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High-performance liquid chromatographic stationary phases based on poly(methyloctylsiloxane) immobilized on silica II. Chromatographic evaluation

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Abstract

This work describes the chromatographic characterization of stationary phases prepared by deposition of poly(methyloctylsiloxane) (PMOS) on silica followed by immobilization using one of several different processes: thermal treatments (120 or 220 °C for 4 h), microwave irradiation (495 W for 15 min), γ radiation (dose of 80 kGy) or self-immobilization. This evaluation was based on the chromatographic parameters of several test solutes. The stationary phases immobilized at 220 °C and which underwent self-immobilization were not appropriate for chromatographic use but the other immobilized phases presented chromatographic performances similar in most respects to a commercial phase (Rainin C_8) while the peak characteristics of the basic probe were significantly better with these phases. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Poly(methyloctylsiloxane)–silica stationary phases; Stationary phases, LC; Immobilization; Poly(methyloctylsiloxane)

1. Introduction

The bonding of organosilane groups onto silica support particles is the most common procedure for preparation of commercial RP-HPLC stationary phases [1–6]. Chemically bonded stationary phases prepared with silica supports are quite stable in neutral pH, but are not stable under more extreme pH conditions. Methods have been developed to improve the stability of these phases when using acidic and basic mobile phases, including endcapping [7], encapsulation [8,9], the use of polar groups embedded in the pendant chains [10] and even modification of the silica support [11]. As an alternative, a stationary phase may be prepared by sorbing and immobilizing a liquid polymer onto an appropriate support surface using temperature [12,13], γ radiation [14–16] or other crosslinking processes [13–15,17,18]. Among the polymers already used in the preparation of such stationary phases are poly(ethylene) [19–21], poly(butadiene) [22–30], poly(styrene) [31,32], poly(dimethylsiloxane) [33], poly(methyloctylsiloxane) [12,14,15,34–37] and poly(methyloctadecylsiloxane) [12,15], while the supports include silica, zirconia, titania and alumina.

Porous silica is the support most widely used for RP-HPLC packings because it is mechanically stable at high pressures and can be easily derivatized. Its structure and properties have been extensively studied and it is commercially available in a variety of forms and sizes with different pore dimensions. On the other hand, phases based on silica tend to be

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limited in two important aspects. One of these is related to the non-homogeneity of its surface, which presents different types of acidic silanol groups [37,38] that can interact strongly with (and adsorb) basic molecules. The second aspect relates to the instability of these stationary phases in acidic and basic mobile phases. As already described in the literature, these problems can be minimized by preparing stationary phases where a polymer is sorbed and immobilized onto the support [12,14,15,39,40]. To extend these observations, we have prepared stationary phases by deposition of PMOS on a spherical 5-μm silica followed by immobilization using several different processes: thermal treatments (120 or 220 °C for 4 h), irradiation with microwaves (495 W for 15 min), γ radiation (dose of 80 kGy) and self-immobilization [41]. A previous paper describes the physical and chemical characterization of these stationary phases [42]. This work describes chromatographic evaluations based on parameters obtained with columns packed with the immobilized stationary phases using two test mixtures and methanol–water (7:3, v/v) as mobile phase. These properties were compared with a laboratory-packed column containing a commercial stationary phase (Rainin C_8, 5 μm).

2. Experimental

2.1. Chemicals and materials

The chromatographic support used to prepare the stationary phases was spherical Kromasil silica (Eka Nobel) having a mean particle diameter of 5 μm, 11.3 nm pore size, 0.89 ml g^{-1} specific pore volume and 330 m^2 g^{-1} specific surface area.

The stationary phase used for comparison was a spherical chemically bonded C_8 (Rainin from Varian), having a mean particle diameter of 5 μm and an 11.0 nm pore size.

Poly(methyloctylsiloxane) (product PS-140), M_r 6200 and weight-average molecular mass (M_w) 16 000, was obtained from Hülls America, USA

Methanol (Omnisolv), chloroform (LiChrosolv), toluene, hexane and ethylbenzene were all from Merck. Distilled and deionized water (Milli-Q Plus, Millipore) was used throughout.

The chromatographic test substances [acetone (Merck), benzonitrile (Riedel-de Haën), benzene (Synth), naphthalene (Vetec), uracil (Aldrich), phenol (Labsynth), *N*,*N*-dimethyilaniline (Fluka), ethylbenzene (Merck) and acenaphthene (Aldrich)] were analytical reagent-grade and not purified further.

