

High-performance liquid chromatographic stationary phases based on poly(methyloctylsiloxane) immobilized on silica

III. Stability evaluations

Edivan Tonhi, Kenneth E. Collins, Carol H. Collins*

Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, CEP 13084-971 Campinas, SP, Brazil

Abstract

Several reversed-phase materials for high-performance liquid chromatography were obtained by deposition of poly(methyloctylsiloxane) (PMOS) on HPLC silica particles, followed by immobilization using different procedures. Each phase had characteristic physicochemical and chromatographic properties. The present work evaluates the stability of these phases with both neutral and basic mobile phases. All of the stationary phases were quite stable to neutral mobile phase, with less stability at higher pH. However, one thermally immobilized phase presented high stability even at an elevated temperature with a pH 10.0 mobile phase.

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1. Introduction

Many chromatographic applications demand acidic ($\text{pH} \leq 3$) or basic ($\text{pH} \geq 8$) conditions while the reversed phases most commonly used in high-performance liquid chromatography (HPLC) present significantly decreased lifetimes under these conditions. Such pH stable phases are of increasing interest for the separation of basic compounds, especially pharmaceuticals, for which symmetrical peaks are a requirement to evaluate product purity. Thus, many attempts have been made to prepare basic-mobile-phase-stable, selective stationary phases which produce symmetrical peaks with basic compounds, with varying degrees of success [1–5].

The stability of columns packed with chemically

bonded stationary phases based on silica is directly related to the type and purity of the silica support and the nature of the bonded phase, but it also depends significantly on analysis conditions such as: pH, temperature, type and concentration of the buffer and the organic modifier used in the mobile phase [6]. Studies have shown that mobile phases above pH 8 that have certain characteristics increase the speed of dissolution of the silica and, therefore, significantly decrease the lifetime of the column [7–10]. These characteristics, of which one or more may be necessary for silica dissolution, are: use of carbonate or phosphate buffers, which are much more aggressive than borate or organic buffers, use of buffer salt concentrations above 0.05 mol/L, use of methanol instead of acetonitrile as the organic modifier and use of analysis temperatures higher than 40 °C.

Methods to evaluate the stability of chemically

*Corresponding author. Fax: +55-19-3788-3023.

E-mail address: chc@iqm.unicamp.br (C.H. Collins).

bonded phases have been an important aspect of many studies. For these studies, several analysis conditions have been used, but there is no established general procedure. The more widely used methods of evaluation of the stability of current stationary phases [7,8,11–13] employ very large volumes of mobile phase, due to the recent increases in stationary phase stability.

When a packing material is stable, it allows the column characteristics to stay unaffected and useful for a long time, reducing the frequency of column substitution. The lifetime of a column is defined by degradation of the chromatographic separation and depends on the amount of loss of both retention and resolution that can be tolerated before the column must be replaced. With conventional reversed phases, the first sign of the degradation of a column is usually a decrease in the retention factor (k) [11]. However, for columns more stable to high pH, k may stay relatively constant, even though dissolution of the silica has started. Since the lifetime of these columns is limited, their overall chromatographic performance, especially the number of plates and the asymmetry factor, soon begin to degrade. Thus, the best indicators for evaluating the stability of a column are a loss in efficiency (N) and an increase in the asymmetry factor (A_s).

In this work, several different reversed-phase materials for HPLC were prepared by deposition of poly(methyloctylsiloxane) (PMOS) on silica particles, followed by immobilization using different procedures: thermal treatment, microwave irradiation, γ radiation and self-immobilization, as described in Parts I [14] and II [15] of this study. These immobilized phases were tested using the usual neutral mobile phase method [13] and also by a new, faster test using more drastic mobile phase conditions [16,17].

2. Experimental

2.1. Materials

The chromatographic support used to prepare the stationary phases was spherical Kromasil silica (Akzo Nobel, Bohus, Sweden) having a mean particle diameter of 5 μm , 0.89 ml g^{-1} specific pore

volume and 330 $\text{m}^2 \text{g}^{-1}$ specific surface area. For comparison, a commercial stationary phase, Rainin C_8 (Varian, Mountain Creek, CA, USA), with a mean particle diameter of 5 μm was also used.

