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Reversed-phase high-performance liquid chromatography of basic compounds at pH 11 with silica-based column packings

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Abstract

These studies describe additional ways in which silica-based column packings can be stabilized for routine use at high pH (pH 9–11). Excellent column stability at high pH is obtained by using densely-bonded, endcapped, longer-chain alkyl column packings with certain organic buffers. Column lifetime can be further extended by using untreated silica precolumns that partially saturate the mobile phase entering the analytical column. Highest stability was obtained with a new densely-bonded, double-endcapped bidentate-C_{18} silane stationary phase. Column packings with this material exhibit superior stability at pH 11, while maintaining the high column efficiency and excellent peak shapes that are characteristic of monofunctional bonded-silane silica-based columns. © 1998 Elsevier Science B.V.

Keywords: Stationary phase, LC; pH effects; Mobile phase composition; Precolumn; Basic compounds

1. Introduction

Separating basic compounds at high pH (>9) as free bases is attractive for routine analyses. Problems of unwanted ionic interactions are minimized as a result of the inability of the free bases to interact by ion-exchange with the totally-ionized, unreacted silanol groups on the silica-based packing. Although separations at high pH result in excellent peak shapes and column efficiency for basic compounds, chromatographers have been reluctant to use silica-based columns with high pH mobile phases because of questions regarding column stability.

Earlier reports have indicated that certain silica-based, bonded-phase columns can be routinely used at least to pH 9–10 in reversed-phase separations [1–3]. Recent studies in our laboratories confirmed these claims, and parameters such as silica support and bonded phase type, buffer type and concentration, and temperature have been systematically defined for optimum column performance, repeatability and stability in high pH use [4–8].

This study provides additional approaches for improving the stability of silica-based columns at high pH for routine separation methods. The use of precolumns (‘presaturators’) has been systematically investigated, and bonded phases useful for high pH tested. A new bonded-phase type exhibits strong potential as a preferred column packing material for high pH applications. This work suggests that silica-based column packings have a wider pH range of

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applicability for developing rugged separation methods than generally perceived.

2. Experimental

2.1. Chromatographic reagents, columns

HPLC-grade solvents were from EM Science (Gibbstown, NJ, USA). All 15×0.46 cm I.D. Zorbax columns were prepared at the Hewlett-Packard Newport Site. The porous silica microsphere support in these columns is a Type B silica formed by aggregating ultra-pure silica sols [9,10]. Type B silicas generally are the newer chromatographic supports that are highly purified and less acidic, leading to superior separations, especially for ionizable compounds. Physical and surface properties of this silica support have been reported [5,10–12]. Zorbax XDB-C8 and XDB-C18 columns are comprised of densely-bonded dimethyl-silane-substituted stationary phases exhaustively double-endcapped with dimethyl- and trimethylsilane groups by a proprietary process [8]. Columns of these packings are available from Hewlett-Packard (Wilmington, DE, USA). Column packings of the bidentate C18 stationary phase were prepared using technology similar to that previously described [13]. All columns were prepared by conventional slurry-packing methods [14].

2.2. Silica support dissolution studies

As in previous studies, columns were continuously purged with eluent using 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). The purge solutions for the dissolution studies were composed of acetonitrile–0.02 *M* potassium phosphate buffer, pH 11 (50:50, v/v).

2.2.1. Procedures

To duplicate actual chromatographic practice, columns were continuously purged at 1.5 ml/min with eluents and not recycled. The procedure is in contrast to 'simulated column aging studies' where packings are immersed in a static volume of mobile phase for a time period. Here, the chromatographic process actually is not simulated since the mobile phase becomes saturated with silica and further support dissolution cannot occur as in actual use. In the present study, dissolution tests were conducted with the mobile phase flowing at 1.5 ml/min with the columns thermostatted at 25°C. All columns were flushed for 10 min with acetonitrile–water (50:50, v/v) prior to the dissolution experiments. After beginning a specific dissolution experiment, we sampled the effluent after ~1 l had passed through the column, using a fraction collector. Column effluent samples for silicate analysis were collected for a 6-min period (9 ml total).

Silica concentrations dissolved from the columns were measured colorimetrically at 410 nm for collected fractions using the well-known silicomolybdate complex method [15]. For these silica measurements, 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 on the colorimetric method was eliminated by removing phosphate prior to silica measurement [5].

