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

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

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

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**Abstract**

This work describes the preparation and characterization of six stationary phases for high-performance liquid chromatography (HPLC) obtained by deposition of poly(dimethylsiloxane) (PDMS) in HPLC silica particles, followed by immobilization using different processes (thermal treatments, thermal treatment + microwave irradiation, self-immobilization + gamma irradiation and self-immobilization + microwave irradiation). The chromatographic parameters of all the phases were evaluated with a mixture of test compounds having varied natures (acid, basic and neutral). The stability of one of these phases was evaluated in both a neutral mobile phase and a higher pH mobile phase used at an elevated temperature, with promising results.


*Keywords:* Reversed phase; Poly(dimethylsiloxane); Immobilization; Stability

## 1. Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely used for qualitative and quantitative analysis. This modality of HPLC presents several advantages, including the use of less noxious and less expensive mobile phases, such as solutions of methanol and water; stable stationary phases; fast equilibration of the column after changing the mobile phase; usefulness with gradient elution; high speed analyses and good repetitivity of retention times. It can also be applied for the separation of compounds having different polarities, molar masses and functionalities.

As the stationary phases for use in RP-HPLC should present non-polar character and the oxide surfaces used as supports are usually polar, it is necessary to introduce non-polar organic groups on the support surfaces. Several procedures exist to obtain non-polar organic layers on the oxide surfaces. The most common method is the introduction of an organic monolayer through reaction with appropriate reagents, producing chemically bonded phases [1–4]. Other procedures involve surface coating of the oxides with organic polymers [3–6].

The objective of producing phases by covering an oxide support with a polymer is to combine the mechanical resistance of the support with the selectivity and chemical inertness of the organic polymer. Several ways of preparing stable polymeric coatings exist. One is to produce a polymer coating by in situ monomer polymerization [3,7], although the most common procedure used for preparing chromatographic phases consists of the physical adsorption of a polymer with a defined chemical composition onto the support [3,4,6–8]. Then, subsequent immobilization processes are induced by peroxide [7–12], thermal treatments [9,13] and gamma irradiation [7,8,14–16] to cause rearrangement on the surface and/or to promote crosslinking or covalent bonds between the polymer chains and between the polymer and the support, resulting in an anchored monolayer [3,7]. For the latter, the polymer should present specific functional groups to react with the support. Among the polymers already used in the preparation of such stationary phases are polyethylene [17–19], polybutadiene [20–28], polystyrene

* Corresponding author. Fax: +55 19 3788 3023.
*E-mail address:* chc@iqm.unicamp.br (C.H. Collins).

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[29,30], poly(methyloctylsiloxane) [7–9,13,14,31,32] and poly(methyloctadecylsiloxane) [8,9].

In spite of its very short organic chains, poly(dimethylsiloxane) – PDMS – is the one of most used stationary phase in capillary GC [33]. It is also one of the most used phases for the solid phase pre-concentration of diverse substrates, especially in solid phase microextraction (SPME) [34–37]. Its use for thin-film microextraction [38], in-tube solid phase microextraction [39,40], stir bar sorptive extraction (SBSE) [34,37,41], coated rod extraction [42,43] has also been described. Recent papers also indicate the use of PDMS fibers [44,45], pré-columns packed with PDMS particles [46,47] and layered into microchips [48], for pre-concentration before GC [38], HPLC [46], capillary eletrophoretic [48], spectrophotometric [47] or mass spectrometric [44] determinations. Since all these procedures retain and then release the substrates with high efficiencies, the use of PDMS as a RP-HPLC phase warrants investigation.

The present paper describes the properties of several RP-HPLC stationary phases based on poly(dimethylsiloxane) immobilized on porous silica surfaces. The immobilization procedures include thermal immobilizations and several sequential procedures involving self-immobilization (the slow rearrangement of polymer on the silica surface to obtain a more compact configuration accompanied by possible bond formation [49,50]) followed by irradiation with microwaves or gamma radiation.

