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Rapid method for evaluating reversed-phase high-performance liquid chromatography column stability

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Abstract

A procedure is presented for the rapid evaluation of HPLC stationary phase stability at pH 8.4 or 10.1 using a temperature of 60 °C. Mobile phase (MeOH–0.1 mol l^{-1} aqueous NaHCO$_3$, 50:50, v/v) is continuously passed through the column with periodic injections of a test solution until the several chromatographic parameters of the resulting chromatograms are degraded. The tests were applied to several commercial and laboratory-made stationary phases. After degradation two of these phases, one commercial and one laboratory-made, were examined by elemental analysis and scanning electron microscopy to elucidate the degradation process.
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1. Introduction

The use of alkaline mobile phases for the analysis of basic compounds requires the development of stationary phases stable in this pH range. Many silica-based phases give high chromatographic performance in the pH range from 3 to 8. However, pH values out of this range present problems of chemical stability if the silica support surface is not properly protected. At a high pH, the chemically bonded stationary phases tend to degrade, as a result of silica dissolution [1] while at acidic pH, the siloxane linkage of conventional bonded phase is susceptible to hydrolysis [2]. Modifications in stationary phase synthesis indicate some alternative ways to improve stationary phase stability at extreme pH values. Thus, phases prepared with alkylsilanes containing bulky side groups are less prone to hydrolysis than phases prepared with dimethyl groups [3,4] while, phases with longer alkyl chains (C$_{18}$) offer higher stability in acidic pH than phases with shorter alkyl chains or cyano groups [5,6].

In basic mobile phase, other approaches are necessary. Stationary phases made with a densely-bonded, double-endcapped bidentate-C$_8$ silane stationary phase have been shown to have high column efficiency and excellent peak shape at pH 11, exhibiting stability superior to that of monofunctional bonded silica-based phases [7,8]. In addition, column-aging procedures performed on C$_8$ stationary phases prepared with xerogel and sol–gel types of silicas showed that silicas made by the sol–gel process are more resistant to dissolution than those made with silicas from the xerogel process [9].

Other approaches to prepare stationary phases stable at high pH involve using other supports, such as zirconia [10–13], alumina [12,14–16] and titania [12,13,17–21]. More recently, mixed oxide supports, such as silica-zirconia [22] and silica–titania [23] have been reported. In addition, immobilized polymer-covered supports have been studied in order to increase surface protection [20,21,24]. Zirconized silica having poly(methyloctylsiloxane) (PMOS) immobilized with gamma radiation showed better chromatographic performance than unmodified supports, presenting a much higher resistance under neutral and basic conditions [25]. Titanized silica with a similar PMOS coating was also shown to be suitable for use as a chromatographic support in the range of pH 7–11 [26].

Several reports have indicated that selected analysis conditions can increase useful column lifetime. These studies suggest that the rate of silica dissolution at high pH is reduced using high concentrations of organic modifier [6,27] and certain types and concentrations of salts in the mobile phase [28,29]. Moreover, the column lifetime is greatly increased using: (a) organic buffers, such as Tris or glycine

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instead of the commonly used phosphate and carbonate buffers, (b) temperatures of 40 °C or lower, (c) buffer concentrations of 0.02–0.05 mol l^{-1} and (d) buffers made with lithium salts instead of sodium salts [9,30,31].

Among the stationary phases that have been evaluated in terms of stability, endcapped phases are the most studied [1,7,8,24]. Many different aging conditions have been tested; a general method for stability evaluation does not exist. However, most current procedures use very large volumes of mobile phases, as the stationary phases now available are more resistant to the attack of mobile phase. For example, aging tests performed on Hypersil ODS, NovaPak C$_{18}$ and Zorbax RX-C$_8$ columns employed 82, 57 and 50 l, respectively, of pH 9 mobile phase at room temperature [1]. These values correspond to approximately 4–7 months of 8 h workdays.

Since these testing procedures are very slow, the objective of the present work is to develop a procedure that would allow faster stability evaluations for a series of projects devoted to development of new stationary phases. By comparison with tests previously reported for some commercial columns, the lower limit of durability for these new columns could then be predicted.

