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High pH mobile phase effects on silica-based reversed-phase high-performance liquid chromatographic columns

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Abstract

Aqueous mobile phases above pH 8 often cause premature column failure, limiting the utility of silica-based columns for applications requiring high pH. Previous studies suggest that covalently bound silane ligands are hydrolyzed and removed by high-pH mobile phases. However, we found that the siloxane bonds for certain monomeric silanes are hydrolyzed very slowly from silica supports at pH 9–10. Therefore, bonded-phase packing degradation at high pH is a result mainly of silica support dissolution. The rate of column degradation for C_{18} columns is influenced not only by the type and purity of silica support, but also by the nature of the silane stationary phase. We found different rates of degradation for several commercial C_{18} columns. The relative rates of silica dissolution for these packings were determined by chemically measuring the silicate formed during column purging at high pH. The type and concentration of mobile phase organic modifier also significantly influences column degradation at high pH. Certain silica-based C_{18} packings can be used for long periods at pH 9 without significant changes in chromatographic properties. Results of this study better define the practical utility and limitations of silica-based columns in high pH environments.

1. Introduction

Based mainly on manufacturers recommendations, most workers do not attempt reversed-phase separations on silica-based bonded-phase columns with mobile phases above pH 8. This practice apparently is based on early studies with columns of certain silica supports [1,2]. These early studies showed that the rate of column degradation was dependent on the type of base and the concentration of organic modifier used in the mobile phase. Other workers also found that the rate of silica dissolution for untreated silica at pH 9–10 was reduced in high concentrations of organic modifier, especially when ammonia was the source of hydroxyl ions [3,4]. Another study showed that dissolution of unmodified chromatographic silica was affected by the type and concentration of salts in the mobile phase [5]. Vigorous treatment of unmodified chromatographic silicas with certain bases also caused significant changes in surface areas and pore structure [6]. More recently, work with a specific untreated chromatographic silica showed that this material could be used for extended periods with pH 9.2 methanol–buffer mobile phase for good results, provided the column inlet was repacked when performance decreased [7].

The study herein reported provides additional insight regarding the utility of silica-based bond-

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ed-phase column packings in high-pH aqueous mobile phases. Although basic compounds often are separated at low pH [8,9] or with ion-pairing agents [9], there are instances when reversed-phase operation at high pH is preferred. Previous studies have not defined how differences in the silica support and different stationary phases influence column stabilities at high pH. Also, the reasons for the degradation of silica-based, bonded-phase columns under various operating conditions were not verified. Therefore, a wider range of information was needed to better define the potential utility of silica-based column packings for reversed-phase HPLC.

2. Experimental

2.1. Chromatographic columns

All 15 × 0.46 cm I.D. Zorbax columns were prepared by Rockland Technologies. Comparable Zorbax SB-C_8 (diisopropyl-C_8), Zorbax Rx-C_{18} (dimethyl-C_{18}), Zorbax SB-C_{18} (diisobutyl-C_{18}), and Zorbax-ODS (dimethyl-C_{18}) columns are available from Mac-Mod Analytical (Chadds Ford, PA, USA). The 15 × 0.39 cm I.D. Novapak C_{18} column was from Waters Assoc. (part No. 86344; lot No. T-32451; Milford, MA, USA). 15 × 0.46 cm I.D. Hypersil ODS (cat. No. 9875; SN 93091196) and Nucleosil C_{18} (cat. No. 89161; SN 93091195) columns were obtained from Alltech (Deerfield, IL, USA). LiChrosorb C_{18} columns were packed at Eindhoven Technical University using packing obtained from Merck (Darmstadt, Germany). Typical physical characteristics of the silicas used in these columns are summarized in Table 1. In Table 1, Type A refers to more acidic, less purified silica supports, while Types B are the less acidic, highly purified silicas [6].

The purity of the silica supports used for the alkyl-bonded phase columns tested is summarized in Table 2. Absolute silica purity suggested by these results may not be totally comparable, since analyses may have been conducted by methods with different levels of detectability for the various elements.

2.2. Silica support solubility study

Apparatus and reagents

Columns were purged with a Model 100A pump (Beckman, Fullerton, CA, USA). Eluent fractions were collected with a Waters P/N 37040 fraction collector (Waters, Milford, MA, USA).

Table 1
Typical physical properties of silica supports for C_{18} columns studied

Column name	Silica type[a]	Pore size (nm)	Surface area (m^2/g)	% Volume porosity[b] (ml/ml)	Ref.
Hypersil ODS	A	12	170	57	10
LiChrosorb C_{18}	A	10	355	71	11
Novapak C_{18}	A/B	6	N/A[c]	N/A	12
Nucleosil C_{18}	B	10	350	69	10
Zorbax-ODS	A	6	300	55	10
Zorbax Rx-C_{18}	B	8	180	50	13
Zorbax SB-C_8, C_{18}	B	8	180	50	13

[a] Based on data in Refs. [9,13].
[b] Calculated as in Ref. [14].
[c] N/A = not available.

Table 2
Typical impurity levels in silica supports

Silica	Na	K	Mg	Al	Ca	Ti	Fe	Zr	Cu	Cr	Zn	Ref.
Hypersil	2900	N/A	40	300	38	65	230	N/A	N/A	N/A	N/A	27
Nucleosil	56	N/A	N/A	nd	130	57	76	nd	N/A	N/A	nd	16
Zorbax-SIL	17	nd	nd	57	9	32	21	88	< 1	nd	88	15
Zorbax Rx-SIL	10	< 3	4	1.5	2	nd	3	nd	nd	nd	1	13,15

Metal concentration, ppm. Data for impurity levels in LiChrosorb and Novapak were not available. N/A = not available. nd = Not detected by the analytical method used.

Absorbance measurements were with a Zeiss MM 12 UV–Vis spectrophotometer (Carl Zeiss, Oberkocken/Württemberg, Germany). All chemicals and solvents were of analytical grade from Merck. Silicate standard solutions also were from Merck. Buffers and reagent solutions were prepared from deionized water from a MILLI-Q purification system (Millipore, Bedford, MA, USA): eluent I: methanol–0.1 *M* sodium carbonate/bicarbonate buffer, pH 10.0 (50:50, v/v) (overall buffer concentration 0.05 *M*); eluent II: acetonitrile–0.084 *M* sodium carbonate/bicarbonate buffer pH 10.0 (40.6:59.4, v/v) (overall buffer concentration 0.05 *M*).

Different concentrations of methanol and acetonitrile were used in these tests to maintain equal-strength mobile phases for the tests —acetonitrile is a stronger modifier [9].

Procedures

Columns were continuously purged at 1.0 ml/min with eluents I or II. To maintain an equivalent linear velocity, a flow-rate of 0.72 ml/min was used for the NovaPak column. To simulate usual chromatographic practice, mobile phases were *not* recycled. Tests were at ambient temperature (about 22°C). All columns were flushed for 10 min with a mixture of methanol–water (1:1) prior to the dissolution experiments. After a specific dissolution experiment was begun, the column effluent was sampled after 1 h. After that, the column effluent was sampled every 8–10 h using a fraction collector. Column effluent samples for silicate analysis were collected for a 5-min period (total: 5 ml; for the Novapak column: 3.6 ml).

