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Stability of silica-based, endcapped columns with pH 7 and 11 mobile phases for reversed-phase high-performance liquid chromatography

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Abstract

The goal of this study was to define practical conditions and limitations of using silica-based, endcapped bonded-phase columns in intermediate and higher pH environments for developing rugged HPLC methods. Bonded-phase degradation in this pH range is a result mainly of silica support dissolution; covalently-bound silane ligands are hydrolyzed very slowly if at all from silica supports at intermediate and higher pH. Based on rates of silica support dissolution determined by chemical measurements and comparable chromatographic studies, we now find that endcapping alkyl-bonded stationary phases increases column longevity at pH 7, compared to non-endcapped columns. As previously determined for non-endcapped packings, we also find that the type of silica support determines the stability of bonded-phase packings. Silicas made by the sol-gel process are more resistant to dissolution than supports made by a silicate-gel (xerogel) process. In addition, endcapping methods apparently affect column stability, with double-endcapping methods apparently superior to single-endcapping approaches. Degradation rates for several endcapped commercial bonded-phase C_8 columns were found to be quite variable in highly aggressive pH 7 accelerated-lifetime tests. Column stability in the pH 7–11 range is enhanced by using buffers other than phosphate in the mobile phase, and by excluding higher column temperatures. Certain silica-based endcapped bonded-phase columns can be used for developing rugged methods to at least pH 11 when used with organic buffers at ≤40°C.

Keywords: Stationary phases, LC; Silica, bonded; Mobile phase composition; Antidepressants, tricyclic

1. Introduction

Recent studies in these laboratories have confirmed previous reports [1–3] that certain silica-based, bonded-phase columns can be routinely used at least to pH 9–10 in reversed-phase separations, providing certain operating conditions are met [4–6]. In these latter studies, the monofunctionally-bonded, non-endcapped columns showed excellent stability when organic (e.g, glycine, Tris) and borate buffers were utilized [6]. Conversely, these same columns degraded rapidly with 0.1 *M* phosphate and carbonate buffers at pH 10, and even with phosphate buffers at pH 7 and 8. The rate of degradation was especially fast at higher operating temperatures and higher buffer concentrations [6]. The type of silica support (silica sol-gel or xerogel) used in the bonded-phase packings also was shown to have a strong influence on column stability [5], as well as the type and method of bonded-phase attachment [4,5].

While many chromatographers have found that samples containing ionizable compounds usually are

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best separated with mobile phases of ≤pH 3 [7,8], some separations are best performed at intermediate (4–8) and higher pH(>9) because [9]: (i) sample components are unstable at low pH, (ii) basic compounds protonated at low pH elute too quickly, (iii) required band spacings are not found at low pH. Therefore, operation at intermediate or higher pH may provide a way that a needed separation can be satisfactorily performed.

Separating basic compounds using mobile phases of pH≥10 is especially attractive. Here, many compounds of interest (e.g., basic drugs) exist as free bases, minimizing problems with deleterious interactions with completely ionized silanol groups on the silica support. While use of silica-based bonded-phase packings at pH≥10 have been reported (e.g., Refs. [4,10]), users often have been reluctant to place such packings into routine use because of questions about column stability. Some column packings have been designed to operate at higher pH (e.g., graphitized carbon, porous polymers, polymeric phases on alumina, or zirconia supports). However, these materials have not reached a high level of acceptance because of problems with reproducibility, efficiency, and limitations in mobile phases that can be effectively used. Therefore, silica-based column packings without these limitations would be especially attractive for use at higher pH, providing columns of such materials are adequately stable.

Most users are aware that silica-based columns usually are not recommended for operation at higher pH (e.g., >pH 8), because of potential dissolution of the silica support with accompanying column failure. However, there are also not-so-widely-recognized problems that can arise in separations that are designed for operation in the pH 6–8 range [9]. Silica support solubility can be significant in this pH range, so that separation reproducibility and column lifetime is less than might be expected. It has now been documented that degradation of silica-based columns at intermediate (and higher) pH is largely a function of dissolution of the silica support, rather than a loss of bonded organic substrate due to hydrolysis [5]. It has also been found that silica support solubility in the pH 6–8 range is greatly increased in the presence of phosphate buffers, particularly at higher temperatures and higher buffer concentrations [6]. For best stability, separation methods based on phosphate buffers at intermediate pH should be limited to concentrations of 10–50 m*M* and column temperatures not exceeding 40°C. However, for best separation reproducibility and lifetime of silica-based columns, the application of organic buffers (e.g., Tris) in the intermediate and higher pH range has been indicated [6].

This study was designed to determine if separation reproducibility and column lifetime in reversed-phase separations at intermediate and higher pH could be further enhanced by using endcapped alkyl bonded-phase columns. We also wanted to gain information about the intermediate-pH stability of certain columns reported by suppliers to be designed specifically for separating difficult basic compounds in this region.

2. Experimental

2.1. Chromatographic reagents, columns

HPLC-grade solvents used for separations were from EM Science (Gibbstown, NJ, USA). All 15× 0.46 cm I.D. Zorbax columns of 5-μm particles were prepared by Rockland Technologies. The spherical porous-silica support in these columns is a less-acidic, highly-purified Type B silica made by aggregating ultra-pure silica sols [11,12]. Physical and surface properties of this silica support were previously given [5,13,14]. The Zorbax XDB-C_8 columns used are comprised of a densely-bonded (3.6 μmol/m^2) dimethyl-C_8 phase on an ultra-pure Type B support (80 Å pores, 180 m^2/g). This stationary phase then was exhaustively double-endcapped with dimethyl- and trimethylsilane using a proprietary process. Columns were prepared by conventional slurry-packing methods [15]. Comparable columns are available from Mac-Mod Analytical (Chadds Ford, PA, USA).

Other columns (15×0.46 cm) of 5-μm particles were obtained from suppliers: Symmetry-C_8 from Waters Associates (Milford, MA, USA); Hypersil BDS-C_8 and Inertsil-C_8 from Alltech Associates (Deerfield, IL, USA); YMC Basic from Y.M.C. (Wilmington, NC, USA); and Supelcosil ABZ+ from Supelco (Bellefonte, PA, USA).

Table 1
Characteristics for silica supports used in bonded-phase packings of this study[a]

Column designation	Silica support[a]				
	Surface type	Silica type[b]	Surface area (m^2/g)	Pore diameter (Å)	Porosity (cm^3/ml)
Hypersil BDS-C_8	A	SolGel	170	130	0.65
Inertsil-C_8	B	SilGel	320	150	NA[c]
Supelcosil ABZ+	A	SolGel	175	120	0.60
Symmetry-C_8	B	SilGel	340	100	0.84
YMC-Basic	B	SilGel	325	120	1.0
Zorbax XDB-C_8	B	SolGel	180	80	0.50

[a] Data taken from commercial literature or manufacturer's sources.
[b] See text for assigned silica type description.
[c] NA=not available.

The physical characteristics of the silica supports for the tested columns are summarized in Table 1.

The data in Table 1 and previous results [6] suggest that two distinctly different types of silica supports are used in commercial columns. The nomenclature for porous silicas is not standard in the literature. Therefore, these two silica types can be defined by their different physical and chromatographic properties. Particles usually made by gelling soluble silicates or coalescing fumed silica (sometimes called xerogels) are characterized by higher surface areas, higher porosities and irregular pore shapes with variable wall thicknesses. These materials have been arbitrarily named "SilGel" silicas [9]. Particles made by aggregating silica-sol particles (sometimes called sol-gel materials) have lower surface areas, lower porosities and more regular pores with thicker walls defined by surrounding solid silica sol microparticles. These particles were arbitrarily termed "SolGel" silicas [9]. The silica types for the columns used in this study have been designated in Table 1, based on physical differences.