In this work, the effects of temperature, pH and mobile phase flow rate on the dissolution rate of polymer-coated silicas were studied. Also, a zirconized silica-based stationary phase was evaluated and compared with commercial stationary phases. The results suggest a rapid procedure for stability evaluation of columns prepared with sorbed, immobilized or chemically bonded packings, requiring less time and smaller quantities of reagents and solvents.

Two different stationary phases subjected to the same very aggressive test conditions were investigated by elemental analysis and scanning electron microscopy. The results confirm that different kinds of stationary phases can have quite different degradation paths.

2. Experimental

2.1. Reagents and materials

Analytical-reagent grade or HPLC-grade solvents were obtained from Mallinckrodt (methanol and chloroform), Merck (acetone, toluene and dichloromethane), Riëdel de Häen (benzonitrile), Synth (benzene), Carlo Erba (naphthalene), Nuclear (sodium hydroxide) and Fischer Scientific (sodium bicarbonate). Water was distilled and then deionized (Milli-Q, Millipore).

Irregular Davisil silica (Alltech) was used as chromatographic support, having a mean particle size of 13 μm, average pore diameter of 8.7 nm, specific pore volume of 0.75 ml g^{-1} and specific surface area of 350 m^2 g^{-1}. The zirconized silica was prepared [32] by an adaptation of the method of Peixoto et al. [33]. PMOS polymer with an average molar mass of 6200 was obtained from Petrarch

Table 1
Columns prepared from the several stationary phases

Stationary phase	Column dimensions (mm)	Carbon (%)
SiO$_2$(PMOS)	150 × 3.9	21.8
Si-Zr(PMOS)	150 × 3.9	19.3
Hypersil ODS	80 × 4.0	11.1
NovaPak C$_{18}$	60 × 4.0	8.2

Systems/Hüls America. Two chemically bonded C$_{18}$ phases, Hypersil ODS, 3 μm and NovaPak C$_{18}$, 4 μm, from Waters, were used for comparison.

2.2. Preparation of the stationary phases and columns

Batches of stationary phases were prepared from both silica and zirconized silica by the solvent evaporation method [34]. The supports were dried in air at 150 °C for 24 h and then added to solutions of PMOS dissolved in dichloromethane. The solvent was then slowly evaporated from the mixture at room temperature to give a 1:1 (m/m) PMOS-loaded silica, SiO$_2$(PMOS). Batches were stored in air at room temperature for at least two months for self-immobilization [35] before slurry-packing into columns. Zirconized silica-based phases, SiO$_2$-Zr(PMOS), were submitted to immobilization by gamma radiation at a dose of 120 kGy, carried out under air in glass ampoules with a cobalt-60 irradiator (IBRAS-CBO, Campinas, Brazil).

Columns were made from type 316 stainless steel tubing. The internal surfaces were polished using a technique developed in our laboratory [36]. Slurry packing of the columns was done at 34.5 MPa with a Haskel packing pump using 10% slurries of the stationary phases in CHCl$_3$–MeOH (90:10, v/v). Methanol was used as propulsion solvent. Columns were conditioned for 4 h with mobile phase, at 0.3 ml min^{-1} prior to testing. Table 1 lists the columns prepared from the different stationary phases.

2.3. Instrumentation

The pH of the mobile phases was measured with a Digimed DC 21 pH meter (São Paulo, Brasil). Column aging and chromatographic tests were performed with a modular HPLC system consisting of a Waters 510 pump, a Rheodyne model 7125 injector (10 μl), an Alltech model 450 UV (254 nm) detector, a Waters model RCM-100 oven and a Waters model TCM temperature controller. Data acquisition was carried out by Chrom Perfect for Windows, version 3.52 and Report-Write Plus software (Justice Innovations).

2.4. Stability evaluations

A test mixture composed of solutes having different polarities (acetone, benzonitrile, benzene, toluene and naphthalene) was dissolved in methanol–water (70:30, v/v). Chromatographic performance was evaluated by means

of efficiency (plates m^{-1}), retention factor (k) and asymmetry (As) at 10% of peak high. The column dead time, t_M, was determined using methanol as an unretained compound. The stability was evaluated using several different mobile phases: methanol–water (70:30 or 60:40, v/v) and methanol–0.1 mol l^{-1} aqueous NaHCO$_3$ (50:50, v/v), at pH 8.4, 9.2 and 10.1. The pH was adjusted with a concentrated NaOH solution before mixing with methanol. The amount of mobile phase that passed through the column is expressed in milliliters.