PMOS (product PS-140), number-average molecular mass (M_n) 6200 and weight-average molecular mass (M_w) 16 000, was obtained from Petrarch/Hüls America (Piscataway, NJ, USA). Methanol (Omnisolv), chloroform (LiChrosolv) and hexane (HPLC-grade) were all from Merck (Rio de Janeiro, Brazil). Distilled, deionized water (Milli-Q Plus, Millipore, Bedford, MA, USA) was used throughout.

The chromatographic test substances uracil (Aldrich, Milwaukee, WI, USA), phenol (Labsynth, Diadema, Brazil), N,N -dimethylaniline (Fluka, Steinheim, Switzerland), naphthalene (Vetec, Rio de Janeiro, Brazil), ethylbenzene (Merck) and acenaphthene (Aldrich) were analytical-reagent grade and not further purified. The sodium hydrogencarbonate and sodium hydroxide used to prepare alkaline mobile phases were analytical-reagent grade from Fisher (Fair Lawn, NJ, USA).

2.2. Instrumentation

The columns were evaluated by a modular HPLC system equipped with a Rheodyne (Rohnert Park, CA, USA) model 8125 injector (5- μl loop), a Shimadzu (Tokyo, Japan) model LC-10AD pump and an Alltech (Deerfield, IL, USA) model 450 UV (254 nm) detector with a 0.8- μl cell. Data acquisition used ChromPerfect for Windows, version 3.52 (Justice Innovations, Mountain View, CA, USA), with the Report-Write Plus option for calculation of the chromatographic parameters, installed in a IBM compatible personal computer.

2.3. Preparation of the stationary phases

The preparation and physical characterization of four of the stationary phases tested here: $\text{SiO}_2(\text{PMOS})$ -1, thermal treatment at 120 $^\circ\text{C}$ for 4 h; $\text{SiO}_2(\text{PMOS})$ -2, irradiation with microwaves at 495 W for 15 min; $\text{SiO}_2(\text{PMOS})$ -3, irradiation with γ radiation to a dose of 80 kGy; and $\text{SiO}_2(\text{PMOS})$ -4, self-immobilization by storage in air for 30 days, were described in a previous paper [14].

A new stationary phase, $\text{SiO}_2(\text{PMOS})$ -5, was

prepared by optimization of the thermal immobilization process, based on the results from ^{29}Si - and ^{13}C -NMR determinations [14], which showed that bonding between the polymer and the support is induced by thermal immobilization, and that these bonds involve both the silanol groups of the silica and the PMOS chains [18]. Aiming to increase the number of available silanol groups for bonding with the polymeric chains, an acid hydrolysis of the silica was promoted by refluxing the silica in 0.01 mol/L nitric acid for 4 h. The silica was then washed sequentially with methanol at 3 ml min^{-1} for 3 h, isopropanol at 2 ml min^{-1} for 30 min and hexane at 2 ml min^{-1} for 2 h, and dried at $40\text{ }^\circ\text{C}$ for 3 h. After silica activation ($120\text{ }^\circ\text{C}/17\text{ h}$), the stationary phase (54.0% initial loading) was prepared by the solvent evaporation method, as previously described [14]. Polymer immobilization was at $120\text{ }^\circ\text{C}$ for 16 h. The excess PMOS, which was not immobilized, was extracted from the stationary phase by pumping a sequence of chloroform (0.5 ml min^{-1} flow rate for 4 h) and methanol (0.5 ml min^{-1} flow rate for 2 h) at room temperature through the material contained in a column-type washing system. The phase was then dried ($40\text{ }^\circ\text{C}$ for 12 h) and stored in a closed container until needed. This phase was characterized both physically and chromatographically as previously described [14,15].

