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Effect of buffers on silica-based column stability in reversed-phase high-performance liquid chromatography

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Abstract

Previous studies have shown that bonded-phase packing degradation at pH 9–10 mainly is due to silica support dissolution, and does not primarily result from the hydrolysis of covalently attaching siloxane bonds. Column stability also is significantly affected by the type and concentration of organic mobile-phase modifier. We now find that silica-based bonded-phase packings variably degrade with buffers containing different anions and cations. This effect is especially apparent with intermediate- and high-pH buffers. Under the same conditions, pH 10 aqueous carbonate and phosphate buffers with 50% methanol degraded bonded-phase packings much faster than borate and glycine buffers. The nature of the buffer cation also influences bonded-phase packing stability, with column lifetime a function of sodium>potassium> ammonium cations. The rate of bonded-phase packing degradation at pH 7–10 increases with higher concentrations of certain buffers, but especially phosphate. Column degradation is very strongly influenced by temperature. Certain mobile phase-buffer conditions can lead to increased column lifetime, so that practical operation up to pH 10 appears possible for some silica-based columns.

Keywords: Column stability; Stationary phases, LC; Mobile phase composition; Buffer composition

1. Introduction

A previous study has shown that degradation of silica-based bonded-phase column packings for reversed-phase high-performance liquid chromatography (HPLC) at high pH mainly is a result of silica support dissolution [1]. Further, this investigation found that column-packing degradation is strongly affected by the type and purity of the silica support, and also influenced by the nature of bonded silane stationary phase. Surprising stability was found for certain C_{18} bonded-phase packings at pH 9–10, so that routine use at pH values higher than those previously indicated in the literature [2,3] (or suggested by many manufacturers) appears feasible [1,4]. These findings confirmed earlier studies indicating that silica-based column packings could be successfully used at pH>8 with high concentrations of organic modifier [5–8], and certain types and concentrations of salts in the mobile phase [9]. The type of silica support especially influences the stability of packed beds of silica-based particles with high-pH mobile phases. Column packings made with chromatographic silica synthesized by the aggregation of silica sols consistently shows greater stability at high pH than xerogel-type silicas [1].

Many workers now perceive that both basic and acidic compounds often are best separated in re-

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versed phase at low pH where both the solutes and the silanols on the silica support are protonated [10,11]. Such separations usually are more stable and reproducible when the mobile phase pH is maintained at pH<3, where retention variations are less pH-sensitive. As a result, a large fraction of applications reported in the literature now use low pH operation.

However, there are occasions where reversed-phase separations at low pH are less desirable than at higher pH. Reasons for developing methods at intermediate (or higher) pH include:

1. Compounds of interest are unstable at low pH
2. Desired selectivity cannot be obtained at low pH
3. Belief that bonded-phase columns are unstable at low pH
4. At low pH, protonated hydrophilic basic compounds are too poorly retained

This study provides additional information on the stability of silica-based bonded-phase packings in both intermediate- and high-pH aqueous mobile phases. It is widely recognized that many silica-based column packings are unstable when operated at high pH. However, it is not well known that silica-based, reversed-phase packings can show less-than-desired stability at intermediate pH (e.g., pH 7), depending on the operating conditions used. We find that careful selection of column type, mobile phase and other experimental variables can significantly improve the stability and reliability of such columns. Of special interest was the influence of mobile-phase buffer type, concentration and temperature on column stability.

2. Experimental

2.1. Chromatographic columns, reagents

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 [12,13]. Physical and surface properties of these bonded-phase columns were summarized in Ref. [1], and the characteristics of the silica support are described in Refs. [13–15]. The dimethyl-C_{18} stationary phase for the Zorbax Rx-C18 column is densely bonded (3.3 μmol/m^2) on an ultra-pure Type B support (80 Å pores, 180 m^2/g), and this packing is not endcapped. Zorbax SB-CN has a densely bonded monofunctional diisopropyl-3-cyanopropylsilane stationary phase at 2.1 μmol/m^2 (not-endcapped) on the same Type B silica support. Comparable columns are available from Mac-Mod Analytical (Chadds Ford, PA, USA). Columns were prepared by conventional slurry-packing methods [16]. HPLC-grade solvents used for separations were from EM Science (Gibbstown, NJ, USA).

2.2. Silica support solubility study

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. After preparation of a buffer of a specific pH, it was diluted with the appropriate amount of organic modifier; pH 10 eluent series: methanol–0.1 *M* buffer pH 10.0 (50:50, v/v); pH 7 eluent series: acetonitrile–0.01 *M* sodium phosphate buffer pH=7.0 (20:80, v/v) and acetonitrile–TRIS buffer, pH=7.1 (20:80, v/v); buffer concentrations: 0.01, 0.05 and 0.25 *M*.

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 (e.g., see [8]). Test were conducted at 25, 40 and 60°C for some systems. All columns were flushed for 10 min with a mixture of 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 5- or 10-min period (total: 5 or 10 ml).

Dissolved silica concentrations were measured colorimetrically in collected fractions using the well known silicomolybdate complex method [17]. 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 was overcome by removing phosphate prior to the silica determination. Phosphate was precipitated by adding calcium chloride to create insoluble calcium phosphate. This precipitate was centrifuged, then filtered from sample solutions prior to silica measurements. The reliability of the colorimetric method was determined by running calibrations with known amounts of silica for several concentrations of phosphate buffers. Fig. 1 shows the reproducibility of duplicate silicate calibrations performed on different days with the acetonitrile–0.25 *M* phosphate buffer system. This level of reproducibility provided credibility that soluble silica could be measured with good accuracy in the presence of phosphate that might otherwise interfere with the molybdate reaction. Individual silicate calibrations were prepared for the different types and concentrations of buffers used in the dissolution experiments.