In the development of the new testing procedure, the following parameters were evaluated: column temperature, flow rate and mobile phase pH.

2.4.1. Effect of the temperature

Using a methanol–water (60:40, v/v) mobile phase at 1.2 ml min^{-1}, the temperature was varied from 30 to 60 °C. After a determined volume of neutral mobile phase had passed, the flow rate was lowered to 0.3 or 0.8 ml min^{-1} for 20 min before injection of the test mixture.

2.4.2. Effect of the pH

Using a flow rate of 0.6 or 1.2 ml min^{-1} with the temperature at 60 °C, different mobile phases, consisting of methanol–0.1 mol l^{-1} aqueous NaHCO$_3$ (50:50, v/v), with pH 8.4, 9.2 or 10.1, were tested. With some columns the chromatographic evaluation was carried out in a methanol–water mobile phase at 0.3 ml min^{-1} while with others the test solutions were injected directly into the basic mobile phase using a flow rate of 1.0 ml min^{-1}.

2.5. Physical characterization after stationary phase degradation

The stability tests terminated when a $\geq$30% decrease in column efficiency had occurred. The columns were then carefully emptied and portions of packing from different regions of the column (top, middle and bottom), as well as unused packings, were taken for physical and chemical characterizations.

The percent carbon in the stationary phases was determined by elemental analysis using a model CHN 2400 Perkin-Elmer analyzer.

Scanning electron microscopy (SEM) was used to study the changes which occurred in the particle and on its surface and to ascertain the particle size distributions of the phases. To obtain the SEM images, the samples were sputter-coated with a thin film of gold (100 Å) and the metallized target was bombarded with atoms of argon under high vacuum. SEM images were obtained using a field-emission gun scanning electronic microscope (JSM-6330 F). The particle size distribution was determined by measuring the particle diameter from the SEM images. In the case of the zirconized irregular silica-based stationary phase, the particle diameter was defined as the larger dimension of each particle. As a representative magnitude of the amount of particle per diameter

the count frequency given by the following expression was used:

$$\text{frequency} = \frac{100 N_i}{N_T}$$

where N_i is the particle number with diameter between d_i and $d_i + \Delta d$ and N_T is the total number of measured particles.

3. Results and discussion

3.1. Stability testing

Fig. 1 shows the behavior of several chromatographic parameters for a self-immobilized stationary phase (stored for 2 months) during stability testing with the MeOH–water mobile phase, as a function of increasing temperature. The most significant changes in efficiency and retention factor occurred at the lowest temperature (30 °C), related to lixiviation of non-immobilized polymer. Once this had been removed, the several temperature increments, to 40, 50 and 60 °C, in steps of 15,000 ml of mobile phase, caused relatively little change in these chromatographic parameters, suggesting that temperature does not significantly affect the results when the MeOH–water mobile phase is used. This stationary phase resisted more than 54,000 ml (30,000 column volumes) of mobile phase, with the last 14,000 ml at 60 °C, while maintaining its good chromatographic characteristics. The time required for this test was about 4 months.

The column was evaluated at three different flow rates (0.3, 0.8 and 1.2 ml min^{-1}), presenting higher efficiency values when using 0.3 ml min^{-1}, in agreement with conventional interpretations of a van Deemter plot.

The degradations of the SiO$_2$(PMOS) stationary phases, using different pH of the mobile phase at 60 °C, are shown in Fig. 2. In the case of the test carried out with pH 10.1, the column failed so quickly that it was not possible to observe an efficiency plateau. However, a longer column lifetime was obtained at pH 8.4, making it possible to study the evolution of stationary phase degradation. In Fig. 2b, an increase in the asymmetry factor, after passing of a certain amount of mobile phase through the column, can be observed. This asymmetry increase is correlated with the efficiency loss. The retention factor remains almost unaffected (Fig. 2c). Since the retention factor is related to solute interaction with the polymeric phase, this means that, even if the silica dissolves, the loss of polymeric phase from inside the column is minimal. Thus, stability is better evaluated by observing the variations in efficiency and asymmetry.