2.4. Preparation of the test columns

The columns ($50\times 4\text{ mm}$) were made from type 303 stainless-steel tubing with highly polished interior surfaces [19] and downward packed using 10% (w/v) slurries of each stationary phase in chloroform. A packing pressure of 34.5 MPa (Haskel Model 51769 packing pump) was used, with methanol as the propulsion solvent. Columns were conditioned for 4 h with mobile phase (methanol–water 7:3, v/v) at 0.2 ml min^{-1} at room temperature prior to the stability tests.

2.5. Chromatographic evaluation

The chromatographic evaluations of the columns packed with stationary phases $\text{SiO}_2(\text{PMOS})$ -1 to $\text{SiO}_2(\text{PMOS})$ -4 are shown in Part II [15]. The evaluation of the column packed with stationary

phase $\text{SiO}_2(\text{PMOS})$ -5 was based on the separation of a text mixture containing acidic, basic and neutral solutes (uracil, phenol, *N,N*-dimethylaniline, naphthalene, ethylbenzene and acenaphthene), dissolved in mobile phase (methanol:water 7:3, v/v). Injection of $5\text{ }\mu\text{l}$ of this mixture produced satisfactory chromatographic peaks with detection at 254 nm. The separation was carried out at room temperature with a flow rate of 0.5 ml min^{-1} . This optimal flow rate was determined by a Van Deemter curve. The column dead time, t_M , was determined by uracil (an unretained compound). The chromatographic parameters (retention factor (k), efficiency from peak width at half height (N) and asymmetry factor at 10% of the peak height (A_s)) were determined for each peak.

2.6. Stability tests

2.6.1. Stability testing using a neutral mobile phase

The columns packed with the stationary phases $\text{SiO}_2(\text{PMOS})$ -1 to $\text{SiO}_2(\text{PMOS})$ -4 were submitted to testing by passing methanol–water (7:3, v/v) at room temperature ($\sim 25\text{ }^\circ\text{C}$) through the column at 1.0 ml min^{-1} and periodically injecting a test mixture (uracil, phenol, *N,N*-dimethylaniline and naphthalene) to evaluate column performance with time. For the chromatographic evaluation, the flow rate of the mobile phase was decreased to 0.5 ml min^{-1} . The chromatographic parameters k , N and A_s were determined for each peak. Column degradation was evaluated by comparing the percentage of the initial efficiency (% initial N), as a function of the time.

2.6.2. Stability testing using an alkaline (pH 8.4) mobile phase and elevated temperature

This test, developed in our laboratory [16,17], consists of pumping an alkaline (pH 8.4) mobile phase, methanol–0.1 mol/L sodium hydrogencarbonate (1:1, v/v), through the columns at 0.6 ml min^{-1} with the columns inside an oven held at $60\text{ }^\circ\text{C}$. After defined time periods of 1 h, the columns were removed from the oven and coupled to the HPLC test system, passing a methanol–water (7:3, v/v) mobile phase at 0.5 ml min^{-1} for 15 min to remove the alkaline mobile phase from within the columns as well as to lower the column temperature.

After this time, the detector was connected and a test mixture, composed of uracil, phenol, *N,N*-dimethylaniline and naphthalene, was injected. After chromatographic evaluation, the columns were again placed inside the oven at 60 °C and submitted to the passage of more alkaline mobile phase (0.6 ml min⁻¹ for 1 h). This test continued until total degradation of each column.

2.6.3. Stability testing using an alkaline (pH 10.0) mobile phase and elevated temperature

This testing procedure was carried out as described for the stability testing with pH 8.4 mobile phase, although for this test, the alkaline mobile phase, methanol–0.1 mol/L sodium hydrogencarbonate (1:1, v/v), had the pH increased to 10.0 with concentrated NaOH and was pumped through the columns at 1.0 ml min⁻¹. These conditions accelerate column deterioration and it is possible to obtain faster evaluations using considerably less mobile phase and instrument time.

2.7. Percent carbon

The percent carbon was obtained by elemental analysis of each stationary phase before and after the stability test using a Perkin-Elmer (Norwalk, CT, USA) Model 2400 CHN analyzer.