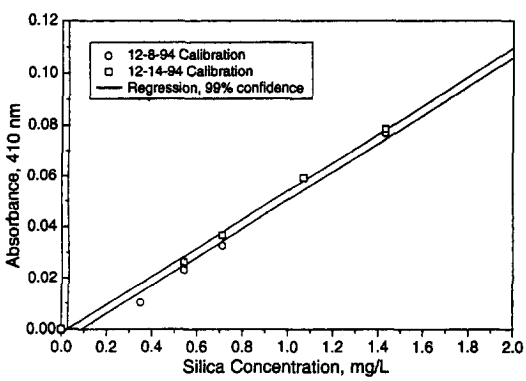

Fig. 1. Calibration reproducibility in phosphate buffer for silicomolybdate colorimetric method; duplicate calibrations acetonitrile–0.25 *M*, pH=7.0 phosphate buffer.

Results from the colorimetric measurements made for the concentration of dissolved silica in the eluents were plotted as a function of column effluent volume. 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

Apparatus and reagents

Analytical-grade methanol, acetonitrile, hydrochloric acid, sodium hydroxide, potassium hydroxide, citric acid, tris(hydroxymethyl)aminomethane-free base (TRIS), 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, and N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulphonic acid-free base) (HEPES). Test solutes were from Chem Service (West Chester, PA, USA) and Sigma (St. Louis, MO, USA), and were used as received. Column purging studies were performed with a Shimadzu Model LC-600 pump (Tokyo, Japan). Chromatographic testing studies for the pH 7 and 8 studies used a DuPont Model 860 pump and a Model 860 UV absorbance detector. Chromatographic samples were injected with a Rheodyne Model 7125 sampling valve (Cotati, CA, USA).

Phosphate buffers were prepared by mixing appropriate 0.25, 0.05 and 0.01 *M* NaH_2PO_4 and Na_2HPO_4 solutions to obtain the desired pH. Citrate buffer at pH 6.5 was made by titrating 0.25 *N* citric acid with 0.25 *N* sodium hydroxide. (At 60°C, the actual pH of this buffer is probably closer to pH 6.7.) TRIS and HEPES buffers were prepared by titrating appropriate concentrations of the free bases to the desired pH with equivalent concentrations of hydrochloric acid solutions.

Column ageing procedures

For the pH 7 study, 15×0.46 cm Zorbax SB-CN columns (Rockland Technologies, Newport, DE, USA) were continuously purged (1.0 ml/min, *not* recycled) with 35% methanol–65% various pH ~7, 0.25 *M* buffers at 60°C. Buffer solutions were prepared at pH values to maintain good buffering capacity (within about one pK_a unit of the buffering agent). These columns then were periodically tested first with toluene solute (uracil as t_0 marker) using a mobile phase of 60% methanol–40% water at ambient temperature, 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 20% acetonitrile–80% 0.01 *M*, pH 7.0 sodium phosphate buffer at 40°C, 1.0 ml/min. 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.

For the pH 8.0 tests, 15×0.46 cm Zorbax Rx-C18 columns were continuously purged (1.0 ml/min, *not* recycled) with 20% methanol–80% of phosphate or TRIS buffers, pH 8.0 at 40 or 60°C. Periodically during the purging, the columns were flushed with about 20 column volumes of 35% methanol–water, then equilibrated with about 20 volumes of the mobile phase for chromatographic testing. Purging was maintained until the columns showed serious chromatographic degradation. The test chromatographic separations were performed at various temperatures with 5 μl injections of a mixture of 0.1, 0.07 and 165 μg/ml, respectively, of secobarbital, doxepin and toluene, using a mobile phase of 40% acetonitrile–60% TRIS, 0.01 *M*, pH 7 at 40°C and 1.0 ml/min. Detection was at 254 nm.

3. Results and discussion

Use of silica-based, bonded-phase columns at high pH eventually results in reduced performance in reversed-phase chromatography because of deterioration of the column packing, largely through solubilization of the silica support [1–4]. However, the rate of degradation appears to depend on several factors: (a) the type of silica support; (b) the type and concentration of bonded phase; (c) mobile phase organic modifier; (d) buffers and additives; and (e) the operating temperature. A previous study has defined the effects of (a)–(c), silica support, bonded phase and organic modifier [1]. This work describes the effects of (d) and (e), buffer type and concentration plus temperature on the stability of silica-based reversed-phase columns at pH>6. With this information, the utility of silica-based, reversed-phase columns is significantly enhanced for intermediate and high pH operation.

As in the previous study [1], two different experimental approaches were used to obtain data needed to define the stability of columns under the desired operating conditions. In one approach, columns were continuously purged with various aqueous-organic mobile phases at different temperatures. Silicate dissolved in the mobile phase was measured with the well-known molybdate colorimetric method. With the second method, columns continuously purged with different buffers at varying temperatures were monitored chromatographically. We found that buffer type and concentration, column type, and temperature all have a strong influence on column stability and lifetime at both intermediate and high pH.

It should be noted that degraded bonded-phase–silica material may be retained within the column during purging experiments, and that this material may not be completely soluble in the purge mobile phase solvent used. As a result, actual column damage may be even worse than indicated by dissolved silica measurements. However, the aim of this part of the study was to measure the dissolved silica in the column effluent, and no attempt was made to determine degraded siliceous material remaining in the column. We expect that the overall practical effect of physically adsorbed, degraded column packing is not significant, since chromatographic results reported in the next section closely follow trends found for dissolution data.