We measured silica concentrations colorimetrically in collected fractions using the well-known silicomolybdate complex method [18]. Absorbance was measured at 410 nm in 1-cm cuvets. For the silica measurement, standard silicate mixtures were prepared in the concentration range of 1.0 to 20.0 mg Si/l. These standard mixtures were prepared in the corresponding buffer–modifier purge solutions used in the dissolution studies. Absorbance values were measured using blank solutions as reference. Results were plotted of the silica concentration in the column effluent as a function of the volume of effluent. The total silica dissolved from the column was first determined by using the silica average of two consecutive fractions. Then, the corresponding intermediate eluent volume was calculated. By multiplying these values and summing the mass of silica over the total effluent volume, cumulative plots then were obtained which represented the mass of silica which had been removed as a function of eluent volume flushed through the column.

2.3. Chromatographic column degradation studies

Apparatus and reagents

Analytical-grade methanol, sodium hydroxide and Na_2HPO_4 were from Baker (Phillipsburg, NJ, USA). EM Science (Gibbstown, NJ, USA) supplied HPLC-grade methanol and acetonitrile for chromatographic measurements. Test solutes were from Chem Service (West Chester, PA,

USA). Column purging ("ageing") studies at pH 9 were performed with a Shimadzu Model LC-600 pump. Column testing for the pH 9 study was with a DuPont Model 860 pump and Model 860 UV absorbance detector. Column ageing and testing at pH 12.3 used a Hewlett-Packard Model 1050 pump/detector system (Wilmington, DE, USA). Chromatographic samples were injected with a Rheodyne Model 7125 sampling valve (Cotati, CA, USA).

Column ageing procedures

For the pH 9 study, methanol–0.01 *M* phosphate solution (60:40) or acetonitrile–0.01 *M* phosphate solution (50:50) was continuously pumped through each column at a flow-rate of 1.0 ml/min at ambient temperature (ca. 22°C). As in the silica solubility study, to simulate actual chromatographic usage, the mobile phase purge was not recycled. The different organic modifier concentrations in both the silica support dissolution and column ageing studies were used to maintain solvent strength approximately constant, according to published solvent strength relationships [9]. The phosphate solution was made by adjusting 0.01 *M* Na_2HPO_4 to pH 9.0 with 4 *M* sodium hydroxide solution. (Note: phosphate solutions do not buffer strongly at pH 9.) Periodically during this purging routine, the columns were flushed with at least 20 column volumes of methanol–water (60:40), and 5 μl of a test solution chromatographed. This test mixture consisted of 0.02, 0.10, 0.033, 0.033, 0.20, 0.20 mg/ml each of uracil (t_0 marker), benzamide, 4-bromoacetanilide, N,N′-dimethylaniline, naphthalene, and N,N′-diethylaniline, respectively, in methanol–water (50:50).

For pH 12.3 tests, methanol–0.02 *M* sodium hydroxide (50:50) was continuously purged through a column of dimethyl-C_{18} (Zorbax Rx-C_{18}) at ambient temperature (ca. 22°C). Periodically the column was injected with 5 μl of a test solution consisting of 0.05, 0.5, 0.01, and 1.0 mg/ml each of uracil (t_0 marker), phenol, N,N′-dimethylaniline, and toluene, respectively, in methanol–water (50:50).

3. Results and discussion

Previous studies have shown that silica-based columns at high pH resulted in eventual deterioration of the silica support [1–5]. However, the rate and extent of degradation appeared to vary, depending on the silica, mobile phase and other conditions. Our interest was to better define the factors controlling deterioration of the silica support for bonded-phase packings at high pH. With more information, it was anticipated that a wider utility of silica-based packings in higher pH environments might be feasible.

We used two different experimental approaches to obtain information on the stability of silica-based bonded-phase packings. First, columns of various packings were continuously purged with pH 10 aqueous–organic mobile phases. The resulting dissolved silicate was measured with the well-known molybdate color reaction. Second, columns were continuously purged with organic-modified pH 9.0 and 12.3 mobile phases, and packed-bed stability and change in bonded stationary phase measured chromatographically. In both approaches, we found that the mobile phase organic modifier, silica support type, and the type of silane bonded phase all can affect the rate of silica support degradation.

3.1. Silica support solubility studies

The repeatability of experiments to measure the rate of silica dissolution was tested by performing duplicate experiments with columns of a monomeric dimethyl-C_{18} bonded-phase on a highly purified silica support (Zorbax Rx-C_{18}). Two columns each were tested with both methanol- and acetonitrile-modified pH 10 mobile phases. The pH 10 environment was chosen to ensure sufficient solubility of the silica supports for precise and meaningful measurements of silica in the collected fractions. Fig. 1 shows the amount of silica dissolved from these four different columns as a function of the volume of eluted mobile phase. These results confirm that

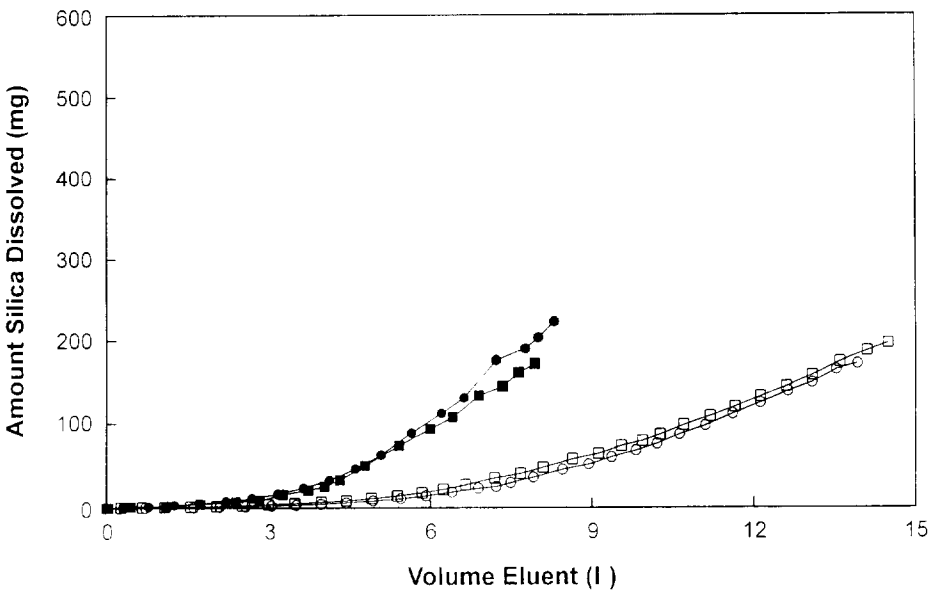

Fig. 1. Reproducibility of tests for silica support dissolution. Columns (A = ○; B = □): 15 × 0.46 cm I.D. Zorbax Rx-C_{18} (dimethyl-C_{18}); duplicate tests with mobile phases: methanol–sodium carbonate/bicarbonate (0.1 *M*) buffer pH 10.0 (50:50, v/v) (solid symbols) and acetonitrile–sodium carbonate/bicarbonate buffer (0.084 *M*) buffer pH 10.0 (40.6:59.4, v/v) (open symbols); flow-rate: 1.0 ml/min; ambient temperature; molybdate colorimetric analysis for dissolved silicate.

the test and analytical measurements method are repeatable. All four experiments were concluded when the columns clogged and exhibited very high back pressures. The data in Fig. 1 further demonstrate that silica support solubility can be considerably higher in methanol compared to acetonitrile.

However, for a sterically protected silane stationary phase with bulky side groups, a different silica solubility pattern occurred. Fig. 2 shows that two columns of a highly purified silica support with a monomeric diisopropyl-C_8 stationary phase gave essentially the same solubility pattern for methanol and acetonitrile mobile phase modifiers. Unfortunately, tests had to be discontinued after about 5.6 l of acetonitrile–buffer purge, since the column bed clogged. (The resultant high column bed back pressure might reflect the precipitation of sodium silicate because of the lower solubility in acetonitrile, compared to methanol modifier). For methanol- or acetonitrile-modified mobile phases, the solubility of the diisopropyl C_8-modified silica was significantly higher than that for the same silica modified with dimethyl-C_{18} groups, shown on Fig. 2 for the two solvents. From this, one might conclude that the type of stationary phase can influence the solubility of a silica support and the ultimate stability of a column at higher pH.

