

Effects of Different High pH Solutions on the Chemical Stability of Immobilized-Polymer Stationary Phases

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Abstract

Many chromatographic separations require high pH mobile phases for the elution of basic compounds with symmetrical peaks appropriate for quantitation. Phosphate and carbonate salts are the most common additives used to achieve these conditions. However, they act in different ways on the stability of silica-based stationary phases. To provide a faster evaluation of the effects of these high pH solutions on the column lifetime of new immobilized-polymer metalized-silica-based phases prepared in our laboratory, an accelerated stability test at a nominal pH of 10 and at 50°C was developed and applied with monitoring of the retention factor, column efficiency, and asymmetry as functions of the volume of high pH mobile phase. The laboratory-made stationary phases presented chemical stabilities in phosphate solution more than three times better their chemical stabilities in carbonate buffer.

Introduction

Many silica-based stationary phases present low stability in mobile phases with pH more than 8 due to the accelerated dissolution of silica under alkaline conditions (1). However, a large number of applications using high-performance liquid chromatography (HPLC) require alkaline mobile phases to separate and to elute some compounds, especially basic ones, with short retention times and resolutions appropriate for quantitation (2–4). The use of inorganic salts as additives in the mobile phase can provide a significant improvement of the resolution and symmetry of these compounds. Phosphate and carbonate are the principal inorganic salts used (5–7), but they act in different ways on the stability of silica-based stationary phases by mechanisms that are not completely elucidated. One of the experimentally evaluated explanations is that after mixing aqueous solutions of these salts, each at the same aqueous pH, with methanol or acetonitrile, the pH of carbonate mobile phases are higher than the pH of those prepared with phosphate salts (6,7). Thus, the column collapse, characterized by abrupt decreases in the plate numbers of the HPLC phases, is faster when using mobile phases buffered with carbonate salts than of similar mobile phases prepared with phosphate salts (1,6).

Nowadays, a number of non-silica-based stationary phases are available to separate basic compounds using high pH buffered mobile phases (8–10). The behaviors of several of these phases with high pH mobile phases having different additives suggest that the lifetime of these stationary phases is extended, although these stationary phases are usually considerably more expensive.

In our laboratory, silica having a metal oxide layer has been used to prepare stationary phases that have shown significant improvement in stability when using high pH mobile phases (11–19). The present paper evaluates the effect of high pH solutions on the column lifetimes of immobilized-polymer metalized-silica-based phases prepared in our laboratory using an accelerated stability test with a temperature of 50°C and a nominal pH 10 mobile phase containing either phosphate or carbonate. The laboratory-made stationary phases were prepared by thermal immobilization of poly(methyltetradecylsiloxane), PMTDS, onto doubly-zirconized silica particles followed by end-capping reactions.

Experimental

Reagents

Milli-Q water (Millipore, Bedford, MA) and HPLC-grade methanol (Mallinckrodt, Rio de Janeiro, Brazil) were used to prepare the mobile phases and filtered using 0.45-mm membrane filters. HPLC-grade chloroform, hexane, and toluene obtained from Mallinckrodt were used after filtration. Zirconium(IV) tetrabutoxide, hexamethyldisilazane, and trimethylchlorosilane were purchased from Aldrich (Milwaukee, WI) and were used as received. The silica support was Kromasil silica from Akzo Nobel (Bohus, Sweden), having a mean particle size of 5 mm, a mean pore size of 11.1 nm, and a 313 m²/g specific surface area. PMTDS was obtained from United Chemicals Technologies (Bristol, PA). Test compounds were analytical reagent-grade and not further purified. KH₂PO₄, K₂HPO₄, K₂CO₃, and KHCO₃ were purchased from Merck (Darmstadt, Germany).

Preparation of packing materials

The zirconized silica was synthesized by a previously described optimized procedure (20). These support particles were dried in air at 150°C for 12 h and added to a 10% (w/v) solution of PMTDS in hexane to give a specific mass (m_{PMTDS}) of 0.9 g_{PMTDS}/g_{Si-Zr}. The mixture was slowly stirred for 3 h at room temperature and

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then was placed in a fume hood, without stirring, for evaporation of the hexane at room temperature for six days (sorbed stationary phase). Sorbed Si-Zr(PMTDS) phases were placed into a stainless steel tube (150 mm × 10 mm i.d.), which was fitted with frits and connectors and placed in a tubular oven to induce thermal immobilization. Thermal treatment was performed at 110°C for 12 h under a slow nitrogen flow.