Results from the colorimetric measurements for the concentration of dissolved silica in the eluents were plotted as a function of effluent volume. First, the volume of eluent (V) between two consecutive fractions was calculated using the relationship ($V_{i+1}-V_i$). With this value and the measured concentrations of silica in the two consecutive fractions, the average silica concentration of two consecutive fractions ($\dot{C}_i$) was determined. Plots of the amount of silica dissolved (m) vs. eluent volume (V) then were obtained by integrating the silica concentration vs. eluent volume ($\Delta m/\Delta V$ vs. V) using the expression $m=\Sigma\dot{C}_i\Delta_i V$, where $\Delta_i V$ represents the effluent vol-

ume difference corresponding to two consecutive fractions.

2.3. Chromatographic column degradation studies

2.3.1. Apparatus and reagents

Analytical-grade methanol, acetonitrile and buffer components were from J.T. Baker (Phillipsburg, NJ, USA). EM Science (Gibbstown, NJ, USA) supplied HPLC-grade methanol and acetonitrile. Test solutes from Sigma (St. Louis, MO, USA) were used as received. Column purging ('aging') studies were carried out with a Shimadzu Model LC-600 pump (Tokyo, Japan). Chromatographic testing was with a Hewlett-Packard Model 1050 instrument and a DuPont Instruments column thermostat (Wilmington, DE, USA). Chromatographic data were recorded and processed with CHROMPERFECT version 6.02 software (Justice Innovation, Palo Alto, CA, USA). Plate height calculations were made using the half-peak-height method (Eq. 5.2 of [14]). Peak asymmetry values were determined by the ratio of trailing vs. leading band widths (at 10% of the peak height) defined by dropping a perpendicular from the peak apex to the baseline (Fig. 5.15 of [14]). Samples were injected with a Rheodyne Model 7125 sampling valve (Cotati, CA. USA). The bidentate C_{18} silane was synthesized at the Newport Site laboratories and reacted with Zorbax Rx-SIL [12] (80 Å, 180 m^2/g; Hewlett-Packard) by a proprietary process [16,17].

The phosphate buffer was prepared by mixing appropriate K_2HPO_4 and KOH solutions to obtain the desired pH 11. Triethylamine buffer was obtained by titrating 0.05 *M* 'Sequenal'-grade triethylamine (Pierce Chemicals, Rockford, IL, USA) with 5 *M* hydrochloric acid to pH 11. The 0.05 *M* 1-methylpiperidine buffer was made by titrating a solution of the free base (Aldrich, Milwaukee, WI, USA) to pH 11 with hydrochloric acid [7].

2.3.2. Column aging procedures, pH 11

Columns were continuously purged at 1.5 ml/min (not recycled) with an acetonitrile–0.017 *M* potassium phosphate pH 11 buffer (50:50, v/v) mixture at ambient temperature (23°C). (Note that all pH values relate to that of the buffer used and not the organic–buffer mixture.) These columns were periodically tested with toluene using a methanol–deionized water (80:20, v/v) mobile phase at 1.0 ml/min at ambient temperature. Tests also were made with a mixture of ß-blocker basic drugs (pindolol, metoprolol, oxyprenolol, propranolol — pK_a=9.5–9.7 — at 0.008, 0.165, 0.413, 0.413 and 0.083 μg/μl, respectively, in methanol–water, 1:1) at 40°C, using a mobile phase of acetonitrile–0.017 *M* potassium phosphate (pH 11) buffer (50:50, v/v) with a flow-rate of 1.0 ml/min. Sample injection volumes were 5 μl. Before chromatographic testing, each column was first flushed with at least twenty column volumes of methanol–water (50:50) before equilibrating with about twenty column volumes of the new mobile phase.

3. Results and discussion

Previous studies in these laboratories have defined preferred parameters for ensuring optimum stability and reproducibility of silica-based columns at intermediate and high pH for separating ionizable compounds [4–8]. These approaches include using: lower-porosity and surface-area sol-gel silica supports; densely-bonded, endcapped longer-chain alkyl stationary phases; organic buffers with concentrations of no more than 50 m*M*; and operating temperatures of 40°C or lower. The present investigations were designed to find ways that would further extent the practicality of operating silica-based columns especially at high pH.