## 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 $m^2$ $g^{-1}$ specific surface area.

Poly(dimethylsiloxane), PDMS (product PS-043), $M_r$ 28,000, was obtained from Petrarch/Hüls America, Piscataway, NJ, USA. Methanol (Omnisolv), chloroform (LiChrosolv) and hexane (HPLC-grade) were all from Merck, Rio de Janeiro, RJ, 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, SP, Brazil), *N*,*N*-dimethylaniline (Fluka, Steinheim, Switzerland), naphthalene (Vetec, Rio de Janeiro, RJ, Brazil) and acenaphthene (Aldrich) were analytical-reagent grade and not further purified. The sodium bicarbonate 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 using 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 aquisition used Chrom Perfect for Windows, Version 3.52 (Justice Innovations, Denville, NJ, USA), with the Report-Write Plus option for calculation of the chromatographic parameters, installed in a PC compatible computer.

### 2.3. Preparation of the $SiO_2$(PMDS) stationary phases

The silica was dried in air at 150 °C for 17 h. The dried silica was then added to a 10% (w/v) solution of PDMS in hexane in the proportion of 1.3 g PDMS to 1 g silica. The mixture was stirred for 3 h at 40 °C and the solvent was then allowed to evaporate, without stirring, at 40 °C.

As soon as evaporation was complete, the stationary phase was divided into six portions. Each portion was submitted to a different procedure for polymer immobilization: (1) thermal treatment at 80 °C for 30 h; (2) thermal treatment at 120 °C for 16 h; (3) thermal treatment at 240 °C for 4 h; (4) thermal treatment at 80 °C for 30 h, followed by microwave irradiation (495 W for 15 min) in a Model QWave 3000 Questron microwave oven (Mississauga, Ontario, Canada); (5) self-immobilization [49,50] by storage in air at room temperature (22 ± 1 °C) for 30 days followed by gamma irradiation to a dose of 80 kGy, carried out under air in glass ampoules with a commercial Cobalt-60 irradiator (IBRAS-CBO, Campinas, SP, Brazil); and (6) self-immobilization by storage in air at room temperature (22 ± 1 °C) for 30 days followed by microwave irradiation (495 W for 15 min).

After each immobilization procedure, the excess PDMS, which was not immobilized, was extracted from the stationary phase by pumping a sequence of chloroform (0.5 mL $min^{-1}$ for 4 h) and methanol (0.5 mL $min^{-1}$ for 2 h) at room temperature through the material contained in a column-type washing system. The phases were then dried (40 °C for 12 h) and stored in closed containers until needed.

### 2.4. Characterization of stationary phases

#### 2.4.1. Percent carbon and specific surface area

The percent carbon of the $SiO_2$(PDMS) phases was obtained through elemental analysis after extraction of the excess polymer to evaluate the loading of each stationary phase. These determinations were made with a Model 2400 Perkin-Elmer CHN analyzer (Boston, MA, USA).

The specific surface area of the stationary phases was measured using the BET (Brunauer, Emmett and Teller) method in a Micromeritics model FlowSorb 2300 instrument (Norcross, GA, USA).

### 2.5. Chromatographic characterization

#### 2.5.1. Preparation of the test columns

The columns (50 mm × 4 mm) were made from type 303 stainless-steel tubing with highly polished interior surfaces

[51] and downward packed using 10% slurries (w/v) of each stationary phase in chloroform. A packing pressure of 34.5 MPa (Haskel Model 5I769 Packing Pump, Burbank, CA, USA) 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 chromatographic tests.

#### 2.5.2. Chromatographic evaluation

The evaluation of the columns packed with the immobilized-extracted $SiO_2$(PDMS) stationary phases was based on the separation of a test mixture containing acidic, basic and neutral solutes (uracil, phenol, *N*,*N*-dimethylaniline, naphthalene and acenaphthene), dissolved in mobile phase (methanol:water 7:3 (v/v)). Injection of 5 μL 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 $min^{-1}$. The optimal flow rate was determined by a van Deemter curve. The column dead time, $t_M$, was determined from uracil (an unretained compound). The chromatographic parameters were retention factor (*k*), efficiency from peak width at half height (*N*) and asymmetry factor at 10% of the peak height (As) for each peak, as well as resolution (Rs) and separation factor ($\alpha$) for adjacent peaks. The chromatographic parameters reported were obtained from one column (several injections) although confirmation of this result was obtained by several injections on two or more other columns packed with the same stationary phase.