3.1. Silica support solubility studies at pH 10

A previous study showed that a variety of commercial C18 columns are rapidly degraded with a mobile phase of 1:1 methanol–0.01 *M* pH 10 sodium

carbonate buffer at ambient temperature [1]. Columns with silica supports made by aggregating silica sols were much more stable under these conditions than those prepared with chromatographic silicas of the xerogel type. We were interested in determining if the stability and lifetime of silica-based, bonded-phase columns also was influenced by the types of anions and cations used in buffers, as occurs in the dissolution of unmodified chromatographic silica [9]. Accordingly, experiments were devised to test the effect of buffer types on bonded-phase columns. Silica support solubility experiments for this phase of the study used a model monofunctionalized dimethyl-C_{18} bonded-phase column with an ultra-pure Type B silica support, Zorbax Rx-C18 (see Sect. 2.1).

Anion effects

Fig. 2 shows the silica dissolved from this densely bonded dimethyl-C_{18} column during continuous purging with 0.1 *M* pH 10 buffers (sodium cation, except glycine). These results clearly show that a very large difference in the solubility of the silica support occurs with different buffer anions, with highest solubility resulting from carbonate. Surprisingly, the widely used phosphate buffer also aggressively dissolves the silica support for this bonded-phase, but less rapidly than carbonate. Borate and glycine buffers were much less aggressive towards the support, with silica dissolutions about ten-fold less than for phosphate buffer.

Why do carbonate and phosphate more vigorously attack silica at this pH than other buffers? The mechanism for this effect needs clarification. However, we speculate that carbonate and phosphate ions may complex with the silica surface at intermediate and high pH, weakening surface silica–siloxane bonds so that they are more readily attacked by hydrated hydroxyl ions. The effect of phosphate ions in solubilizing silica at pH 7–10 apparently is entirely different than for low pH, where the addition of only 0.06% H_3PO_4 greatly reduces the solubility of silica [18]. These effects suggest that only the di- and tri-ionic forms of phosphate may be responsible for the strong tendency for silica dissolution (also see data in Sect. 3.2 below).

The data in Fig. 2 strongly indicate that column lifetime for silica-based, bonded-phase columns can be measurably improved by using borate or glycine buffers (and other organic-based buffers), rather than commonly used phosphate and carbonate buffers. The results in Fig. 2 also suggest that densely bonded monofunctionalized C_{18} columns with supports made from the aggregation of silica sols might be safely used with borate or glycine buffers up to pH 10. This conclusion is supported by previous studies indicating that certain silica-based C_{18} columns can be used successfully up to at least pH 9 with some mobile phases [1,4,7].

Cation effects

The influence of various pH 10 phosphate buffer cations on the solubility of the silica support for Zorbax Rx-C18 columns is illustrated in Fig. 3.

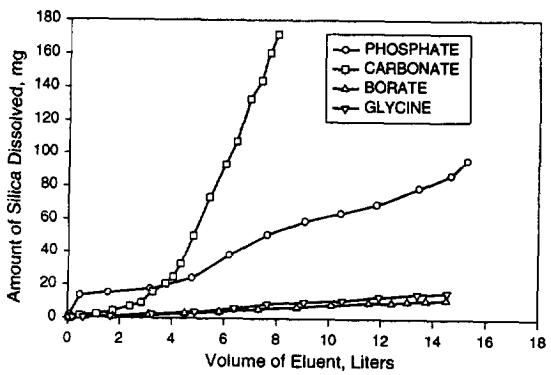

Fig. 2. Effect of pH 10 purge buffer anions on silica support dissolution. Columns: Zorbax Rx-C18, 15×0.46 cm; purge: 50% methanol–50% 0.1 *M* buffers, pH=10; 1.0 ml/min; 25°C.

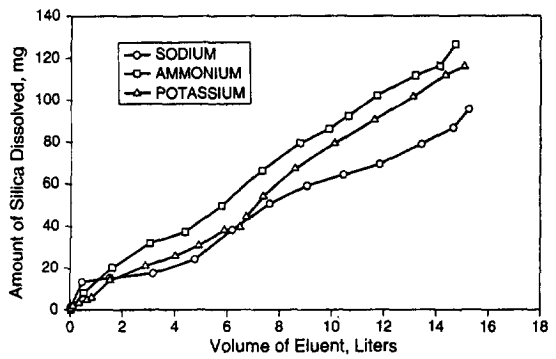

Fig. 3. Effect of pH 10 phosphate buffer cation on silica support dissolution. Conditions same as for Fig. 2, except different buffer cations.

While differences due to the different cations are not gross, lowest solubility was shown for sodium phosphate at this pH, followed by potassium, then ammonium. Interestingly, this pattern directly correlates with the solubility of many salts in water: ammonium>potassium>sodium, which may provide a reasonable explanation for the observed differences in the solubility of the silicate from silica support dissolution.

The effect of certain cations on support solubility in the presence of borate buffer is shown in Fig. 4. Initially, lithium borate buffer causes significantly lower silica solubility than sodium borate. However, after significant purging the amount of silica dissolved is about equal for the two cations. The lower solubility of lithium silicate would suggest that the lithium borate buffer would always result in slower silica support solubility. Therefore, these data suggest that silicate solubility may not be the only mechanism controlling degradation of the silica support.