3.1. Effect of organic modifier on column stability

Previous studies have suggested that the type of organic modifier may influence the dissolution of silica-based column packings at intermediate pH [6,8]. Mobile phases with phosphate buffer were shown to be especially aggressive in the dissolution of silica at pH 7, resulting in column degradation. Tests at pH 11 with aggressive phosphate buffer also indicate differences in organic modifier type on column stability. Fig. 1 shows the effect of purging densely-bonded, double-endcapped columns with mobile phases modified with acetonitrile or methanol. Significant difference occurred with acetonitrile modifier as compared to methanol, using plate heights of the highly-basic propanolol solute as a

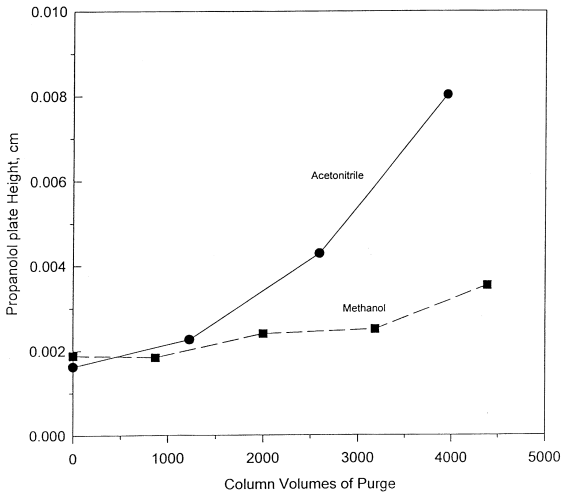

Fig. 1. Effect of organic modifier on column stability at pH 11. Columns: 15×0.46 cm Zorbax XDB-C8; Aging: 50% acetonitrile or methanol–0.017 *M* potassium phosphate buffer, pH 11; flow-rate: 1.5 ml/min; temperature: 23°C; chromatographic test: acetonitrile–0.017 *M* potassium phosphate buffer, pH 11 (50:50, v/v): flow-rate: 1.0 ml/min; temperature: 40°C; solute: propanolol.

measure of column degradation. Similar results also were found for the neutral molecule, toluene (results not shown). Since silicates from the dissolution of silica support are expected to be more soluble in the methanol- rather than acetonitrile-modified mobile phase as confirmed in our earlier studies with non-endcapped bonded phases [5], another effect apparently dominates. We speculate that the difference is due to the aqueous mobile phase containing the highly-hydrophilic methanol molecule being less able to 'wet' and penetrate this particular highly hydrophobic stationary phase than the less-hydrophilic acetonitrile-modified mobile phase. The result would be that the silica support is less-exposed to the aggressive pH 11 phosphate mobile phase for dissolution. It is also likely that the effect of organic modifier on column lifetime at high pH is influenced by the nature of the stationary phase.

3.2. Effect of buffer type

Previous studies showed that certain buffers based on organic amines (i.e., 1-methyl-piperidine and pyrrolidine) exhibit surprisingly low solubility of silica supports at pH ~11, thus significantly enhancing column stability under this normally aggressive condition [7,8]. It was speculated that the reason for this is that the nitrogeneous portion of these rather large organic molecules is sorbed to the silica surface, leaving the hydrophobic portion of the structures to further 'shield' the silica surface from dissolution. Assuming that this is the correct mechanism, we proposed that an organic buffer based on a smaller, less hydrophobic molecule may not be as effective in protecting the silica surface. To test this postulation, columns with double-endcapped dimethyl-C_{18} stationary phases were purged with methanol-modified pH 11 buffers made with potassium phosphate, 1-methyl-piperidine and triethylamine, with the results shown in Fig. 2. Based on plate heights found for propanolol, the buffer made with the more hydrophobic amine, 1-methyl-piperidine, resulted in the most stable column, with very little column change after more than 32 000 column volumes of mobile phase purge. (This corresponds to about 3 months of 8-h column usage). The buffer based on the less-hydrophobic, smaller triethylamine molecule was clearly less effective in maintaining column stability, supporting the postulation of an

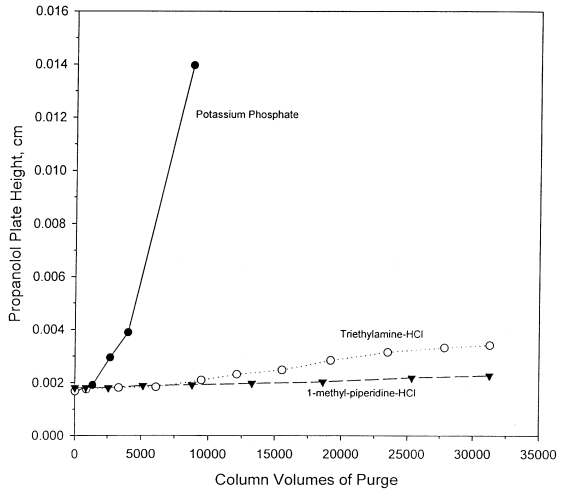

Fig. 2. Effect of buffer type on column stability at pH 11. Columns: 15×0.46 cm Zorbax XDB-C8; aging: methanol–0.017 *M* potassium phosphate, trimethylamine–HCl or 1-methyl-piperidine–HCl buffers, pH 11 (55:45, v/v); flow-rate: 1.5 ml/min; temperature: 23°C; chromatographic test: same, except: flow-rate: 1.0 ml/min; temperature: 40°C.

adsorbed hydrophobic amine structure protecting the silica surface. Note in Fig. 2 that rapid column degradation occurs when a phosphate buffer is used under the same pH 11 conditions. For this reason, phosphate-based buffers are not recommended for routine use at high (and intermediate) pH values [6,8].