Fig. 3 shows the influence of the mobile phase flow rates of 0.6 and 1.2 ml min^{-1} during the stability test in two columns prepared with the same stationary phase. A lesser stability was observed when a flow rate of 0.6 ml min^{-1} was used. This indicates that the contact time was greater at lower flow rate, promoting faster dissolution of the silica. Correlating these results with those shown in Fig. 1, we in-
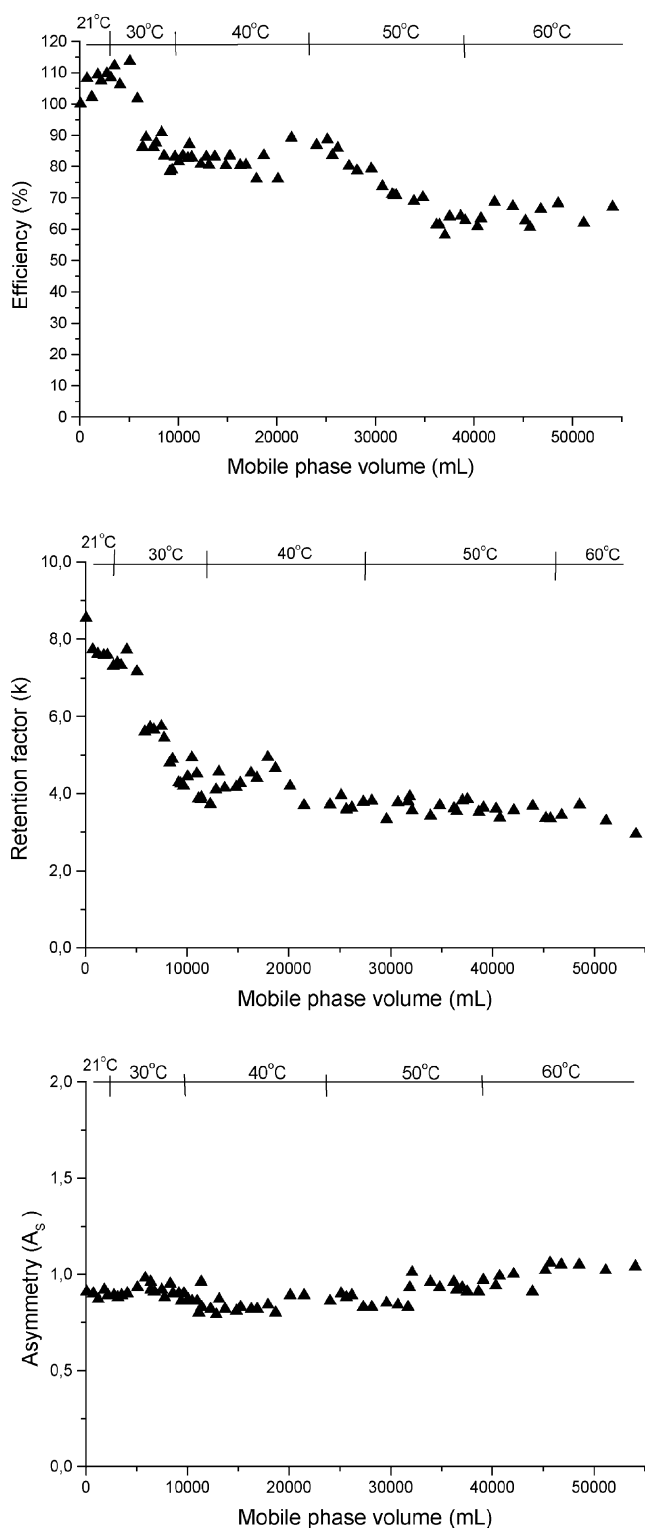
Fig. 1. Performance (efficiency, retention factor and asymmetry factor) of a $SiO_2(PMOS)$ phase stored for 2 months before packing as a function of column temperature. Stability and evaluation mobile phase: MeOH–water (60:40, v/v), stability and evaluation flow rates: 1.2 and $0.3\,ml\,min^{-1}$, respectively. The parameters were calculated from the naphthalene peak.
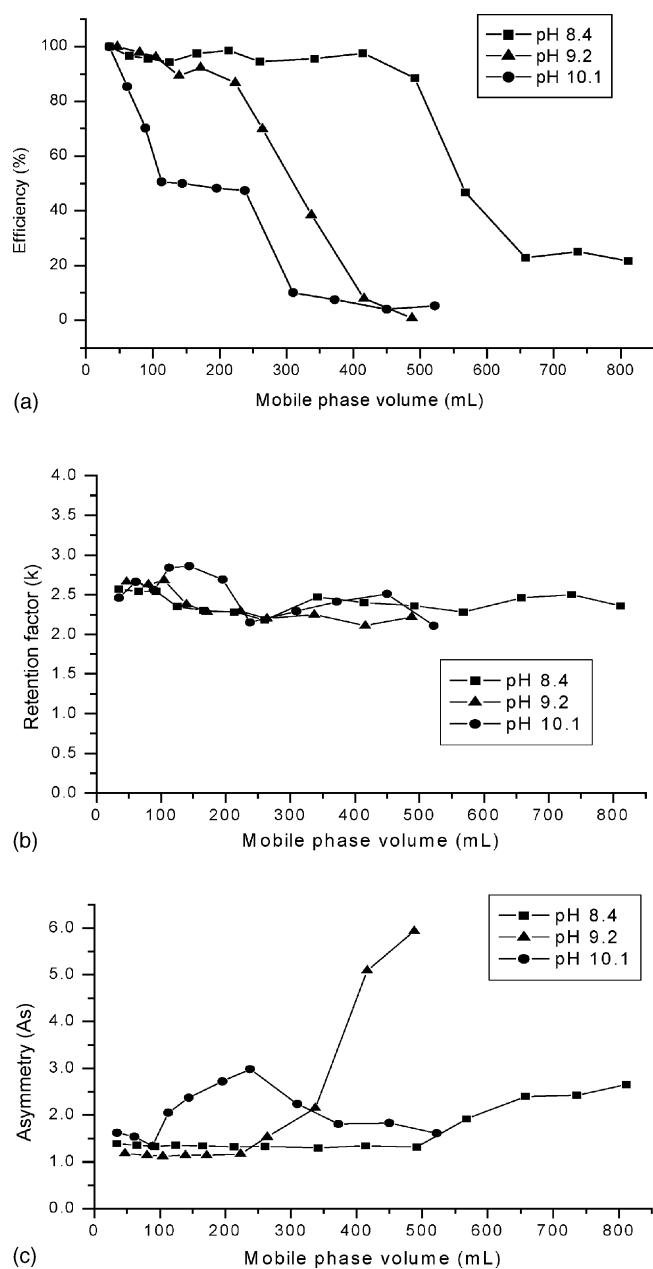