Fig. 3 shows the silica dissolution found for several representative commercial C_{18} columns when continuously purged with a methanol–pH 10 buffer (50:50) mixture. Nucleosil C_{18} showed the highest rate of silica dissolved. This silica previously was shown to be weaker than some other silicas tested in column packing and crush-test studies [6,19]. The silica from LiChrosorb C_{18} also dissolved quickly, but at a somewhat slower rate than Nucleosil. Silicas from Novapak C_{18}, Zorbax Rx-C_{18}, Zorbax-ODS and Hypersil C_{18} dissolved more slowly, with the latter showing the slowest rate of those columns tested. Again, all experiments were terminated when columns clogged and exhibited very high back pressures.

Three factors probably dictate the differences

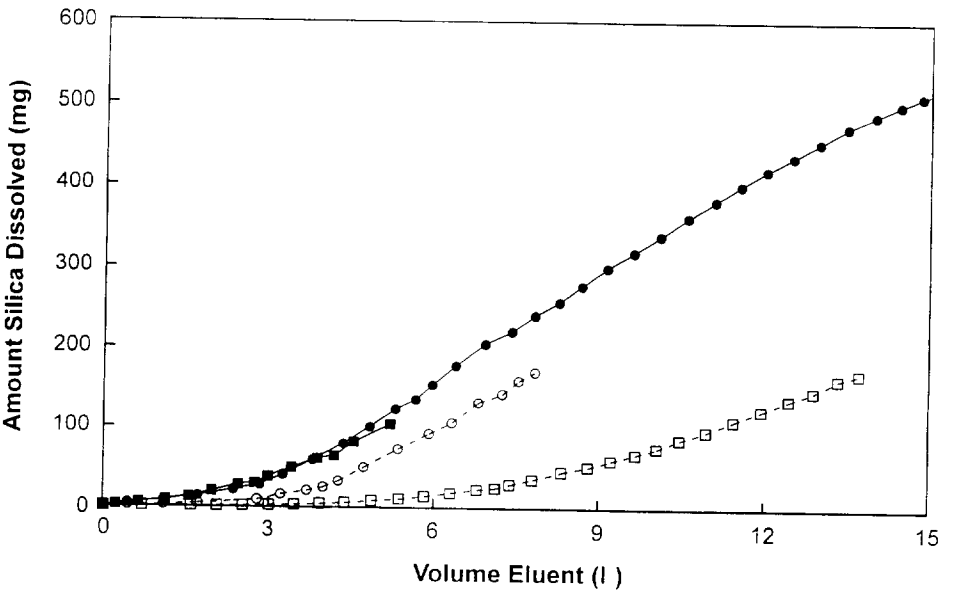

Fig. 2. Silica support dissolution for diisopropyl-C_8 column. Columns: 15 × 0.46 cm I.D. Zorbax SB-C_8 (diisopropyl-C_8; solid symbols) and Zorbax Rx-C_{18} (dimethyl-C_{18}; open symbols); same mobile phases and pH 10 buffer conditions as in Fig. 1. ○ = Methanol, □ = acetonitrile.

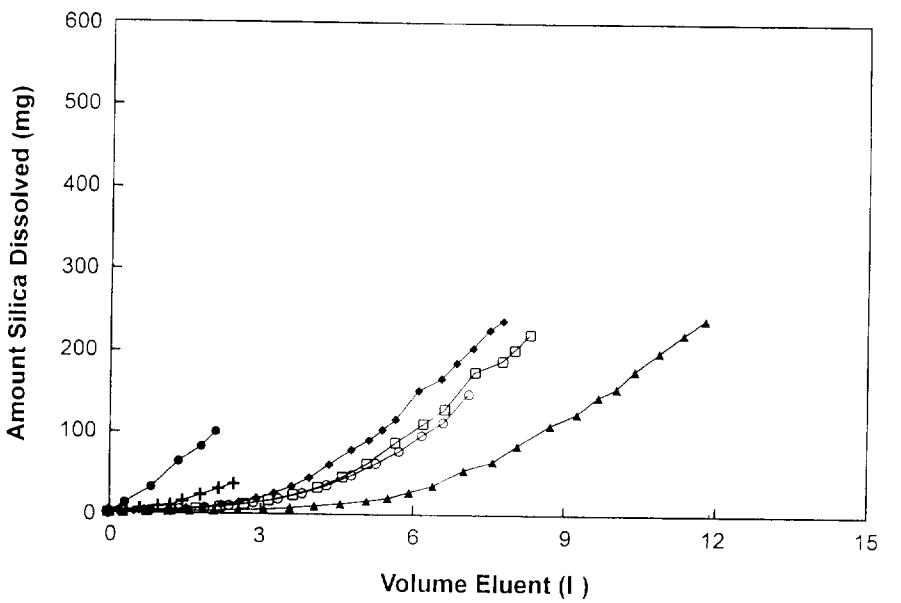

Fig. 3. Comparison of silica support dissolution for some commercial C_{18} columns. Columns: 15 × 0.46 cm I.D., except 15 × 0.39 cm I.D. for Novapak C_{18}; conditions of Fig. 1, except methanol–pH 10 buffer mobile phase and flow-rate 0.72 ml/min for the Novapak column. ● = Nucleosil C_{18}; ▲ = Hypersil ODS; ◆ = Novapak C_{18}; + = LiChrosorb C_{18}; ○ = Zorbax-ODS; □ = Zorbax Rx-C_{18}.

in silica dissolution rates shown in Fig. 3: silica pore structure, silica purity, and nature of the stationary phase. We believe that both of the silicas showing highest dissolution rates (Nucleosil, LiChrosorb) are made by a xerogel process [20], resulting in high surface areas (Table 1) and pores with variable wall thicknesses. The silicas with slower dissolution rates (e.g., Zorbax, Hypersil) are made by silica sol-gel or silica sol coacervation methods [20]. These silicas all have lower surface areas, but more importantly, the walls of the pores are quite thick. Particles formed from silica sols have cusp-shaped pores, whose walls are formed by neighboring silica sol particles. The result is that the degradation of pores within these particles requires a much larger amount of dissolved silica than particles having pores with thinner, more randomly shaped walls.

We speculate that differences in the dissolution rates from particles formed from silica sols also may be related to silica purity. It is well-known that silicas containing even small quantities of certain elements such as aluminum, iron, etc. show much lower solubility in aqueous systems than comparable highly purified silica [21]. These contaminating elements not only affect solubility, but they also increase the acidity of the silica surface [6,22,23]. Based on chromatographic properties and the published specifications in Table 2, the sol-based silica supports for Hypersil ODS and Zorbax-ODS are less pure; silicas for Nucleosil C_{18} and Zorbax Rx-C_{18} more highly purified [13,15,17]. Therefore, bonded phases from these more highly purified silicas contain less solubility inhibiting impurities and might be expected to dissolve more readily at high pH. This general trend appears to be supported by data in Fig. 3, where Hypersil column with the highest impurity levels of Al, Fe, etc., shows the lowest level of solubility. However, this effect is complicated by the fact that the type of stationary phase also may influence the silica support solubility (see following discussion). Silica support particle size, pore size and bonded phase type and concentration probably also affect silica support solubility.

