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Titanized silica-based stationary phases prepared with thermally and microwave-immobilized poly(methyloctylsiloxane)

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Abstract

Silica supports having their surface modified with titanium oxide were prepared and coated with poly(methyloctylsiloxane) (PMOS). Subsequently, immobilization of the polysiloxane was induced by thermal treatment or microwave radiation. The thermal treatment was carried out for different times (4, 8, 16 and 24 h) at temperatures ranging between 100 and 220 °C. For PMOS immobilization by microwave radiation, 452, 520 and 586 W power levels and exposure times of 5, 15 and 30 min were used. After extraction of non-immobilized polymer, the chromatographic properties of the phases were evaluated. The phase immobilized at 120 °C for 8 h presented the best chromatographic parameters, suggesting that the quantity of acidic hydroxyl groups on the support surface was reduced, resulting in fewer undesirable interactions of a basic solute with the silanols not removed or covered on the support surface.

Keywords: Stationary phases, LC; Silica, titanized; Titanized silica; Poly(methyloctylsiloxane)

1. Introduction

To date, many stationary phases have been developed and a large variety are commercially available. Silica-based bonded-phase chromatographic columns are the most widely employed for the analysis of a great diversity of compounds [1]. On the other hand, several approaches have been taken to minimize some drawbacks presented by these silica-based materials. These include the production of stationary phases with increased surface coverage, reduced residual silanols and improved chemical stability [2]. The latter is obtained by protecting the alkyl bonded-phase ligands from possible hydrolysis at low pH and the silica from dissolution at high pH. Thus, different modifications are carried out with respect to support type, nature of the organosilane reagent and bonding or immobilization conditions.

The use of trialkylsilanes with bulky isopropyl or *tert*-butyl side groups [3,4] and longer alkyl chains (C_8, C_{18}) [5,6] has increased the stability of the stationary phases at low pH. Horizontally polymerized [7–10], bidentate [4], encapsulated [11–13] and immobilized [14–19] stationary phases have shown reduced silanophilic interaction and improved alkaline stability. Other porous inorganic oxide-based packings have also been developed [20–23]. Among them, zirconia and titania are significantly more stable that silica and are becoming popular chromatographic supports [24,25].

Extensive studies with zirconia-based normal and reversed phases have been made, showing that this support is stable over the pH range 0–14 and to temperatures of 100 °C [23,24,26–28]. This material presents retention properties comparable to conventional silica-based phases when coated with polybutadiene (PBD) [26,27] or bonded to an octadecyl group [28]. Titania also is stable under most acidic and basic conditions, allowing separations under conditions where silica-based phases are less stable. This material has been studied as a support for normal, reversed and ion exchange phases [24,25,29–31], but not as extensively as zirconia. Recent investigations have been performed using silica modified with zirconium or titanium as chromatographic supports [32–36]. Stationary phases having poly(methyloctylsiloxane) (PMOS) immobilized by γ-radiation on zirconized silica or titanized silica showed better chromatographic performance than unmodified silica-based phases [32,34] and significant improvement of stability when using basic mobile phases [33,35].

In this paper, we evaluate the chromatographic behavior of titanized silica-based reversed phases prepared by immobilization of a poly(methyloctylsiloxane) layer by

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thermal treatment or microwave radiation. Looking for the best immobilization conditions, different thermal treatment temperatures (100, 120, 140 and 220 °C) and times (4, 8, 16 and 24 h) were tested. In the case of immobilization by microwave radiation, different power levels (452, 520 and 586 W) for 5, 15 or 30 min exposures were evaluated. The immobilized phases were characterized by thermogravimetric analysis (TGA), infrared (IR) spectroscopy and reversed-phase liquid chromatography. The results indicate that thermal treatment provides phases with better chromatographic properties than those immobilized by microwave radiation for the analysis of neutral, acidic and basic solutes.

