SiOH groups on the surface was increased, but the DRIFT peak for the isolated silanols was not shifted to lower frequencies. This result suggests that it is not the intensity of the adsorption peak for isolated or associated silanols that can be used to evaluate their chromatographic behavior. Rather, it is necessary to measure carefully the *frequency* of the peak maximum as an indication of the activity of the isolated silanols. Results with DRIFT on silica samples, heated at increasing temperatures, substantiate this interpretation[1]. A shift to higher frequencies (fewer bonded and more isolated SiOH groups) was observed with increasing heating temperature. This increase in unbonded or isolated SiOH groups can be correlated directly with increased chromatographic adsorption of basic organic compounds.

A close correlation of DRIFT data with chromatographic adsorptivity can be observed, as illustrated in Table V. This correlation is apparent, even though the range between strong (S), moderate (M), and no (N) adsorption of N,N-DEA is only six wavenumbers and the precision of the IR measurement is ±1 wavenumber.

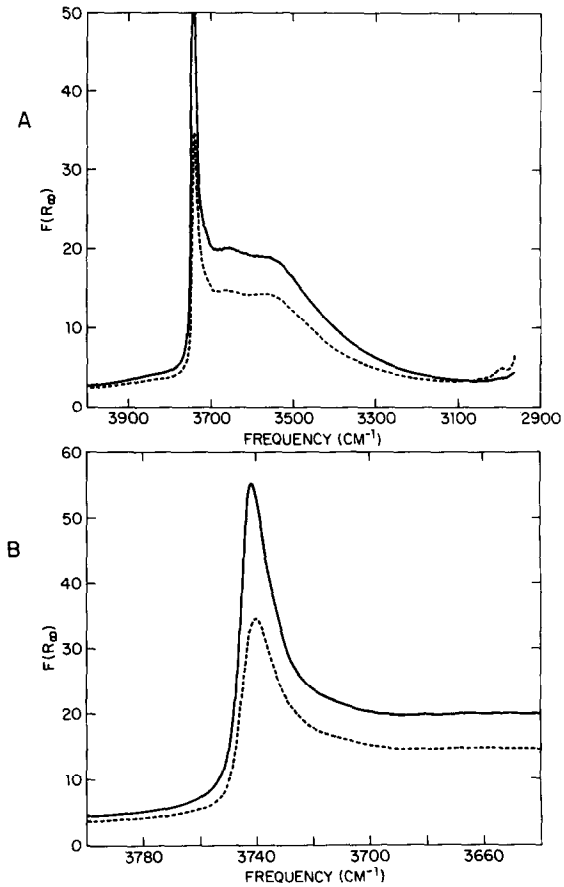

Fig. 13. DRIFT spectra of reacted Type A silicas. Samples: (---) Zorbax® PSM-60, starting silica 1; (——) Zorbax® PSM-60 after reaction with silicon tetrachloride, rehydroxylated with water, silica 33 (A) SiOH fundamental absorption region; (B) expanded frequency range for isolated silanols.

Strongly adsorbing silicas have peak maxima for their unbonded or isolated silanols at 3740 cm^{-1} and higher; moderately or non-adsorbing silicas show a maximum at a lower frequency, between 3740 and 3737 cm^{-1}. The DRIFT spectra strongly project that there is a virtual continuum of SiOH on the surface of silica between the limits of completely hydrogen bonded or associated SiOH groups and essentially unbonded or isolated SiOH groups.

Our studies indicate that the method of silica preparation, thermal history, and perhaps purity in regard to certain surface impurities (*e.g.*, alumina), all can contribute to the degree of desirable association that can occur between SiOH groups on silica surfaces (see ref. 1). Our studies further suggest that the desired surface concentration of about 8 $\mu mol/m^2$ SiOH must be arranged in a relatively homogeneous pattern to allow essentially all of these groups to associate strongly. Desirable silica supports for chromatography apparently have only a few unbonded SiOH groups for undesirable adsorptive interaction.

TABLE V

DRIFT DATA RELATED TO CHROMATOGRAPHIC ADSORPTIVITY

*Silica**	*Wave number of isolated SiOH*	*Adsorptivity***
1	3741	S
4	3738	N
19	3739	N
24	3742	S
27	3741	–
31	3740	M
32	3741	S
33	3743	S
34	3738	N
35	3742	M
36	3739	N
37	3737	N
38	3738	N
39	3739	N

* Compare Table I.
** S = strong, M = moderate, N = none.