3.3. Effect of bonding on silica support solubility

Bonding the silica support with a stationary phase greatly affects the rate of silica support dissolution, as illustrated in Fig. 3. Here, the amount of silica dissolved with an acetonitrile–phosphate buffer (pH 11) mobile phase for an unmodified silica support is compared to that for the same silica densely-bonded with a double-endcapped dimethyl-C_8 silane. The bonded stationary phase greatly retards the rate of silica support dissolution, indicating protection of the silica surface by the hydrophobic bonded silane.

3.4. Effect of stationary phase chain length

The length and bulk of the stationary phase also has a significant effect on column stability at high pH, as shown in Fig. 4. Purging of comparable double-endcapped dimethyl-C_8 and C_{18} columns with a highly-aggressive methanol–phosphate buffer (pH 11) mobile phase indicated a significant degradation of the C_8 column after about 5000 column volumes of purge, as measured by the peak asymmetry for toluene. On the other hand, the C_{18} showed little change with 9000 column volumes of purge, after which the test was arbitrarily terminated. The indications are that the steric bulk of the C_{18} group assists in reducing silica support dissolution, presumably by additional shielding of the silica surface.

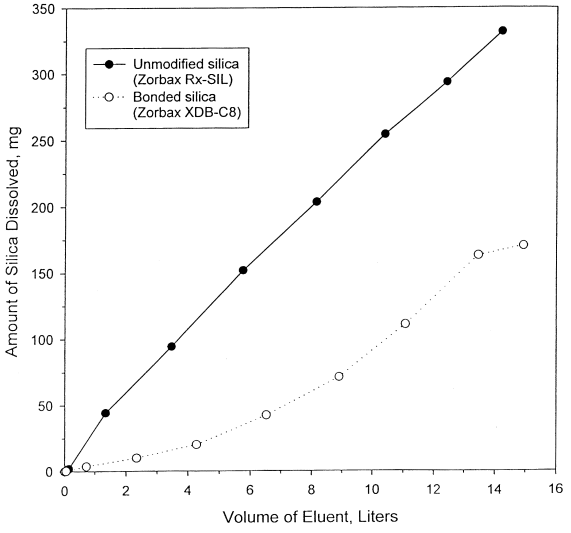

Fig. 3. Effect of bonding on silica support solubility. Columns: 15×0.46 cm Zorbax Rx-SIL and Zorbax XDB-C8; Purging: acetonitrile–0.02 *M* potassium phosphate buffer, pH 11, (50:50, v/v); flow-rate: 1.5 ml/min; temperature 25°C; silicate concentration by silicomolybdate color reaction.

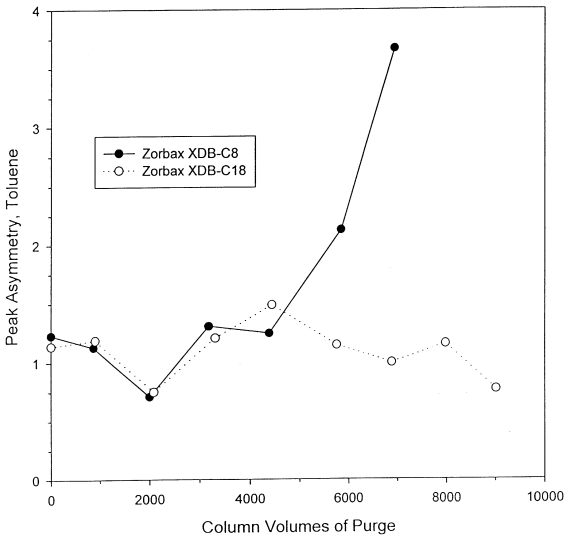

Fig. 4. Effect of stationary phase on column stability at pH 11. Columns: 15×0.46 cm Zorbax XDB-C8 and Zorbax XDB-C18; aging and chromatographic tests same as for Fig. 2.