After that, the stainless steel tubes (150 × 10 mm) were connected to a Waters 510 pump (Milford, MA) for extraction of non-immobilized PMTDS by passing hexane at 0.5 mL/min at 50°C for 4 h. Portions of the extracted Si-Zr(PMTDS) phase, previously dried with nitrogen flow for 20 min, were submitted to the end-capping reaction, which was performed with 50 mL of a TMCS and HMDS mixture (30:70, v/v) and 100 mL of dry toluene under nitrogen for 48 h under reflux. The solid was then filtered, washed with toluene, and dried under vacuum at 60°C for 5 h prior to packing the columns made of highly polished (21) type 316 stainless steel tubing with dimensions of 60 mm × 3.9 mm i.d.

The endcapped, Si-Zr(PMTDS)ec, and non-endcapped, Si-Zr(PMTDS), stationary phases were slurry packed using 5% slurries (w/v) of the stationary phases in chloroform. A constant packing pressure of 40 MPa (Haskel packing pump, Burbank, CA) was used with methanol as propulsion solvent. Columns were conditioned for 50 min with mobile phase at 0.4 mL/min prior to analysis.

Chromatographic evaluations

Firstly, a laboratory-made test mixture (uracil, phenol, *N,N*-dimethylaniline, naphthalene, and acenaphthene) was used for evaluation of chromatographic performance of the Si-Zr(PMTDS) and Si-Zr(PMTDS)ec stationary phases. The chromatographic evaluations were performed using a modular HPLC system from Shimadzu (Kyoto, Japan) equipped with an LC-10AD liquid chromatography pump, a SPD-10A UV-Vis detector, a CTO-10AS column oven, a SIL-10AD automatic injector, and a SCL-10A system controller. Data were acquired and processed using the CLASS-VP program (Shimadzu).

For the stability tests, Si-Zr(PMTDS) and Si-Zr(PMTDS)ec stationary phases were eluted with 70:30 (v/v) MeOH:0.05 mol/L K₂CO₃/KHCO₃, pH 10 or with 70:30 (v/v) MeOH:0.05 mol/L K₂HPO₄/KH₂PO₄, pH 10 as mobile phases. The mobile phases were prepared volumetrically from individually measured amounts of each solvent. All solvents were filtered and degassed before use. The pH of the inorganic salt solutions were adjusted to 10 with concentrated KOH using a DM-21 pH meter (Digimed, São Paulo, Brazil) before mixing with methanol. The flow rates through the columns were 1.0 mL/min at 50°C. These conditions were used to accelerate column deterioration, obtaining faster evaluations with considerably less mobile phase and instrument time (22). The columns were periodically tested for the separation of the same laboratory-made test mixture to evaluate retention factors, efficiencies, and asymmetries.

Characterizations of the stationary phases

The extent of degradation of the stationary phases was determined by elemental analyses before and after the stability tests. Portions were analyzed on a Model CHN-2400 Perkin-Elmer

Analyzer (Shelton, CT). The percent carbon was used to calculate the quantities of PMTDS (in grams per gram of support) present on the respective phases using equation 1,

$$m = \frac{\%C}{(70 - \%C)} \quad \text{Eq. 1}$$

where *m* is the specific mass, % C is the percent carbon, and 70 is the percent carbon in the PMTDS molecule.

The specific surface areas of the stationary phases were determined by nitrogen adsorption in the dry state following the conventional BET (Brunauer, Emmet and Teller) method (23) with a Model 2300 Micromeritics Flow Sorb II instrument (Norcross, GA).

Results and Discussion

The metalized silica-based stationary phases had their performance evaluated by chromatographic separation of two test mixtures containing polar, apolar, acidic and basic compounds.

