



Chromatographic evaluation of self-immobilized stationary phases for reversed-phase liquid chromatography

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Abstract

The preparation of stationary phases for HPLC using polymers deposited on silica usually includes an immobilization step involving cross-linking by free radicals induced by ionizing radiation or by other radical initiators. The present paper reports changes which occur at ambient temperature in the character of poly(methyloctylsiloxane) deposited on porous silica particles as a function of the time interval between particle loading and column packing. Column performance and retention factors increase with time and these changes are attributed to rearrangement (self-assembly) which result in “self-immobilization” of the polymer molecules on the silica surface.

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1. Introduction

Development of new stationary phases and the knowledge about their features are of importance to high-performance liquid chromatography (HPLC) because more stable and more selective phases can increase the usefulness of HPLC for many analytical applications. The preparation, properties and availability of newly developed packings for HPLC usually involve the reaction of surface silanol groups with a functional group (e.g. alkoxy or chloro) of an organosilane reagent [1]. The preparation of chemically bonded phases has been described in numerous papers [2–9]. Although silanized bonded reversed-phases usually have good chromatographic performance and selectivity in the separation of different types of analytes, they have problems related to the

residual silanol groups, which may cause alteration of the retention mechanism during a chromatographic separation, especially for basic solutes. Many are also limited to use at pH between 3 and 8 due to instability of the silica support, which can be dissolved in basic media, promoting loss of the silica and, consequently, of the column packing material. At very low pH loss of silane bonded phases can occur due to hydrolysis of the Si–O–Si bond that binds the silane to the support.

One approach to overcome the problems related to many of today's bonded phases lies in the use of polymer-coated silica. There are various ways to attach polymeric material to the surface of chromatographic silica particles. Petro and Berek [10], Unger and co-workers [11,12] and Jardim and collaborators [13–15] describe several of these. The present work involves the deposition of a pre-synthesized polysiloxane [poly(methyloctylsiloxane), (PMOS)] in the silica pores and the phenomenon of self-immobiliza-

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tion [16]: the development of a kinetically stable adsorbed layer at ambient temperature in the absence of conventional immobilization procedures.

The main objective of the present study is the determination of chromatographic parameters of the self-immobilized stationary phases, in order to evaluate this phenomenon of redistribution of the liquid polymer on the silica surface and the concomitant appearance of new more stable stationary phases.

2. Experimental

2.1. Materials

HPLC-grade methanol, Mallinckrodt (Rio de Janeiro, Brazil) and Milli-Q water, Millipore (Bedford, USA) were used to prepare the mobile phases. HPLC-grade dichloromethane, EM Science (Gibbstown, USA), analytical reagent-grade chloroform, Merck (Darmstadt, Germany) and HPLC-grade hexane, Mallinckrodt (Rio de Janeiro, Brazil) were used without further purification. The eluent solutions were filtered using 0.22- μm membrane filters. Most of the compounds used for the chromatographic testing (acenaphthene, phenol, naphthalene and uracil) were analytical reagent grade and not further purified. *N,N*-Dimethylaniline (analytical-reagent grade) was purified by distillation. The silica supports were Kromasil silica from Akzo Nobel (Bohus, Sweden), having a mean particle size of 6 μm , a mean pore size of 11.1 nm and a 320- $\text{m}^2 \text{g}^{-1}$ specific surface area and Davisil-630 from Alltech (Deerfield, IL, USA), having irregular porous 13 μm particles, with an average pore diameter of 8.7 nm and a specific surface area of 350 $\text{m}^2 \text{g}^{-1}$. The PMOS polymer (weight-average molar mass, M_w , of 6200) was obtained from United Chemical Technologies (Bristol, USA).