2. Experimental

2.1. Reagents and materials

Methanol, isopropanol (both HPLC grade), and chloroform (analytical-reagent grade) were from Mallinckrodt, dichloromethane (analytical-reagent grade) was from Merck, titanium tetrabutoxide from Aldrich and nitric acid from Quimex. Water was distilled and then deionized (Milli-Q Plus, Millipore). The compounds used for preparing the test mixtures were analytical-reagent grade (acetone, acenaphthene, benzene, benzonitrile, phenol, naphthalene, toluene, uracil and *N*,*N*-dimethylaniline). The silica used for modification with titanium was Astrosil (Stellar Phases Inc.) having a mean particle size of 5 μm, average pore diameter of 13.2 nm, specific pore volume of 0.9 ml g^{-1} and specific surface area of 261 m^2 g^{-1}. The PMOS polymer, with an average molar mass of 6200, was obtained from Petrarch Systems/Hüls America.

2.2. Modification of silica with titanium (SiO_2–Ti)

First, an acid hydrolysis was carried out on the silica in order to reduce superficial impurities [37]. For this, the silica was treated with 0.01 mol l^{-1} nitric acid under reflux for 4 h. Later, successive washings were made with bidistilled water, methanol, isopropanol and hexane. After drying at 90 °C for 2 h, the silica was exposed to humid (50% relative humidity) air by placing the silica in a closed glass system containing a saturated solution of calcium nitrate in water for 60 h. A solution of 1.0 mol l^{-1} $Ti(OBu)_4$ in toluene was prepared and added to a flask containing the humid silica at a ratio of 3 ml of $Ti(OBu)_4$ solution for each gram of silica. This mixture was sonicated for 10 min and then maintained at 2 °C for 8 h. After that, the solid was washed with toluene (to remove excess reagent), isopropanol and water. Then, hydrolysis of the remaining butoxide groups was carried out with a 1×10^{-3} mol l^{-1} nitric acid solution. This mixture was stirred for 2 h. Finally, the solid was washed with bidistilled water and isopropanol. The resulting titanized silica had a specific surface area of 260 m^2 g^{-1}, pore diameter of 11.3 nm and pore volume of 0.8 ml g^{-1}. The amount of titanium present on the silica was 8.4%, as determined by X-ray fluorescence analysis in a Tracer model Spectrace 5000 instrument.

2.3. Preparation of the stationary phase

Batches of SiO_2–Ti(PMOS) stationary phase were prepared by mixing titanized silica support, usually activated by heating in air at 150 °C for 24 h, and PMOS, present initially in dichloromethane solution, to obtain 40 or 50% loadings of PMOS. The mixture was covered with aluminum foil and remained at rest for 3 h, after which the dichloromethane was evaporated, without stirring, at room temperature, in a fume hood. The freshly prepared sorbed phases were stored in air at room temperature for 6 days before immobilization.

2.4. Immobilization of the stationary phases

2.4.1. Thermal treatment

Portions of the sorbed stationary phases prepared with activated titanized silica were placed in stainless steel tubes (150 mm × 10 mm) inside a model EDG 10P-S tubular oven. A continuous nitrogen flow was maintained through the stationary phase. In this study, the influence of the time (4, 8, 16 and 24 h at 120 °C) and the immobilization temperature (100, 120, 140 and 220 °C for 8 h) were evaluated.

2.4.2. Microwave radiation

PTFE flasks containing sorbed phases prepared both from activated and non-activated titanized silica were placed inside a model Qwave 3000 Questron microwave oven. For the phase with 50% loading, irradiations at 452, 520 and 586 W for 15 min and at 520 W for 5 and 30 min were evaluated. The phases with a 40% loading of PMOS were irradiated at 520 and 586 W for 15 min.

2.5. Extraction procedure

After the immobilization treatments, the phases were placed in stainless steel tubes and the tubes were connected to a Waters 510 pump for extraction of soluble non-immobilized PMOS. This was performed by passing 120 ml of chloroform and, subsequently, 120 ml of methanol at a flow rate of 1.0 ml min^{-1} at room temperature through the tubes.