Extensive studies with non-endcapped silica supports have shown that higher surface area SilGel silicas dissolve much more rapidly than SolGel silicas in the pH range 6.5–10 [5,6]. Therefore, non-endcapped columns made with SolGel supports demonstrate higher chromatographic stability in this pH range [2–6]. A specific goal of this study was to determine the effect of endcapping on the stability of silica-based columns in the intermediate pH range. Two experimental approaches were used: (a) chemically measuring the rate of silica dissolution of the silica from the columns in a continuously flowing, non-recycled mobile phase, and (b) determining column stability by appropriate chromatographic measurements after comparable purging.

2.2. Silica support solubility study

2.2.1. Apparatus and reagents

Columns were continuously 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). Absorbance measurements were with a Pye Unicam LC3 detector (ATI Unicam, Cambridge, UK). All chemicals and solvents were of analytical grade from Merck (Darmstadt, Germany). Silicate standard solutions also were from Merck. Buffers and reagent solutions were prepared with deionized water from a Milli-Q purification system (Millipore, Bedford, MA, USA); pH 7 eluent series: acetonitrile–0.25 *M* sodium phosphate buffer pH 7.0 and 0.25 *M* Tris buffer, pH 7.1 (20:80, v/v).

2.2.2. Procedures

To simulate the usual chromatographic practice, columns were continuously purged at 1.0 ml/min with eluents and *not* recycled. This approach is in contrast to column ageing studies where packings are immersed in a static volume of mobile phase for a time period. Dissolution tests were conducted at 60°C. All columns were flushed for 10 min with methanol–water (50:50, v/v) prior to the dissolution experiments. After beginning a specific dissolution

experiment, we sampled the effluent after about one liter had passed through the column, using a fraction collector. Column effluent samples for silicate analyses were collected for a 10-min period (total: 10 ml).

Dissolved silica concentrations were measured colorimetrically in collected fractions using the well-known silicomolybdate complex method [16]. Absorbance was measured at 410 nm. For the silica measurement, standard silicate mixtures were prepared in the corresponding buffer–modifier purge solutions used for the dissolution studies. Absorbance values were measured using blank solutions as reference. The potential interference of phosphate buffer on the colorimetric method was overcome by removing phosphate prior to the silica measurement [5]. Individual silicate calibrations were prepared for the different types of buffers used in the dissolution experiments.

Results from the colorimetric measurements made for the concentration of dissolved silica in the eluents were plotted 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. From this, 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 representing the mass of silica which had been dissolved as a function of eluent volume flushed through the column.

2.3. Chromatographic column degradation studies

2.3.1. Apparatus and reagents

Analytical-grade methanol, acetonitrile, hydrochloric acid, sodium hydroxide, citric acid, Tris (free base), NaH_2PO_4 and Na_2HPO_4 were from J.T. Baker (Phillipsburg, NJ, USA). EM Science (Gibbstown, NJ, USA) supplied HPLC-grade methanol and acetonitrile. Test solutes from Chem Service (West Chester, PA, USA) and Sigma (St. Louis, MO, USA) were used as received. Pyrrolidine (99%) was used as received from Aldrich (St. Louis, MO, USA). Column purging studies were performed with a Shimadzu Model LC-600 pump (Tokyo, Japan). Chromatographic testing studies used a DuPont Model 860 pump and a Model 860 UV absorbance detector or 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).

Phosphate buffers were prepared by mixing appropriate NaH_2PO_4 and Na_2HPO_4 solutions to obtain pH 7.0. Citrate buffer at pH 6.5 was made by titrating a 0.083 *M* citric acid with 0.25 *M* sodium hydroxide. (At 60°C, the actual pH of this buffer is probably closer to pH 6.7). Tris buffer of pH 7.1 and pyrrolidine buffer of pH 11.5 were prepared by titrating an appropriate concentration of the free bases with hydrochloric acid solution.

2.3.2. Column ageing procedures, pH 7

Columns were continuously purged (1.0 ml/min, *not* recycled) either with acetonitrile–pH≈7, 0.25 *M* buffers (20:80) at 60°C, or methanol–0.05 *M* sodium phosphate buffer, pH 7.0 (30:70) at 40°C, for two different experiments. Buffer solutions were prepared at pH values to maintain good buffering capacity (within about one pH unit of the buffering agent pK_i). The columns purged at 60°C were periodically tested first with toluene solute (uracil as t_0 marker) using a mobile phase of methanol–water (60:40) at ambient, 1.0 ml/min, then with a mixture of tricyclic antidepressants (doxepin, trimipramine, amitriptyline and nortriptyline at 0.025, 0.25, 0.025 and 0.25 mg/ml, respectively) using a mobile phase of acetonitrile–0.01 *M*, pH 7.0 sodium phosphate buffer (20:80) at 40°C, 1.5 ml/min. Columns purged at 40°C were periodically tested with the tricyclic antidepressant mixture. Injected sample solution volumes were 5 μl. Before chromatographic testing, each column was first flushed with at least 20 column volumes of methanol–water (60:40), before equilibrating with about 20 column volumes of the mobile phase.

2.3.3. Column ageing procedure, pH 11.5

A Zorbax XDB-C_8 column was continuously purged (not recycled) at 1.5 ml/min with a mobile phase of methanol–0.05 *M* pyrrolidine·HCl buffer, pH 11.5 (55:45) at 40°C. These columns were periodically tested under the same conditions at a flow-rate of 1.0 ml/min, using a mixture of highly-basic β-blocker drugs (pK_a=9.5–9.7).

2.4. Bonded phase identification studies

Since a goal of this study was to determine the effect of endcapping, there was a need to gain information about the endcapping method used on the various columns studied. Such data usually are not available from manufacturers, so we attempted to develop this on some popular commercial column packings. For this, we used a variation of the method for degrading the silica-based support with hydrofluoric acid, followed by identification of the liberated fluoroalkylsilane derivatives [17].

A 10-mg sample of the designated column packing was placed in a 15×45 mm polyethylene screw-cap vial. To this was added 0.1 ml of 50% hydrofluoric acid solution, and after closure with a PTFE-covered butyl rubber-lined cap, the vial was then lightly agitated to dissolve the sample. Hexane (1.0 ml) was added, and the vial shaken to extract the hydrophobic fluoro-derivatives into the hexane layer. A sample (0.5 μl) of the separated hexane layer was removed with a micro syringe and injected into a Hewlett-Packard GCD 1800A GC–MS instrument fitted with an electron ionization detector (Wilmington, DE, USA). The GC–MS inlet system was operated in the split mode (20:1) at a temperature of 200°C, using a glass-lined inlet tube. For the separation, a 30-m, 0.25-mm capillary column with a 0.25-μm-thick stationary phase (HP-5, Hewlett-Packard) was initially held at 40°C for 2 min, then programmed linearly to 160°C at 20°C/min with a column flow-rate of 1.0 ml/min. The MS detector mass range was set at 45:425 m/z, and data were analyzed with the instrument computer software.

3. Results and discussion

3.1. Bonded-phase identification

Based on data obtained by the HF-degradation–GC–MS method, characteristics of the bonded-phase for columns used in this study were assigned, as summarized in Table 2.

Hypersil BDS-C_8, Symmetry-C_8, YMC Basic and Zorbax XDB-C_8 all appeared to contain monofunctionally-bonded dimethyl-*n*-octyl ligands. There was evidence for small amounts of dimethyl-C_6 ligands in the YMC Basic packing, which is in keeping with the manufacturer's claim that this is a mixed alkane stationary phase. Inertsil-C_8 packing appeared to contain a difunctional methyl-*n*-octyl ligand, with evidence of C_8 dimers. Results on Supelcosil ABZ+ suggest a trifunctional silane attachment. A GC pyrolysis study gave evidence of an amide group. The presence of an amide functionality was confirmed by the manufacturer.