3.2. Silica support solubility studies at pH 7

While the potential for appreciable solubility of silica supports at high pH (e.g., pH 10) is well known, it is not readily recognized that the silica support solubility also can be significant at intermediate pH values with some operating conditions. Therefore, we investigated the effect of phosphate buffer concentration and temperature at pH 7 to illustrate the magnitude of support solubility differences. Chromatographic results for similar pH studies are given in following sections.

Phosphate buffer concentration effects

Other reports on silica solubility suggest that the concentration of salts can significantly affect the solubility of silica [19]. Literature accounts have suggested that the rate of solution is enhanced at higher temperatures, and in solutions containing a minimum of organic solvents [1,5,6]. Accordingly, we tested the solubility of the silica support for Zorbax Rx-C18 columns with different sodium phosphate buffer concentrations, using low organic modifier concentrations and higher temperatures to magnify effects by increasing silicate solubility.

Results in Fig. 5 show that the solubility of the silica support does increase with buffer concentration, as might be expected. However, the effect is not drastic, with 0.25 *M* buffer dissolving about one-third more than 0.01 *M* in this experiment. Mobile phases with methanol cause greater silica support solubility than an equivalent concentration of acetonitrile [1]. These results suggest that better column lifetime can be expected at lower buffer concentrations. However, mobile phase buffer concentrations always should be adequate to ensure that the pH does not vary during the separation as sample components pass through the column. For most separations, buffer concentrations of 0.01–0.05 *M* usually are adequate.

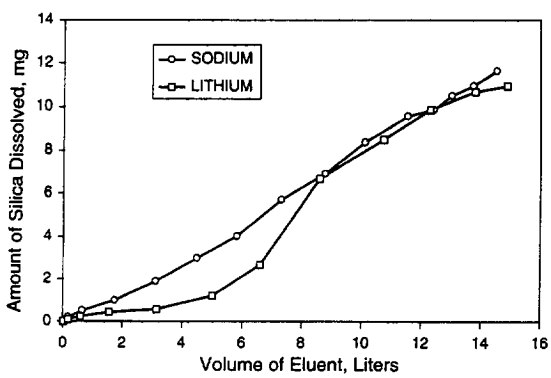

Fig. 4. Effect of pH 10 borate buffer cations on silica support dissolution. Conditions same as for Fig. 2, except sodium and lithium borate buffers.

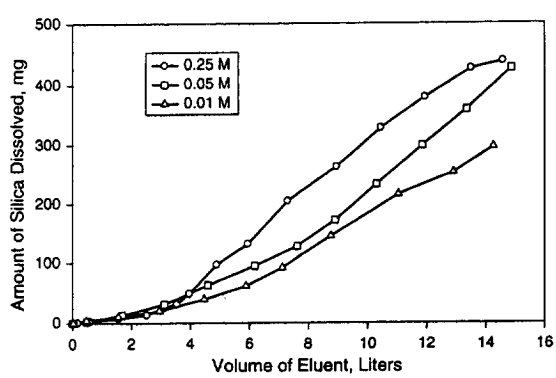

Fig. 5. Effect of pH 7 phosphate buffer on silica support dissolution. Columns: Zorbax Rx-C18, 15×0.46 cm; purge: 20% acetonitrile–80% buffer, pH=7.0; 1.0 ml/min; 60°C.

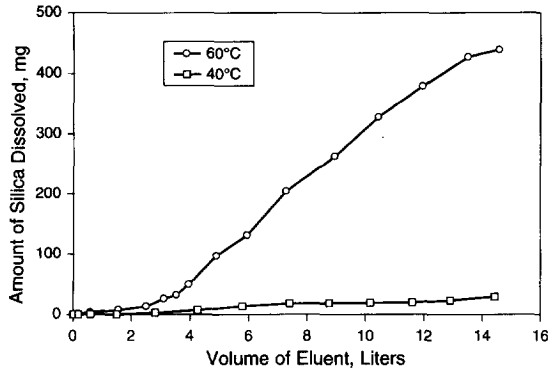

Fig. 6. Effect of temperature with pH=7.0 phosphate buffer on silica support dissolution. Conditions same as Fig. 5, except buffer, 0.25 *M*; 60 and 40°C.

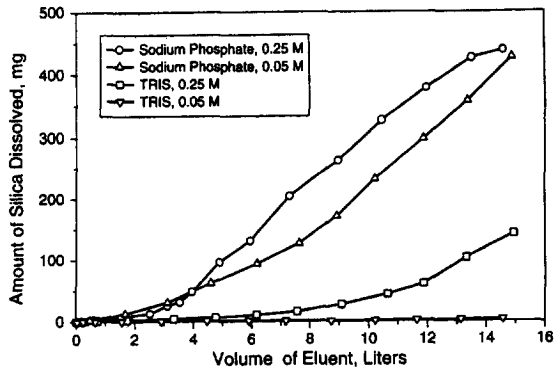

Fig. 7. Effect of buffer type and concentration on silica support dissolution. Columns: Zorbax Rx-C18, 15×0.46 cm; purge: 20% acetonitrile–80% sodium phosphate and TRIS buffers, pH=7; 1.0 ml/min; 60°C.

Temperature effects

The solubility of unmodified silica increases substantially with temperature increase [19]. Fig. 6 shows the change in the solubility for the silica support of a bonded-phase packing (Zorbax Rx-C18 columns) with pH 7 sodium phosphate buffer at 60°C versus 40°C. The surprisingly large increase in solubility at 60°C strongly suggests that separations with pH 7 phosphate buffer should be maintained at no more than 40°C for better column lifetime and more rugged separations.