2.2. Preparation of the stationary phases

The silica was dried in air at 150 °C for 17 h. It was then added to a 10% (w/v) solution of PMOS in hexane in the proportion of 1.22 g PMOS for 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.

The stationary phase obtained by evaporation of the solvent was divided into five portions. Each portion was submitted to a different procedure for polymer immobilization: (1) thermal treatment at 120 °C for 4 h; (2) thermal treatment at 220 °C for 4 h; (3) irradiation with microwaves (potency of 495 W for 15 min) in a Model Qwave 3000 Questron microwave oven; (4) irradiation with γ radiation to a dose of 80 kGy, carried out under air in glass ampoules with a commercial cobalt-60 irradiator (IBRAS-CBO, Campinas, Brazil); and (5) self-immobilization by storage in air at room temperature (22±1 °C) for 30 days.

After each immobilization procedure, excess PMOS (not immobilized) was extracted from the stationary phase by passing hexane through the material contained in a column-type washing tube at 0.5 ml min^{-1} flow rate for 4 h at ambient temperature. The phase was then dried (40 °C for 12 h) and stored in closed containers until needed.

Each phase was characterized by several physical and chemical tests, as described in a previous paper [42].

2.3. Preparation of columns

Columns (50×4 mm) were made from type 303 stainless-steel tubing with highly polished interior surfaces [43]. The columns were downward packed using 10% (w/v) slurries of each stationary phase in chloroform. A packing pressure of 34.5 MPa (Haskel packing pump) was used, with methanol as propulsion solvent. Columns were conditioned for 4 h with

mobile phase (methanol–water, 7:3, v/v) at 0.2 ml min^{-1} prior to testing.

2.4. Instrumentation

The chromatography was performed with 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 acquisition used Chrom Perfect for Windows, version 3.52 and Report–Write Plus software (Justice Innovations) installed in an IBM-compatible personal computer.

2.5. Evaluation of columns

All separations were carried out at room temperature with flow rates of 0.4 or 0.5 ml min^{-1}. These optimal flow rates were determined by individual Van Deemter curves. Two test mixtures were used in this study. The first contained acidic, basic and neutral solutes: (1) uracil, phenol, *N,N*-dimethylaniline (*N,N*-DMA), naphthalene, ethylbenzene and acenaphthene; and the second contained neutral solutes with varying polarities; (2) uracil, acetone, benzonitrile, benzene, toluene and naphthalene. Both mixtures were dissolved in the mobile phase (methanol–water, 7:3, v/v). Injections of 5 μl of these mixtures produced satisfactory chromatographic peaks with detection at 254 nm. The column dead time, t_M, was determined with uracil (an unretained compound). The asymmetry (A_s) was calculated at 10% of the peak height and efficiency (N) from peak width at half height. Retention factors (k) and resolution (R_s) were also determined.

3. Results and discussion

3.1. Text mixture 1: uracil, phenol, N,N-dimethylaniline, naphthalene, ethylbenzene and acenaphthene

The chromatograms obtained using columns packed with the different immobilized stationary phases and with a commercial phase (Rainin C_8) using test mixture 1 are shown in Fig. 1.

The column packed with the stationary phase immobilized by a 220 °C thermal treatment (Fig. 1a) did not separate the substances of this test mixture. This may be related to the large amount of PMOS immobilized onto the outer surface of the silica, as shown in Table 1, which promotes agglomeration of the particles [42]. It was not possible to obtain the chromatographic parameters of the test solutes for this column.

The column packed with the self-immobilized stationary phase was also not able to completely separate all the solutes of the test mixture (Fig. 1e). This result is due to an effect opposite to that of the phase heated at 220 °C for 4 h. In other words, the very low quantity of polymeric covering for this phase (Table 1) is not capable of retaining the solutes well, thus there is little separation. Perhaps, if a longer self-immobilization time were used, this phase would present a higher polymeric covering and better chromatographic performance, as has been seen with PMOS self-immobilized on other silicas [44]. Nevertheless, it was possible to obtain some of the chromatographic parameters.

The other columns (Fig. 1b–d) were efficient for the separation of the test mixture, presenting chromatograms similar to that of the commercial stationary phase (Fig. 1f).

The chromatographic parameters obtained from the peaks of the test mixture solutes (retention factor, asymmetry and resolution) are shown in Tables 2–4. The different values for retention factor (Table 2) show that the columns present different separation characteristics as a function of the degree of polymer loading promoted by each immobilization process (Table 1).