The results in Table 2 further suggest that all packings had been endcapped with trimethylsilyl groups except Supelcosil ABZ+. Zorbax XDB-C_8 was the only packing that gave definite evidence of being doubly-endcapped with dimethylsilyl and trimethylsilyl groups (also confirmed by the manufacturer). A separate head-space analysis of the Hypersil

Table 2
Characteristics of column packings evidenced by HF degradation–derivatization–GC–MS

Column packing	Apparent bonded functionality	Endcapping agents identified	
		Trimethylsilyl	Dimethylsilyl
Hypersil BDS-C_8	Mono; normal C_8	Yes	?[a]
Inertsil-C_8	Di; normal C_8[b]	Yes	No
Supelcosil-ABZ+	Trifunctional silane[c]	No	No
Symmetry-C_8	Mono; normal C_8	Yes	No
YMC Basic	Mono; normal C_8[d]	Yes	No
Zorbax XDB-C_8	Mono; normal C_8	Yes	Yes

[a] Not positive; separate head-space analysis suggests some dimethylsilyl.
[b] GC retention also indicated some branched C_8.
[c] Insoluble reaction products; evidence of amide group in bonded ligand.
[d] Evidence for small amounts of short-chain dimethyl alkylsilyl groups.

BDS-C_8 reaction suggested the presence of a small amount of difluorodimethylsilane, but this was not taken as proof of a double-endcapping procedure.

3.2. Silica support dissolution tests

3.2.1. Column tests

Aggressive support dissolution tests were conducted on candidate columns using the conditions of 80% 0.25 *M* sodium phosphate buffer, pH 7.0, 60°C as applied in a previous study [6]. Since the solubility of silica support is not at equilibrium during passage of a mobile phase, the concentration level of dissolved silica is a function of the surface area and porosity of the support, as well as support purity (impurities such as alumina decrease solubility [5]). Tests were terminated when the back pressure of the columns was too high for the pumping system to operate effectively, signifying gross destruction of the column packed bed. In those cases where columns still could be operated, experiments arbitrarily were terminated after 15 l of eluent were used. Fig. 1 summarizes the results of these aggressive dissolution studies. The resulting dissolution data suggest that the column packings tested belong to two distinct groups: (a) a group in which the silica support was more rapidly dissolved (Inertsil-C_8, Supelcosil-ABZ+, Symmetry-C_8, and YMC Basic, and (b) those in which dissolution was much slower (Hypersil BDS-C_8 and Zorbax XDB-C_8). Information on the supports for these columns in Table 1 shows that the more rapidly degrading columns of the first group were of the higher surface area, higher porosity type which have been termed SilGel silicas [9]. The exception was the Supelcosil-ABZ+ column which is made from a lower surface area, lower porosity silica support. However, this column packing does not have an alkyl bonded phase like the other columns, but appears to use a trifunctionally-bonded silane derivative containing an amide group. We speculate that this bonded phase with a polar functionality does not protect the underlying silica support from dissolution as well as the lower surface energy (higher surface tension) alkyl ligands for the other columns of this group.

The second, less soluble group of columns in Fig. 1 (Hypersil BDS-C_8 and Zorbax XDB-C_8) are made from SolGel particles that have lower surface areas and porosities. The Hypersil column shows slightly less silica support solubility than the Zorbax column,

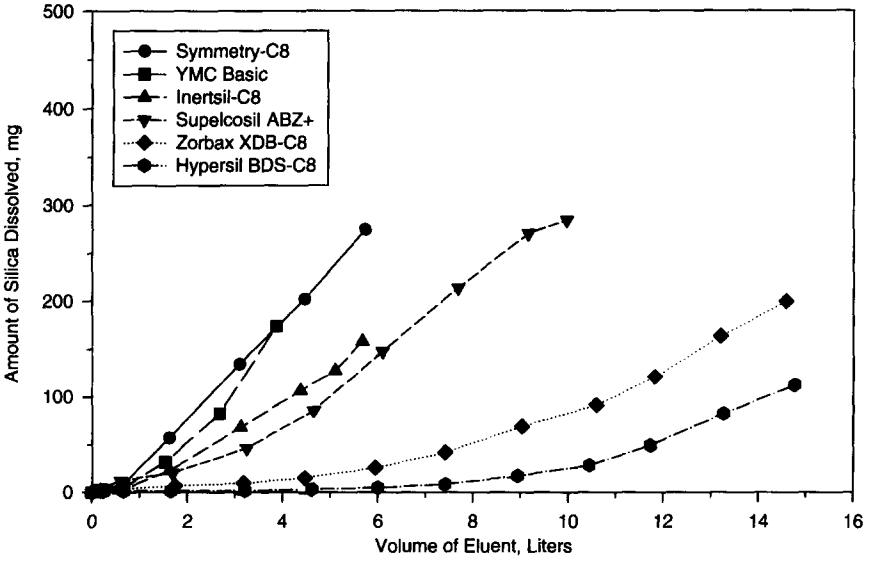

Fig. 1. Silica support dissolution tests. Columns: 15×0.46 cm; purge eluent: acetonitrile–0.25 *M* sodium phosphate buffer, pH 7.0 (20:80); 60°C; 1.0 ml/min; measured by molybdate–silicate color reaction.

perhaps because of a lower surface area and a higher level of silica impurities that decrease silica solubility [5].

3.2.2. Effect of organic modifier type

Previous studies have shown that organic solvent modifier type has a substantial influence on silica support solubility for non-endcapped columns; silica support solubility was higher with methanol modifier than with acetonitrile [5]. The results of similar tests with an endcapped column (Zorbax XDB-C_8) are shown in Fig. 2. Contrary to that previously found for non-endcapped columns [5], an acetonitrile-modified mobile phase initially showed somewhat higher solubility for the silica support, compared to methanol. After continued purging, however, the silica solubility becomes comparable for the two organic modifiers when the column packing is sufficiently degraded.

3.2.3. Effect of buffer type on silica support dissolution

Non-endcapped bonded-phases columns have shown much higher solubility and shorter column lifetime with pH≥7 mobile phases containing phosphate, compared to those with organic-based or borate buffers [6]. It was proposed that at higher pH, phosphate interacts with siloxane groups on the silica support surface, facilitating hydrolysis by hydroxyl ions and enhancing silica solubility [6]. We now find that the large differences in silica support solubility for phosphate and organic-based buffers also holds for endcapped column packings, as indicated in Fig. 3. Here, solubility plots are given at pH 7 phosphate- and Tris-buffered mobile phases for two C_8 units: a double-endcapped SolGel column and a single-endcapped SilGel column. Again, the Tris-buffered mobile phase shows much lower silica support solubility than for phosphate buffer with this aggressive test system. As noted in our previous report [6], for maximum column stability and lifetime, such results suggest that at pH≥6, mobile phases should contain phosphate at ≤50 m*M*, and column temperature should be maintained at ≤40°C. The results in Fig. 3 also show the superior stability of SolGel-based silica supports against the dissolution that causes packed bed degradation. Double endcapping also may contribute to additional stability versus single endcapping, but definite proof is not available to support this possibility.