Rehydration of large-pore-size silica

Rehydration of wide-pore silica can be carried out in the same manner as described above for small-pore silicas. A commercial 300-Å Type A silica with a surface area of 56 m^2/g (silica 3, Table I) was sintered at 850°C, and then hydroxylated by 75 ppm (silica 28) and 400 ppm hydrofluoric acid (silica 38) reactions. The results showed that the 75-ppm-hydrofluoric acid treatment led to a satisfactory rehydroxylation of this material. According to TGA data, the 75-ppm-hydrofluoric acid treatment approximately doubled the observable weight loss (see Table IV), compared to the starting silica. Note, however, that TGA measurements are not very precise for lower-surface-area silicas, due to the small weight losses that occur during heating. This successful rehydroxylation was further documented by the good chemical stability of the alkyl ligand exhibited during hydrolysis, and by very low k' values and good peak shapes for N,N-DEA. Fig. 14 compares the separation of a test mixture on a TMS-modified commercial Type A (300-Å) material with that of the 75-ppm-hydrofluoric acid-hydrolyzed and TMS-modified silica 28. Not only is the retention time of the bases decreased and the peak shape improved by the hydrofluoric acid treatment, but the strength of the supported particle was even further improved, as suggested by the lower column back-pressure for the hydrofluoric acid-hydrolyzed silica.

Particle strength

The physical strength of small particles used in HPLC is especially important because of the high pressures and flow-rates used in the loading of these particles into columns. Weak particles usually produce columns with relatively high back pressures because of "fines" created during the packing process. Chromatographic use and physical tests have previously shown that Zorbax® porous silica microspheres

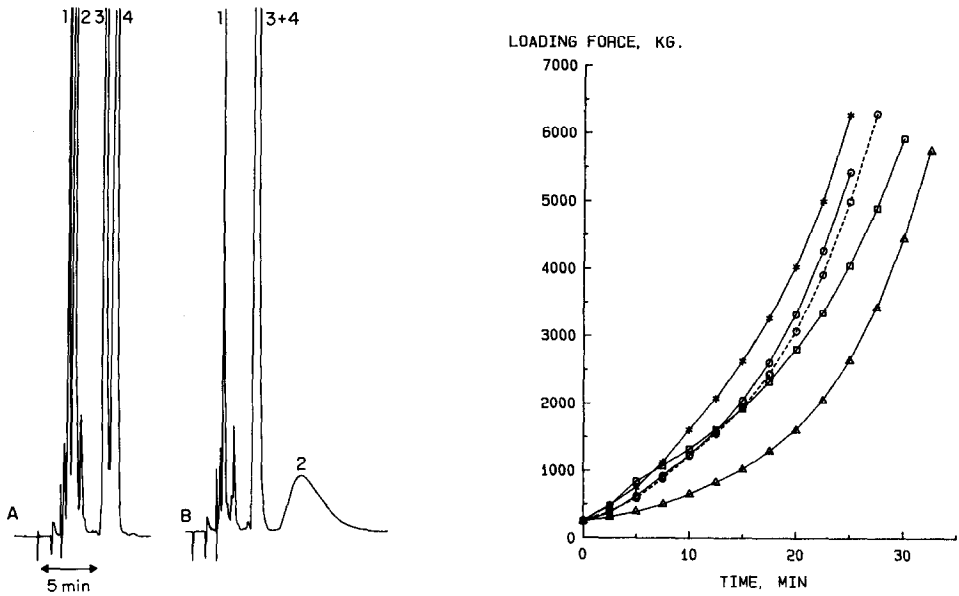

Fig. 14. Chromatographic comparison of TMS-modified wide-pore silicas. Columns, 150 × 4.6 mm; mobile phase, 60% aq. methanol; 50°C; flow-rate, 1.0 ml/min; 254 nm. (A) Zorbax® PSM-300, dehydroxylated and rehydroxylated with 75 ppm of hydrochloric acid, silica 28, Tables I–III; 50 bars. (B) Zorbax® PSM-300; 80 bars. Peaks: 1 = 5-phenylpentanol; 2 = N,N-diethylaniline; 3 = 2,6-di-*tert.*-butylpyridine; 4 = 1-phenylheptane.

Fig. 15. Crush-strength test for porous silicas. 0.5-in. diameter plunger; loading rate 0.01 in./min; sample, 1.0 g. (○) Zorbax PSM-60; (□) Nucleosil 5-100; (△) Vydac 5-80; (*) Zorbax PSM-60 (rehydroxylated); (—○—) Zorbax PSM-60 (repeat).

(E. I. du Pont de Nemours & Company, Wilmington, DE, U.S.A.) consist of very strong silica particles. We have found that Zorbax®, rehydrolyzed by the methods described above, has even greater resistance to crushing than commercial Zorbax® particles. A definitive comparison of various chromatographic silica with 300-Å pores has been demonstrated by a pressure-strength or crushing test. For these tests, samples of the porous silica were loaded into a stainless-steel die with a 0.5 in. diameter plunger. This device is normally used for preparing potassium bromide disks for infrared spectroscopy studies by high-pressure loading. All samples were initially compacted (or pre-loaded) to a firm homogeneous bed with a load of 250 kg, and then continuously loaded to a total of about 6000 kg. The data in Fig. 15 differentiates the crushing strength of silica particles by this pressure-strength test. In this presentation a steep curve represents the ability of stronger particles to accept the pressure load readily; the pressure increases rapidly as crush-resistant particles are loaded. Conversely, a more shallow curve indicates that the particles are crushed more easily; the pressure increases more slowly as particles crumble under the load. The data in this curve show that Zorbax® PSM-60 rehydrolyzed with 200 ppm hydrofluoric acid, showed the highest crush-resistance of all measured. Duplicate tests on commercial Zorbax® PSM-60 produced essentially the same data, even though several days elapsed between these tests, indicating the analytical reproducibility.