The effect of stationary phase type on silica support solubility is more difficult to determine. Stationary phases for the columns in Fig. 3 probably were variously monomeric or polymeric, depending on the manufacturer. Published specifications are incomplete, but our NMR measurements showed that Nucleosil C_{18} and Hypersil-ODS are bonded with trifunctional silanes and endcapped. NMR measurements also revealed that LiChrosorb C_{18} is difunctionally modified. The rest of the stationary phases in Fig. 3 are monomeric, including the Zorbax products. While Zorbax ODS with the less-purified silica shows lower solubility than Zorbax Rx-C_{18} with highly purified silica, Zorbax ODS is end-capped, while Zorbax Rx-C_{18} is not. Therefore, from the tests reported in Fig. 3, it is difficult to isolate effects of pore structure and particle purity from effects solely due to stationary phase differences.

To gain information regarding the effect of stationary phase type, a series of solubility tests were conducted on the same silica with four densely covering monomeric stationary phases, three with conformational differences. Fig. 4 shows dissolution results with the methanol–pH 10 buffer (50:50) purge. The rate of silica solubility was slowest for columns with dimethyl-C_{18} stationary bonded phases. The silica for the dimethyl-C_{18} column on the Type A silica (Zorbax-ODS) appears to be similarly solubilized than for the highly purified Type B silica (Zorbax Rx-C_{18}) with the same stationary phase. However, the effect again may be complicated in that Zorbax-ODS is end-capped, while Zorbax Rx-C_{18} is not. Support solubility rates in Fig. 4 were comparable for silicas with sterically protected diisopropyl-C_8 (Zorbax SB-C_8) and diisobutyl-C_{18} (Zorbax SB-C_{18}) bonded phases.

These results suggest that the bulky side groups of the sterically protected bonded silanes affect the posture of the organic stationary phase. Packings with these bulky side groups apparently leave a larger surface area of unmodified silica support exposed for dissolution at high pH, compared to that for the dimethyl-C_{18} bonded phases. Presumably, densely bonded dimethyl-C_{18} groups more effectively cover the surface of the silica support. This outcome is in

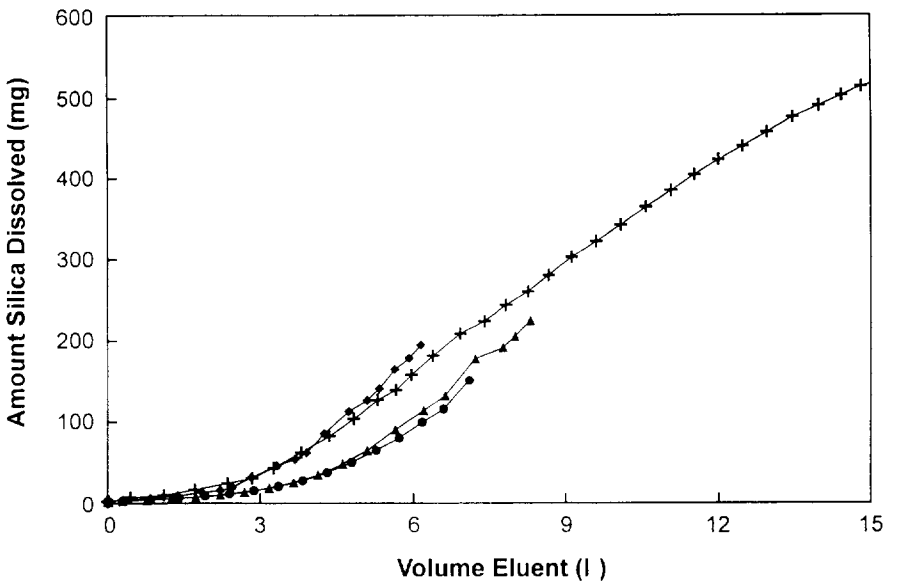

Fig. 4. Effect of bonded stationary phase type on silica support dissolution: methanol modifier. Columns: 15 × 0.46 cm I.D., (●) Zorbax-ODS (dimethyl-C_{18}; Type A silica), (▲) Zorbax Rx-C_{18} (dimethyl-C_{18}; Type B silica), (◆) Zorbax SB-C_{18} (diisobutyl-C_{18}; Type B silica) and (+) Zorbax SB-C_8 (diisopropyl-C_8; Type B silica); conditions of Fig. 1, except methanol–pH 10 buffer mobile phase.

strong contrast to low pH effects where bulky side groups much more effectively protect the bonded phase-connecting siloxane group against hydrolysis [24,25]. It also is likely that the essentially equivalent solubility for packings with the two sterically protected stationary phases occurs because the concentration of organic groups on the surface is essentially the same; steric effects from the bulky side groups limit and define the concentration of densely bonded ligands [26]. The results in Fig. 4 suggest that the nature of the stationary phase affects silica support solubility at high pH. However, the purity of the underlying silica support also may be a factor.

Previous studies have indicated that bare silica support dissolves more quickly than silica bonded with alkyl-silane functional groups [17]. With bonded phases, the concentration of the organic modifier also has a significant effect on chromatographic silica solubility [2–4]. However, the influence of the nature of the organic modifier on silica support dissolution has not been previously totally clarified. Data in Fig. 1 showed that the silica support for a dimethyl-C_{18} column (Zorbax Rx-C_{18}) was much more rapidly dissolved with methanol than with acetonitrile. Conversely, Fig. 2 showed that the silica support solubility for a diisopropyl-C_8 column with the same silica support (Zorbax SB-C_8) was only slightly less for the acetonitrile organic modifier. These limited results suggest that the stationary phase type can influence whether the silica support displays differences in solubility with different organic modifiers.

On the other hand, data in Figs. 4 and 5 suggest that stationary phase functionality causes larger silica solubility differences when acetonitrile is the mobile phase modifier, compared to methanol. With acetonitrile modifier, larger differences in silica solubility occurred for the dimethyl-substituted C_{18} packings than for the packings with bulky side groups, compared to results with methanol modifier (Fig. 4). These results suggest that use of acetonitrile modifier can reduce silica support solubility, especially for certain stationary phase functionalities.

Following the silica support dissolution tests,

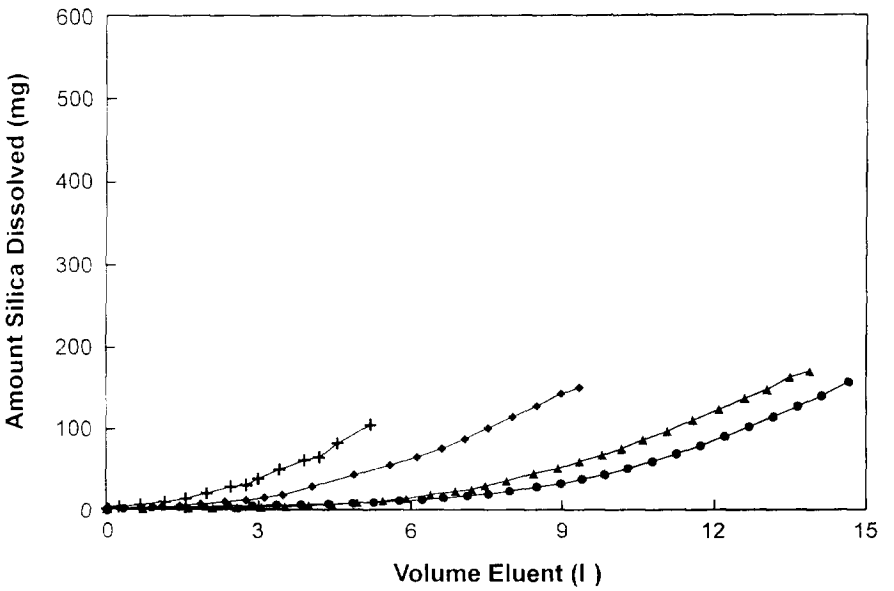

Fig. 5. Effect of bonded stationary phase type on silica support dissolution: acetonitrile modifier. Same conditions as Fig. 4, except acetonitrile–pH 10 buffer mobile phase of Fig. 1.