### 2.6. Stability tests using a neutral mobile phase and an alkaline (pH 8.4) mobile phase at elevated temperature

Columns packed with the $SiO_2$(PDMS) stationary phases immobilized by thermal treatment at 120 °C for 16 h were submitted to testing by passing 7:3 (v/v) methanol:water at room temperature (~22 °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 as a function of time. For the chromatographic evaluation, the flow rate of the mobile phase was decreased to 0.5 mL $min^{-1}$. The chromatographic parameters (retention factor (*k*), efficiency (*N*) and asymmetry factor (As)) were determined for each peak. Column degradation was evaluated using the changes in the percent of the initial efficiency (% initial *N*), as a function of time.

The test using alkaline (pH 8.4) mobile phase and elevated temperature was developed in our laboratory [52,53] and consists of pumping an alkaline (pH 8.4) mobile phase, 1:1 (v/v) methanol:0.1 mol $L^{-1}$ sodium bicarbonate, through the columns at 0.6 mL $min^{-1}$ with the columns inside an oven held at 60 °C. After defined time periods of 1 h, the column was removed from the oven and coupled to the HPLC test system, passing a 7:3 (v/v) methanol:water mobile phase at 0.5 mL $min^{-1}$ for 15 min to remove the alkaline mobile phase from within the column 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 column was 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 for approximately 1600 column volumes ($V_c$).

## 3. Results and discussion

### 3.1. Percent carbon, loading, specific mass and specific surface area

The immobilized and non-immobilized stationary phases were submitted to elemental analyses. The phase loadings (Table 1) were obtained from the percent carbon through the following equation:

$$\text{loading}\,(\%) = \left(\frac{m_{\text{PDMS}}}{m_{\text{SP}}}\right) \times 100$$

where

$m_{SP}$ = mass of the stationary phase = $m_{SiO_2} + m_{PDMS}$,
$m_{PDMS}$ = mass of the PDMS = (% $C_{SP} \times m_{SP}$)/32.5,
32.5 = constant, considering that 32.5% of the total mass of PDMS is carbon,

Table 1
Percent carbon, percent loading, specific mass and specific surface area of non-immobilized and immobilized-extracted $SiO_2$(PDMS) stationary phases

| | Stationary phase | | | | | | |
|---|---|---|---|---|---|---|---|
| | 80 °C/30 h | 120 °C/16 h | 240 °C/4 h | 80 °C/30 h + microwave irradiation | Self-immobilized + microwave irradiation | Self-immobilized + gamma irradiation | Not immobilized[a] |
| Percent Carbon | 9.1 ± 0.5 | 10.5 ± 0.5 | 11.9 ± 0.5 | 10.1 ± 0.5 | 8.6 ± 0.5 | 12.0 ± 0.5 | 17.7 ± 0.5 |
| Loading (%) | 28 ± 2 | 32 ± 2 | 37 ± 2 | 31 ± 2 | 26 ± 2 | 37 ± 2 | 55 ± 2 |
| Specific mass (g/g) | 0.39 ± 0.02 | 0.47 ± 0.02 | 0.59 ± 0.02 | 0.45 ± 0.02 | 0.35 ± 0.02 | 0.59 ± 0.02 | 1.22 ± 0.02 |
| Specific surface area ($m^2\ g^{-1}$) | 87 | 73 | 48 | 75 | 95 | 48 | 0.3 |

[a] This phase was not extracted before these determinations.

% $C_{SP}$ = measured percent carbon of the stationary phase,

while the specific mass of polymer on the silica is obtained from:

$$\text{specific mass} = \frac{\%\, C_{SP}}{(32.5 - \%\, C_{SP})}.$$

The non-immobilized stationary phase presented a high loading because this phase was not submitted to an extraction process to remove excess PDMS. The different processes of polymer immobilization promoted stationary phases with varied loadings, although some were quite similar. The most effective immobilization processes were heating at 240 °C for 4 h and self-immobilization + gamma radiation, both of which retained 67% of the initial polymer, while the phases prepared by self-immobilization + microwave irradiation and by heating at 80 °C for 30 h retained only 47–51% of the initial polymer. The phases heated at 80 °C for 30 h + microwave irradiation and heated at 120 °C for 16 h gave intermediate loadings.

The specific surface areas decreased linearly with the increases in loadings for the immobilized-extracted phases (Table 1).