3.5. Effect of precolumns ('saturator columns') on column stability

Precolumn or 'saturator' columns placed prior to the sampling valve can be used to precondition a mobile phase before entering a column. Precolumns usually are required to saturate an incoming mobile phase with the stationary phase when liquid–liquid chromatographic separations are attempted [18]. In this way, the concentration of the stationary phase is maintained in the mobile phase so that solute retentions are reproduced. The use of silica-based

precolumns also have been reported for intermediate and high pH mobile phases to minimize degradation of the analytical silica-based column [e.g., see [19]]. Here, the intent is to pre-saturate the mobile phase with silica before passing into the analytical column, so that dissolution of the silica support in the analytical column is reduced or eliminated. However, to our knowledge, there has not been a study in which the proposed beneficial effects of a precolumn were quantitated and documented. To determine level of potential benefit of using a precolumn in high pH applications, we performed experiments measuring the effect of using both untreated and bonded-phase precolumns in dissolution and chromatographic tests.

3.5.1. Untreated silica precolumn

Column aging studies were carried out on densely-bonded, double-endcapped dimethyl-C_8 columns with the aggressive acetonitrile–phosphate buffer (pH 11) mobile phase, with and without a precolumn of untreated silica support of the same type. The results in Fig. 5 clearly show the column stability advantage of using such an arrangement. As measured with the basic drug, propanolol, and the neutral solute, toluene, column stability was significantly improved with the precolumn, compared with no precolumn. Without a precolumn, the analytical column quickly failed (after 1000–2000 column volumes of purge) and tests were terminated after about 5000 column volumes. With a silica precolumn, there was only an indication of a slight change in the analytical column after almost 11 000 column volumes of purge, after which the test was arbitrarily terminated. As reported in previous studies [6,8], plate height (Fig. 5B) and peak symmetry (Fig. 5C) measurements are much more sensitive in defining column stability than solute *k* values. Stationary phase eroded from aged columns largely is captured by the remaining stationary phase [5]; retention apparently changes only when the freed stationary phase is eluted from the column, as suggested in Fig. 5A.

3.5.2. Bonded-phase precolumn

Some workers have used another bonded-phase column as the precolumn for protecting the analytical column during high pH applications. To test this

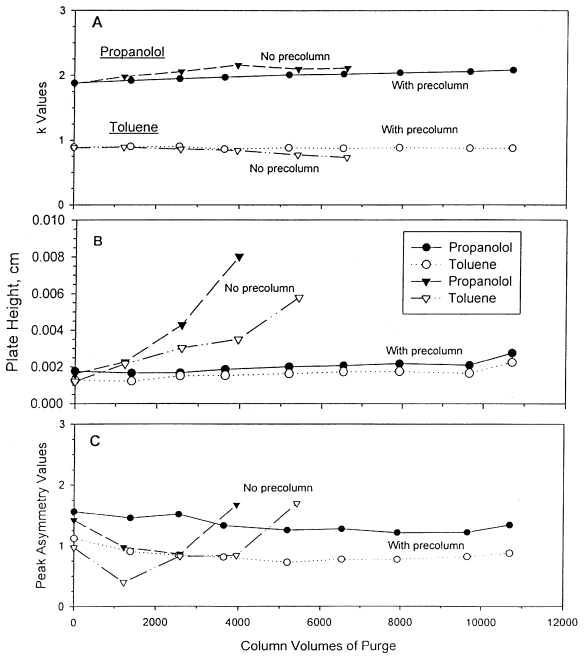

Fig. 5. Effect of silica precolumn on bonded-phase column stability at pH 11. Columns: 15×0.46 cm; precolumn: Zorbax Rx-SIL; analytical column: Zorbax XDB-C8; Aging: acetonitrile–0.017 *M* potassium phosphate buffer, pH 11 (50:50, v/v); flow-rate: 1.5 ml/min; temperature: 23°C; chromatographic tests: propanolol, same mobile phase, flow-rate: 1.0 ml/min, 40°C; toluene, methanol–water (80:20, v/v) flow-rate: 1.0 ml/min, 23°C.

approach, a densely-bonded double-endcapped dimethyl-C_8 column packing was used in a pH 11 dissolution study as a precolumn for an analytical column of the same type. Fig. 6 shows the amount of silica dissolved for this arrangement, compared to the silica dissolved for the same analytical column type without a precolumn. The results indicate that the bonded-phase precolumn actually increased the level of silica dissolved, suggesting that the analytical column probably received some protection by this approach.