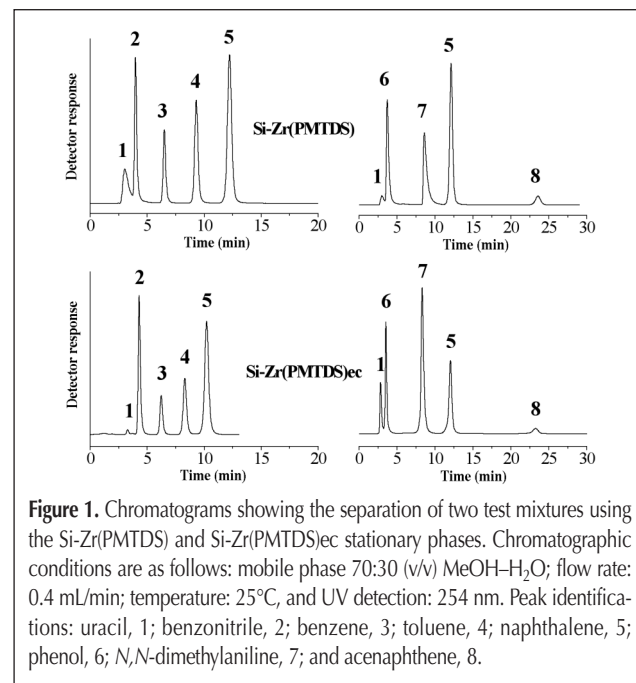


Figure 1. Chromatograms showing the separation of two test mixtures using the Si-Zr(PMTDS) and Si-Zr(PMTDS)ec stationary phases. Chromatographic conditions are as follows: mobile phase 70:30 (v/v) MeOH–H₂O; flow rate: 0.4 mL/min; temperature: 25°C, and UV detection: 254 nm. Peak identifications: uracil, 1; benzonitrile, 2; benzene, 3; toluene, 4; naphthalene, 5; phenol, 6; *N,N*-dimethylaniline, 7; and acenaphthene, 8.

Table I. Chromatographic Performance of Metalized Silica-Based Stationary Phases

Stationary phases	Chromatographic performance		
	N/m	As	k
<i>Si-Zr(PMTDS)</i>			
Phenol	32700	1.6	1.0
<i>N,N</i> -dimethylaniline	19100	2.2	3.3
Acenaphthene	77800	1.1	11.1
<i>Si-Zr(PMTDS)ec</i>			
Phenol	44100	1.3	1.1
<i>N,N</i> -dimethylaniline	64500	1.1	3.1
Acenaphthene	81200	1.0	10.9

Figure 1 shows the chromatograms obtained in the separation of these mixtures. *N,N*-dimethylaniline, the basic compound, was eluted with a more symmetrical peak on the endcapped phase, which shows a significant reduction of residual hydroxyl groups

Stationary phases	Properties				
	S_{BET}^* (m ² /g)	V_{p}^{\dagger} (cm ³ /g)	$d_{\text{pore}}^{\ddagger}$ (nm)	% PMTDS	% Zr
Si-Zr(PMTDS)	126	0.37	6.2	22.1	20.6
Si-Zr(PMTDS)ec	141	0.36	6.1	23.1	20.6

* S = specific surface area;
 $\dagger V_{\text{p}}$ = pore volume;
 $\ddagger d_{\text{pore}}$ = mean pore diameter.

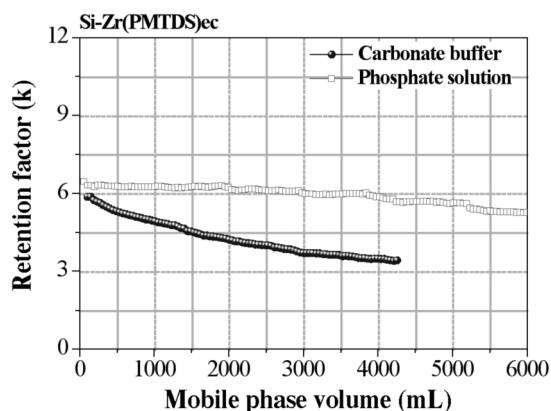


Figure 2. Variation of the retention factor of the acenaphthene peak on the Si-Zr(PMTDS)ec phase during the passage of the mobile phases (□) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, pH 10 and (●) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{CO}_3/\text{KHCO}_3$, pH 10. Flow rate: 1.0 mL/min; temperature: 50°C, and UV detection: 254 nm.

