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# High-performance liquid chromatographic stationary phases based on polysiloxanes with different chain lengths thermally immobilized on silica supports

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**Abstract**

Reversed phases for high-performance liquid chromatography (RP-HPLC) were obtained by thermal immobilization of polysiloxanes having different length chains (C1, C8 and C14) onto HPLC silica particles. The importance both of percent loading of the stationary phase promoted by each immobilization procedure and of the length of the lateral chain of the polymer on the chromatographic performances of the phases obtained is compared and discussed.


*Keywords:* Reversed phase; Polysiloxane; Immobilization

## 1. Introduction

Using a pre-formed polymer to cover an oxide support has become an important means for obtaining reversed phases for HPLC. The main advantages of these phases with polymer coatings, compared to chemically bonded phases, are: higher covering of active sites of the support and the possibility of greater selectivity of the stationary phase from an appropriate choice of the polymer.

There are several different ways to promote polymer immobilization onto the oxide support. The methods most commonly mentioned in the literature are thermal treatment, with or without the presence of free radical-inducing agents [1–3], use of high energy (gamma) radiation [1,3–5] or use of specific reagents to induce chemical cross linking [1,3]. Besides these, there are other possibilities, such as the use of microwave irradiation [6] and self-immobilization [7,8].

Most early work using thermal treatments to immobilize polymers onto a chromatographic support were carried out in the presence of radical-generating agents, such as azobisisobutyronitrile, dicumylperoxide or allylmethacrylate, to promote cross linking and the consequent immobilization of the polymer onto the support [3,9,10].

The use of the thermal immobilization of polymers on chromatographic supports, without the presence of radical inducing agents, has also been reported. Schomburg et al. [1] immobilized a polysiloxane on silica by heating to 180 °C. A more recent study [7] used temperatures of 80–280 °C. It was shown that the phases obtained using temperatures below 150 °C promoted the formation of a polymer monolayer on the support and that these phases may be useful in HPLC. However, the phases immobilized using temperatures higher than 180 °C showed the formation of multilayers on the support surface, and the columns obtained with these phases were not adequate for chromatographic use.

In this work a comparison between stationary phases based on polysiloxanes with different lateral chains, immobilized by thermal treatment onto porous silica particles, is described.

## 2. Experimental

### 2.1. Materials

The chromatographic support used to prepare the stationary phases was spherical Kromasil silica (Akzo Nobel) 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, poly(methyloctylsiloxane) (PMOS) (product PS-140),

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$M_r$ 6200 and poly(methyltetradecylsiloxane), PMTDS, (product PS-134), $M_r$ 9400 were obtained from Petrarch/Hüls America. Methanol (Omnisolv), chloroform (LiChrosolv) and hexane (HPLC-grade) were all from Merck. Distilled, deionized water (Milli-Q Plus, Millipore) was used throughout.

The chromatographic test substances uracil (Aldrich), phenol (Labsynth), *N*,*N*-dimethylaniline (Fluka), naphthalene (Vetec), ethylbenzene (Merck) and acenaphthene (Aldrich) were analytical-reagent grade and not further purified.

### 2.2. Preparation of the stationary phases

The silica was dried in air at 150 °C for 17 h. Then it was added to a 10% (w/v) solution of the polysiloxane in hexane in the proportion of 1.30 g of PDMS, 1.22 g of PMOS or 1.20 g PMTDS to 1 g silica, respectively. The objective of these polysiloxane/silica proportions was to ensure excess polymer in order to fill the pores of the silica for the preparation of the initial adsorbed stationary phase. The mixtures were stirred for 3 h at 40 °C and the solvent was then allowed to evaporate, without stirring, at 40 °C.

The stationary phases obtained by evaporation of the solvent were divided into several portions and each portion was then submitted to a specific thermal immobilization treatment. The stationary phase $SiO_2$(PDMS) was immobilized at: (1) 80 °C for 30 h; (2) 120 °C for 16 h; and (3) 240 °C for 4 h. The stationary phase $SiO_2$(PMOS) was immobilized at: (1) 120 °C for 4 h; (2) 120 °C for 16 h; and (3) 220 °C for 4 h. The stationary phase $SiO_2$(PMTDS) was immobilized at: (1) 80 °C for 30 h and (2) at 120 °C for 16 h. All thermal immobilizations were carried out in the presence of air.

After each immobilization procedure, the excess polysiloxane that was not immobilized was extracted from the stationary phase by passing hexane or chloroform (0.5 mL min$^{-1}$ for 4 h at room temperature), followed by 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.

Each immobilized phase was characterized both physically and chromatographically, as previously described [11–13].

### 2.3. Preparation of the test columns

The columns (50 mm × 4 mm) were made from type 303 stainless-steel tubing with highly polished interior surfaces [14] 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) 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.

### 2.4. Chromatographic evaluation

The columns were evaluated using a modular HPLC system equipped with a Rheodyne model 8125 injector (5 μL loop), a Shimadzu model LC-10AD pump and an Alltech model 450 UV (254 nm) detector with a 0.8 μL cell. Data aquisition used Chrom Perfect for Windows, version 3.52 (Justice Innovations), with the Report-Write Plus option for calculation of the chromatographic parameters, installed in a PC compatible computer.

The evaluations of the columns packed with immobilized-extracted $SiO_2$(polysiloxane) stationary phases were based on the separation of a test 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 μL of this mixture produced satisfactory chromatographic peaks with detection at 254 nm. The separations were carried out at room temperature with a flow rate of 0.5 mL min$^{-1}$, optimized by means of a van Deemter curve. The column dead time, $t_M$, was determined using uracil (an unretained compound). The retention factor ($k$) was determined for each peak and the separation factor ($\alpha$) was determined for adjacent peaks.

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

Columns packed with several of the stationary phases were submitted to stability 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) at 10% of peak height) were determined for each peak.

The test using alkaline (pH 8.4) mobile phase at 60 °C, developed in our laboratory [15], 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 (1 h), the column is removed from the oven and coupled to a 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 is connected and the test mixture is injected. After chromatographic evaluation, the column is 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 ($V_c$).

### 2.6. Percent carbon

The percent carbon of each $SiO_2$(polysiloxane) phase was obtained through elemental analysis after polymer immobilization to evaluate the loading of the stationary phases. These determinations were made with a Model 2400 Perkin-Elmer CHN analyzer. From these values, the phase loadings (Table 1) were obtained through the following equation:

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