### 3.2. Chromatographic evaluation

The chromatograms obtained with the packed columns having different immobilized-extracted $SiO_2$(PDMS) stationary phases are shown in Fig. 1. All columns separated the test mixture in a short time interval (less than 7 min). The chromatographic parameters of the peaks (retention factor, asymmetry factor, efficiency, resolution and separation factor) are shown in the Tables 2 and 3.

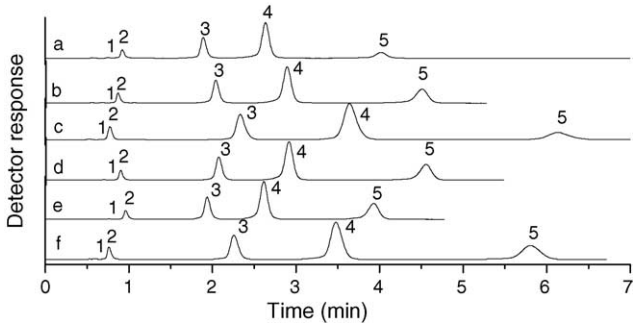


Fig. 1. Chromatograms of $SiO_2$(PDMS) stationary phases immobilized by: (a) heat treatment at 80 °C for 30 h; (b) heat treatment at 120 °C for 16 h; (c) heat treatment at 240 °C for 4 h; (d) heat treatment at 80 °C for 30 h + microwave irradiation; (e) self-immobilization (30 days) + microwave irradiation; and (f) self-immobilization (30 days) + gamma irradiation. Conditions: columns: 50 mm × 4 mm, mobile phase methanol:water 7:3 (v/v), flow rate 0.5 mL min$^{-1}$. Peaks: 1—uracil, 2—phenol, 3—*N*,*N*-dimethylaniline, 4—naphthalene, 5—acenaphthene.

The different values for the retention factor (Table 2) show that the columns packed with the immobilized $SiO_2$(PDMS) stationary phases present different separation characteristics as a function of the degree of polymer loading obtained from each immobilization process (Table 1).

The asymmetry factors (Table 2) show that all phases provide chromatograms with quite symmetrical peaks for the neutral and basic (*N*,*N*-dimethylaniline) compounds, show-

Table 2
Chromatographic parameters for acidic, basic and neutral solutes of the test mixture for columns packed with immobilized-extracted $SiO_2$(PDMS) stationary phases

| Solutes | Stationary phase | | | | | |
|---|---|---|---|---|---|---|
| | 80 °C/30 h | 120 °C/16 h | 240 °C/4 h | 80 °C/30 h + microwave irradiation | Self-immobilized + microwave irradiation | Self-immobilized + gamma irradiation |
| Retention factors | | | | | | |
| Phenol | 0.2 | 0.3 | 0.5 | 0.3 | 0.3 | 0.3 |
| *N*,*N*-DMA[a] | 1.5 | 2.0 | 3.5 | 2.0 | 1.6 | 2.7 |
| Naphthalene | 2.5 | 3.3 | 6.0 | 3.2 | 2.4 | 4.7 |
| Acenaphthene | 4.4 | 5.7 | 10.8 | 5.5 | 4.2 | 8.5 |
| Asymmetry factors | | | | | | |
| Phenol | 1.6 | 1.6 | 1.5 | 1.6 | 1.6 | 1.6 |
| *N*,*N*-DMA[a] | 1.2 | 1.1 | 1.3 | 1.1 | 1.1 | 1.3 |
| Naphthalene | 1.0 | 1.0 | 1.3 | 1.0 | 0.9 | 1.1 |
| Acenaphthene | 1.0 | 1.0 | 1.2 | 1.0 | 1.0 | 1.0 |
| Efficiencies (plate number) | | | | | | |
| Phenol | 41800 | 38400 | 20400 | 38600 | 43000 | 24000 |
| *N*,*N*-DMA[a] | 70400 | 72400 | 46000 | 73200 | 72000 | 48800 |
| Naphthalene | 80200 | 82200 | 51400 | 83200 | 81600 | 56000 |
| Acenaphthene | 86000 | 94000 | 54000 | 89600 | 83400 | 59200 |

Columns: 50 mm × 4 mm; mobile phase: methanol:water 7:3 (v/v); flow rate: 0.5 mL min$^{-1}$.
[a] *N*,*N*-dimethylaniline.