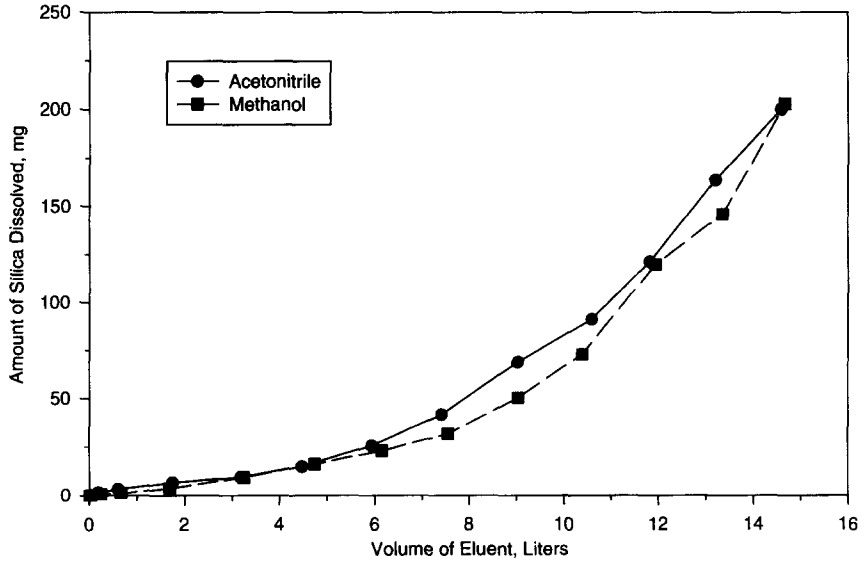

Fig. 2. Effect of organic modifier type on silica support dissolution. Columns: 15×0.46 cm, Zorbax XDB-C_8; purge conditions same as Fig. 1, except also methanol–0.25 *M* sodium phosphate buffer, pH 7.0 (20:80).

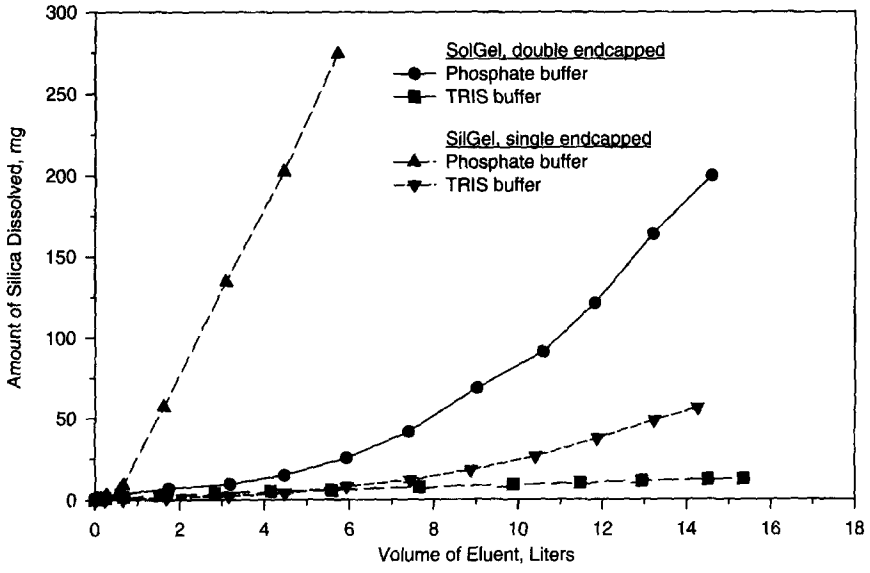

Fig. 3. Effect of buffer type on silica support dissolution for different bonded-phase C_8 columns. Columns: 15×0.46 cm; purge eluent: acetonitrile–0.25 *M* buffers, pH 7 (20:80); 60°C; 1.0 *ml/min*.

3.2.4. Elemental analysis of aged columns

Carbon analysis of the column packings before and after the various treatments described above further confirmed conclusions reached from the dissolution studies. Following the ageing experiments on Zorbax XDB-C_8 (double-endcapped dimethyl-C_8) columns, these units were sampled at the inlet, middle and outlet. These sections then were homogenized and subjected to elemental analysis as summarized in Table 3.

The aggressive ageing in phosphate/acetonitrile at 60°C resulted in significantly higher-than-initial carbon values for the aged packing, with the carbon content decreasing from column inlet to outlet. Bonded alkylsilanes apparently are slowly (if at all) hydrolyzed under pH 7 conditions [5,6]. Therefore, carbon concentration is then higher at the inlet because of the less-dense packing caused by silica dissolution. This means that the carbon content is higher for aged packing caused by lower-density silica where the support has been most dissolved.

Table 3
Elemental analysis of aged Zorbax XDB-C_8 column (double-endcapped dimethyl-C_8)

Mobile phase for ageing	Column section	% Carbon
Untreated (initial)	–	7.71
0.25 *M* phosphate–ACN (80:20)	Inlet	9.09
	Middle	8.53
	Outlet	8.28
0.25 *M* Tris–ACN (80:20)	Inlet	7.64
	Middle	7.69
	Outlet	7.65
0.25 *M* phosphate–MeOH (80:20)	Inlet	8.45
	Middle	8.18
	Outlet	7.90

On the other hand, Table 3 shows that the carbon content of the packing was hardly affected under the same conditions with Tris–acetonitrile ageing. No variation of carbon content down the column was found, within experimental error. These results indicate substantially higher silica support dissolution and lower column stability with phosphate buffers at pH 7 for endcapped columns, just as was previously found for non-endcapped columns [6]. The data in Table 3 and Fig. 2 further suggest that, contrary to that found for non-endcapped columns, methanol-modified mobile phases cause somewhat less column degradation by silica support dissolution than do acetonitrile-modified mobile phases.

3.3. Chromatographic studies

3.3.1. Endcapping effects

Direct comparison of the stability of endcapped

vs. non-endcapped columns is shown in Fig. 4. Here, the aggressive purge system with 0.25 *M* phosphate buffer at 60°C was used on non-endcapped and double-endcapped dimethyl-C_8 bonded on SolGel silica support. When tested with the neutral toluene solute, the non-endcapped column was degraded much faster than the endcapped column, as shown in Fig. 4A. Solute k' values for trimipramine in this ageing test are shown in Fig. 4B. The k' values for this highly basic amine are higher at the start for the non-endcapped column, suggesting a more acidic packing surface. The k' values for the non-endcapped column then increase at a faster rate than for the endcapped column, indicating a more rapid degradation of the non-endcapped packing surface to a more acidic and solute-retaining state. Results such as those in Fig. 4 suggest that endcapping ligands provide an additional barrier (lower surface energy, higher surface tension) that retards silica support dissolution in the intermediate (and higher) pH range.

3.3.2. Column comparisons

This part of the study was devised to compare the chromatographic properties and stability of various commercial bonded-phase columns reportedly designed for separating basic and highly polar compounds with a minimum of mobile phase additives. To compare the residual silanol activity of the