2.6. Column packing

Columns (60 mm × 3.9 mm) were made from type 316 stainless steel tubing. The internal surfaces were polished using a technique developed in our laboratory [38]. Slurry packing of the columns was made at 34.5 MPa with a Haskel packing pump using 10% slurries of the stationary phases in $CHCl_3$. Methanol was used as propulsion solvent. Columns were conditioned for 3 h with a methanol–water (70:30, v/v) mobile phase at 0.3 ml min^{-1}, prior to the testing.

2.7. Chromatographic evaluation

Two test mixtures were used for the stationary phase evaluation using the chromatographic parameters: retention factor (k), efficiency ($N\,m^{-1}$), resolution (R_s) and asymmetry (A_s) at 10% (b/a) for selected compounds. Mixture 1 contained uracil, acetone, benzonitrile, benzene, toluene and naphthalene and mixture 2 had uracil, phenol, *N*,*N*-dimethylaniline, naphthalene and acenaphthene. The column dead time (t_M) was determined using uracil as unretained compound. Injections of 10 μl of appropriate concentrations of these mixtures produced satisfactory chromatographic peaks with detection at 254 nm. The mobile phase was methanol–water (70:30, v/v) at 0.3 ml min^{-1}. HPLC separations were performed with a modular system consisting of a Waters 510 pump, a Rheodyne model injector and an Alltech model 450 UV detector. Data acquisition was carried out by Chrom Perfect for Windows, version 3.52 and Report-Write Plus software (Justice Innovations).

2.8. Physical characterization

After chromatographic evaluation, portions of the stationary phases were subjected to TGA and IR spectroscopy. The thermal stability of the immobilized phases was evaluated by TGA in an argon (inert) atmosphere using a model 2050 TA instrument with the temperature range from 25 to 1000 °C at a heating rate of 10 °C min^{-1}. The IR spectra were obtained using a Perkin-Elmer model FT-IR 1600 spectrometer in order to evaluate the presence of residual silanols.

3. Results and discussion

3.1. Thermally immobilized stationary phases

Table 1 summarizes the chromatographic parameters, obtained with mixture 1, of the phases immobilized at 120 °C, while varying the immobilization time. Fig. 1 shows the corresponding chromatograms. As shown in Table 1, the phases immobilized for 4 and 8 h present excellent chromatographic parameters, showing higher efficiency values than phases immobilized using longer times (16 and 24 h).

Table 1
Chromatographic parameters of the phases immobilized at 120 °C for different times, evaluated with mixture 1[a]

Time (h)	Efficiency ($N\,m^{-1}$)[b]	Asymmetry (A_s)[b]	Retention factor (k)[b]	Resolution (R_s)[c]
4	68000	1.0	3.8	2.9
8	73000	1.1	3.5	2.6
16	45000	1.8	3.6	2.1
24	61300	1.2	3.3	2.7

[a] Average of duplicate values.
[b] Naphthalene.
[c] Toluene–naphthalene.

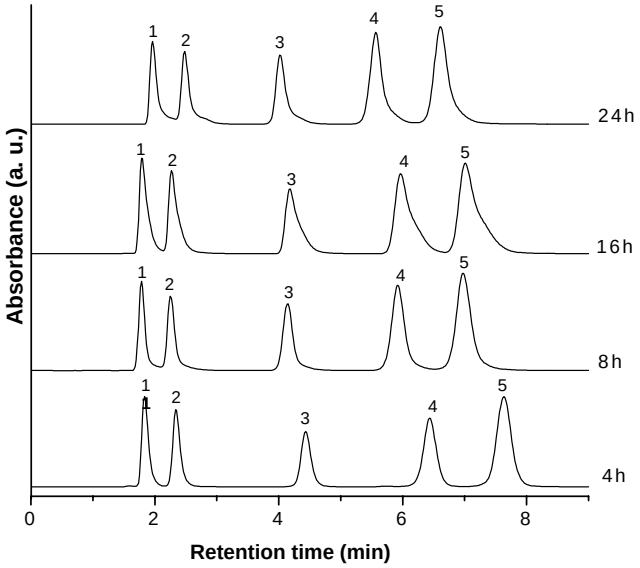

Fig. 1. Chromatograms of the phases immobilized at 120 °C for different immobilization times. Mixture 1: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