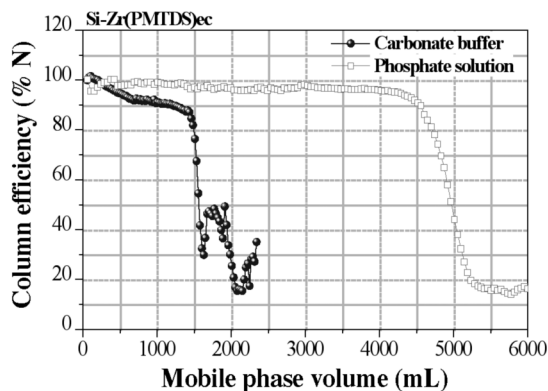


Figure 3. Variation of the column efficiency of the acenaphthene peak on the Si-Zr(PMTDS)ec phase during the passage of the mobile phases (□) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, pH 10 and (●) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{CO}_3/\text{KHCO}_3$, pH 10. Flow rate: 1.0 mL/min; temperature: 50°C, and UV detection: 254 nm.

due to the incorporation of trimethylsilyl groups in the end-capping step. The performance of metalized silica-based phases, especially for the more retained compound, acenaphthene, and the order of elution of all compounds are similar to those obtained by conventional commercial phases, such as the chemically bonded C_{18} phases with the same particle size, characterizing these phases as true reversed phases with acceptable performance.

The chromatographic data obtained from the chromatograms are shown in Table I. The end-capping was a fundamental step in the preparation of metalized silica-based phases because the presence of zirconium in the support structure increases the acidic character of residual hydroxyl groups (20), which more strongly retains basic compounds (asymmetry factor > 2).

Some textural and physicochemical properties of metalized silica-based phases were also evaluated. This information is compiled in Table II. No significant changes are observed in the textural or physicochemical properties of metalized silica-based stationary phases after the end-capping step.

Metalized silica-based HPLC phases have already demonstrated higher stability than chemically bonded silica-based phases (18,19,24). These chemical stability evaluations for metalized

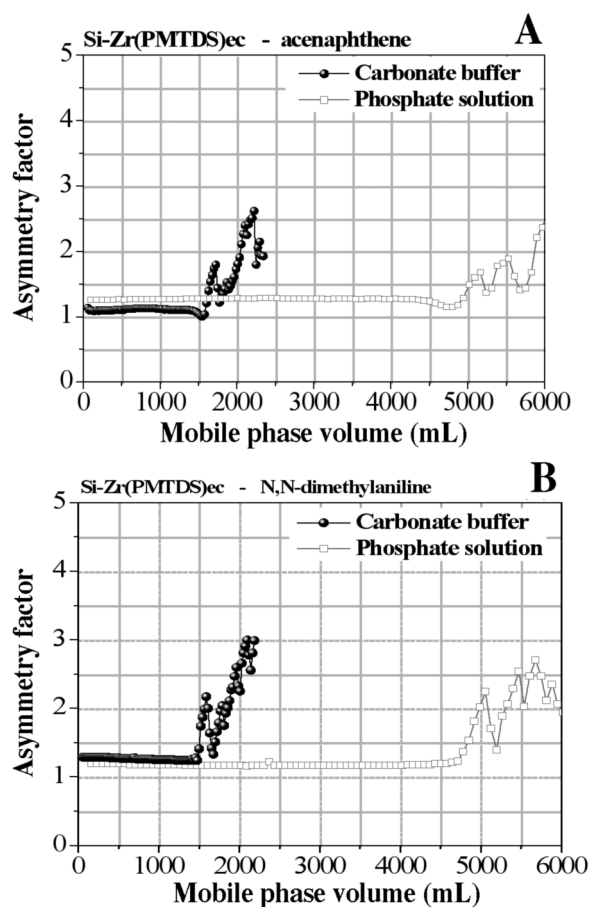


Figure 4. Variations of the asymmetry factor on the Si-Zr(PMTDS)ec phase during the passage of the mobile phases (□) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, pH 10 and (●) 70:30 (v/v) MeOH/0.05 mol/L $\text{K}_2\text{CO}_3/\text{KHCO}_3$, pH 10. Flow rate: 1.0 mL/min; temperature: 50°C, and UV detection: 254 nm. Test probes: (A) acenaphthene and (B) *N,N*-dimethylaniline.