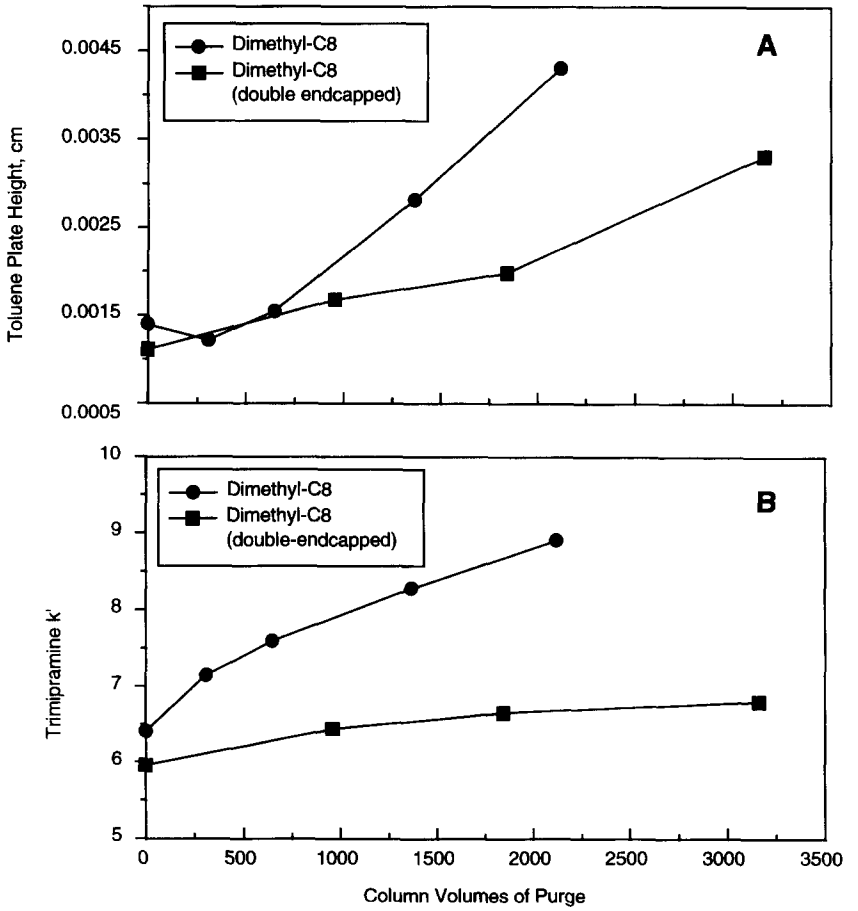

Fig. 4. Stability tests for non-endcapped versus endcapped C_8 columns. Columns: 15×0.46 cm; purge – mobile phase: methanol–0.25 *M* sodium phosphate buffer, pH 7.0 (20:80); 60°C; flow-rate: 1.0 ml/min; Test with toluene – mobile phase: methanol–water (80:20); flow-rate: 1.0 ml/min; 22°C; Test with trimipramine – acetonitrile–0.01 *M* sodium phosphate buffer, pH 7.0 (60:40); flow-rate: 1.5 ml/min; 40°C; sample: 5 μl containing 0.125 μg trimipramine.

selected columns, a modified "Engelhardt" test [18] was used. This test monitors the elution and peak shapes for basic and acidic compounds without buffer or other modifiers in the mobile phase (only methanol–water). Also, with this test, *m*- and *p*-toluidine should co-elute for "good" columns to indicate a low order of unwanted silanol interaction [18]. Fig. 5 shows the results with this test on the columns of this study. All columns showed that basic pyridine solute eluted prior to phenol, which is indicative of a surface of reduced acidic activity. The Symmetry-C_8 column co-eluted *m*-and *p*-toluidine, but pyridine showed a broad band that tailed into the aniline peak, suggesting the presence of some acidic sites on the packing surface. Hypersil BDS-C_8 produced very broad peaks for all solutes, suggesting poor wetting of the bonded phase with this mobile phase of 30% methanol in water. YMC-Basic overlapped pyridine and aniline, and also did not co-elute the sensitive *m*- and *p*-toluidine pair; acidic sites on the packing are suspected. Supelcosil ABZ+ produced excellent peak shapes, and co-eluted *m*- and *p*-toluidine; however, for unexplained reasons, phenol did not elute in this test. Inertsil-C_8 showed co-elution of the toluidine pair (with some band tailing), with also some tailing of pyridine. Zorbax XDB-C_8 eluted all peaks with good symmetry, with co-elution of the toluidine pair. Results of this unbuffered test may have questionable practical value since mobile phase buffers are recommended for methods with ionic compounds [9]. Nevertheless, the chromatograms of Fig. 5 show large differences in the columns, with some apparently having superior potential for separating basic and polar compounds under these conditions.

Since rugged and reproducible separation methods for ionic compounds should always be performed with a buffered mobile phase [9], a test with a buffered mobile phase was initiated to compare the relative stability of these columns. Fig. 6a shows the separation of a mixture of highly-basic tricyclic antidepressants prior to column ageing, using typical operating conditions for such basic compounds. To reduce the time and effort required, aggressive operating parameters were used in continuously purging (or "ageing") these columns: a mobile phase of acetonitrile–0.25 *M* sodium phosphate buffer, pH 7.0 (20:80), at 60°C. Periodically during this aggressive ageing, the columns were re-tested with the tricyclic antidepressant mixture. Fig. 6b shows the chromatograms obtained after about 2000 column volumes (3 l) of purging mobile phase. Note that two columns made with SolGel silica supports (Hypersil BDS-C_8 and Zorbax XDB-C_8) showed the least degradation in this aggressive test. Although Supelcosil ABZ+ column apparently was also made

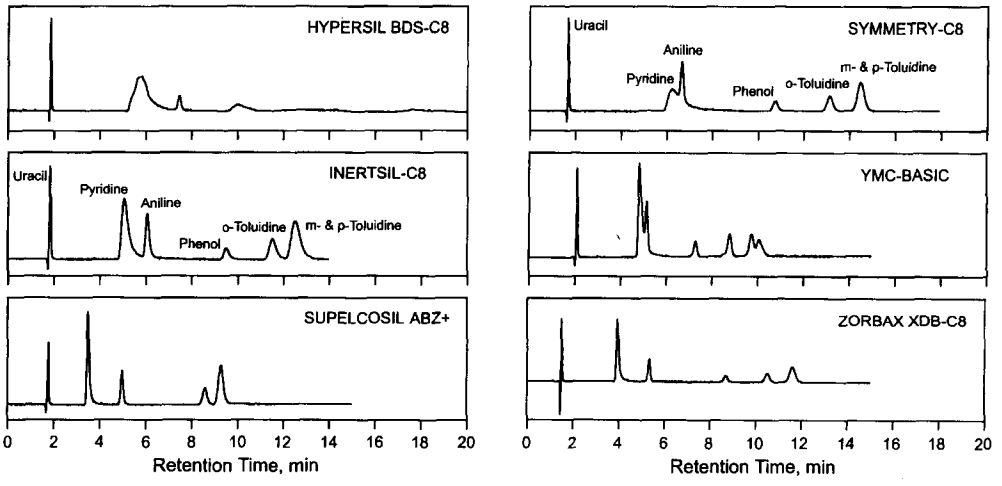

Fig. 5. Comparative separations with unbuffered mobile phase. Columns: 15×0.46 cm; mobile phase: methanol–water (30:70); flow-rate: 1.0 ml/min; ambient temperature; sample: 5 μl containing 0.025 μg uracil, 0.25 μg aniline, pyridine and phenol, and 0.25 μg each of *o*-, *m*- and *p*-toluidine.