certain column packings were extruded from columns and thoroughly washed with methanol–water prior to elemental analysis. Table 3 shows carbon analysis results obtained in the silica dissolution tests, compared to % carbon present on the starting packing material. Also, for some columns, the inlet, middle and outlet one-third portions were separately extruded and sampled for elemental analysis. In all cases for the C_{18} columns, the % carbon after ageing actually is higher than for the original packing. We believe that this is due to significant dissolution of the silica under these aggressive conditions, leaving the organic coating essentially intact (as indicated by virtually unchanged k' values —see later data such as in Fig. 6). This increase in the organic–silica ratio with resultant decrease in particle density, increases the weight % carbon in the elemental analysis. This result is additional evidence that the Si–O–Si bond binding the silane to the silica is not significantly hydrolyzed at pH 10.

Table 3 also shows that the % carbon values actually decreased for the diisopropyl-C_8 columns in the pH 10 tests, especially when acetonitrile was used as the organic modifier. This effect is counter to the increase in % carbon for the diisobutyl-C_{18} packing, which displayed essentially identical solubility characteristics in methanol–pH 10 buffer (Fig. 4). Consequently, a change in the density of the support does not explain this trend. We speculate that the loss in carbon is a function of the shorter C_8 ligand. The % carbon decrease may involve a faster spalling off and subsequent elution of bonded silane than a decrease in support density can describe. Or, the decrease in % carbon may reflect a cleavage of the Si–O–Si bond connecting the shorter-chain silane to the silica support. Such effects were not seen for the longer-chain diisobutyl-C_{18} packing.

Finally, from the data in Table 3 for the inlet, middle and outlet samples from the aged columns, it is clear that the rates of silica dissolution and ligand hydrolysis are different for the packings investigated. For example, compare the results for Zorbax ODS with those for Nucleosil C_{18}, Hypersil ODS and Novapak C_{18}. Zorbax ODS shows little change in carbon content along the aged column, while the others exhibit changes that often are large. These differences probably are associated with the type of bonded

Table 3
Elemental analysis of column packings from silica support dissolution tests

Column type	Mobile phase purge	Carbon content (%, w/w)				
		Initial	After test			
			Final	Inlet	Middle	Outlet
Dimethyl-C_{18} (Zorbax Rx-C_{18})						
Column A	MeOH/pH 10	12.30	16.17	N/A	N/A	N/A
Column B	MeOH/pH 10	12.30	14.52	N/A	N/A	N/A
Dimethyl-C_{18} (Zorbax Rx-C_{18})						
Column A	ACN/pH 10	12.30	13.66	N/A	N/A	N/A
Column B	ACN/pH 10	12.30	14.73	N/A	N/A	N/A
Diisobutyl-C_{18}	MeOH/pH 10	10.07	12.38	N/A	N/A	N/A
(Zorbax SB-C_{18})	ACN/pH 10	10.07	11.03	N/A	N/A	N/A
Dimethyl-C_{18}	MeOH/pH 10	16.84	20.30	19.92	20.67	20.31
(Zorbax-ODS)	ACN/pH 10	16.84	20.18	19.75	20.61	20.18
Diisopropyl-C_8	MeOH/pH 10	6.28	5.98	N/A	N/A	N/A
(Zorbax SB-C_8)	ACN/pH 10	6.28	5.06	N/A	N/A	N/A
Nucleosil-C_{18}	MeOH/pH 10	N/A	N/A	17.86	14.14	13.23
Hypersil ODS	MeOH/pH 10	N/A	N/A	14.47	13.58	12.19
Novapak C_{18}	MeOH/pH 10	N/A	N/A	13.72	10.54	9.43
LiChrosorb C_{18}	MeOH/pH 10	20.18	21.07	N/A	N/A	N/A

N/A = Not available.

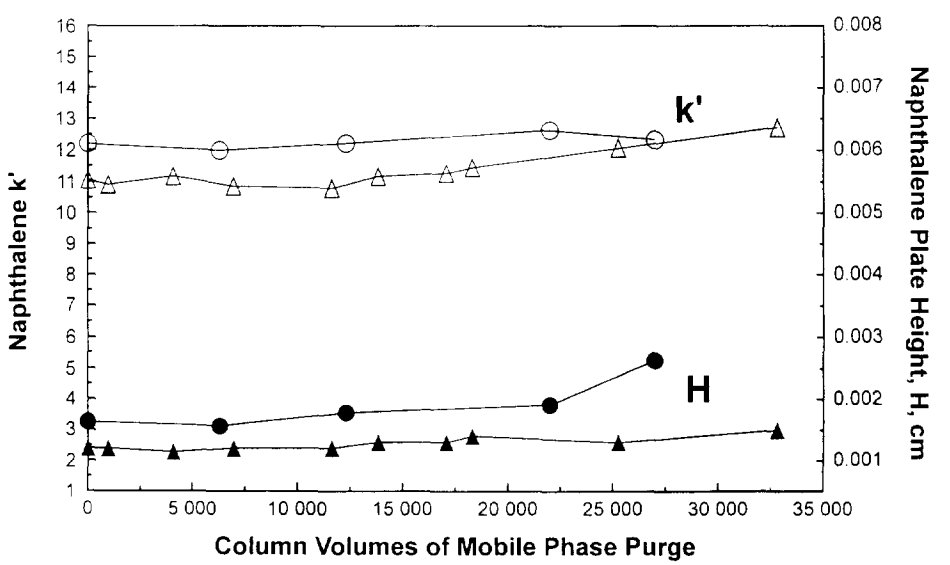

Fig. 6. Repeatability of chromatographic column pH 9 ageing tests. Columns: duplicate 15 × 0.46 cm I.D. Zorbax Rx-C_{18} (dimethyl-C_{18}); mobile phase purge: methanol–0.01 *M* phosphate, pH 9.0 (60:40); flow-rate: 1.0 ml/min; solute: naphthalene; ambient temperature.

phase used (i.e., monomeric, polyfunctional), and differences in the pore configuration and purity of the silica support.

3.2. Chromatographic column ageing tests

We also studied the degradation of silica-based, bonded-phase packings using chromatographic ageing tests. As given in Experimental, columns were continuously purged with pH 9.0 phosphate solution and periodically tested chromatographically for changes in retention, peak shape and column efficiency. Both neutral and basic solute probes were used in these tests. Naphthalene was used as the neutral solute to measure the loss of organic reversed-phase ligands from the silica surface; k' values for neutral solutes are related to the amount of bonded organic stationary phase [24]. The basic solute, N,N'-dimethylaniline (DMA) was selected as a solute to detect possible changes in the acidity and the type and number of residual silanols of the silica support. Data from the other test solutes used are not presented, since they gave no additional insights over the results herein given.

Fig. 6 data illustrate the repeatability of the chromatographic degradation tests. In June 1992 and October 1992 dimethyl-C_{18} columns with the same silica support were continuously purged with methanol–0.01 *M* pH 9.0 phosphate solution (50:50), and tested periodically. The neutral solute, naphthalene was plotted as the test solute, but similar results were seen for the other compounds in the test mixture. The June 92 column showed essentially no change for about 25 000 column volumes of purge, then suddenly failed. The October 92 column remained stable for more than 34 000 column volumes when the stability test was arbitrarily terminated. Plate height comparisons for the two columns indicate that the October 92 column was better packed, suggesting the reason for the better stability during the tests. Slight differences in the k' values are ascribed to different instruments and mobile phases used for the experiments after a four-month delay.