Fig. 2. Stability of a $SiO_2(PMOS)$ phase at $60\,°C$ as a function of mobile phase pH. Stability test conditions: mobile phase: MeOH–$0.1\,mol\,l^{-1}$ aqueous $NaHCO_3$ (50:50, v/v) at different pH, using a $1.2\,ml\,min^{-1}$ flow rate. Evaluation conditions: mobile phase MeOH–water (70:30, v/v), flow rate $0.3\,ml\,min^{-1}$. The parameters were calculated from the naphthalene peak.

fer that degradation of this sorbed stationary phase after the use of 350 ml of pH 8.4 mobile phase at $60\,°C$ represents a column lifetime greater than 4 months under neutral mobile phase conditions.

Using a basic mobile phase at $60\,°C$ reduces evaluation time to several days. However, the test would be more efficient if the chromatographic evaluation could be carried out directly in the aggressive mobile phase being used for the stability test. Fig. 4 shows the chromatographic param-
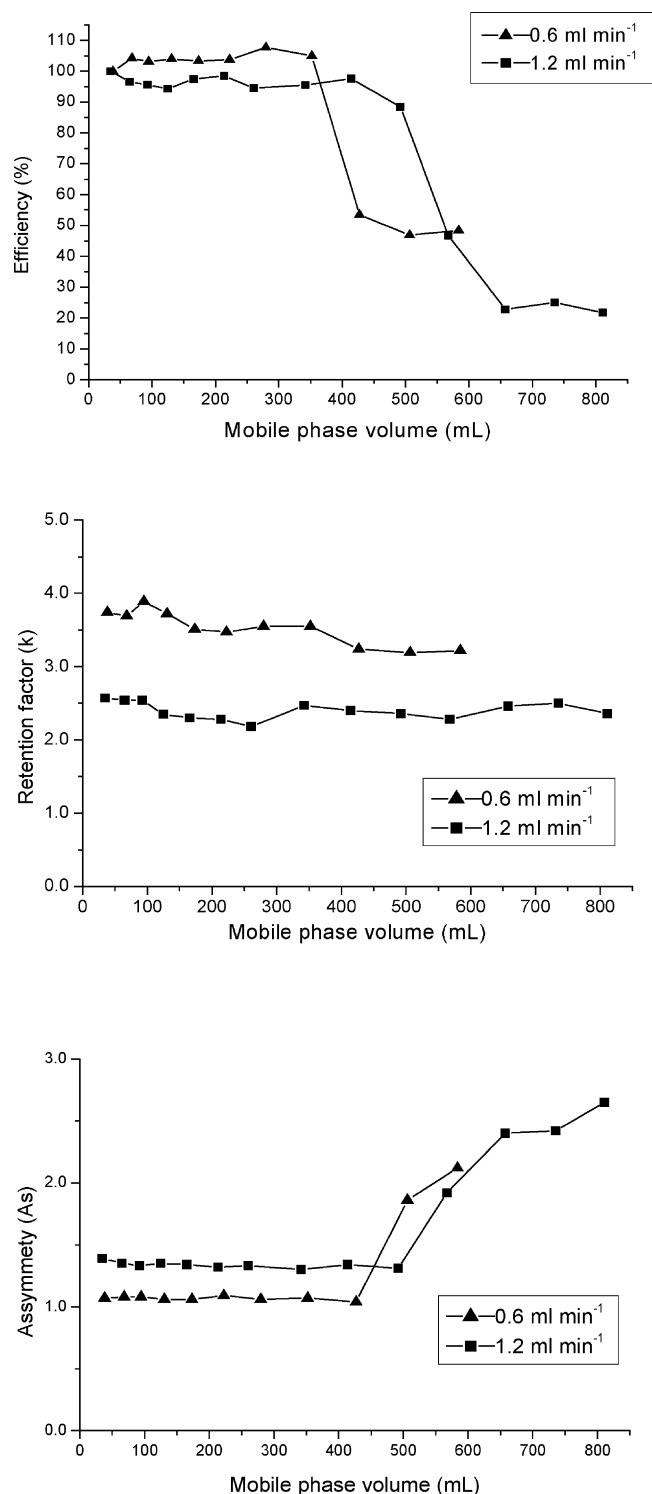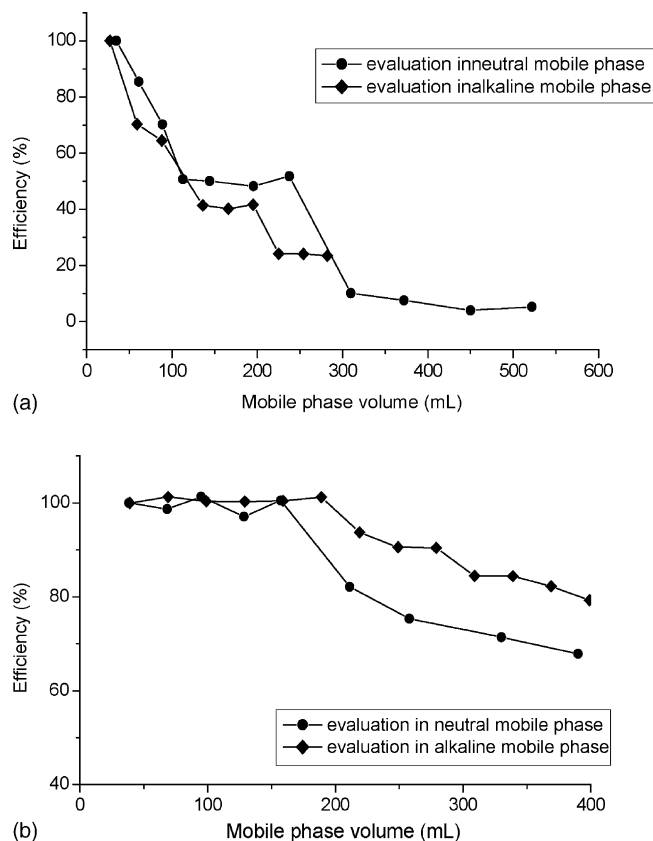
Fig. 4. Evaluation of (a) SiO$_2$(PMOS) and (b) Hypersil ODS stationary phases, in MeOH–water (70:30, v/v) at 0.3 ml min^{-1} and MeOH–0.1 mol l^{-1} aqueous NaHCO$_3$, pH 10.1 (50:50, v/v) at 1.0 ml min^{-1}; temperature 60 °C.