2.2. Preparation of polymer-coated stationary phases

Batches of PMOS-loaded silica having a specific mass (\bar{m}_{PMOS}) of nearly 1.0 $\text{g}_{\text{PMOS}}/\text{g}_{\text{silica}}$ were prepared by addition of a determined quantity of silica (dried at 150 °C for 24 h) to a solution of PMOS in dichloromethane [1 g (PMOS + silica)

+12 ml CH_2Cl_2]. This mixture was slowly agitated at room temperature for 3 h, after which the dichloromethane was evaporated, without stirring, at room temperature.

2.3. Solvent extraction

Samples of prepared phase (~3.5 g) were placed in stainless steel extraction columns (150×10 mm) for extraction with hexane at 50 °C in an Eldex Model CH-150 oven from Alltech. The columns were connected to a Waters 510 pump (Milford, MA, USA) for extraction of all the non-immobilized PMOS by passing hexane at a flow-rate of 0.5 ml min^{-1} for 4 h. After each extraction, the sample contained in the column was removed; the solvent was evaporated and dried in oven at 45 °C for 2 h prior to CHN analysis and column packing.

2.4. Column packing

Columns (60×4 mm) were made from type 316 stainless steel tubing. The internal surface was polished using a technique developed in our laboratory [17]. The columns were downward slurry packed using 10% (w/v) slurries of the stationary phases in chloroform. A constant packing pressure of 34.5 MPa with a Haskel packing pump (Burbank, USA) was used, with methanol as propulsion solvent. Columns were conditioned for 3 h with mobile phase at 0.2 ml min^{-1} prior to testing.

2.5. Chromatographic evaluation

The chromatographic mixture used in this study contained phenol, *N,N*-dimethylaniline, naphthalene and acenaphthene. HPLC separations were performed with a Waters 510 pump, a Rheodyne (Cotati, USA) model 7125 injection valve (5 μl loop) and a Waters 486 UV-Vis detector (at 254 nm). The mobile phase was methanol–water (70:30, v/v) and/or methanol–water (50:50, v/v) at 0.2 ml min^{-1} . The column dead time, t_M , was determined using uracil as unretained compound. Data collection and treatment was carried out by ChromPerfect for Windows, version 3.52, and Report Write Plus from Justice Innovations (Mountain View, USA). Chro-

matographic performance was evaluated by means of efficiency [plates per meter (N/m) and reduced plate height (h)], retention factor (k), resolution (R_s) and asymmetry (A_s) measured at 10% of the peak height.

2.6. Physical characterization

Portions of all extracted samples were subjected to elemental analysis on a Model CHN-2400 Perkin-Elmer analyzer. From these data, the specific mass of PMOS, \bar{m}_{PMOS} , which represents the mass of polymer per gram of silica, was calculated using the formula: $\bar{m}_{\text{PMOS}} = \%C / (62 - \%C)$, since 62% of the PMOS refers to carbon. With the data of specific mass the polymer layer thickness was calculated, as follows:

$$\tau = -\left(\frac{\sqrt{d^2 - Fd^2} - d}{2}\right)$$

where the F is self-immobilized fraction, $F = \bar{m}_{(\text{self-imm})} / \bar{m}_{(\text{fullpores})}$, and d is the mean pore diameter. It is assumed for this calculation that the pores of the silica have a constant diameter and that the self-immobilized polymer is characterized by a layer of constant thickness, τ , on the pore walls [18]. The specific pore volume, vp , for the Kromasil silica is 0.88 ml/g of SiO_2 and the density of PMOS is 0.91

g ml⁻¹. From this pore volume, the mass of polymer that fills the pore system is 0.80 g_{PMOS}/g_{silica}.

3. Results and discussion

Earlier work with Davisil silica (13 μm particle size, 8.7 nm pore size) has shown that 1 day after preparation of the coating of silica with polysiloxane nearly 90% of the poly(methyloctylsiloxane) is extracted from SiO_2 (PMOS) while only 60% is extracted after 20 days [16]. In this work, the phenomenon of self-immobilization was also characterized by ²⁹Si NMR experiments [16].