Phases immobilized for 4 and 8 h at 120 °C were then evaluated with mixture 2, in order to study their behavior towards acidic (phenol) and basic (*N*,*N*-dimethylaniline) solutes. The results are presented in Table 2 and Fig. 2. Differences in peak shape of the *N*,*N*-dimethylaniline are observed, with the phase immobilized for 8 h showing a more symmetrical peak. This indicates that the undesirable interactions of the basic solute with residual silanols and titanols were significantly reduced using an immobilization time higher than 4 h.

Other tests were carried out with phases immobilized at different temperatures for 8 h. The results are shown in Table 3 and Fig. 3. The results confirm that phases immobilized at 120 °C for 8 h present the best chromato-

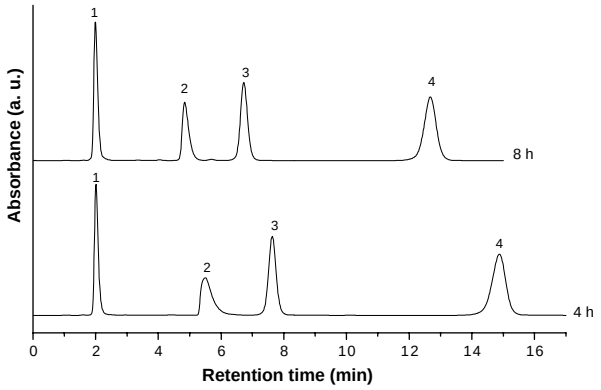

Fig. 2. Chromatograms of the phases immobilized at 120 °C for 4 and 8 h. Mixture 2: 1 = phenol; 2 = *N*,*N*-dimethylaniline; 3 = naphthalene; 4 = acenaphthene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

Table 2
Chromatographic parameters of the phases immobilized at 120 °C for 4 and 8 h, evaluated with mixture 2

Time (h)	Efficiency ($N\,m^{-1}$)		Asymmetry (A_s)[a]				Retention factor (k)[b]	Resolution (R_s)[c]
	Naphthalene	Acenaphthene	1	2	3	4		
4	65400	77100	1.2	1.8	1.0	0.9	3.8	4.0
8	67100	83000	1.2	1.6	1.1	1.0	3.2	4.2

[a] 1: Phenol; 2: *N*,*N*-dimethylaniline; 3: naphthalene; 4: acenaphthene.
[b] Naphthalene.
[c] Naphthalene–acenaphthene.

Table 3
Chromatographic parameters of the phases immobilized at different temperatures for 8 h, evaluated with mixture 1[a]

Temperature (°C)	Efficiency ($N\,m^{-1}$)[b]	Asymmetry (A_s)[b]	Retention factor (k)[b]	Resolution (R_s)[c]
100	39200	1.1	1.9	1.2
120	73000	1.1	3.5	2.6
140	59300	1.2	2.7	1.9
220	43000	0.4	8.8	2.5

[a] Average of duplicate values.
[b] Naphthalene.
[c] Toluene–naphthalene.

graphic values. As seen in Fig. 3, the resolution loss of the mixture 1 solutes, when the phase is immobilized at 100 °C indicates that an insufficient polymeric layer was formed. On the other hand, the phase immobilized at 220 °C presented greater retention of the more hydrophobic solutes as result of the formation of a thicker polymeric layer.