2.8. Scanning electron microscopy

A morphologic study of the stationary phases before and after the stability test was made using a JEOL (Peabody, MA, USA) Model JSM-T300 scanning electron microscope at 20 kV. The samples were sputter-coated with gold and then examined with 20 000× magnification.

3. Results and discussion

3.1. Chromatographic characterization of the SiO₂(PMOS)-5 stationary phase obtained by optimization of thermal immobilization

The stationary phase obtained by the optimization of thermal immobilization (SiO₂(PMOS)-5), was chromatographically evaluated using methanol–

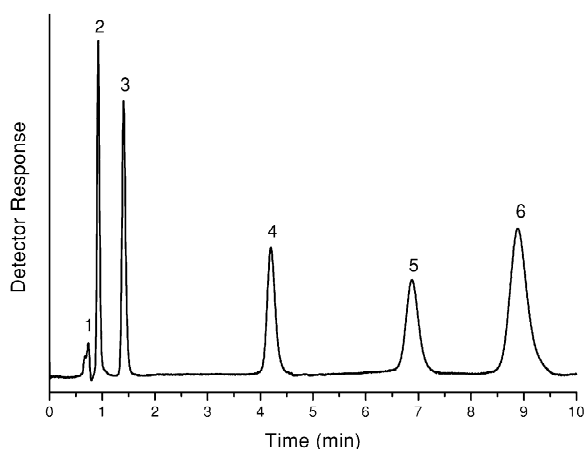


Fig. 1. Chromatogram using the SiO₂(PMOS)-5 stationary phase, immobilized by heating at 120 °C for 16 h. Conditions: column: 50×4 mm, mobile phase: methanol–water (7:3, v/v), flow rate: 0.5 ml min⁻¹. Peaks, in elution order: (1) uracil, (2) acetone, (3) benzonitrile, (4) benzene, (5) toluene and (6) naphthalene.

water (7:3, v/v) mobile phase. The column was efficient for the separation of the test mixture, as shown in the chromatogram presented in Fig. 1.

The chromatographic parameters obtained from the peak of acenaphthene (efficiency, retention factor, asymmetry and resolution) are shown in Table 1. For comparison, Table 1 repeats the results of the columns packed with phases SiO₂(PMOS)-1 to SiO₂(PMOS)-4 [15]. The column packed with optimized stationary phase (thermal treatment at 120 °C for 16 h) showed higher efficiency than the other columns, although the peak of acenaphthene appears less symmetrical. The retention factor of the acenaphthene peak and the resolution between the ethylbenzene–acenaphthene pair were also higher for the column packed with the optimized phase, due to the increase of polymeric covering promoted by this immobilization procedure, as shown by the percent carbon and specific mass given in Table 2.

3.2. Stability testing using neutral mobile phase

The plots of the chromatographic parameters for the naphthalene peak as a function of the mobile phase volume which has passed through the column are shown in Fig. 2. Throughout this work the mobile phase volume which passed through the column is expressed in column volumes (v_c), as is

Table 1

Comparison of the chromatographic parameters from the acenaphthene peak for columns packed with SiO₂(PMOS) stationary phases immobilized by different processes

Chromatographic parameter	Stationary phase				
	(1) Heated at 120 °C for 4 h	(2) Irradiated with microwaves	(3) Irradiated with γ radiation	(4) Self-immobilized	(5) Heated at 120 °C for 16 h
N	3035	2670	2515	2835	3430
A_s	1.00	0.94	0.95	0.96	1.26
k	8.30	3.07	5.38	1.61	29.13
R_s	6.27	4.33	4.79	3.25	9.07

The resolution was calculated for the ethylbenzene–acenaphthene pair. Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.5 ml min⁻¹.

Table 2

Percent carbon and specific masses of PMOS for the SiO₂(PMOS) stationary phases immobilized by different processes

	Stationary phase				
	(1) Heated at 120 °C for 4 h	(2) Irradiated with microwaves	(3) Irradiated with γ radiation	(4) Self-immobilized	(5) Heated at 120 °C for 16 h
% Carbon	14.5	9.5	12.4	5.8	23.3
Specific mass (g_{PMOS}/g_{SiO_2})	0.30	0.18	0.25	0.10	0.60

commonly done in the literature. The column volume was calculated from the retention time of an unrestrained compound (uracil). For the columns used in this work v_c is 0.40 ml.