The data in Fig. 6 suggest that this dimethyl-C_{18} column packing can be safely used at pH 9 for method development. These results also indicate that well-packed columns of this material should be useful for more than 100 8-h work days, providing other conditions (contaminations, etc.) are not limiting. As discussed below, depending on bonded-phase functionality and silica support type, other packings may not provide this level of stability.

Results in Fig. 7 show striking differences in the stability of column packings with different bonded-phase functionalities. After about 12 000 column volumes of pH 9 purge, both the diisopropyl-C_8 and the diisobutyl-C_{18} columns failed almost simultaneously. This compares to the $>34\,000$ column volumes of stability exhibited by the dimethyl-C_{18} column. These results also suggest, however, that the diisopropyl-C_8 and diisobutyl-C_{18} columns could be used at pH 9 without problems for more than 30 8-h working days, compared to >100 days for the dimethyl-C_{18} packing.

Fig. 8 shows the effect of methanol and acetonitrile modifiers on k' values in pH 9 mobile phase with diisobutyl-C_{18} and dimethyl-C_{18} columns (same Type B silica support). Values for naphthalene k' slowly and continuously decreased at about the same rate for the diisobutyl-C_{18} column with both organic modifiers, indicating a slow loss in stationary phase. This decrease in k' is largely attributed to a loss in organic stationary phase as the silica support is eroded by the basic mobile phase [26]. (Absolute differences in k' values for the two columns is because of imperfections in adjusting % organic volume for the two organic modifiers to obtain the same k' values —acetonitrile is a stronger modifier [9]). After about 27 000 column volumes of purge, the k' values had decreased only about 10% from initial for columns tested with both organic modifiers. At this point the diisobutyl-C_{18} column k' test was discontinued.

Also shown in Fig. 8 is that the naphthalene k' values for the dimethyl-C_{18} are unchanged after almost 35 000 column volumes of pH 9.0 buffer with methanol and $>33\,000$ column volumes for acetonitrile (tests arbitrarily terminated). These results again suggest that the bonded dimethyl-

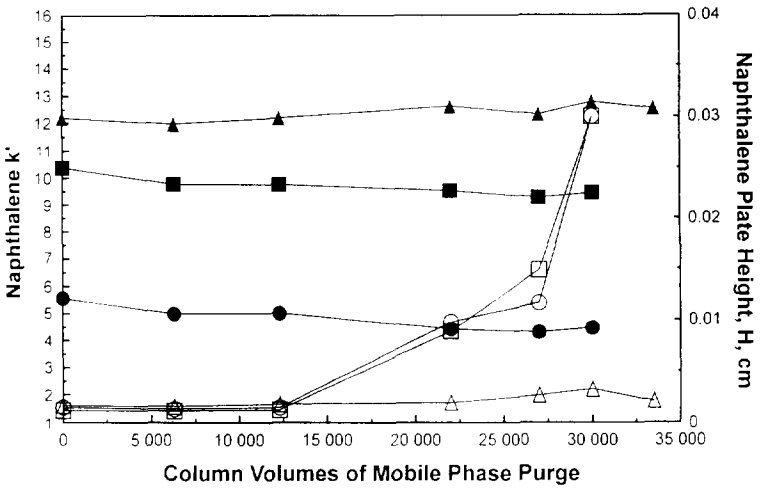

Fig. 7. Effect of stationary type on column ageing at pH 9. Columns: 15 × 0.46 cm I.D. Zorbax SB-C_8 (diisopropyl-C_8, ○), Zorbax SB-C_{18} (diisobutyl-C_{18}, □) and Zorbax Rx-C_{18} (dimethyl-C_{18}, △); open symbols, H; solid symbols, k'; conditions as in Fig. 6.

C_{18}-substituted silane protects the silica support from attack better than the diisobutyl-C_{18} phase. This postulation is directly in keeping with the silica solubility results given in Figs. 4 and 5, and the chromatographic results of Fig. 7.

Fig. 9 shows the same packing degradation tests with DMA as the test solute. Opposite to the retention pattern for the neutral solute, naphthalene, in Fig. 8, the retention pattern for the basic solute, DMA, is controlled by the number and acidity of accessible silanol groups. Again, the diisobutyl-C_{18} column exhibits a continuous slow decrease in k' values for DMA over the test period, just as in Fig. 8 for the

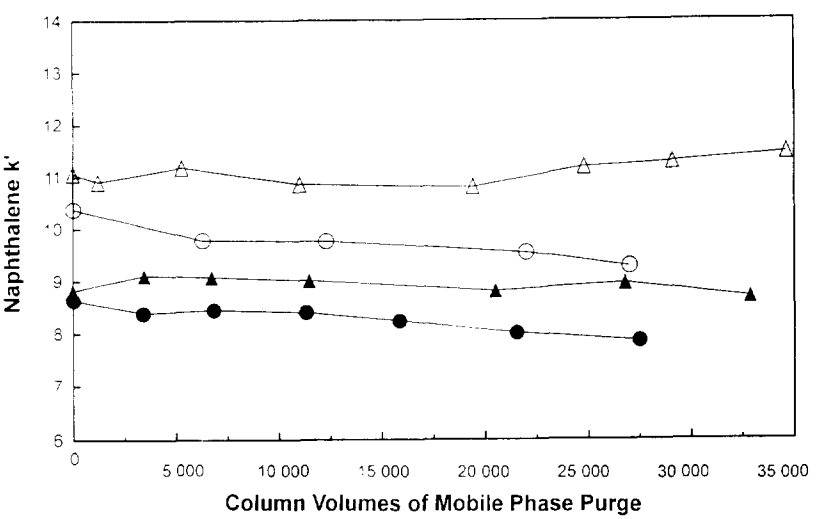

Fig. 8. Effect of organic modifier on k' for column ageing at pH 9: naphthalene solute. Columns: 15 × 0.46 cm I.D. Zorbax SB-C_{18} (diisobutyl-C_{18}, ○) and Zorbax Rx-C_{18} (dimethyl-C_{18}, △); mobile phase purge: methanol–0.01 M phosphate, pH 9.0 (60:40); flow-rate: 1.0 ml/min; solute: naphthalene; ambient temperature. Open symbols, methanol; closed symbols, acetonitrile.

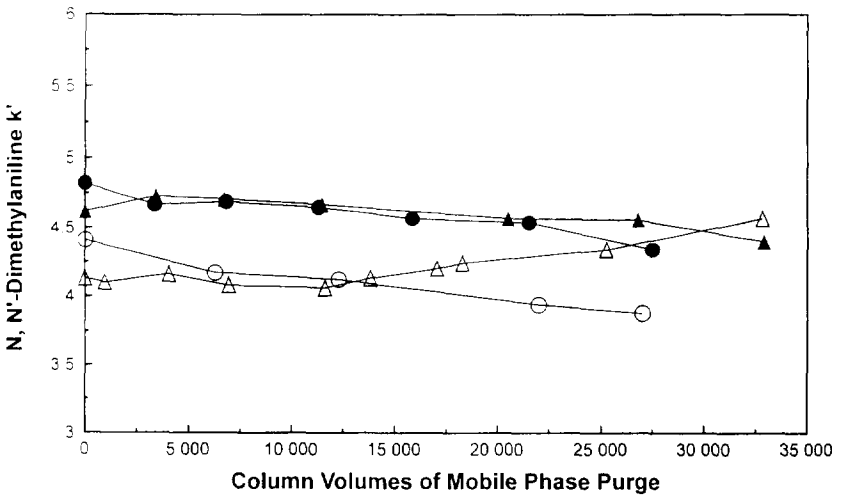

Fig. 9. Effect of organic modifier on k' for column ageing at pH 9: N,N'-dimethylaniline solute. Conditions and symbols as in Fig. 8, except solute: N,N'-dimethylaniline.

neutral solute, naphthalene. The final k' values for DMA again were about 10% below initial for both organic modifiers. With the dimethyl-C_{18} packing, however, a different pattern is evident. For about 20 000 column volumes of purge, k' values were constant. Afterwards, these values slightly increased over the remaining test period. We speculate that this trend could be due to changes in the silica support surface by this treatment, perhaps in the arrangement of silanol groups that influence retention of the basic solute. Finally, it should be noted that absolute k' values for N,N'-dimethylaniline were comparable for both the dimethyl-C_{18} and diisobutyl-C_{18} columns.