In the present work, a similar behavior was observed for the stationary phases prepared with Kromasil silica (6 μm particle size, 11.1 nm pore size). These phases were characterized by their chromatographic parameters. An initial batch was prepared with loading exceeding the mass of PMOS necessary to fill the pores. Portions of the batch were then extracted with hexane after various time periods to remove PMOS that had not self-immobilized. Table 1 shows the amount of polymer extracted from the same batch after different periods of time. As can be seen in Table 1, the amount of PMOS removed by extraction is reduced with time, as is also seen in the case of the Davisil silica (Table 2). This effect corresponds to self-immobilization. The thickness

Table 1
Percent carbon, specific mass, \bar{m}_{PMOS} , and layer thickness, τ , from extraction experiments, as a function of time, with Kromasil silica

Time before extraction (days)	% C	$\bar{m}_{\text{PMOS,retained}}$ (g _{PMOS} /g _{silica})	% self-immobilization	τ (nm)
01	2.39	0.040	4.1	0.1
02	2.40	0.040	4.1	0.1
03	2.70	0.045	4.6	0.1
06	4.57	0.079	8.1	0.3
08	3.98	0.068	6.9	0.2
09	5.54	0.098	10.1	0.4
16	5.34	0.094	9.6	0.3
20	5.79	0.103	10.6	0.4
31	6.12	0.109	11.2	0.4
51	8.90	0.167	17.1	0.6
59	9.89	0.189	19.4	0.7
70	10.16	0.196	20.1	0.7
105	12.99	0.265	27.2	1.0
184	15.86	0.344	35.3	1.4

Initial specific mass: 0.975 g_{PMOS}/g_{silica}.

Table 2

Percent carbon, specific mass, \bar{m}_{PMOS} , and layer thickness, τ , from extraction experiments, as a function of time, with Davisil silica

Time before extraction (days)	% C	$\bar{m}_{\text{PMOS}_{\text{retained}}}$ ($\text{g}_{\text{PMOS}}/\text{g}_{\text{silica}}$)	% self-immobilization	τ (nm)
06	7.82	0.144	15.5	0.5
20	11.90	0.237	25.5	0.8
41	13.59	0.281	30.1	1.0
58	11.59	0.230	24.7	0.8
330	18.65	0.430	46.2	1.7

Initial specific mass: $0.932 \text{ g}_{\text{PMOS}}/\text{g}_{\text{silica}}$.

expected for a monolayer of PMOS is 1.1 nm [18]. Thus, after 100 days, the self-immobilization produced a complete monolayer of PMOS on the Kromasil silica surface. However, the final specific mass is different from the specific mass seen with Davisil silica [16]. The differences are attributable mainly to the different specific surface areas but the surface acidities also contribute. Kromasil silica is a more highly purified, less-acidic silica than Davisil. It can be speculated that the self-immobilization process is faster for Davisil, because trace metallic impurities are a probable source of adsorption sites

on silica and, although they may not interact directly, they increase the activity of neighboring silanols. On the other hand, the mean pore diameter of Kromasil silica is 11.1 nm whereas for Davisil silica, it is 8.7 nm. The slower retention of PMOS on the Kromasil silica may be due to this larger pore diameter where the molecular rearrangements that occur during self-immobilization within the plugs takes place more slowly [19].