Effect of buffer anion

Results from the pH 10 studies above showed that phosphate buffers aggressively dissolved the silica support from a dimethyl–*n*-octadecylsilane bonded-phase packing. Much lower silica support solubility resulted with an organic buffer at the same pH (Fig. 2). We found that the same effect also occurs at pH 7, as shown in Fig. 7. At 60°C and pH 7, much larger amounts of silica support were solubilized with sodium phosphate buffer than for TRIS, a model organic buffer. While only moderate differences were found between 0.25 *M* and 0.05 *M* phosphate buffer concentrations (see also Fig. 5), larger silica solubility differences were seen for 0.25 *M* and 0.05 *M* TRIS buffers. These data support the conclusion that, relative to widely used phosphate buffers, silica-based column lifetime at pH 7 can be prolonged significantly by using organic based-buffers (i.e., TRIS) at lower concentrations (i.e., ≤0.05 *M*) and at temperatures no more than 40°C.

3.3. Chromatographic studies at pH 7: cyano column

Effect of buffer anion

To study the chromatographic effect of buffer anions at pH 7, the highly aggressive conditions of 80% 0.25 *M* buffer concentration and 60°C operation were selected. In addition, a short-chain bonded phase (Zorbax SB-CN) was used as the test column. {Previous studies have shown that the length of the bonded-phase ligand chain significantly affects the stability of the stationary phase—longer bonded phases (e.g., C_{18}) are more stable than shorter phases (e.g., CN, C_3; [20]).} These conditions shortened the experiment time needed for significant differences to be seen in column performance. Fig. 8 shows toluene retention time results for columns continuously purged with various pH ~7 buffers under these aggressive conditions. Previous studies have shown that the retention of neutral hydrophobic solutes (e.g., toluene) are an accurate measure of the relative amount of organic stationary phase remaining on the surface of degraded bonded-phases columns [20]. These purging conditions were so aggressive for this column type that k' values significantly decreased with all buffers, indicating gross dissolution of the silica support and a loss of stationary phase. (This loss in stationary phase probably occurred by me-

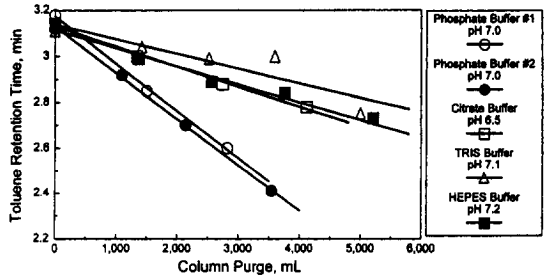

Fig. 8. Effect of buffer type on columns purged at pH ~7, 60°C. Columns: Zorbax SB-CN, 15×0.46 cm; purge: 20% acetonitrile–80% 0.25 *M* buffer at 1.0 ml/min, 60°C; chromatographic test: 60% methanol–40% water, 1.0 ml/min, 22°C, UV at 254 nm; solute: toluene.

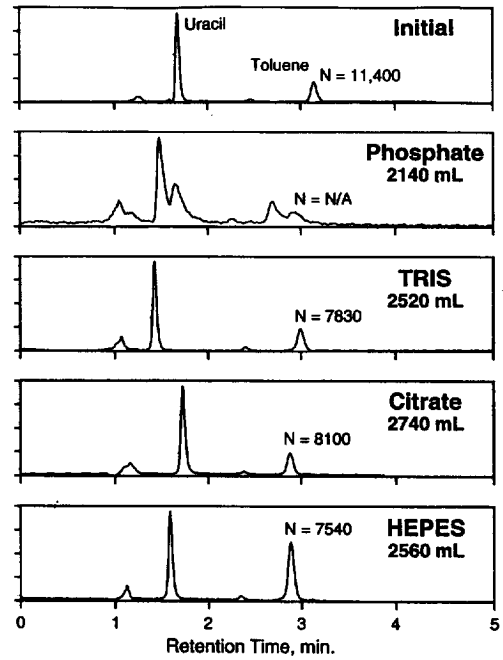

Fig. 9. Chromatograms with columns purged at pH ~7, 60°C with different buffers. Columns: Zorbax SB-CN, 15×0.46 cm; purge conditions same as for Fig. 8; chromatographic test: same as for Fig. 8.

chanical attrition, since other studies have shown that the siloxane bond is not hydrolyzed appreciably even at pH 9 [1,4].) More importantly, duplicate experiments showed that phosphate buffer much more aggressively dissolves the silica support than citrate, TRIS and HEPES buffers, all of which gave similar results.

Fig. 9 shows chromatograms for these columns after about 2 l of purge with these aggressive pH ~7 conditions. For phosphate buffer, the neutral solute, toluene, and t_0 marker, uracil, showed double peaks after purging, compared to the chromatogram before purging. TRIS, citrate, and HEPES showed minor changes, but much less than phosphate. Even more dramatic differences occurred with a mixture of highly basic tricyclic antidepressants in the same test, as shown in Fig. 10. Fig. 10A gives chromatograms after about 1 and 3 l of purge with phosphate and TRIS under these aggressive conditions. Gross changes occurred with the phosphate-purged column after only about 1 l of purge, compared to the initial chromatogram. Changes were so great that no attempt was made to identify peaks. After about 3 l of purge, the column was completely destroyed. When purging with TRIS, we found much less column degradation under the same conditions, as shown in Fig. 10A. Fig. 10B shows that citrate and HEPES buffers gave results similar to TRIS, with the HEPES-purged column probably exhibiting the least change of the buffers tested.