This effect is substantiated by the chromatographic stability data in Fig. 7, where a bonded-phase precolumn was used prior to the analytical bonded-phase column in the same pH 11 stability test described for Fig. 5. The analytical bonded-phase column with a bonded-phase precolumn showed some improvement in stability when tested separately, but much less than that afforded by an

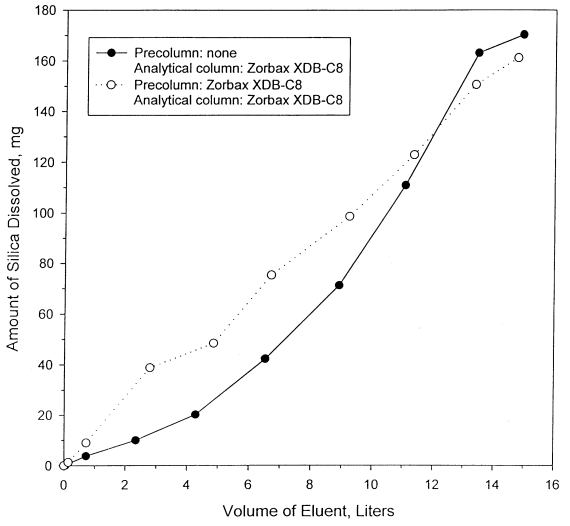

Fig. 6. Effect of bonded-phase precolumn on bonded-phase silica support dissolution at pH 11. Columns: 15×0.46 cm; precolumn and analytical columns: Zorbax XDB-C8; conditions same as Fig. 3.

unmodified silica precolumn (Fig. 5). This might be anticipated, since the much reduced rate of silica dissolution expected from the bonded-phase precolumn would not create the silicate concentration to significantly reduce the dissolution of silica support

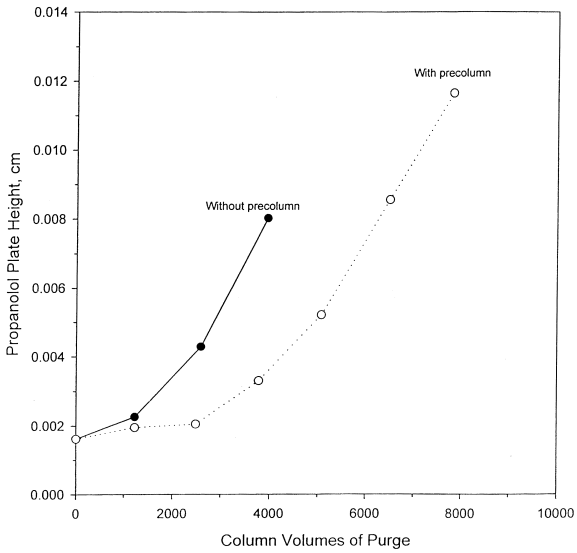

Fig. 7. Effect of bonded-phase precolumn on aging of bonded-phase analytical column at pH 11. Columns: 15×0.46 cm Zorbax XDB-C8; conditions: same as Fig. 5 except no toluene data.

in the analytical bonded-phase column. We conclude that a preferred approach is to use a column of untreated silica as the precolumn for maximum protection against silica support dissolution, as suggested in Section 3.5.1. Since the effluent from such a precolumn apparently is not completely saturated with silicate, this result suggests that a longer precolumn is preferred over a short precolumn to ensure the highest possible level of silicate concentration and resultant analytical column protection at high pH.

Although these studies confirm the capability of precolumns to extend the lifetime of silica-based columns in high pH operation, this approach should be used with caution because of potential limitations. First, precolumns generally preclude the possibility of gradient elution separations. The large dwell volume associated with the precolumn greatly distorts the gradient mixing. Also, the reproducibility of silica support dissolution is difficult to maintain with the changing of organic modifier concentration and the resulting effective change in mobile phase pH. Second, precolumns tend to sorb impurities from the incoming mobile phase, and these eventually can elute into the analytical column to cause baseline perturbations. Third, degradation of the silica in the precolumn can cause a large increase in system back pressure, and such particle degradation also can plug the inlet of the analytical column. An in-line filter may be of aid if this proves to be a problem. Generally, however, the best approach is to use a fresh, unmodified silica column as a precolumn, and periodically replace this unit before silica dissolution becomes severe.

3.6. Bidentate stationary phase

Polymeric [20] and self-assembled, horizontally-polymerized silane [21] stationary phases on silica supports have been proposed as stable column chromatographic packings for high pH applications. The claimed stability for both of these materials apparently is based on the premise that the component silanes are bonded to the silica support by more than one covalent bond. Presumably, such an arrangement reduces loss of the stationary phase as the silica support is dissolved by the high pH mobile phase. On the other hand, a known disadvantage of

both of these approaches is the problem of reproducing the polymeric nature of these phases for repeatable separations from batch to batch of column packing material.