Fig. 3. Stability of a SiO$_2$(PMOS) phase at 60 °C as a function of MeOH:0.1 mol l^{-1} aqueous NaHCO$_3$, pH 8.4 (50:50, v/v) mobile phase flow rate. Evaluation conditions: mobile phase MeOH–water (70:30, v/v), flow rate 0.3 ml min^{-1}. The parameters were calculated from the naphthalene peak.

eters for both a SiO$_2$(PMOS) stationary phase and a commercial chemically-bonded stationary phase (Hypersil ODS) evaluated using either a methanol–water (70:30, v/v) mobile phase at 0.3 ml min^{-1} or a methanol–0.1 mol l^{-1} aqueous NaHCO$_4$, pH 10.1 (50:50, v/v) mobile phase at the same flow rate as used for the stability test. In both cases, the temperature was held at 60 °C. Similar behaviors and degradation times were observed using both evaluation mobile phases. This result suggests that chromatographic evaluation can be made directly in the test phase, eliminating pre-evaluation column conditioning and, thus, reducing the analysis time.

The behavior of PMOS immobilized on zirconized silica and of two commercial bonded phases (Hypersil ODS and NovaPak C$_{18}$) with continual passing of a pH 10.1 mobile phase at 1.0 ml min^{-1} at 60 °C with periodic evaluation in the same mobile phase is shown in Fig. 5. The commercial stationary phases degraded after about 250 ml of pH 10.1 mobile phase has passed, while degradation of the SiO$_2$-Zr(PMOS) phase occurred after about 600 ml. This shows the effect of combining a zirconia layer on the silica with cross-linking of the polymer, preventing fast dissolution of the silica support even with the use of both high temperature and high pH.
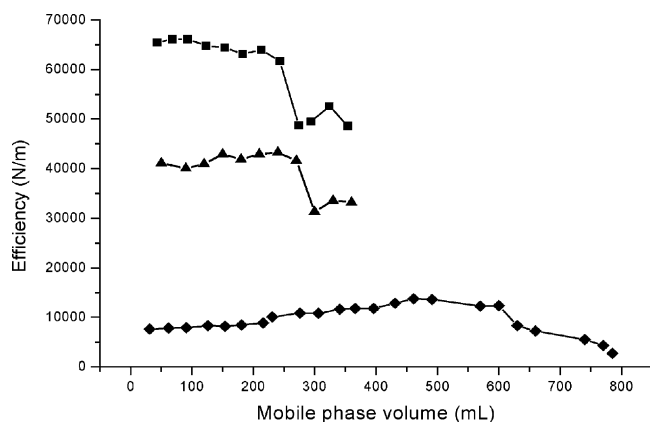
Fig. 5. Chromatographic parameters for columns tested by the new procedure. Stability and evaluation conditions: mobile phase MeOH–0.1 mol l^{-1} aqueous NaHCO$_3$, pH 10 (50:50, v/v) at 1.0 ml min^{-1}; temperature 60 °C. Hypersil ODS (▲), SiO$_2$-Zr(PMOS) (◆) and NovaPak C$_{18}$ (■).

Earlier stability studies [1] made with commercial columns showed that Hypersil ODS resists the passage of 33,000 column volumes (82,200 ml) of pH 9 mobile phase at room temperature, corresponding to approximately 7 months of 8 h workdays, while NovaPak C$_{18}$ resists the passage of 23,000 column volumes (57,300 ml), equivalent to around 5 months of 8 h workdays. In our test, both columns degraded after similar volumes of pH 10.1 mobile phase. This result suggests that stationary phases that degrade after passing approximately 250 ml of aggressive mobile phase at elevated temperature will have average lifetimes greater than 6 months, when routine chromatographic conditions are used.