Plate height data for the dimethyl-C_{18} and diisobutyl-C_{18} columns at pH 9 with methanol and acetonitrile modifiers give a somewhat different picture of packing stability, as shown in Fig. 10. The dimethyl-C_{18} packing showed little loss in efficiency after $>33\,000$ column volumes of purge with methanol, and only a slightly

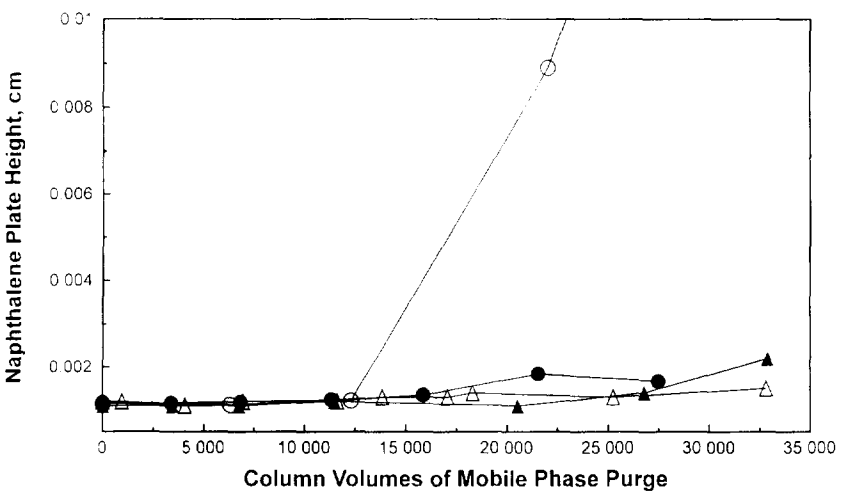

Fig. 10. Effect of organic modifier on plate height for column ageing at pH 9. Columns, conditions and symbols as for Fig. 8.

greater efficiency loss after >27 000 column volumes of purge with acetonitrile as organic modifier. In contrast, the diisobutyl-C_{18} column failed after about 12 000 column volumes of purge with methanol–pH 9 buffer, but remained stable for >27 000 column volumes when purged with acetonitrile-modified mobile phase. However, since only one column was tested in methanol, the earlier failure may have been due to a more poorly packed bed, rather than bed degradation because of silica support dissolution (see data in Fig. 4).

Peak asymmetry data also is useful for defining when packed bed stability is compromised. However, information on peak symmetry degradation closely follows that of plate height data. Therefore, although available for all studies, peak symmetries generally are not reported since results are redundant with plate height data.

Several commercial C_{18} columns were tested with the phosphate pH 9–acetonitrile system for stability, with the results shown in Fig. 11. As described previously in Figs. 8 and 9, Zorbax Rx-C_{18} (dimethyl-C_{18}) packing exhibits stability for >27 000 column volumes of purge. Here, plate height data suggest that this column fails after about 30 000 column volumes under the test conditions used. Tests with other commercial columns produced similar results. Hypersil ODS showed higher stability (wider pores? —see Table 1), while Novapak C_{18} was only slightly less stable, based on plate height data. But, Nucleosil quickly failed in this test. This result is in strong contrast to previous tests on laboratory-synthesized bonded phases made with this silica [17]. It also should be noted, however, that packing stability is a strong function of silica type and purity, as discussed above. Therefore, it is difficult to isolate the influence of bonded phase type when the type of silica support is varied.

3.3. Sodium hydroxide column-flush studies

Workers analyzing protein and peptides often like to flush their columns periodically with high-pH (>12) sodium hydroxide solution to purge strongly retained extraneous material from the separating system. Zorbax Rx-C_{18} (dimethyl-C_{18}) columns have demonstrated interesting characteristics for analyzing peptides and small proteins with conditions normally used for separating these materials. Therefore, we were interested in determining the stability of this column with sodium hydroxide washing procedures that might be required for some applica-

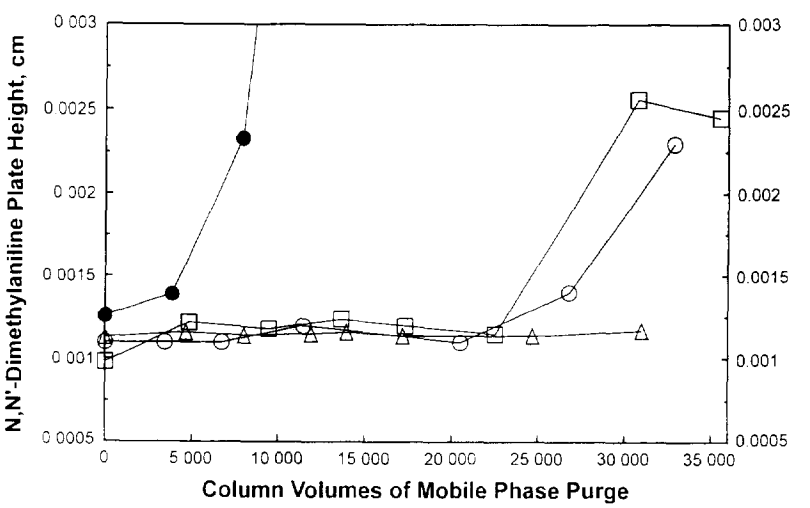

Fig. 11. Comparison of ageing at pH 9 for some commercial dimethyl-C_{18} columns. Columns: 15 × 0.46 cm I.D. Zorbax Rx-C_{18} (○, k' = 4.3), Hypersil ODS (△, k' = 4.3), and Nucleosil C_{18} (●, k' = 4.5); 15 × 0.39 cm I.D. Novapak C_{18} (□; k' = 4.1); mobile phase purge: acetonitrile–0.01 *M* phosphate, pH 9.0 (50:50); solute: N,N'-dimethylaniline.

tions. Accordingly, a 1:1 mixture of methanol–0.02 *M* sodium hydroxide (pH 12.3) was passed continuously through a Zorbax Rx-C_{18} column at 1.0 ml/min. Periodically, k' values, plate heights and peak asymmetry values (not shown) were measured for a neutral solute, toluene, and a basic solute, N,N′-dimethylaniline. The plots in Fig. 12 indicate that the packed bed was unchanged after about 15 h of this continuous treatment (ca. 900 ml). The column then exhibited a modest change, then stabilized in performance for an additional 17 h before apparent bed collapse. Inspection of the column after removing the frit showed about a 1-mm void at the column inlet. This pH 12.3 study suggests that a column of this silica and functional group type probably could be purged more than 60 times with ten column volumes (15 ml total) of pH 12.3 sodium hydroxide solution without objectionable dissolution of the silica support. Previously, such a treatment for silica-based columns was believed to invite an early catastrophic column failure.