Table 3
Resolution and separation factors between adjacent peaks of the acidic, basic and neutral solutes of the test mixture for columns packed with immobilized-extracted $SiO_2$(PDMS) stationary phases

| Solutes | Stationary phase | | | | | |
|---|---|---|---|---|---|---|
| | 80 °C/30 h | 120 °C/16 h | 240 °C/4 h | 80 °C/30 h + microwave irradiation | Self-immobilized + microwave irradiation | Self-immobilized + gamma irradiation |
| Resolution | | | | | | |
| Phenol–*N*,*N*-DMA[a] | 9.3 | 11.0 | 10.7 | 10.8 | 9.3 | 11.1 |
| *N*,*N*-DMA–naphthalene | 5.1 | 5.4 | 5.5 | 5.3 | 4.6 | 5.5 |
| Naphthalene–acenaphthene | 6.7 | 7.3 | 6.6 | 7.3 | 6.5 | 6.8 |
| Separation factors | | | | | | |
| Phenol–*N*,*N*-DMA[a] | 6.6 | 6.8 | 7.3 | 6.8 | 6.0 | 10.8 |
| *N*,*N*-DMA–naphthalene | 1.7 | 1.6 | 1.7 | 1.6 | 1.6 | 1.7 |
| Naphthalene–acenaphthene | 1.7 | 1.7 | 1.8 | 1.7 | 1.7 | 1.8 |

Columns: 50 mm × 4 mm; mobile phase: methanol:water 7:3 (v/v); flow rate: 0.5 mL $min^{-1}$.
[a] *N*,*N*-dimethylaniline.

ing that there was a considerable covering of the silanol groups of the support. On the other hand, the peak of the acidic compound (phenol) was less symmetrical for all the columns (As > 1.5).

Good separations between the peaks are shown by the resolution between adjacent peaks (Table 3) for all columns, although the column immobilized by self-immobilization + gamma irradiation was a little better than