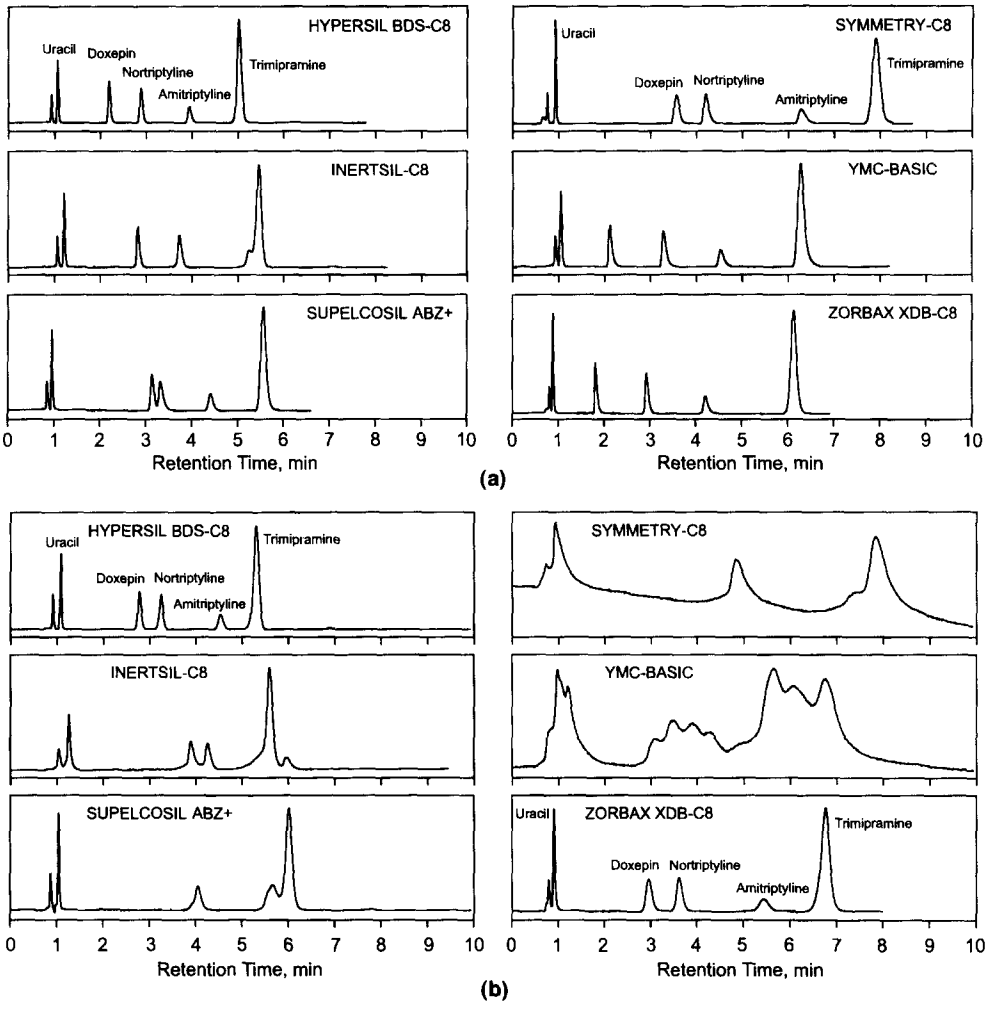

Fig. 6. Comparative separations with tricyclic antidepressant drug mixture. Columns: 15×0.46 cm. (a) Initial–mobile phase: acetonitrile–0.01 *M* sodium phosphate buffer, pH 7.0 (60:40); flow-rate: 1.5 ml/min; 40°C; sample: 5 μl containing 0.025 μg uracil, 0.125 μg doxepin, 0.25 μg nortriptyline, 0.125 μg amitriptyline and 0.25 μg trimipramine. (b) After ~2000 column volumes of purge with mobile phase: acetonitrile–0.25 *M* sodium phosphate buffer, pH 7.0 (20:80); flow-rate: 1.0 ml/min; 60°C; chromatographic test as in A.

with a SolGel support (Table 1), it was significantly degraded during this test. This result suggests that the polar amide stationary phase functionality and lack of endcapping may have contributed to a higher rate of silica support solubility (see also Fig. 1).

3.3.3. Effect of buffer type on column stability

As documented in Section 3.2.3 and in previous studies for non-endcapped alkyl bonded-phase columns [6], use of an organic (or borate) rather than a phosphate buffer greatly decreases silica support dissolution during column use. This trend is further verified in Fig. 7 for a double-endcapped dimethyl-C_8 column, comparing plate heights resulting from the purge of columns with phosphate, citrate and Tris buffers. Fig. 7A shows the plate height plots for the neutral solute, toluene, as a function of the column volumes of purge. Under the severe conditions of this test, the column quickly failed with the phosphate buffer. Better column stability and longer column lifetime were seen with the citrate buffer. By far the greatest column stability was experienced

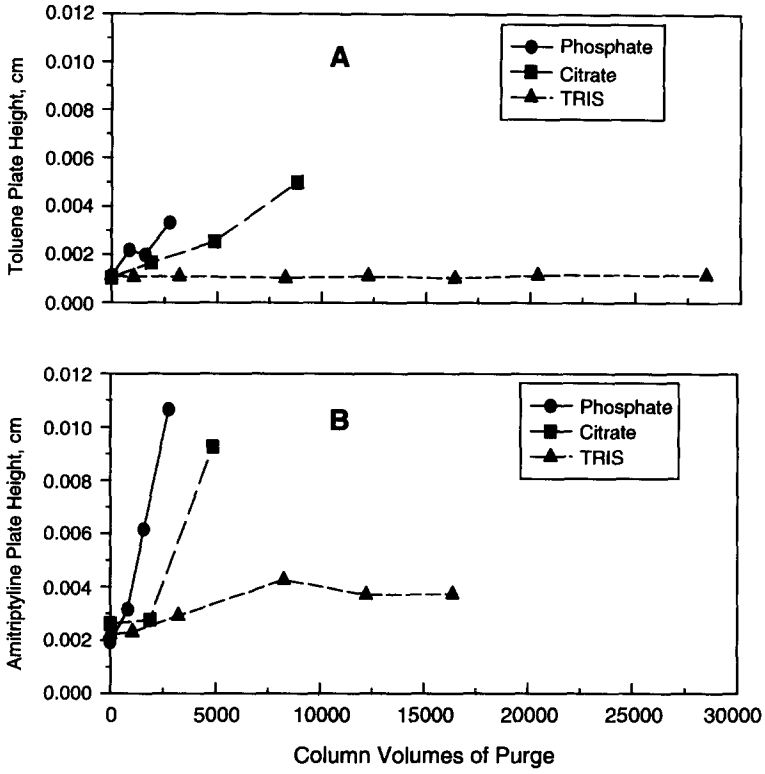

Fig. 7. Effect of buffer type on column stability. Columns: 15×0.46 cm Zorbax XDB-C_8; purge: acetonitrile–0.25 *M* buffer, pH 7 (20:80); 60°C; 1.0 ml/min; test: acetonitrile–0.01 *M* sodium phosphate buffer, pH 7.0 (60:40), 22°C; 1.5 ml/min; sample: 5 μl. (A) toluene, 220 μg. (B) amitriptyline, 0.125 μg.

using Tris buffer, with no change noted after almost 30 000 column volumes of purging. Fig. 7B shows comparable data for amitriptyline during this purging procedure. Poorest stability again was found for phosphate buffer, with citrate slightly better. For the Tris buffer, a ~50% plate height increase in the highly basic amitriptyline solute was observed, presumably because of minor changes in the silica support (becoming more acidic) during the test.

Curiously, with the endcapped columns in Fig. 7, citrate buffer did not show as much improvement in stability as with non-endcapped columns [6]. We speculate that the reason might involve the inability of highly-hydrophilic citrate to protect more highly hydrophobic endcapped surface sites. On the other hand, the more hydrophobic and cationic Tris molecule seems able to bind tightly to the silica-based packing surface (both non-endcapped and endcapped) to provide effective protection against attack of hydrated hydroxyl groups that dissolve the silica support. Therefore, the use of basic organic buffers such as Tris, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), etc. [6] at pH 6 and higher for both non-endcapped and endcapped columns appears to be an effective approach for prolonging the lifetime of silica-based columns.