All the stationary phases were reasonably stable when tested with the neutral mobile phase: there was no significant alteration of the chromatographic parameters after passing 7500 v_c of methanol–water

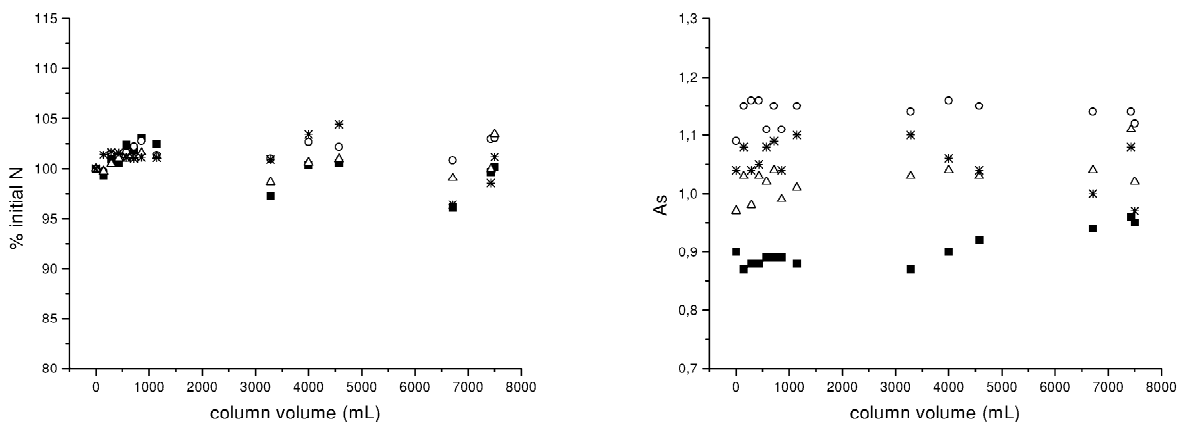


Fig. 2. Chromatographic parameters for the naphthalene peak as a function of the neutral mobile phase volume passed through columns packed with SiO₂(PMOS) stationary phases immobilized by: “■” heat treatment at 120 °C for 4 h; “○” microwave irradiation; “△” γ irradiation; and “*” self-immobilization.

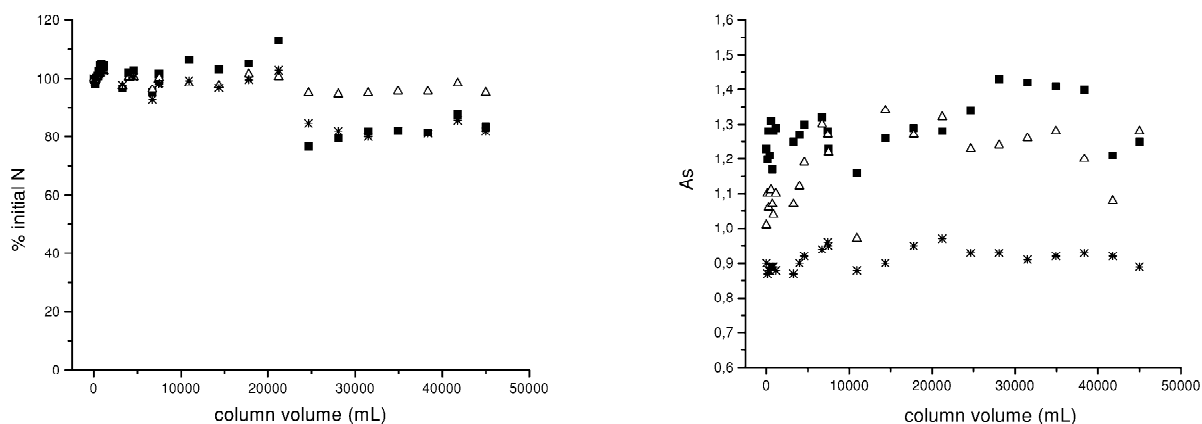


Fig. 3. Chromatographic parameters as a function of the neutral mobile phase volume passed through the columns packed with $\text{SiO}_2(\text{PMOS})$ -1 stationary phase, immobilized by heat treatment at 120°C for 4 h. Test solutes: “■” phenol; “△” *N,N*-dimethylaniline; and “*” naphthalene.