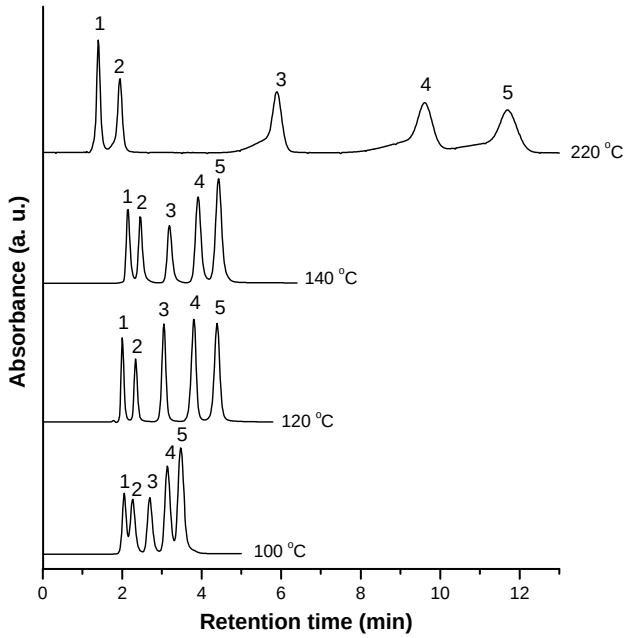

Fig. 3. Chromatograms of the phases immobilized at different temperatures for 8 h. Mixture 1: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

Table 4
Chromatographic parameters of the phases immobilized by microwave radiation for 15 min, varying the power, evaluated with mixture 1[a]

Radiation power (W)	PMOS (%)	Efficiency ($N\,m^{-1}$)[b]	Asymmetry (A_s)[b]	Retention factor (k)[b]	Resolution (R_s)[c]
520	40	37200	1.4	2.2	1.5
586	40	34500	1.3	2.2	1.4
452	50	45200	0.9	2.2	1.5
520	50	60300	1.1	3.0	2.3
586	50	32800	1.2	2.3	1.1

[a] Average of duplicate values.
[b] Naphthalene.
[c] Toluene–naphthalene.

Portions of the phases immobilized at different temperatures were subjected to thermogravimetric analysis under argon. The mass loss is directly related to the amount of PMOS retained by the support. Thus, the mass loss increases as the immobilization temperature increases. This is in agreement with the chromatographic results (Fig. 3), indicating that the phase immobilized at 220 °C contains a larger amount of polymer, some 29%, on the support surface, compared to 8–10% for the phases immobilized at lower temperatures.

The IR spectra showed the characteristic band of the PMOS methyl groups that appears around 2929 cm^{-1}. The intensity of this band increases for higher amounts of immo-

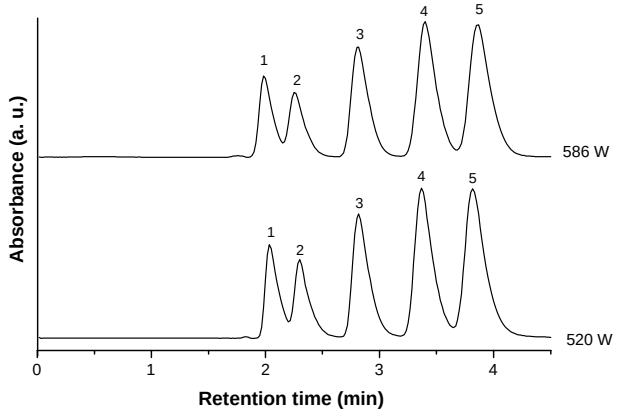

Fig. 4. Chromatograms of the phases, with a 40% loading of PMOS, subjected to microwave radiation for 15 min, varying the irradiation power. Mixture 1: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

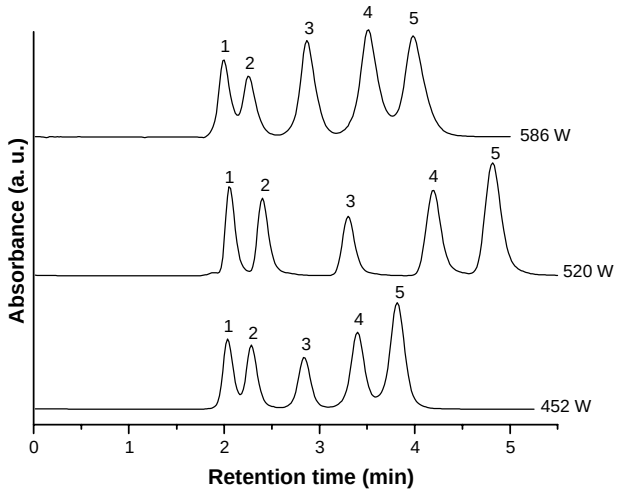

Fig. 5. Chromatograms of the phases, with a 50% loading of PMOS, subjected to microwave radiation for 15 min, varying the irradiation power. Mixture 1: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