silica-based phases were performed using high pH mobile phases buffered with carbonate salts. Tindall and Perry (6) have shown that after the addition of 50 % methanol to 50% carbonate buffer solution, the pH of the mobile phase increases c.a. 1.4 units from its nominal pH of 10 to pH 11.4. However, with a mobile phase using phosphate solution as the aqueous phase the pH increases only about 0.6 units to pH 10.6. Thus, the chemical stability of an endcapped immobilized PMTDS phase, Si-Zr(PMTDS)_{ec}, was evaluated, varying the inorganic salt used as additive, carbonate or phosphate, with both aqueous solutions adjusted to pH 10 before mixing with methanol. The chromatographic parameters monitored during the test were the variations in column efficiency and retention factor for the acenaphthene peak and peak asymmetries for both acenaphthene and dimethylaniline, using the accelerated degradation test.

Figure 2 shows the variation of the retention factor for acenaphthene on the Si-Zr(PMTDS)_{ec} stationary phase during the passage of alkaline mobile phase prepared with carbonate buffer and phosphate solution. The retention factor for acenaphthene has a negative variation of about 8% after passing ca. 4.2 L of mobile phase prepared with phosphate solution. Passing the same volume of methanol-carbonate buffer solution resulted in a reduction of about 50% in its retention factor.

As illustrated in Figure 3, the chemical stability of the end-

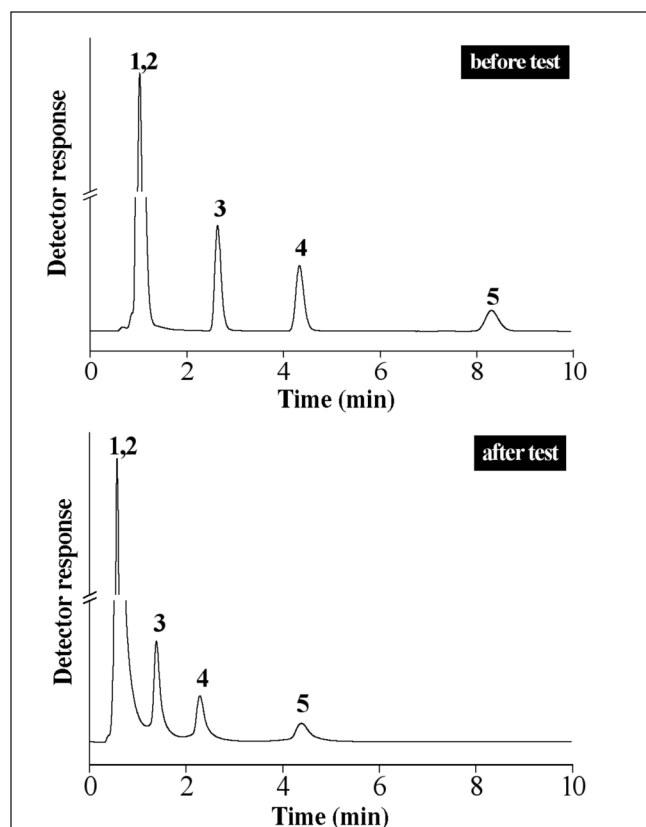


Figure 5. Chromatograms showing the separation of a test mixture using the Si-Zr(PMTDS)_{ec} stationary phase before and after (6000 mL) stability testing. Chromatographic conditions: mobile phase 70:30 (v/v) MeOH/0.05 mol/L K₂HPO₄/KH₂PO₄, pH 10. Flow rate: 1.0 mL/min; temperature: 50°C, and UV detection: 254 nm. Peak identifications: uracil, 1; fenol, 2; *N,N*-dimethylaniline, 3; naphthalene, 4; and acenaphthene, 5.