3.3.4. Effect silica support and endcapping

As indicated previously, the type of silica support and the bonding and endcapping procedures all have a strong influence on the stability of silica-based columns when used at intermediate (and higher) pH. Fig. 8 compares results for a single-endcapped dimethyl-C_8 column prepared with a SilGel silica support and a double-endcapped dimethyl-C_8 column made with a SolGel silica support. This study, performed under conditions that are typical of many separations, found that the single-endcapped SilGel-

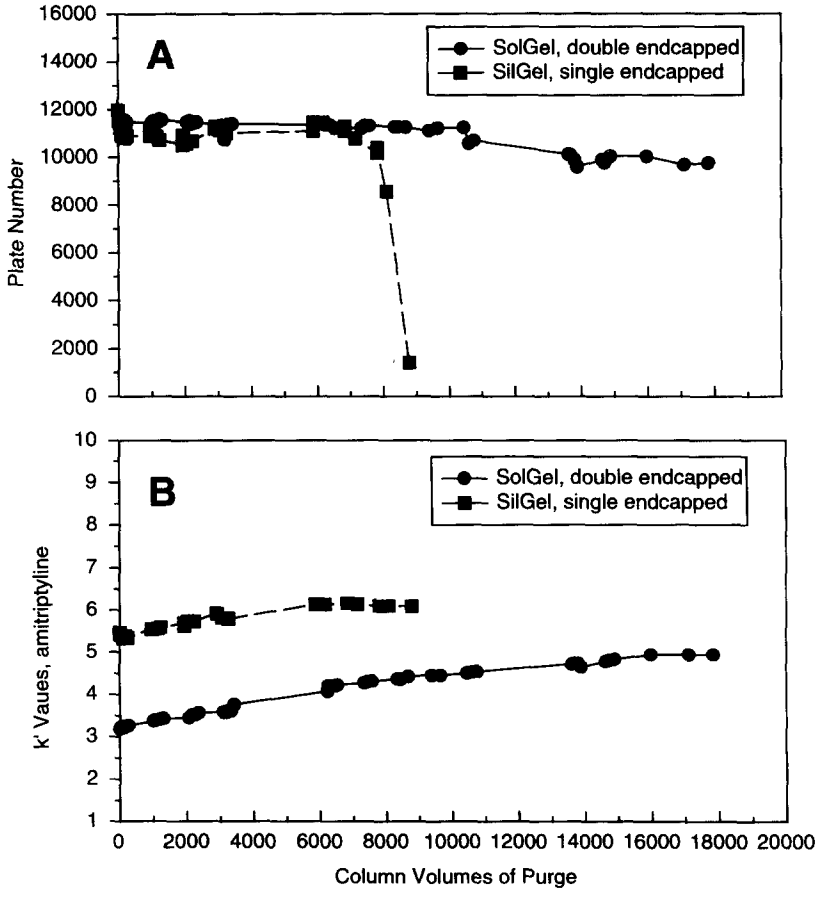

Fig. 8. Comparative stability of SilGel- and SolGel-based columns. Columns: 15×0.46 cm; mobile phase: acetonitrile–0.02 *M* sodium phosphate buffer, pH 7.0 (60:40); flow-rate, purge and test: 1.0 ml/min; 40°C; sample: 5 μl containing 0.125 μg amitriptyline. (A) Plate heights. (B) *k'* values.

based column failed after about 8 000 column volumes of continuous purge with acetonitrile-0.02 *M* sodium phosphate buffer, pH 7.0 (60:40) at 40°C, based on plate height values for amitriptyline, as shown in Fig. 8A. On the other hand, the double-endcapped column made with SolGel silica support still was functional under the same conditions after 18 000 column volumes, when the experiment was terminated arbitrarily. Note in Fig. 8B that the increase in *k'* values for the two columns during this purging experiment are initially somewhat comparable for the column volumes tested. The slight but steady increase in *k'* for this highly basic drug (pK_a=9.5) is suggestive of the exposure of more acidic silanol groups on the support surface during purging. This effect has been noted for a variety of silica-based columns used at intermediate pH [6,19]. The increased effectiveness of double versus single endcapping against column failure also has been noted for cyano bonded-phase columns [20].

3.3.5. Column stability studies at pH>11

Samples containing ionizable compounds, including basic solutes, are usually best separated with mobile phases of pH≤3 [7,8]. However, use of high pH (pH>9) mobile phases for separating highly basic compounds (e.g., basic drugs) may be desirable in some cases because of possible problems at low pH, as discussed in the Introduction. With high pH mobile phases, basic compounds are in the free (non-ionized) state, and unreacted silanol groups on the silica support are completely ionized. Therefore,

this high pH condition minimizes any unwanted ionic interactions between basic solutes and the silica support that might occur at intermediate pH where partial ionization of solutes and silica surface can co-exist. While high pH operation should promote good peak shapes and column efficiency, the perceived instability of bonded-phase silica-based columns above pH 8 has been a major deterrent to use under these conditions.

Previous studies in these laboratories have confirmed earlier reports [1–3] that certain silica-based, bonded-phase columns can be used routinely for long periods to at least pH 9–10, if particular operating conditions are used. We previously showed that monofunctionally bonded, non-endcapped columns exhibit excellent stability at high pH when organic (e.g., Tris, glycine) and borate buffers are used [5]. Conversely, silica-based columns are rapidly degraded when carbonate and phosphate buffers are used at pH 10, and phosphate buffers can cause rapid degradation even as low as pH 7–8, especially at higher temperatures and higher buffer concentrations [5,6].

Failure of silica-based, bonded-phase columns at both intermediate and high pH is a result of support dissolution, causing collapse of the packed bed [5,6,10]. Therefore, a strong factor in the stability of silica-based columns at both intermediate and high

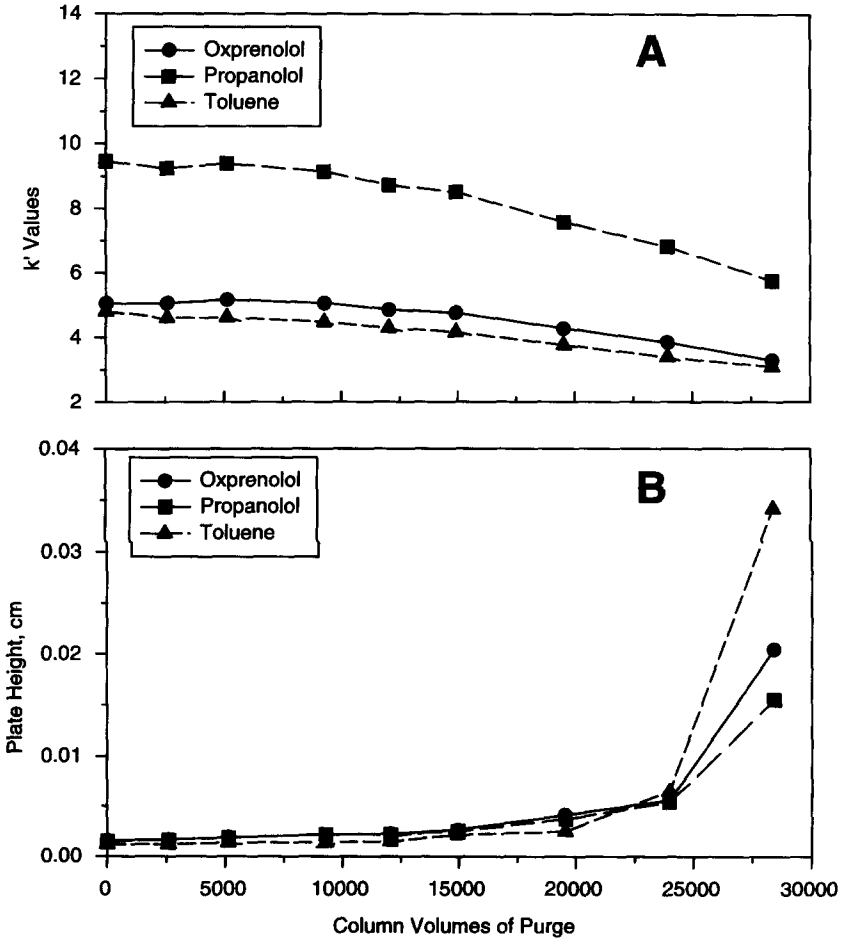

Fig. 9. Silica-based bonded-phase column degradation at pH 11.5. Column: 15×0.46 cm Zorbax XDB-C_8 (double-endcapped dimethyl-C_8 on SolGel silica support); mobile phase: methanol–0.05 *M* pyrrolidine·HCl buffer, pH 11.5 (55:45); flow-rate, purge and test: 1.5 ml/min; 40°C; UV, 215 nm; sample: 5 μl containing 2.1 μg oxprenolol and 0.42 μg propanolol. (A) *k'* values. (B) Plate heights.

pH is the type of silica support used [5,6]. Greatest resistance to dissolution is shown by the lower surface area, lower porosity SolGel packings (made by aggregating silica sols–see Table 1).