The columns in this study all exhibited voids in the inlets when the inlet frit was removed, strongly indicating that the silica support had dissolved during purging. Fig. 11 shows the measurement of voids created in the column inlets as a function of the buffer used. Columns purged with phosphate showed much larger voids than those purged with the other buffers. (The two phosphate-purged columns were purged with different volumes, with the larger purge volume creating the larger void). These results with different pH 7 buffers correspond closely with the silica support solubility data shown in Fig. 2 and 7. Widely-used phosphate buffers clearly are more aggressive towards silica supports than comparable organic-based buffers.

Citrate buffers have been mentioned as being aggressive towards some stainless-steel hardware used in HPLC equipment. However, in these and other studies spanning a period of several months, there have been no obvious problems associated with the use of citrate buffers in the pH range herein used.

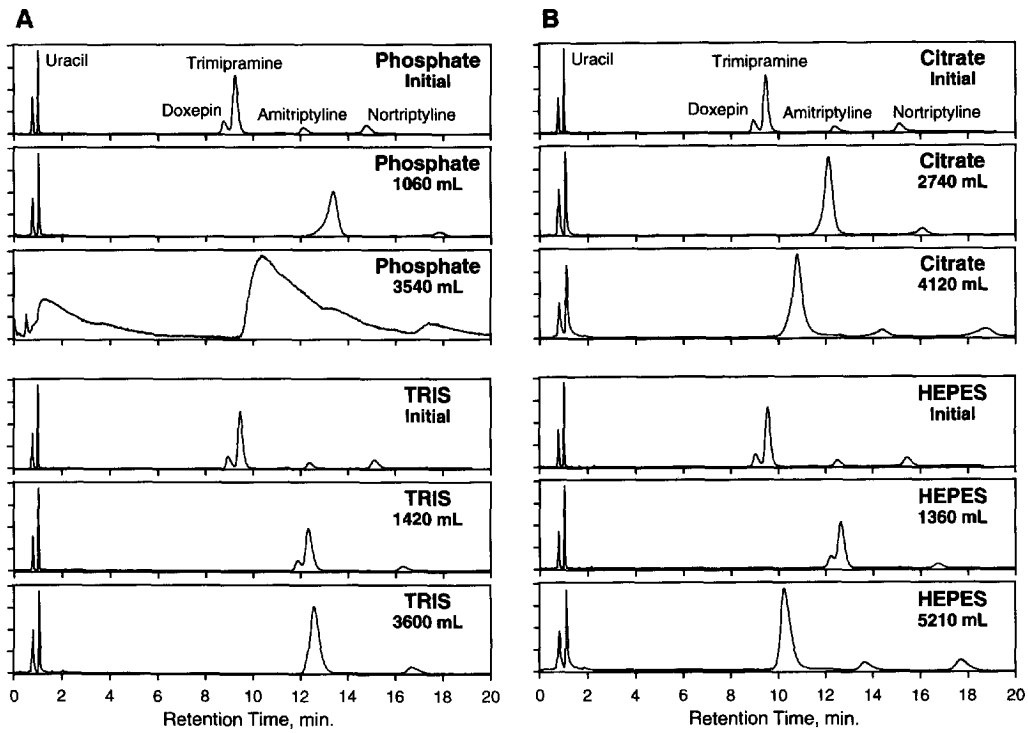

Fig. 10. Separations with columns purged at pH 7, 60°C with different buffers. Columns: Zorbax SB-CN, 15×0.46 cm; purge conditions same as Fig. 8; chromatographic test: 20% acetonitrile–80% 0.01 *M* buffer, pH ~7.0, 40°C; solutes: tricyclic antidepressants. (A) Sodium phosphate and TRIS buffers. (B) Citrate and HEPES buffers

3.4. Chromatographic studies at pH 8: C_{18} column

Chromatographic column stability studies were conducted using conditions similar to those described above for silica support dissolution, including the same non-endcapped, monofunctionalized, dimethyl–*n*-octadecylsilane-bonded column, Zorbax Rx-C18. The only difference was that the buffer was set at pH 8, to gain wider information regarding the effect of pH on column stability. For these chromatographic tests, a mixture of secobarbital (acidic), doxepin (basic) and toluene (neutral) was used. Both secobarbital and doxepin are ionized in the pH 8.0 purge and subsequent pH 7.0 chromatographic tests. Under the test conditions used (no basic mobile phase additive), doxepin produces a tailing peak with the non-endcapped C_{18} column used. In these studies, column performance was compared after purging with phosphate and TRIS buffers, the latter again used as a model organic buffer.

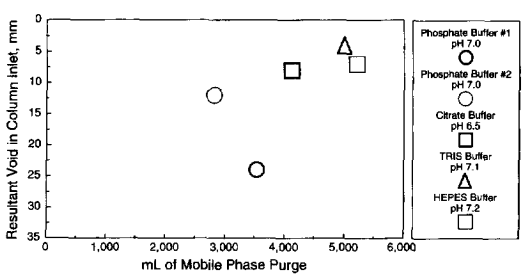

Fig. 11. Voids created in column inlets by buffer purge. Conditions same as Fig. 8.