capped phase was characterized by column collapse, indicated by abrupt decreases in plate numbers after passing mobile phases prepared with carbonate and phosphate salts. Column collapse at high pH is directly associated with silica dissolution and consequent formation of voids in the packed column bed, which resulted in a loss of column efficiency. The Si-Zr(PMTDS)_{ec} stationary phase presented higher chemical stability in phosphate solutions, resisting the passage of 4600 mL of mobile phase, which is more than three times the chemical stability seen with carbonate buffer (1400 mL of mobile phase of MeOH-K₂CO₃/KHCO₃).

As expected, the asymmetry factor also presented a longer plateau (~ 4600 mL) when using the phosphate mobile phase than with the carbonate mobile phase, which caused significant change after 1500 mL. Tailing for both the acenaphthene and the dimethylaniline peaks occurred after these plateaus, coinciding with column collapse (Figure 4). The variations of the asymmetry factor data after the start of column collapse can be attributed to rearrangements of the degraded packing in the chromatographic column.

Carbon loadings after stability tests also indicated that the carbonate mobile phases accelerate dissolution of the metalized silica-based phases more than with the phosphate mobile phases. The percent PMTDS present on the stationary phases after the end of the stability test was about 20% lower when using the car-

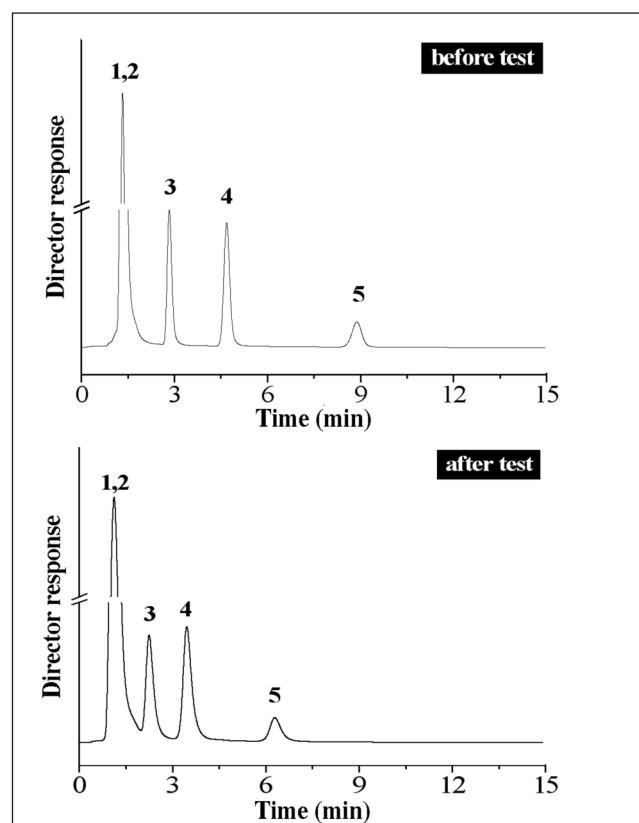


Figure 6. Chromatograms showing the separation of a test mixture using Si-Zr(PMTDS)_{ec} stationary phase before and after (2000 mL) stability testing. Chromatographic conditions: mobile phase 70:30 (v/v) MeOH:0.05 mol/L K₂CO₃/KHCO₃, pH 10. Flow rate: 1.0 mL/min; temperature: 50°C, and UV detection: 254 nm. Peak identifications: uracil, 1; fenol, 2; *N,N*-dimethylaniline, 3; naphthalene, 4; and acenaphthene, 5.