(7:3, v/v) mobile phase through the columns at room temperature (Fig. 2).

An extended test with neutral mobile phase, comparable to many shown in the literature [13], was carried out with one of the prepared phases, $\text{SiO}_2(\text{PMOS})$ -1, immobilized at 120°C for 4 h. This test used $45\,000\ v_c$ of the mobile phase, at room temperature. The results are shown in Fig. 3. The abrupt change at $22\,500\ v_c$ coincides with a subtle change in the chromatographic system (new connection tubing). Excepting this, it is noted that, even after passing $45\,000\ v_c$, there was little alteration of the chromatographic parameters of the test solutes.

3.3. Stability testing using an alkaline (pH 8.4) mobile phase and elevated temperature

The $\text{SiO}_2(\text{PMOS})$ -1 to $\text{SiO}_2(\text{PMOS})$ -4 phases were also submitted to a test with alkaline (pH 8.4) mobile phase at a temperature of 60°C . The plots of the chromatographic parameters for the naphthalene peak as a function of the mobile phase volume which has passed through the column are shown in Fig. 4.

It can be noted from the plot of % initial *N* as a function of mobile phase volume (Fig. 4) that the columns packed with phases immobilized by the different processes had different stabilities. The

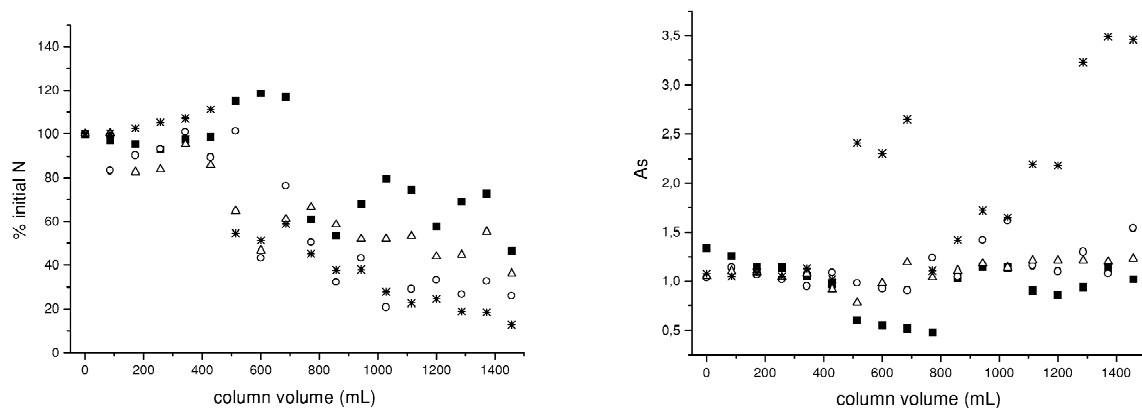


Fig. 4. Chromatographic parameters for the naphthalene peak as a function of the alkaline (pH 8.4) mobile phase volume passed through columns packed with $\text{SiO}_2(\text{PMOS})$ stationary phases immobilized by: “■” heat treatment at 120°C for 4 h; “○” microwave irradiation; “△” γ irradiation; and “*” self-immobilization.

phases immobilized by thermal treatment or by γ radiation present somewhat greater stability, when compared with the phases immobilized by microwave irradiation or using self-immobilization, an effect which is related to the amount of PMOS which has been immobilized. With respect to the asymmetry factor, only the column packed with self-immobilized stationary phase showed significant change.