Effect of phosphate buffer concentration

Phosphate buffer concentration has a significant effect on the solubility of silica-based, bonded-phase

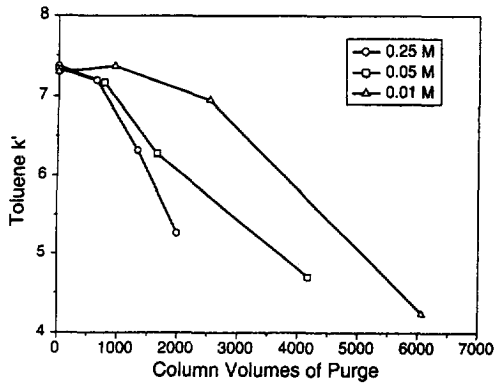

Fig. 12. Effect of pH 8 phosphate buffer concentration. Columns: Zorbax Rx-C18, 15×0.46 cm; purge: 20% methanol–80% sodium phosphate buffer, pH 8 at 1.0 ml/min, 60°C; chromatographic test: 40% acetonitrile–60% TRIS, 0.01 *M*, 40°C, 1.0 ml/min; solute: toluene.

supports, as previously described (Fig. 5). This effect also is found in chromatographic tests, as shown in Fig. 12. Purging the Zorbax Rx-C18 column with the aggressive 0.25 *M* phosphate system caused faster column degradation relative to lower concentrations, based on changes in toluene *k'*. (This very large decrease in retention for 0.25 *M* phosphate indicates gross solubility of the silica support.) Closely similar results were found for plate heights and peak asymmetry (not shown here).

Effect of buffer type, concentration and temperature

Data in Fig. 13 give the effect of buffer type and concentration on the stability of Zorbax Rx-C18 columns as measured by toluene at 60°C. In Fig. 13A, *k'* values decrease almost at the beginning of the phosphate buffer purge with 0.25 and 0.05 *M* buffers, with faster degradation at the higher concentration. With TRIS buffer, toluene *k'* values only begin to show a decrease after more than 5000 column volumes of purge with 0.25 *M* buffer. For the 0.05 *M* TRIS buffer, at least 10,000 column volumes produced no noticeable *k''* decrease. In Fig. 13B the same trend holds for toluene plate height values. Closely similar results also were found for secobarbital (not shown). Again, results show that phosphate buffers attack silica supports much more aggressively than TRIS under these conditions.

Temperature and concentration effects with phosphate and TRIS buffers are compared in Fig. 14. In Fig. 14A the *k'* values for secobarbital in phosphate buffers rapidly decrease with purge of the Zorbax Rx-C18 column. As expected, higher temperatures much more rapidly degrade the column because of the higher rate of silica support solubility. However, changes in *k'* with TRIS buffer are much slower. The plate height data in Fig. 14B closely correlate with the *k'* results of Fig. 14A. These results indicate that phosphate buffers much more quickly degrade column performance than TRIS, and higher temperatures dramatically increase the rate of deterioration. The neutral solute, toluene, gave closely similar results to those found for the acidic secobarbital (not shown).

The decisive influence of temperature compared to buffer concentration on column degradation was further illustrated by column purge tests as in Fig.

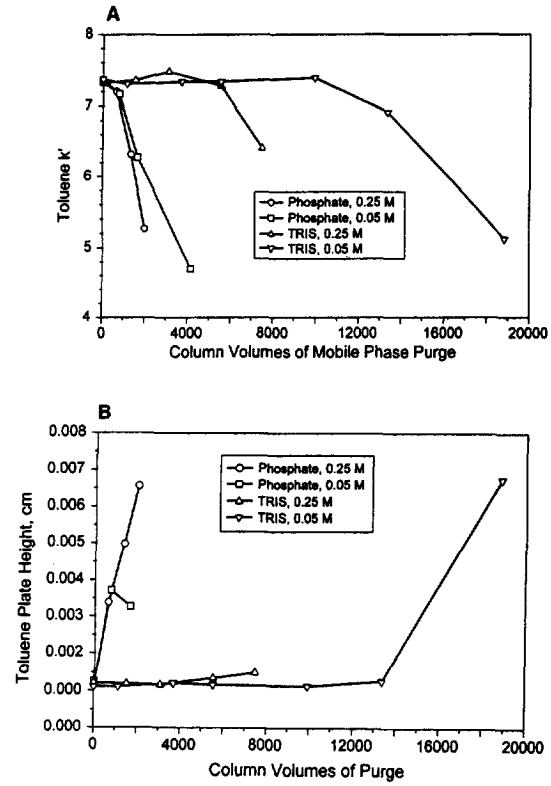

Fig. 13. Effect of pH 8 buffer type and concentration. Conditions same as Fig. 12, except sodium phosphate and TRIS buffers at indicated concentrations; solute: toluene. (A) *k'* values. (B) Plate height values.

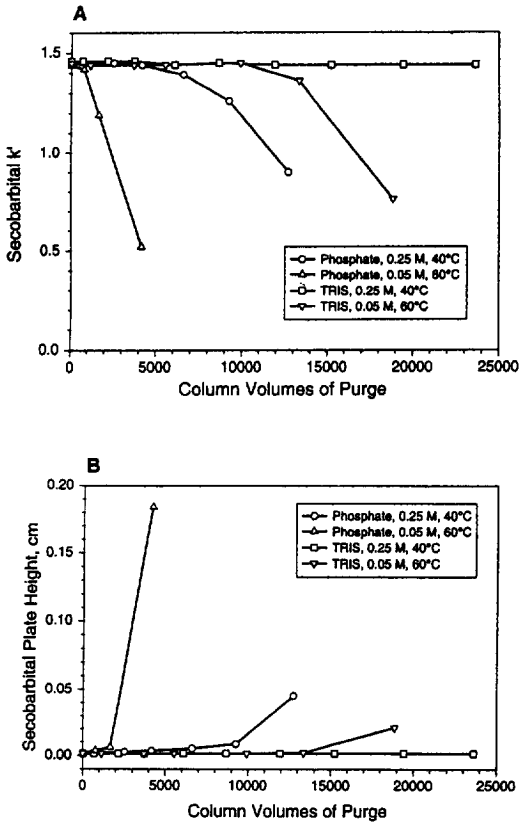

Fig. 14. Buffer type, concentration and temperature effects: secobarbital Conditions same as Fig. 12, except variable temperature; solute: secobarbital. (A) k' values. (B) Plate height values