We find that another approach for obtaining superior stability of silica-based column packings is to use bidentate silane stationary phases. Column packings with bidentate phases have shown superior stability at low pH, presumably based on the covalent attachment of the silane in two places; both attaching siloxane bonds must be hydrolytically broken before the bonded phase is lost from the silica surface [13,22]. In contrast to polymeric phases, a distinct advantage of the bidentate approach is that the attached silane can be reproducibly reacted to the silica surface: one equivalent of bidentate silane reacts with one equivalent of silanol groups. Therefore, the repeatability of products from properly-conducted bidentate synthesis is equivalent to that of materials with the widely-used monofunctional silane chemistry.

We now have determined that the stability advantage of bidentate phases extends beyond the low pH range to high pH. Again, the apparent reason for increased stability at high pH is the attachment of the silane to the silica support by two siloxane groups. Silane loss by dissolution of the silica support is minimized because of this two-fold attachment. Of particular interest in the present studies was the C_{18} bidentate structure shown in Fig. 8. This bonded phase was found to have superior stability at high pH while maintaining all of the advantages of the reproducibility, efficiency and excellent band shape of monofunctional stationary phases. Description of the synthesis and chromatographic properties of this and other bidentate-based stationary phases are given in other publications [16,17].

Comparison of the stability of the bidentate bonded phase with comparable monofunctional densely-bonded, double-endcapped dimethyl-C_8 and

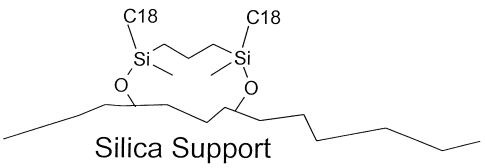

Fig. 8. Structure of bidentate C_{18} bonded phase.

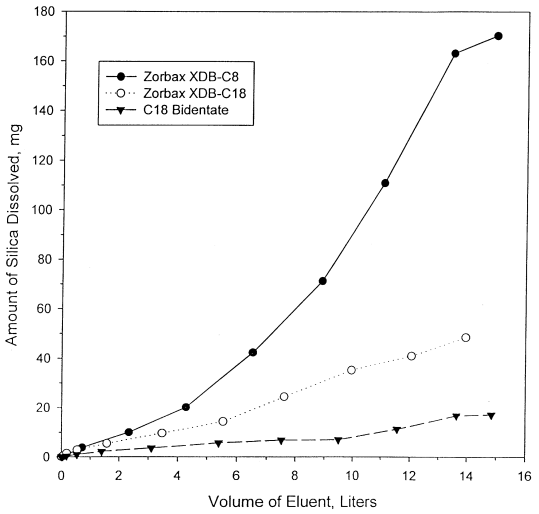

Fig. 9. Effect of bonded phase type on silica support dissolution at pH 11. Columns: 15×0.46 cm, Zorbax XDB-C8, Zorbax XDB-C18 and bidentate-C_{18}; conditions same as for Fig. 3.

-C_{18} stationary phases is shown in the pH 11 dissolution studies of Fig. 9. The double-endcapped bidentate phase showed a strikingly slower silica solubility rate with this aggressive pH 11 phosphate-based mobile phase than the C_8 and C_{18} phases produced from the same Type B silica support. As might be predicted, the C_{18} packing showed a lower rate of solubility than the shorter-chain C_8 packing, suggesting a better shielding of the silica support from attack by hydroxyl ions.

Similar results were found in chromatographic tests, as illustrated in Fig. 10. When purged with an acetonitrile–phosphate (pH 11) mobile phase, the new bidentate phase showed slower degradation than comparable monofunctional C_8 and C_{18} phases. Interestingly, even with the highly-aggressive phosphate (pH 11) mobile phase, the bidentate packing showed only a modest increase in the plate height for the highly-basic propanolol test probe after about 10 000 column volumes of mobile phase (about 1 month of 8-h day use). The monofunctional C_{18} packing also showed increased stability over the C_8 packing, which correlates with the dissolution data of Fig. 9. Fig. 11 shows the initial chromatogram and the separation for the bidentate-C_{18} column after purging with almost 10 000 column volumes of aggressive pH 11 phosphate mobile phase.