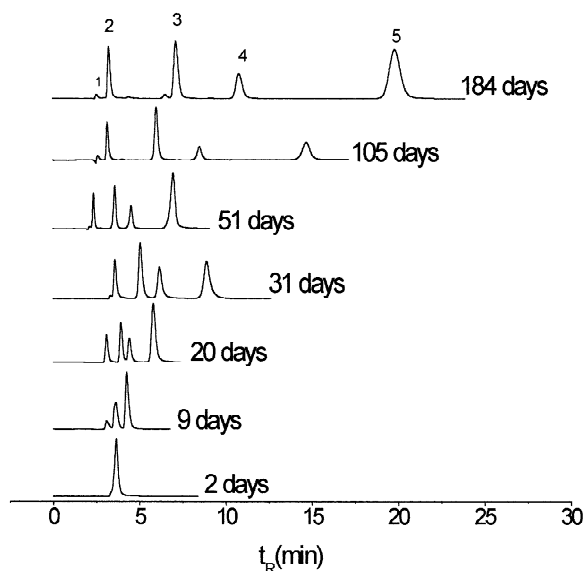


Fig. 1. Chromatograms obtained from non-extracted $\text{SiO}_2(\text{PMOS})$, as a function of time since preparation. Chromatographic mixture: 1, uracil; 2, phenol; 3, *N,N*-dimethylaniline; 4, naphthalene; and 5, acenaphthene. Chromatographic conditions: mobile phase: methanol–water (70:30, v/v); flow-rate: 0.2 ml min^{-1} ; volume of injected sample: $5 \mu\text{l}$; detection: UV at 254 nm.

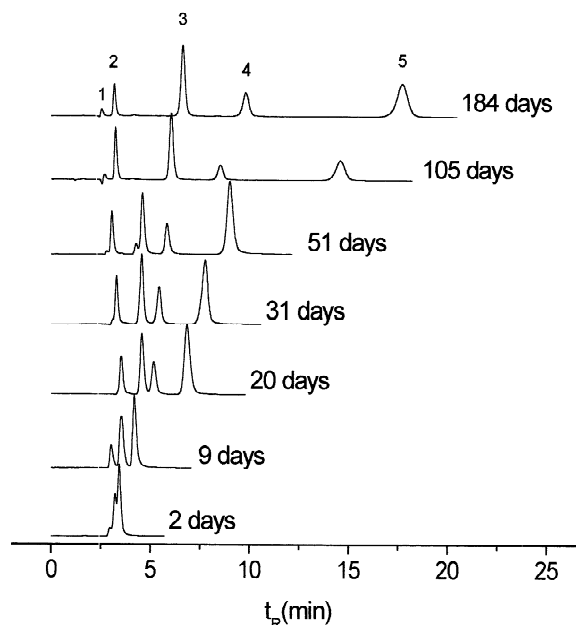


Fig. 2. Chromatograms obtained from $\text{SiO}_2(\text{PMOS})$ extracted with hexane just before column packing, as a function of time since preparation. Chromatographic mixture: 1, uracil; 2, phenol; 3, *N,N*-dimethylaniline; 4, naphthalene; and 5, acenaphthene. Chromatographic conditions: mobile phase: methanol–water (70:30, v/v); flow-rate: 0.2 ml min^{-1} ; volume of injected sample: $5 \mu\text{l}$; detection: UV at 254 nm.

Table 3

Chromatographic parameters obtained from extracted and non-extracted SiO₂(PMOS) as a function of time before column packing with Kromasil silica^a

Extraction	Time before packing (days)	N/m^b	$h^{b,c}$	A_s				$k^{b,g}$
				2 ^d	3 ^e	4 ^b	5 ^f	
Extracted	20	40 600	4.1	1.4	1.3	1.2	1.3	0.5
	31	51 040	3.2	0.9	1.1	1.0	0.8	0.8
	51	58 340	2.8	1.7	1.3	1.4	1.2	1.1
	105	72 140	2.3	1.6	1.2	1.0	0.9	2.2
	184	79 520	2.1	1.4	1.0	1.0	0.9	2.9
Non-extracted	20	43 820	3.8	1.5	1.7	1.3	1.5	0.5
	31	50 593	3.3	1.8	1.6	1.7	1.6	0.8
	51	53 710	3.1	1.3	0.9	0.8	0.7	1.1
	105	81 100	2.0	1.6	1.3	1.2	1.0	2.2
	184	64 700	2.6	1.6	1.4	1.3	1.2	3.2

Initial specific mass: 0.975 g_{PMOS}/g_{silica}.

^a Average values from at least three different chromatographic runs with two or three different columns.