We believe that the improved crush resistance of the rehydroxylated Zorbax® PSM-60 is based on the fact that, during the hydrolysis reaction, silica is dissolved and reprecipitated at the points of contact of the colloidal particles making up the aggregate structure. Thus, this fully hydrolyzed, reprecipitated silica further binds the colloidal particles together within the aggregate structure, increasing the strength of the aggregate macroparticle.

Additional data on the strength of wide-pore silica particles was obtained by a test involving stress in an ultrasonic bath. In these studies, 200 mg of Nucleosil 5-300 (5 μm, 300-Å pores, Machery Nagel, F.R.G.), Vydac TP-300 (5 μm, 300-Å pores, Separations Group, Hesperia, CA, U.S.A.), Zorbax® PSM-300 (5 μm, 300-Å pores, Du Pont) and Zorbax® PSM-300, rehydrolyzed with 75 ppm of hydrofluoric acid, each were suspended in a vial containing 15 ml of water and sonicated for 4 h in a small ultrasonic bath. The suspensions of these wide-pore particles were allowed to settle; "fines" produced by this treatment remained as a "mist" in the liquid. The largest number of "fines" were observed for Nucleosil. The hydrolyzed Zorbax® sample exhibited the smallest amount of "fines", suggesting the highest strength in this test.

Separation of biomolecules

Silica-based reversed-phase packings with 30-nm pores and low surface adsorptivity are especially desirable for separating many biomolecules. Fig. 16 compares the separation of a mixture containing the basic peptide, mellitin (MW = 2600) on a commercial C_8 column specifically designed for peptides (Fig. 16B), with a completely hydroxylated TMS-modified Zorbax® PSM-300 column (Fig. 16A) (silica 28, Tables I–III). The data show that all of the components in the mixture were totally adsorbed on the commercial column during gradient chromatography; no peaks were

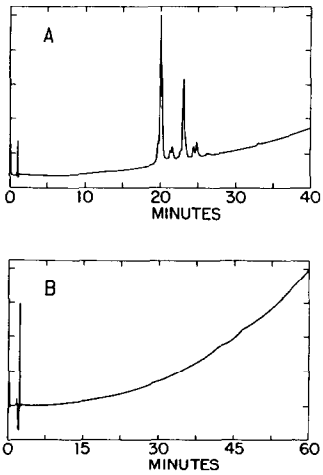

Fig. 16. Chromatographic comparison of wide-pore silicas. Sample, peptide mixture containing mellitin (MW 2600, 26 amino acids). (A) TMS-Modified Type A silica, rehydroxylated with 75 ppm of hydrofluoric acid (silica 28, Tables I–III); (B) C_8 peptide column. Columns, 150 × 4.6 mm; gradient, 20% acetonitrile and 0.1% trifluoroacetic acid to 100% acetonitrile and 0.1% trifluoroacetic acid in 60 min; flow-rate, 1.0 ml/min; 220 nm; 35°C.

noted even when 100% organic solvent was used as the eluent. On the other hand, a successful separation was obtained with the fully hydroxylated silica packing. These results are entirely in keeping with our observations regarding the level and homogeneity of SiOH groups on a silica surface, since we determined that the commercial peptide column is based on a silica that is not fully hydroxylated. A companion paper reports on the desirable properties of reversed-phase packings made from fully hydroxylated, wide-pore silica for the HPLC of proteins[5].

After several days of use with the pH 2 mobile phase containing trifluoroacetic acid, the Zorbax® PSM-300, hydrofluoric acid hydrolyzed, TMS-modified column was retested with the polarity mixture and exhibited retention strikingly different from those initial tests. The non-polar solutes of the polarity test mixture were eluted near t_0, and N,N-DEA was eluted as a relatively broad peak. Subsequent elemental analysis showed that only about 50% of the TMS ligand remained on the support. Curiously, however, the basic peptide, mellitin, and basic proteins, such as lysozyme and ovalbumin were still eluted normally without significant loss in column efficiency. We are now intensively investigating this surprising observation[5].

Two important conclusions have been drawn from this part of the study: (1) N,N-diethylaniline is an effective and sensitive test sample for the measurement of adsorption on silica supports. Apparently, this compound is an even more sensitive probe than basic proteins, because it is smaller and sterically more able to interact with surface SiOH groups that are partially shielded by alkyl ligands; (2) proteins should be chromatographed on reversed-phase packings that are based on fully rehydroxylated silicas with low adsorption for small basic compounds (*e.g.*, N,N-DEA). However, some as yet unidentified concentration of bonded-phase ligand apparently is desired to modify the few residual, deleterious, acidic SiOH groups that remain even on fully hydroxylated silica surfaces.

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