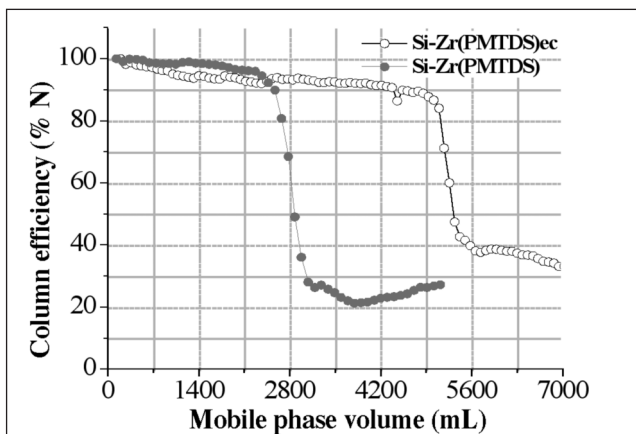


Figure 7. Variation of the column efficiency for the acenaphthene peak on (○) Si-Zr(PMTDS)ec and (●) Si-Zr(PMTDS) thermally immobilized at 110°C for 12 h after passage of 70:30 (v/v) MeOH/0.05 mol/L K_2HPO_4/KH_2PO_4 , pH 10 mobile phase. Flow rate: 1.0 mL/min; Temperature: 50°C, and UV detection: 254 nm.

bonate mobile phase (15.4% PMTDS) compared to when using the phosphate mobile phases (19.8% PMTDS).

Figure 5 shows the separations of the test mixture on the Si-Zr(PMTDS)ec stationary phase before and after the degradation test using the methanol:0.050 mol/L K_2HPO_4/KH_2PO_4 mobile phase. The separations of same test mixture by methanol:0.050 mol/L $K_2CO_3/KHCO_3$ present similar results after the stability test (Figure 6). The main characteristics of the stationary phases during the tests are poorer resolutions and more asymmetric peaks for all compounds.

Furthermore, the degradation tests indicated that the endcapped phase provides a significant improvement in the chemical stability of the Si-Zr(PMTDS) stationary phase (Figure 7). Column collapse for Si-Zr(PMTDS)ec occurred after passing about 4500 mL of MeOH:0.05 mol/L K_2HPO_4/KH_2PO_4 , pH 10, mobile phase at 50°C, whereas for the Si-Zr(PMTDS), column collapse occurred after approximately half this volume of the same mobile phase. End-capping protects some of the exposed residual silanols of the stationary phase by blocking the access of mobile phase components to the chromatographic support and reducing the dissolution ratio of silica.

Conclusions

The use of carbonate buffer as an additive to prepare high pH mobile phases leads to faster degradation of the chromatographic support and, consequently, to faster column collapse than when phosphate solutions are used. Thus, the column lifetime of the laboratory-made metalized silica-based immobilized-polymer stationary phases in high pH mobile phase conditions, which is required for several applications, can be significantly enhanced when phosphate solutions are used as the aqueous component of the mobile phase. Additionally, the chemical stability of metalized silica-based phases in basic mobile phases is also enhanced when these phases are submitted to an end-capping reaction.