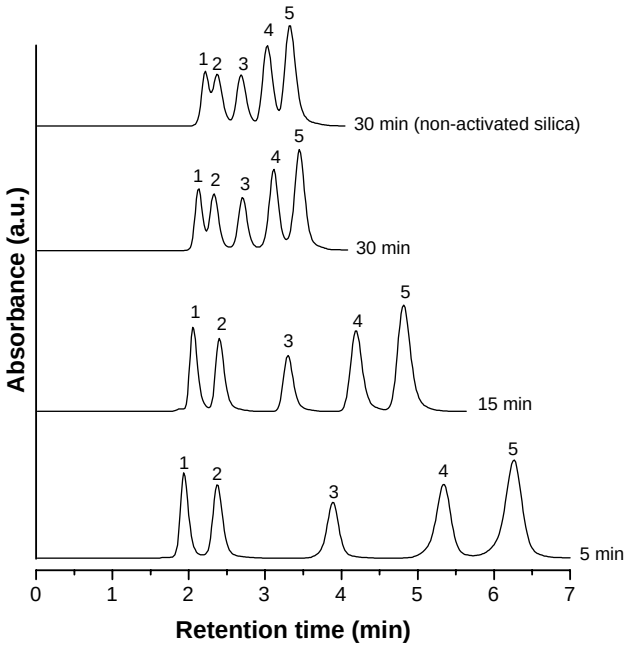

Fig. 6. Chromatograms of the phases immobilized by microwave radiation using a power level of 520 W for different time periods. Mixture 1: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

bilized PMOS. The band situated around 970 cm^{-1}, which corresponds to the free silanols, becomes less noticeable after higher temperature immobilization, suggesting a higher coverage of these active groups.

3.2. Microwave radiation immobilized stationary phases

The chromatographic parameters obtained with phases subjected to microwave radiation for 15 min, varying the irradiation power, are shown in Table 4. Figs. 4 and 5 show the corresponding chromatograms. Phases prepared with 40% PMOS loading presented similar chromatographic parameters for the power levels used (520 and 586 W). When a higher amount of polymer (50%) is loaded onto the support, an improvement in the chromatographic parameters is observed, mainly when a power level of 520 W is used. The power level of 586 W gave the lowest efficiency values with both PMOS loadings, indicating that, under these conditions, the polymer was degraded, part of it being removed during the extraction procedure. This fact provoked the loss of resolution for the test solutes.

Stationary phases immobilized at 520 W for different time periods were also studied. The chromatographic results are shown in Table 5 and Fig. 6. It is observed that short periods of 5 and 15 min are enough to obtain efficient columns. For 30 min of immobilization, the retentions of the solutes decrease, with a consequent loss of resolution and efficiency, as also seen when a higher power level was used. The phase prepared from silica that was not activated presented poorer results, as the dipole rotation of the water molecules induced by the microwave radiation promoted overheating.

Evaluations made with mixture 2 of the phases having a 50% loading and immobilized for 5 and 15 min at 520 W are shown in Table 6 and Fig. 7. The *N*,*N*-dimethylaniline peak presents a lower asymmetry after immobilization for 15 min. However, this value is still high, suggesting that this phase would not efficiently separate basic solutes.

The mass losses, as determined by TGA, of the phases immobilized for different times by microwave radiation show that the quantity of polysiloxane decreases as the immobilization time increases, which indicates the presence of a

Table 5
Chromatographic parameters of the phases immobilized by microwave radiation, using 520 W for different periods, evaluated with mixture 1[a]

Time (min)	Efficiency (N m^{-1})[b]	Asymmetry (A_s)[b]	Retention factor (k)[b]	Resolution (R_s)[c]
5	59100	1.0	4.1	2.4
15	60300	1.1	3.0	2.3
30	48400	1.0	1.9	1.4
30 (Non-activated silica)	38100	1.3	1.8	1.1

[a] Average of duplicate values.
[b] Naphthalene.
[c] Toluene–naphthalene.