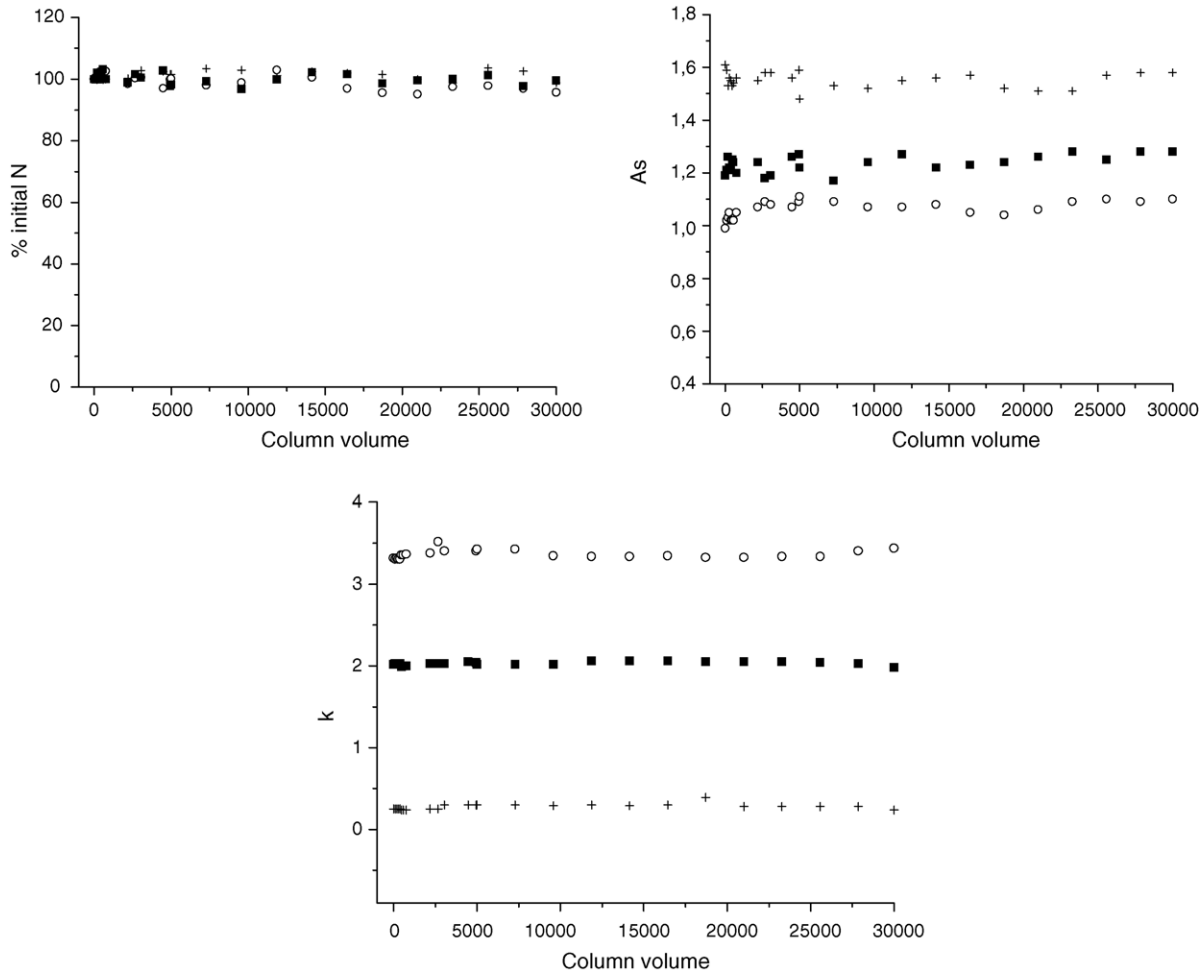


Fig. 2. Chromatographic parameters as a function of the neutral mobile phase volume passed through the columns packed with the $SiO_2$(PDMS) stationary phase immobilized by a heat treatment of 120 °C for 16 h. Test solutes: (+) phenol; (■) *N*,*N*-dimethylaniline; (◯) naphthalene.