^b Calculated for the naphthalene peak.

^c Calculated: $h = L/(Nd_p)$.

^d Calculated for phenol peak.

^e Calculated for *N,N*-dimethylaniline peak.

^f Calculated for acenaphthene peak.

^g Column dead time was measured with uracil.

Columns were prepared from extracted and non-extracted packing materials. Figs. 1 and 2 shows the chromatograms obtained at specific time periods after preparation for extracted and non-extracted portions of SiO₂(PMOS), respectively. The plate number per meter, N/m , reduced plate height, h , retention factor, k , and asymmetry measured at 10% of the, A_s , were calculated from the chromatograms and the results are summarized in Table 3. For comparison, Table 4 shows N/m , h and k calculated for the naphthalene, obtained with portions of the Davisil SiO₂(PMOS).

Test compounds should be selected to demonstrate the differences in chromatographic properties of the stationary phases. Numerous tests are discussed in the literature [20–25]. The mixture used in this work contains compounds of acidic (phenol), basic (*N,N*-dimethylaniline) and neutral (naphthalene and acenaphthene) character. Phenol is a weak acid and its presence can test for polar interactions or hydrogen bonding capacity. *N,N*-Dimethylaniline is a basic compound used to test for silanophilic properties and naphthalene and acenaphthene are used to show hydrophobic properties. Uracil was added in the test mixture to obtain the t_M value, the time necessary for

the passage of mobile phase through the column, a useful value for calculations of the retention factor, k .

From chromatograms obtained at specific times after preparation, it is evident that the time after polymer deposition significantly affects all the chromatographic parameters of the columns. These results reinforce those that were obtained from the

Table 4

Chromatographic parameters obtained from non-extracted SiO₂(PMOS) as a function of time before column packing with Davisil silica^a

Time before packing (days)	N/m^b	$h^{b,c}$	$k^{b,d}$
6	25 360	3.0	4.4
20	29 460	2.6	8.8
41	24 970	3.0	14.5
58	28 180	2.7	13.6
330	41 430	1.8	19.6

Initial specific mass: 0.932 g_{PMOS}/g_{silica}.

^a Average values from at least three different chromatographic runs with two or three different columns.

^b Calculated for the naphthalene peak.

^c Calculated: $h = L/(Nd_p)$.

^d Column dead time was measured with uracil.

elemental analysis, which show increasing quantities of retained polymer with time (Table 1).

As it can be seen in Figs. 1 and 2, the phases corresponding to days 2 and 9 could not be evaluated as stationary phases for Kromasil silica, due to poor resolution, using either (70:30, v/v) or (50:50, v/v) methanol–water as the mobile phase. Only one peak containing all compounds was eluted, confirming the low amount of organic layer available for the separation of compounds. Starting from 20 days, it was possible to calculate all the chromatographic parameters. In contrast, for the stationary phase prepared from Davisil silica, the resolution of compounds was observed even after a few days, due to the larger $\bar{m}_{\text{PMOS}_{\text{retained}}}$ with this silica.

An interesting aspect to be observed is the improvement of the asymmetry factor with time (Table 3). *N,N*-Dimethylaniline is a strongly basic compound but it did not show the tailing indicative of exposed silanol groups. Thus, we believe that the insoluble polymeric layer that is formed during the self-immobilization process effectively blocks the silanol groups of the surface silica.

When we compare the chromatograms of the Fig. 1 with those of Fig. 2, we observe that the chromatographic behavior of the extracted phases follows the same tendency as that of the non-extracted phases, indicating that extraction with hexane before column packing is an unnecessary step. In other words, the solvents used in column packing seem to be equally effective in the extraction of non-immobilized polymer.

4. Conclusions

The self-immobilization process takes considerable time to complete; this time depends on the specific characteristics of the silica support. On the other hand, once complete, a self-immobilized stationary phase shows chromatographic characteristics very similar to those prepared using chemical initiators to induce the immobilization procedures.

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