Table 6
Chromatographic parameters of the phases immobilized by microwave radiation using 520 W for different periods, evaluated with mixture 2

Time (min)	Efficiency ($N\,m^{-1}$)		Asymmetry (A_s)[a]				Retention factor (k)[b]	Resolution (R_s)[c]
	Naphthalene	Acenaphthene	1	2	3	4		
5	57200	64500	1.3	2.1	0.9	0.8	4.1	3.6
15	55600	72600	1.4	2.0	1.2	1.0	3.1	2.2

[a] 1: Phenol; 2: *N*,*N*-dimethylaniline; 3: naphthalene; 4: acenaphthene.
[b] Naphthalene.
[c] Naphthalene–acenaphthene.

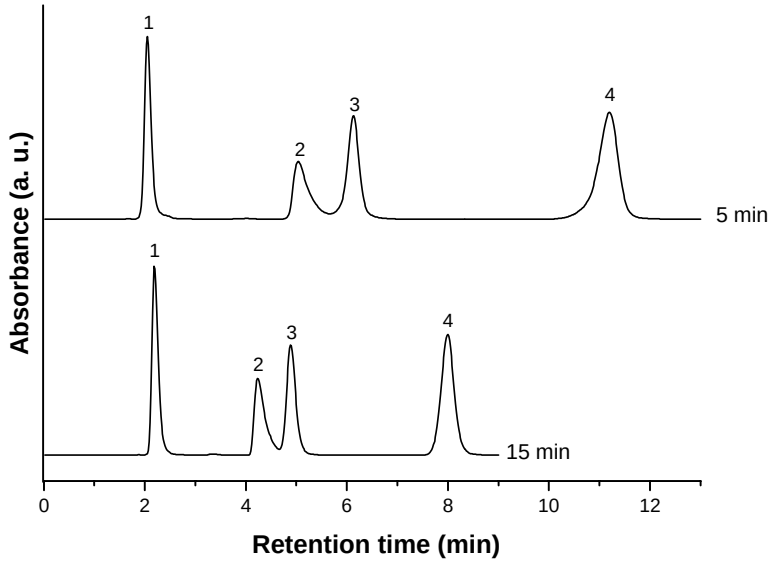

Fig. 7. Chromatograms of the phases immobilized by microwave radiation for 5 and 15 min at 520 W. Mixture 2: 1 = phenol; 2 = *N*,*N*-dimethylaniline; 3 = naphthalene; 4 = acenaphthene. Column: 60 mm × 3.9 mm. Analysis conditions: mobile phase, MeOH–water (70:30, v/v); flow rate, 0.3 ml min^{-1}; UV detection at 254 nm.

thinner polymeric layer, consistent with the lower retention values of the several solutes (Fig. 6).

The IR spectra for phases after different immobilization times at 520 W showed that the band of the free silanols is less noticeable after shorter immobilization periods, with an increased intensity of the characteristic bands of the PMOS methyl group. This is in agreement with the results of thermogravimetric analysis that indicate that higher amounts of PMOS are retained when shorter immobilization times are used.

4. Conclusions

Stationary phases prepared by immobilization of PMOS onto titanized silica for 8 h at temperatures higher than 120 °C showed a reduction in efficiency, due to formation of a thicker polymeric layer that impedes efficient mass transfer of the solutes between the stationary and mobile phases. For lower temperatures (100 °C), the layer formed was not sufficient, resulting in fewer interactions. This interpretation is confirmed through thermogravimetric and IR spectroscopic analyses. The use of microwave radiation for immobilization of PMOS provided stationary phases with lower efficiencies than those obtained using the thermal treatments. The immobilized stationary phase with the best chromatographic properties was obtained by a thermal treatment of 120 °C for 8 h, a result similar to that observed when PMOS is immobilized on bare silica [19].

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