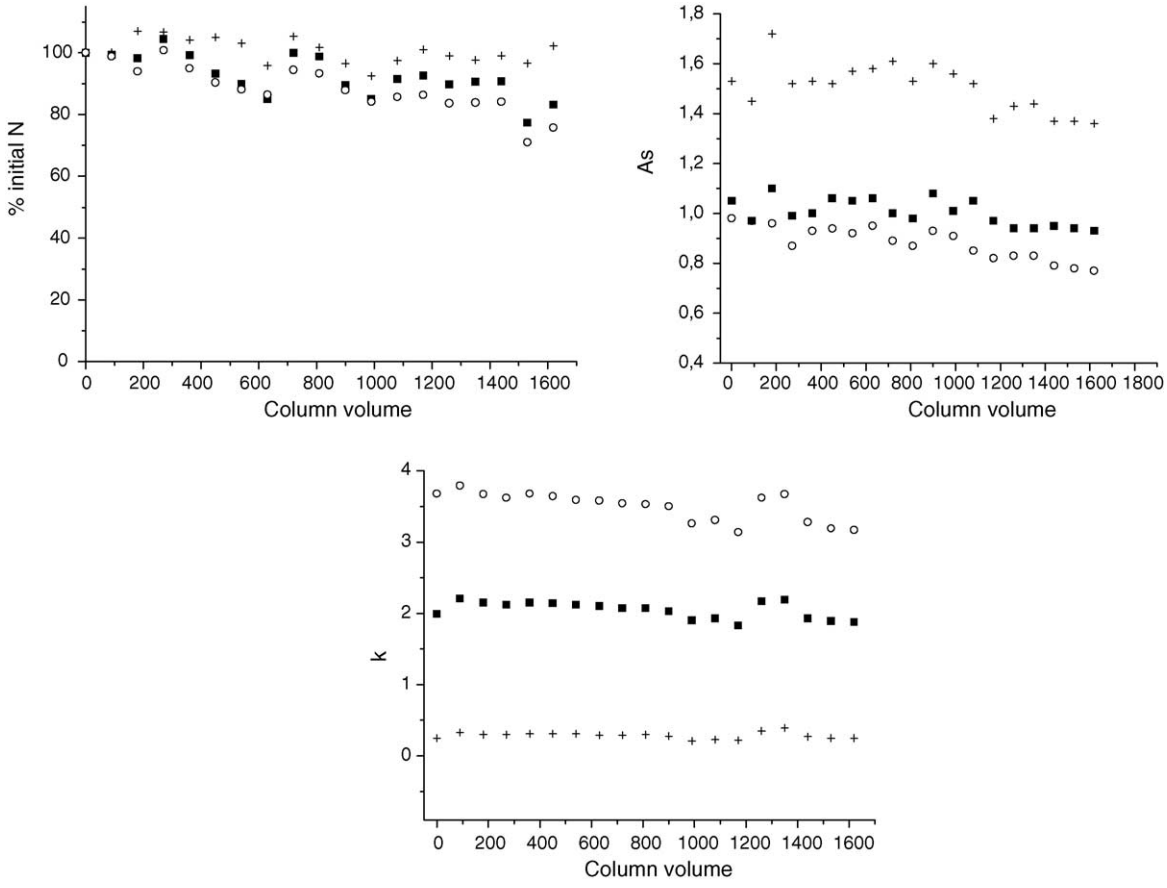


Fig. 3. Chromatographic parameters as a function of the alkaline (pH 8.4) mobile phase volume passed at 60 °C through a column packed with the $SiO_2$(PDMS) stationary phase immobilized by a heat treatment of 120 °C for 16 h. Test solutes: (+) phenol; (■) *N*,*N*-dimethylaniline; (◯) naphthalene.

the others. Even the columns with the lowest percent polymer showed very good performance in the separation of the compounds of the test mixture.

The separation factor between adjacent peaks (Table 3) shows little variation for most of the stationary phases, being somewhat greater for the phase with the highest polymeric covering (self-immobilization + gamma irradiation).

The efficiencies for the peaks of the test mixture (Table 2) show some variations, although this property is somewhat influenced by the column packing. In general the two phases having the highest loadings showed lower efficiencies than phases having less PDMS. This may be related to the significant loss of surface area (Table 1). The stationary phases immobilized by heating for 120 °C for 16 h and heating for 80 °C for 30 h, with or without subsequent microwave irradiation, presented the highest efficiencies.

### *3.3. Stability testing using a neutral mobile phase or an alkaline (pH 8.4) mobile phase at elevated temperature*

The $SiO_2$(PDMS) stationary phase used in the stability tests with neutral and alkaline (pH 8.4) mobile phases was that immobilized by heating at 120 °C for 16 h. Initially this phase was tested with a neutral mobile phase, methanol:water 7:3 (v/v), at room temperature. The results are shown in Fig. 2. In this work the mobile phase volume which passed through the column is expressed in column volumes ($V_c$), as commonly used in the literature. The column volume was calculated from the retention time of an unretained compound (uracil). For the columns used in this work $V_c$ is 0.40 mL.

It can be noted from Fig. 2 that, after passing 30,000 $V_c$ of neutral mobile phase, there was no significant alteration of the chromatographic parameters for any compounds of the test mixture, showing the very good stability of this phase under these, relatively mild conditions, similar to those used for most RP-HPLC separations.

As this phase withstood the test with neutral mobile phase, it was also submitted to a more rigorous evaluation with alkaline (pH 8.4) mobile phase at 60 °C. Fig. 3 shows the chromatographic parameters for the compounds of the test mixture as a function of the alkaline mobile phase passed through the column. A very slow decrease of the chromatographic parameters for the different compounds of the test mixture is seen, as a function of the mobile phase volume passed through the column. However, the stationary phase is moderately resistant to these more drastic conditions. These results are similar to those obtained with other polysiloxanes immobilized by heating onto chromatographic silica using

similar immobilization procedures [53,54], as well as to the results from similar measurements with several commercial phases.

After passing 1600 column volumes of this pH 8.4 mobile phase thorough the column, the percent carbon on the phase had apparently increased from 10.5 (before the test) to 10.7 as a result of a small increase of the polymer/silica ratio, observed when only the support is dissolved, without loss of the polymeric phase. This results in a redistribution of the polymer chains on the support and, thus, changes in efficiency and retention.

## 4. Conclusions

The $SiO_2$(PDMS) stationary phases obtained by the different immobilization procedures present different physicochemical and chromatographic properties as a function of the different amounts of polymer loading. All the phases were shown to be efficient in the separation of test compounds having varied chemical properties, with quite symmetrical peaks for a basic compound (*N*,*N*-dimethylaniline). Another important aspect is the separation of all compounds of the test mixture in a short interval of time. Stability tests done on one of the immobilized phases (immobilized at 120 °C for 16 h) indicated that this phase shows good stability in both neutral and alkaline (pH 8.4) mobile phases.

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