The asymmetry values (Table 3) show that all the phases provided chromatograms with symmetrical peaks for the well separated neutral solute acenaphthene while the less well resolved neutral compounds, naphthalene and ethylbenzene, appear less symmetrical. For the acidic solute (phenol) only the phase immobilized by microwave irradiation presented a symmetrical peak while, for the basic solute (*N,N*-dimethylaniline) all the immobilized phases showed peaks which were more symmetrical than that seen with the chemically bonded commercial phase, demonstrating that there was significant coverage of the superficial silanol groups of the support. It was not possible to evaluate the

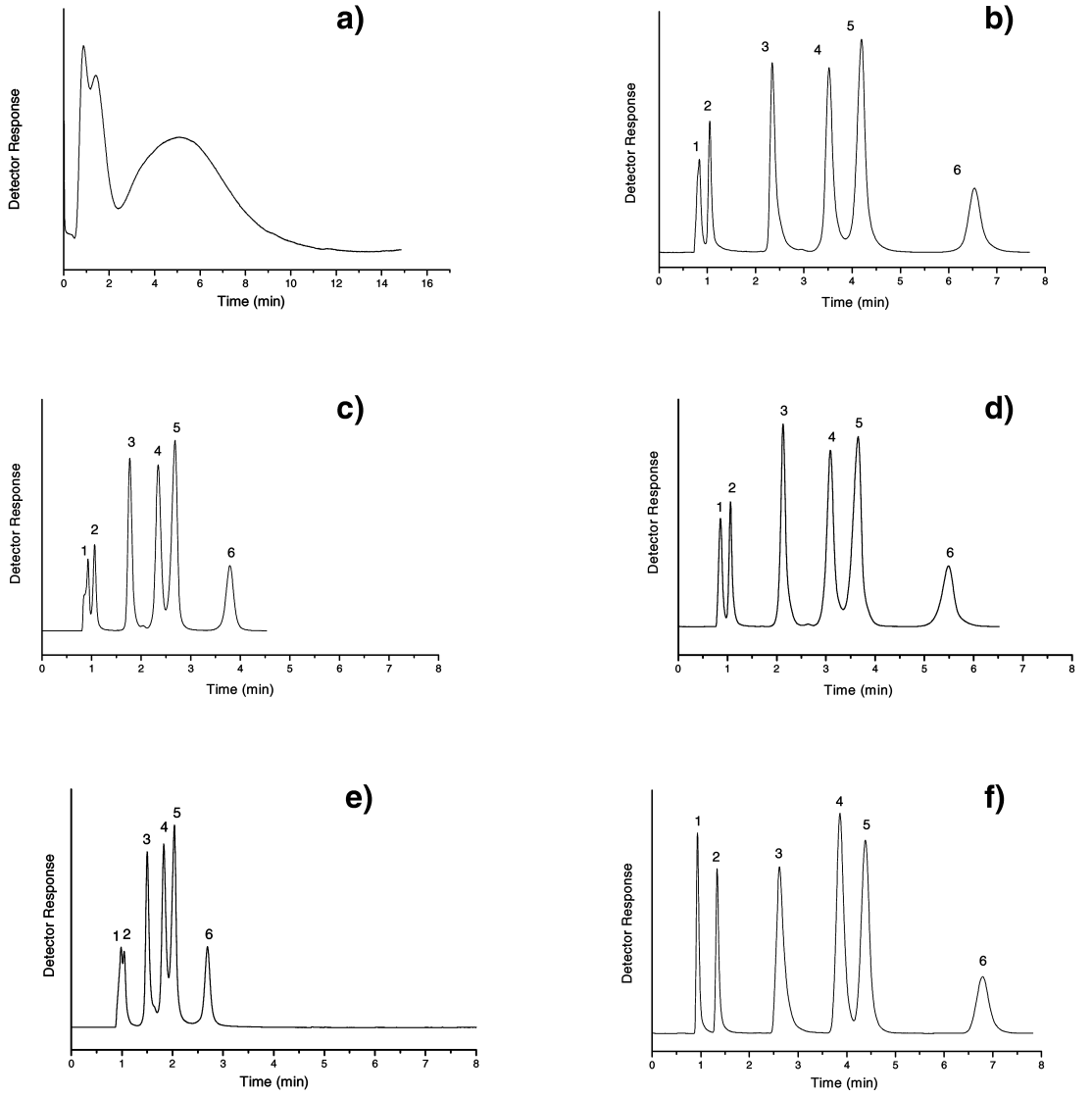

Fig. 1. Chromatograms of SiO_2 (PMOS) stationary phases immobilized by: (a) heat treatment at 220 °C for 4 h; (b) heat treatment at 120 °C for 4 h; (c) microwave irradiation; (d) γ irradiation; (e) self-immobilization; and (f) commercial stationary phase (Rainin C_8). Conditions: columns: 50×4 mm, mobile phase methanol–water (7:3, v/v), flow rate 0.5 ml min^{-1}. Peaks in elution order: 1-uracil, 2-phenol, 3-*N*,*N*-dimethylaniline, 4-naphthalene, 5-ethylbenzene and 6-acenaphthene.

asymmetry for most of the test solutes of the self-immobilized phase, because there was insufficient separation of the peaks. This is confirmed by the resolution values of the adjacent peaks (Table 4), where all the columns separate the test solutes, except for the column packed with self-immobilized stationary phase, which shows very poor resolution of most of the compounds.

The silanol activity of the columns was also determined by the ratio of the retention factors of the basic (*N*,*N*-dimethylaniline) and hydrophobic (acenaphthene) solutes, as described by Neue et al. [45].

Table 1
Percent carbon, loadings and specific mass of the immobilized SiO_2 (PMOS) stationary phases

	Stationary phase				
	Heated at 220 °C	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized
Percent carbon	21.0	14.5	9.5	12.4	5.8
Loading (%)	34	23	15	20	9
Specific mass (m_{PMOS}/m_{SiO2})	0.51	0.30	0.18	0.25	0.10

Table 2
Retention factors of the test solutes of mixture 1 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	*k*				
	Stationary phase				
	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Phenol	0.28	0.14	0.23	0.04	0.44
N,*N*-DMA	2.08	0.90	1.48	0.48	1.82
Naphthalene	3.82	1.52	2.59	0.79	3.15
Ethylbenzene	4.79	1.88	3.24	1.00	3.71