3.2. Physical characterization of the degraded stationary phases

The percentages of carbon present in the SiO$_2$-Zr(PMOS) phase and the NovaPak C$_{18}$ stationary phase, before slurry-packing and after column degradation, are shown in Table 2. After the stability test, the NovaPak C$_{18}$ stationary phase shows a decrease in the percent carbon at the top and in the middle of the column, in relation to untested phase, indicating that the carbon distribution within the column increases from top (inlet) to bottom (outlet). This result is similar to that reported by Kirkland et al. [1], suggesting that the silane stationary phase that is removed from the top, as a result of silica dissolution, is captured by the column packing as it passes down the column. On the other

Table 2
Percent carbon in the stationary phases before and after degradation

Portions of stationary phase	NovaPak C$_{18}$	SiO$_2$-Zr(PMOS)
Untested packing	8.2	19.3
Top of column	7.6	24.1
Middle of column	7.8	18.4
Bottom of column	8.1	18.2

hand, the SiO$_2$-Zr(PMOS) phase showed a different behavior, resulting in a significantly higher percent of carbon at the column inlet than seen in the untested phase, with the carbon content decreasing from the column inlet to the outlet. As a result of the cross-linking of the polymer chains of PMOS, it is not washed down the column. Thus, the polymer/silica ratio increases at the top when some of the silica is dissolved.

SEM images of the untested stationary phases show that the particles of the NovaPak C$_{18}$ stationary phase have spherical form and the size dispersion is small, while in the zirconized silica-based stationary phase the particles are irregular with larger size dispersion. Some SiO$_2$-Zr(PMOS) particles present surface cracks, possibly arising during the process of silica modification with zirconium.

SEM images from the top, middle and bottom of the NovaPak C$_{18}$ phase after degradation appear similar to those without degradation, although a small displacement of the distribution to smaller diameters can be observed in the degraded phases, compared with the untested phase. However, no appreciable differences exist along the column (top, middle and bottom), suggesting that the silica dissolution occurs at about the same rate throughout the column.

The particle size distributions for the SiO$_2$-Zr(PMOS) phase indicate a similarity in size distribution between untested phase and the column outlet portion. For the top and middle portions, the distribution shifts to smaller sizes, with this change being more notable at the top of the column, suggesting that silica dissolution occurs in a different way along the chromatographic column. This is probably related to the fact that the silica used to prepare the zirconized support was an irregular, xerogel silica [9]. It is also possible that the presence of the cracks aid the dissolution.

The SEM images of the packings at different positions of the SiO$_2$-Zr(PMOS) column after degradation reveal that particles at the top of the column do not have surface cracks. This suggests that the increase in the quantity of somewhat smaller particles, in the range between 5 and 10 μm, comes from breaking some larger particles. This breaking may occur if silica dissolution is enhanced in the cracks.

This study thus provides additional information about the silica dissolution process in different stationary phases. With a bonded phase it was observed that, as a result of silica dissolution, silane groups are displaced from the top to the exit of the column. Silica dissolution occurred in essentially the same way throughout the column. However, in the immobilized polymer phase, the polymer remained in place even with silica dissolution. In this case, however, the silica dissolution is not uniform, being more accentuated at the top of the column. The behavior of the particle size distributions, together with the results of percent carbon, permit the conclusion that the two different stationary phases, one based on a sol–gel silica and the other on an irregular, xerogel silica, undergo different degradation mechanisms.

4. Conclusions

A new procedure to evaluate the stability of chemically bonded and immobilized stationary phases is described. The test conditions involve a carbonate-based mobile phase at pH 8.4 or 10 (methanol–0.1 mol l^{-1} aqueous NaHCO$_3$, 50:50, v/v), a temperature of 60 °C and a flow rate of 0.6 or 1.0 ml min^{-1}, with periodic test injections made directly into the same mobile phase.

Through the comparison of the results reported here with stability tests reported in literature for two commercial columns (Hypersil ODS and NovaPak C$_{18}$), the use of 250 ml of pH 10 mobile phase is equivalent to the mobile phase volume used in approximately 6 months of routine chromatographic work (pH $\leq$ 9 with a salt concentration of 0.01 mol l^{-1} and room temperature). On the other hand, for self-immobilized polymer-coated stationary phases the use of 350 ml of pH 8.4 mobile phase is equivalent to the mobile phase volume used in about 4 months.

Since the stability evaluations are made without modifying the mobile phase, this new test procedure can easily be automated, providing a rapid evaluation of the relative stabilities of new stationary phases, while saving both time and reagents.

Acknowledgements

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