Previous studies have shown that silica-based columns can be used routinely at pH 11, providing proper operating conditions are maintained [21]. Densely-bonded, endcapped columns made from SolGel silica showed little change in plate numbers or peak asymmetry for highly-basic β-blocker drugs (pK_a=9.5–9.7) after use with about 30 000 column volumes of methanol–pH 11.0 1-methyl-piperidine buffered mobile phase at ambient temperature. Separation α values were also essentially unchanged. This test corresponded to more than 3 months of 8-h work days under these conditions.

Even more stringent tests with an organic buffer at pH 11.5 have now confirmed the capability of densely-bonded SolGel columns to operate effectively at high pH. Fig. 9 shows k' and plate height values for a densely bonded, double-endcapped dimethyl-C_8 (SolGel) column for two highly-basic β-blocker drugs and toluene after continuously (not recycled) purging with methanol–0.05 *M* pH 11.5 pyrrolidine·HCl buffer at 40°C. This column showed little change after about 10 000 column volumes, then slowly degraded with continued purging until it failed at about 25 000 column volumes under these conditions. Fig. 10 shows the initial chromatogram for this test, and the separation after about 15 000 column volumes of purge. Column efficiencies and band shapes were excellent at the beginning, and even after 15 000 column volumes, more than half the plate numbers remained and peak asymmetries were still adequate. More importantly, separation α values for these highly basic solutes remained essentially unchanged.

These and previous [21] results suggest that

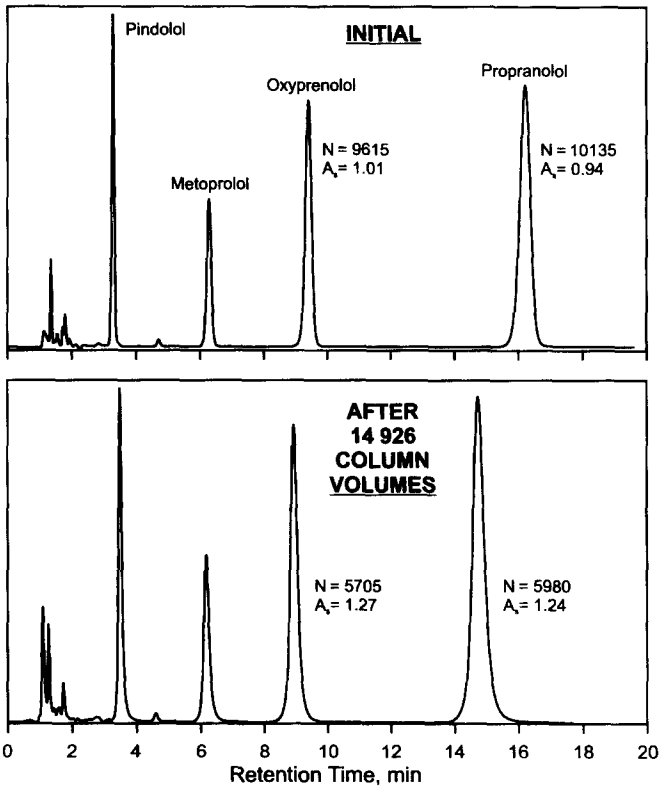

Fig. 10. Stability of silica-based bonded-phase column at pH 11.5. Conditions same as Fig. 9, except chromatograms at 1.0 ml/min; sample: 5 μl containing 0.40 μg uracil, 0.82 μg pindolol, 2.1 μg metoprolol and oxprenolol and 0.42 μg propanolol. (A) Initial chromatogram. (B) After 14 926 column volumes of continuous purge with pH 11.5 buffer at 40°C.

rugged methods for basic compounds at high pH (at least pH 11) are feasible, provided the following conditions are used: (i) columns of endcapped, densely alkyl-bonded SolGel silica support, (ii) organic-based buffers at a concentration of ≤50 m*M*, (iii) operating temperatures of ≤40°C.

4. Conclusions

As with previous studies with non-endcapped columns, these studies strongly suggest that columns made with silica supports derived from aggregating silica sols are more durable than SilGel silicas at intermediate and higher pH because of lower silica support solubility. These studies also show that endcapping the alkyl stationary phase further protects the silica support from dissolution that ultimately results in column failure. Wide variations in the stability of illustrative commercial C_8 columns with pH 7 phosphate-containing mobile phases were found. The functionalities associated with the stationary phase/endcapping methods used to prepare these commercial columns were identified by HF dissolution-derivatization, followed by GC–MS. Our dissolution and chromatographic experiments indicate significant differences in silica support solubility as a result of the type of silica support and the method of bonding/endcapping used. Densely bonded, double-endcapped stationary phases also appear to further increase column stability, presumably by reducing the solubility rate of the silica support. As with previous studies with non-endcapped columns, strikingly longer column lifetime at intermediate pH (e.g., pH 7) is obtained by using Tris (and other organic) mobile phase buffers, rather than phosphate-based buffers. Finally, rugged methods with mobile phases to at least pH 11 appear feasible if certain silica-based, bonded-phase columns are used at ≤40°C with organic-based buffers.

References

[1] J.G. Atwood, G.J. Schmidt and W. Slavin, J. Chromatogr., 171 (1979) 109.
[2] B. Wheals, J. Chromatogr., 187 (1980) 65.
[3] B. Law and P. F. Chan, J. Chromatogr., 467 (1989) 267.
[4] J.J. Kirkland and J.W. Henderson, J. Chromatogr. Sci., 32 (1994) 473.
[5] J.J. Kirkland, M.A. van Straten and H.A. Claessens, J. Chromatogr. A, 691 (1995) 3.
[6] H.A. Claessens, M.A. van Straten and J.J. Kirkland, J. Chromatogr. A, 728 (1996) 259.
[7] L.R. Snyder, J.L. Glajch and J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, 1988, Ch. 9.
[8] M.A. Stadalius, J.S. Berus and L.R. Snyder, LC·GC, 6 (1988) 494.
[9] J.J. Kirkland, LC·GC, 14 (1996) 486.
[10] J.J. Kirkland, J. Chromatogr. Sci., 34 (1996) 309.
[11] J. Köhler and J.J. Kirkland, J. Chromatogr., 385 (1987) 125.
[12] J.J. Kirkland, C.H. Dilks, Jr. and J.J. DeStefano, J. Chromatogr., 635 (1993) 19.
[13] J.J. Kirkland and J. Köhler, US Patent, 4 874 518 (Oct. 17, 1989).
[14] J.J. Kirkland and J. Köhler, US Patent, 5 032 266 (July 16, 1991).
[15] R.K. Iler, The Chemistry of Silica, Wiley, New York, 1979, p. 97.
[16] L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, Wiley, 1979, Ch. 5.
[17] S.D. Fazio, S.A. Tomellini, H. Shik-Hsien, J.B. Crowther, T.V. Raglione, T.R. Floyd and R.A. Hartwick, Anal. Chem., 57 (1985) 1559.
[18] H. Engelhardt and M. Jungheim, Chromatographia, 29 (1990) 59.
[19] J.J. Kirkland, Rockland Technologies, unpublished studies, 1995.
[20] J. Fiorianti, M. Debellis, J. Arraiano, and A.W. Salotto, Book of Abstracts, 210th ACS National Meeting, Chicago, IL, August 20–24, Issue Pt. 1, CHED-146. American Chemical Society, Washington, DC, 1995.
[21] J.J. Kirkland and J.J. DeStefano, CIT Special-Chromatography International, GIT, Darmstadt, June, 1996, p. 62.