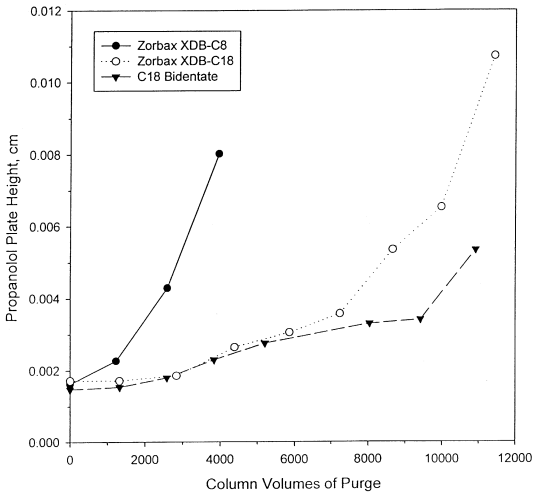

Fig. 10. Effect of bonded-phase type on column stability at pH 11. Columns: 15×0.46 cm, Zorbax XDB-C8, Zorbax XDB-C18 and bidentate-C_{18}; conditions same as Fig. 5 except no toluene data.

As discussed above, silica-based columns demonstrate much higher stability at high pH with organic buffers, compared to when phosphate-based buffers are used [6,7]. In previous studies, we found that the C_8 column packing of Fig. 10 showed very little change after more than 30 000 column volumes of purging with an acetonitrile–1-methyl-piperidine (pH 11) mobile phase [7]. We speculate that the new bidentate C_{18} column packing should show even further stability against change when a recommended organic-based high-pH buffer is used.

As a result of the increased stability of the bidentate C_{18} stationary phase, we speculate that it should be possible to safely purge silica-based columns of this stationary phase periodically with highly-aggressive treatments (such as 0.1 *M* sodium hydroxide) to clean unwanted, highly-retained materials (e.g., endotoxins) from the column bed. Such a treatment seems feasible, since the bidentate C_{18} materials of this study are much more resistant to dissolution and degradation than the densely-reacted non-endcapped dimethyl-C_{18} columns previously described as surprisingly resistant to such treatments [23].

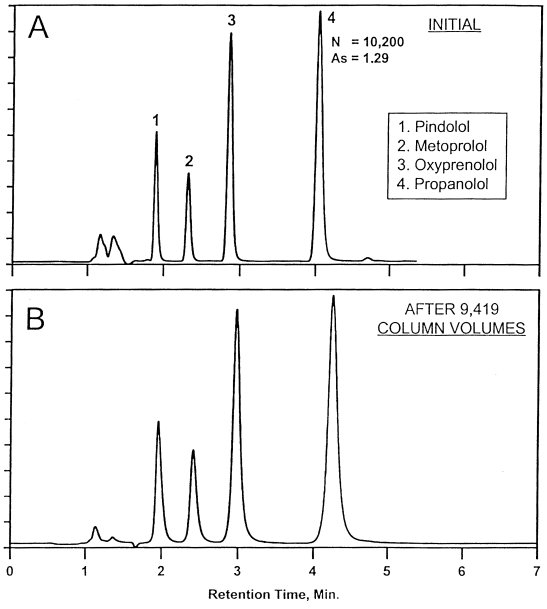

Fig. 11. Stability of bidentate-C_{18} column at pH 11. Column: 15×0.46 cm; conditions same as Fig. 10; solutes: ß-blocker drugs (A) initial separation (B) separation after 9419 column volumes of purge with pH 11 mobile phase.

4. Conclusions

Further studies with bonded-phase packings at high pH have revealed additional ways in which silica-based column packings can be stabilized for repeatable routine separations without sacrificing the desirable advantages of column efficiency and peak shape. Densely-bonded, double-endcapped dimethyl-C_8 phase shows higher stability at pH 11 in methanol-based mobile phase compared to acetonitrile. Silica-based columns apparently are more stable at pH 11 with more hydrophobic organic-based buffers, compared to shorter-chain, less hydrophobic organic buffers and phosphate-based buffers. Both of these organic buffers show plate heights that are comparable to those for phosphate buffers. Precolumns of unmodified silica greatly extend the lifetime of columns used at high pH (e.g., phosphate buffer, pH 11); bonded-phase precolumns appear less effective. Densely-bonded, double-endcapped, silica-based C_{18} bonded-phase column packings are more stable at pH 11 than comparable C_8 packings. A new densely-bonded, double endcapped bidentate C_{18} stationary phase exhibits superior stability at pH 11, while maintaining the advantages of high efficiency and

excellent peak shapes characteristic of monofunctionalized, silica-based bonded-phase columns.

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