Table 4 gives % carbon analysis data for certain column packings analyzed after various chromatographic ageing studies. Little loss in carbon for the bonded-phase columns was found as a result of the pH 9 tests, which is in keeping with the k' data in Figs. 6–9. Contrary to results at pH 10 (Table 3), % carbon values did not increase —the density of the silica particles does not appear to have been significantly altered at pH 9. The packing for the dimethyl-C_{18} column aged at pH 12.3 was extruded so that the top, middle and bottom third could be analyzed separately. The total carbon content of the packing was unchanged by this treatment, as suggested by the constant k' values in Fig. 12. But, higher carbon values were found down the bed, suggesting that silane removed from the top was captured by the stationary phase as it passed down the column.

4. Conclusions

Several important conclusions can be derived from these high-pH chromatographic and dissolution tests. First, densely bonded monomeric dimethyl-C_{18} ligands better protect the silica support from dissolution than bulky diisopropyl- and diisobutyl-substituted bonded silanes. These results suggest that the silica support is more exposed when bulky groups are used on the

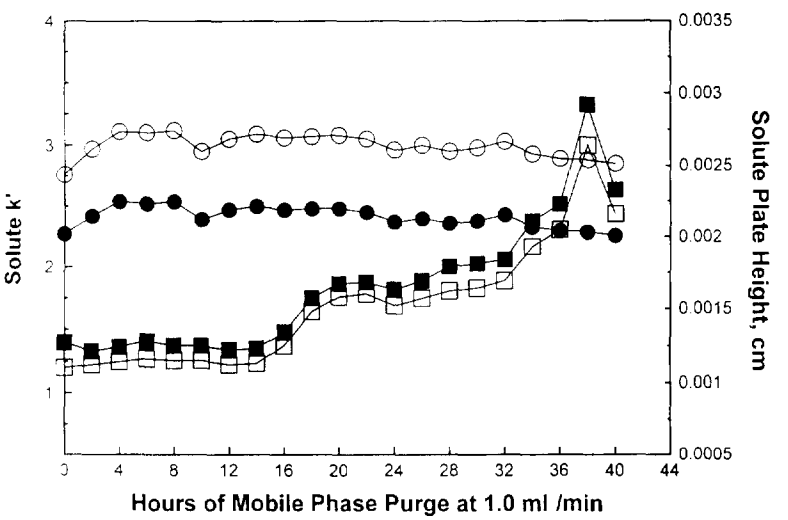

Fig. 12. Stability of dimethyl-C_{18} column at pH 12.3. Column: Zorbax Rx-C_{18}; mobile phase: methanol–0.02 *M* sodium hydroxide, pH 12.3 (50:50); flow-rate: 1.0 ml/min; ambient temperature; solutes: toluene (open symbols) and N,N′-dimethylaniline Solid symbols). ○ = k'; □ = H.

Table 4
Carbon analysis of column packings from chromatographic ageing tests[a]

Column type	Mobile phase purge	Initial % carbon	Final % carbon	After tests, % carbon		
				Inlet third	Middle third	Outlet third
Diisopropyl-C_8 (Zorbax SB-C_8)	pH 9	5.91	5.56	N/A	N/A	N/A
Diisobutyl-C_{18} (Zorbax SB-C_{18})	pH 9	9.90	9.30	N/A	N/A	N/A
Dimethyl-C_{18} (Zorbax Rx-C_{18})	pH 9	12.77	12.12	N/A	N/A	N/A
Dimethyl-C_{18} (Zorbax Rx-C_{18})	pH 12.3	12.21	12.29	12.03	12.39	12.49

Mobile phases with 50% methanol modifier. N/A = Not available.
[a] Average of duplicate values.

silane; the dimethyl-substituted silane groups apparently are more tightly arranged on the surface. This feature is indirectly supported by studies which showed superior peak shapes and column efficiency with diisopropyl- and diisobutyl-C_{18} packings for highly basic drugs whose retention at pH 7 is largely based on interaction with silanol groups [26]. Here, the densely reacted dimethyl-C_{18} phase on highly purified, low-acidity silica support produced poorer results, presumably because a smaller number of surface silanol groups were available for needed interaction with basic drugs ionized at this pH.

Second, failure of silica-based columns at high pH is a direct result of solubilizing the silica support, with ultimate collapse of the packed bed. Columns tested during the pH 10 silica-dissolution studies often showed several centimeters of void at the inlet after the tests. But, after bed failure of columns in the pH 9 chromatographic tests, only a small decrease in k' values (ca. 10%) for solutes was observed. This result confirms that column failure results from loss of silica support, and *not* in the hydrolysis of the Si–O–Si siloxane bond of the bonded silane as has been proposed [2]. This is contrary to effects at low pH, where hydrolysis of the covalently bonding Si–O–Si group is the mechanism of bonded-phase column degradation. Here, bonded phases with bulky, sterically protecting groups show excellent stability at low pH and high temperatures [24,25].

Third, the nature of the bonded C_{18} stationary phase (whether monomeric or polymeric) does not significantly influence column degradation at high pH. The dominating feature is the nature of the silica support, since siloxane (Si–O–Si) hydrolysis for the C_{18} bonded silane either does not occur, or occurs very slowly at high pH. Loss of bonded phase (and retention) at high pH likely is a result of silica dissolving around the covalently attached silane, eventually causing mechanical attrition and a spalling off of the bonded organic phase.

Fourth, the rate of silica dissolution is a strong function of the nature of the porous silica support. All three of the more stable columns in Fig. 11 apparently were prepared from silicas made by consolidating silica sols, although by different methods. Here, the similar pore structure is a series of cusp-shaped passages, with thick outer walls represented by the outer surface of neighboring individual sol particles. Pore volumes of these more stable silicas also are somewhat comparable. Conversely, some silica supports apparently are prepared by a two-phase xerogel process that does not start with a silica sol. The resultant material has a higher pore volume and differently shaped pores. These

silicas apparently have a more random pore structure containing a high population of pores with thin walls that are readily dissolved at high pH. Rapid dissolution apparently causes individual particles to break down, then the whole packed bed to collapse.

Fifth, purity of the support affects the stability of silica-based bonded-phase packings. More highly purified less-acidic Type B silicas dissolve more rapidly; less-pure Type A silicas with significant contamination from aluminum, iron, zinc and other elements appear to solubilize more slowly and are more stable at high pH. In keeping with a previous studies [2,7], these results suggest that certain C_{18} bonded-phase column packings prepared from silica sols are useful at pH 9 operation. C_{18} packings with less-pure Type A silicas made from silica sols appear most stable; pH 10 operation seems feasible for some of these materials. *In all cases, however, potential lifetime of silica-based bonded-phase packings will always be reduced with high aqueous pH operation*. Use of a precolumn of silica support (located between the pump and sample injector) could enhance column lifetime. Such a precolumn would partially saturate the mobile phase with silica, diminishing the tendency of the silica in the separating column to dissolve. However, use of such a precolumn would make gradient elution impractical.

Sixth, in keeping with previous studies, we found that acetonitrile organic modifier often prolongs column life at high pH compared to methanol. Other reports have shown that silica support dissolution is reduced with increasing organic modifier concentrations.

Finally, certain silica-based C_{18} columns can be safely purged with 0.02 *M* sodium hydroxide solution to clean unwanted highly retained materials from the column bed. Bed stability under these conditions is strongly influenced by the type and purity of silica support and the type of C_{18} bonded-phase functionality.

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