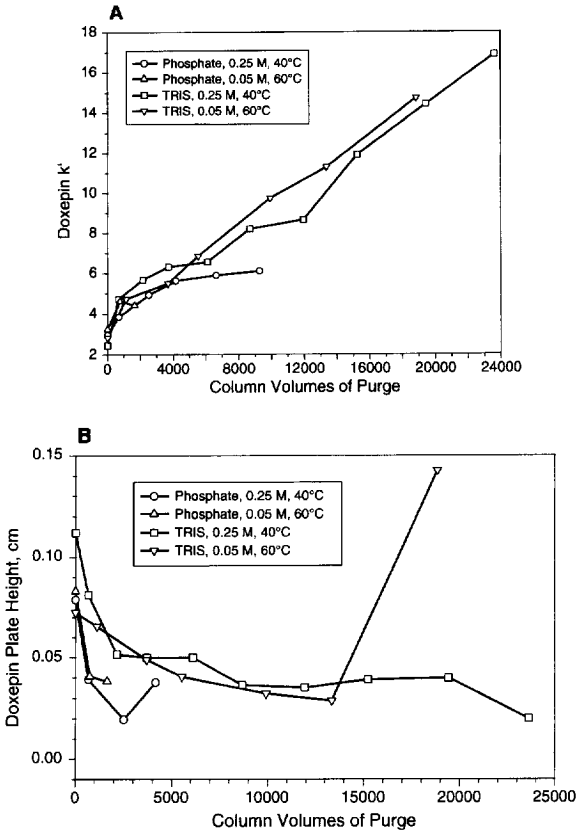

Fig. 15. Buffer type, concentration and temperature effects: doxepin. Conditions same as Fig. 12, except variable temperature; solute: doxepin. (A) k' values. (B) Plate height values.

14, but performed with 0.25 *M* phosphate and TRIS buffers at ambient temperature (22°C). After 35,000 column volumes of methanol–phosphate purge, k' values for toluene decreased by only 3% (secobarbital k' decrease, 5%), compared to no detectable decrease in k' values when methanol–TRIS purge was used. As shown in Fig. 14, these levels of degradation compare with the 29% and 38% k' decrease in toluene and secobarbital, respectively, when the temperature was raised to 40°C (Fig. 14).

Different effects were found for the basic doxepin solute, as shown in Fig. 15. Fig. 15A shows that as this non-endcapped column was purged with both buffers, the k' values for the strongly tailing doxepin peak increased, with only modest differences occurring with the different buffers, concentration and temperature. Little difference between buffer types is noted. Curiously, plate heights decrease under the purge conditions used, as shown in Fig. 15B. We speculate that with this C_{18} column, the buffer purge alters the silica support surface and the residual silanol groups on the bonded-phase packing. The noted decrease in plate height for doxepin suggests that the resulting combined hydrophobic–silanol-interaction adsorption isotherm becomes more linear with this treatment. It should be noted, however, that the precision for doxepin data is poor, because of the broad peak and the strong peak asymmetry found for this highly basic solute in the test system used.

In direct correlation with the silica support dissolution data given above, these chromatographic studies clearly show that silica is more aggressively attacked by phosphate, compared to organic-based buffers. Note that the densely bonded Zorbax Rx-C18 column used as a model in these experiments is

one of the more stable C_{18} commercial columns, as the silica support is formed by the aggregation of silica sol. Other silica supports based on xerogel silicas are less stable, and columns made from these materials might be expected to degrade much more rapidly [1]. Phosphate buffer concentration is a significant factor, but temperature is much more important. Our studies strongly indicate that phosphate buffers should be used at lower concentrations and lower temperatures for optimum lifetime of silica-based columns and maximum method ruggedness. Column lifetime and separation method ruggedness can be increased at both intermediate and higher pH by using other buffers such as TRIS, rather than phosphate.

4. Conclusions

Independent silica-support dissolution and chromatographic-lifetime studies show remarkable agreement in defining the degradation of silica-based column packings at pH 7–10. Silica support dissolution studies are useful in predicting the lifetime (and stability) of silica-based columns at intermediate and high pH operation.

Both silica dissolution and chromatographic studies indicate the following:

1. At pH 10, carbonate and phosphate buffers more aggressively dissolve the silica support and degrade bonded-phase packings faster than organic-based (e.g., glycine) and borate buffers.
2. At pH 7–8, bonded-phase packings also are more rapidly degraded by phosphate buffers than organic-based (e.g., TRIS) and citrate buffers.
3. At pH 7–10, bonded-phase packings are more rapidly degraded by higher column temperatures than higher buffer concentrations.
4. Silica supports for bonded-phase packings are more rapidly dissolved with buffer cations: NH_4>K>Na.
5. Short-chain bonded-phase packings (e.g., CN) are rapidly degraded at pH 7 with high concentration of phosphate buffers at higher temperatures (60°C).
6. Certain densely-bonded silica-based C_{18} columns can be routinely operated at pH≥9 with optimum mobile phases.

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