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References

- J.J. Kirkland, J.W. Henderson, J.J. DeStefan, M.A. van Straten, and H.A. Claessens. Stability of silica-based, endcapped columns with pH 7 and 11 mobile phases for reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **762**: 97–112 (1997).
- J.J. Kirkland, M.A. van Straten, and H.A. Claessens. Reversed-phase high-performance liquid chromatography of basic compounds at pH 11 with silica-based column packings. *J. Chromatogr. A* **797**: 111–120 (1998).
- H.A. Claessens and M.A. van Straten. Review on the chemical and thermal stability of stationary phases for reversed-phase liquid chromatography. *J. Chromatogr. A* **1060**: 23–41 (2004).
- H.A. Claessens, M.A. van Straten, and J.J. Kirkland. Effect of buffers on silica-based column stability in reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **728**: 259–270 (1996).
- G.W. Tindall. Mobile-Phase Buffers, Part I – The interpretation of pH in partially aqueous mobile phases. *LC-CC N.A.* **20**: 1028–1032 (2002).
- G.W. Tindall and R.L. Perry. Explanation for the enhanced dissolution of silica column packing in high pH phosphate and carbonate buffers. *J. Chromatogr. A* **988**: 309–312 (2003).
- X. Subirats, E. Bosch, and M. Rosés. Retention of ionisable compounds on high-performance liquid chromatography XVII: Estimation of the pH variation of aqueous buffers with the change of the methanol fraction of the mobile phase. *J. Chromatogr. A* **1138**: 203–215 (2007).
- C.J. Dunlap, C.V. Mcneff, D. Stoll, and P.W. Carr. Zirconia stationary phases for extreme separations. *Anal. Chem.* **73**: 598A–607A (2001).
- J. Nawrocki, C. Dunlap, J. Li, C.V. McNeff, A. McCormick, and P.W. Carr. Part II. Chromatography using ultra-stable metal oxide-based stationary phases for HPLC. *J. Chromatogr. A* **1028**: 31–62 (2004).
- Z. Jiang, Y. Zuo, R. Li, and J.C. Yu. Chromatographic retention of polybutadiene coated titania stationary phase using quantitative structure-retention relationships. *Chem. Anal. (Warsaw)* **49**: 551–560 (2004).
- D.A. Fonseca, K.E. Collins, and C.H. Collins. Titanized silica-based stationary phases prepared with thermally and microwave-immobilized poly(methyltetradecylsiloxane). *J. Chromatogr. A* **1030**: 209–215 (2004).
- L.S.R. Morais and I.C.S.F. Jardim. Characterization of a new stationary phase based on microwave immobilized polybutadiene on titanium oxide-modified silica. *J. Chromatogr. A* **1073**: 127–135 (2005).
- C.R. Silva, C.H. Collins, K.E. Collins, and C. Airolidi. An overview of the chromatographic properties and stability of C18 titanized phases. *J. Sep. Sci.* **29**: 790–800 (2006).
- A.M. Faria, I.C.S.F. Jardim, K.E. Collins, and C.H. Collins. Immobilized polymeric stationary phases using metalized silica supports. *J. Sep. Sci.* **29**: 782–789 (2006).
- A.M. Faria, K.E. Collins, and C.H. Collins. New stationary phases for HPLC based on poly(methyltetradecylsiloxane) thermally immobilized onto zirconized silica. *J. Chromatogr. A* **1122**: 114–122 (2006).
- A.M. Faria, K.E. Collins, and C.H. Collins. Preparation and characterization of poly(methyltetradecylsiloxane) stationary phases immobilized by gamma radiation onto zirconized silica. *J. Chromatogr. A* **1156**: 51–59 (2007).
- A.M. Faria, K.E. Collins, and C.H. Collins. Enhanced stability stationary phases based on poly(methyltetradecylsiloxane) immobilized onto doubly-titanized silica particles as reversed stationary phases for high-performance liquid chromatography. *Chromatographia* **67**: 357–363 (2008).
- C.R. Silva, C. Airolidi, K.E. Collins, and C.H. Collins. A new generation of more pH stable reversed phases prepared by silanization of zirconized silica. *J. Chromatogr. A* **1191**: 90–98 (2008).
- A.M. Faria, C.R. Silva, C.H. Collins, and I.C.S.F. Jardim. Development of a polymer-coated stationary phase with improved chemical stability in alkaline mobile phases. *J. Sep. Sci.* **31**: 953–960 (2008).
- A.M. Faria, D.R. Magalhães, K.E. Collins, and C.H. Collins. Characterization of zirconized silica supports for HPLC. *Anal. Chim. Acta* **550**: 137–143 (2005).
- K.E. Collins, A.C. Franchon, and I.C.S.F. Jardim, E. Radanovic, M.C. Gonçalves. The effects of inner surface roughness of microbore column blanks on column performance. *LC-CC N.A.* **18**: 106–117 (2000).
- D.A. Fonseca, H.R. Gutiérrez, K.E. Collins, and C.H. Collins. Rapid method for evaluating reversed-phase high-performance liquid chromatography column stability. *J. Chromatogr. A* **1030**: 149–155 (2004).
- S. Brunauer, P.H. Emmet, and E. Teller. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **60**: 309–319 (1938).
- A.M. Faria, E. Tonhi, K.E. Collins, and C.H. Collins. Stability studies of stationary phases from poly(methyltetradecylsiloxane) sorbed and immobilized onto metalized and unmodified silicas. *J. Sep. Sci.* **30**: 1844–1851 (2007).

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