In general, all the immobilized stationary phases begin to degrade after the passage of $\sim 450 v_c$ of pH 8.4 mobile phase at 60 °C. The phase immobilized by thermal treatment (120 °C/4 h) was more stable than the others, supporting larger volumes of alkaline mobile phase (600 v_c).

The cause of the low stability of the immobilized stationary phases is related to the dissolution of the silica support with the passage of the alkaline mobile phase [10], which can be shown by the increase of the percent carbon of all phases after the stability test (Table 3), as a result of the increase of the polymer/silica ratio, observed when only the support is dissolved without loss of the polymer phase. This results in a redistribution of the polymer chains on the support and, thus, changes in efficiency and retention.

3.4. Stability test of the $\text{SiO}_2(\text{PMOS})$ -5 stationary phase using alkaline (pH 8.4 and 10.0) mobile phases and elevated temperature

The stationary phase developed by optimization of the thermal treatment (120 °C for 16 h) was also tested using the pH 8.4 mobile phase at 60 °C. Plots

of the chromatographic parameters for the test solutes peaks as a function of the volume of the pH 8.4 mobile phase are shown in Fig. 5. The chromatographic parameters show essentially no variation during this stability test, except for the asymmetry factor for the phenol peak. Since the column supported a stability test with pH 8.4 mobile phase at 60 °C, it was then submitted to a test with pH 10.0 mobile phase at 60 °C, conditions much more drastic than the previous ones. For comparison, a column packed with a commercial stationary phase (Rainin C_8) also was submitted to this test. The chromatographic parameters for the naphthalene peak, as a function of the mobile phase volume which has passed through the column, are shown in Fig. 6.

The column containing $\text{SiO}_2(\text{PMOS})$ -5 was stable to $\sim 1100 v_c$ with this very aggressive (pH 10.0) mobile phase, before starting to degrade. After this it was no longer possible to obtain the chromatographic parameters, due to overlap of the peaks in the chromatogram. The column packed with commercial stationary phase showed a slow decrease of the efficiency values throughout the test; there was essentially no variation to the asymmetry factor during this stability test.

Again, the degradation of the $\text{SiO}_2(\text{PMOS})$ -5 phase is due to dissolution of the silica support, as shown by the results of the percent carbon determination (Table 3), which was higher at the end of the stability test. This result was confirmed by a morphologic study using scanning electron microscopy (data not shown), which shows that the silica particle is spherical before the test and distorted (partially dissolved) after the test.

Table 3

Percent carbon of the stationary phases immobilized by different processes before and after the stability tests with alkaline mobile phases (pH 8.4 and 10.0)

	Mobile phase pH 8.4				Mobile phase pH 10
	(1) Heated at 120 °C for 4 h	(2) Irradiated with microwaves	(3) Irradiated with γ radiation	(4) Self- immobilized	(5) Heated at 120 °C for 16 h
%C before the test	14.5	9.5	12.4	5.8	23.3
%C after the test	16.7	10.4	14.0	5.9	24.4

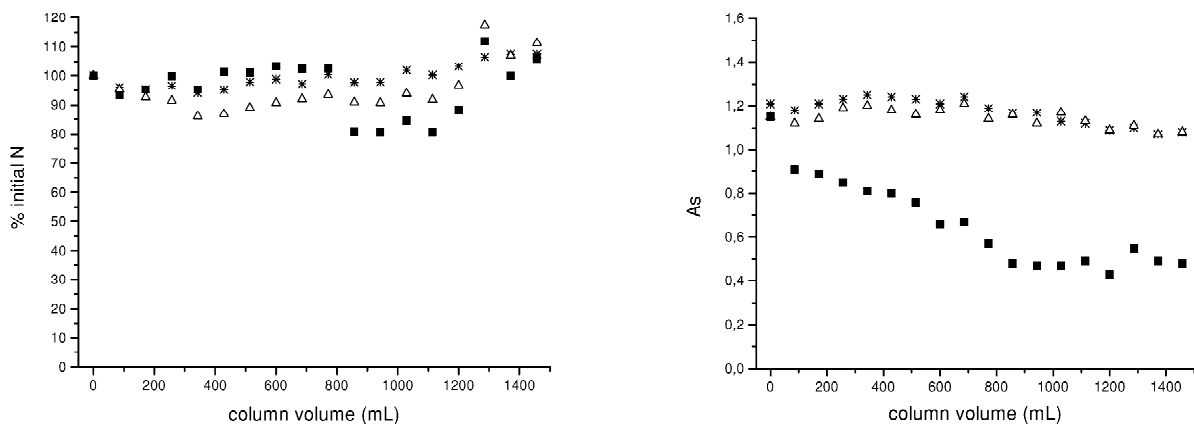


Fig. 5. Chromatographic parameters as a function of the alkaline (pH 8.4) mobile phase volume passed through column packed with $\text{SiO}_2(\text{PMOS})$ -5 stationary phase, immobilized by heat treatment at 120°C for 16 h. Test solutes: “■” phenol; “△” *N,N*-dimethylaniline; and “*” naphthalene.

4. Conclusions

All the $\text{SiO}_2(\text{PMOS})$ stationary phases, immobilized by the different processes developed in this work, are quite acceptable for use under neutral conditions. On the other hand, these phases, except one, were less stable when tested with pH 8.4 mobile phase at 60°C . To resist these drastic conditions, a stationary phase, prepared by thermal immobilization of PMOS on silica at 120°C for 16 h, was de-

veloped. The resulting stationary phase was stable to testing with pH 8.4 mobile phase at 60°C and also resisted a test with pH 10.0 mobile phase at 60°C for a significant period of time.

The tests with pH 8.4 and pH 10 mobile phases at 60°C may be recommended for enhanced-rate stability testing of silica-based reversed-phase packing materials. Even with these aggressive mobile phases, the optimized thermally immobilized PMOS stationary phase $\text{SiO}_2(\text{PMOS})$ -5 was shown to have

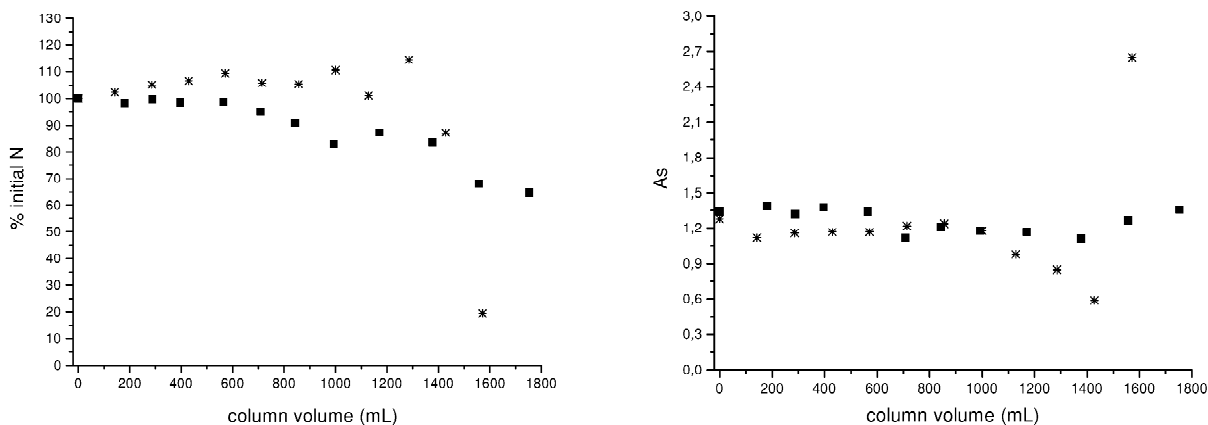


Fig. 6. Chromatographic parameters as a function of the alkaline (pH 10.0) mobile phase volume passed through column packed with: “*” $\text{SiO}_2(\text{PMOS})$ -5 and “■” Rainin C_8 stationary phases.

significant stability. This indicates the promise of these phases for the separation of real samples using basic mobile phases.

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