Acenaphthene	8.26	3.07	5.38	1.61	6.30

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.5 ml min^{-1}.

A curve of relative retention factors between *N*,*N*-dimethylaniline and acenaphthene, as a function of the percent carbon of the stationary phases, is shown in Fig. 2. The silanol activity decreased proportional to the increase in the percent carbon of the stationary phases. This is related to the progressive covering of the silanol groups with the increase in percent loading of the phases.

Curves of efficiency versus flow rate of the mobile phase were obtained for each column (Fig. 3). The maximum efficiencies were similar for all columns at the Van Deemter-optimized flow rates, although the

Table 3
Asymmetry factors for the test solutes of mixture 1 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	A_s				
	Stationary phase				
	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Phenol	1.56	1.04	1.45	–	1.80
N,*N*-DMA	1.66	1.51	1.40	1.38	2.06
Naphthalene	1.28	1.41	1.28	–	1.44
Ethylbenzene	0.78	0.57	0.72	–	1.09
Acenaphthene	1.00	0.94	0.95	0.96	1.24

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.5 ml min^{-1}.

Table 4
Resolution between adjacent peaks for the test solutes of mixture 1 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	R_s				
	Stationary phase				
	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Phenol–*N*,*N*-DMA	9.87	5.33	7.40	2.63	6.42
N,*N*-DMA–naphthalene	5.84	3.24	4.43	2.14	4.35
Naphthalene–ethylbenzene	2.40	1.64	1.95	1.22	1.73
Ethylbenzene–acenaphthene	6.27	4.33	4.79	3.25	6.00

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.5 ml min^{-1}.

column with commercial phase presents a higher efficiency at higher flow rates, indicating better mass transfer and a smaller *C* term in the Van Deemter equation.

3.2. Test mixture 2: uracil, acetone, benzonitrile, benzene, toluene and naphthalene

The chromatograms obtained using columns packed with the immobilized PMOS stationary phases and with a commercial stationary phases (Rainin C_8) using test mixture 2 are shown in Fig. 4.

As observed with text mixture 1, the stationary phase heated at 220 °C and the self-immobilized phase did not separate this test mixture although it was possible to obtain some of the chromatographic parameters for the self-immobilized column. The chromatographic parameters (retention factor, asymmetry and resolution) obtained from the chromatograms for the test solutes of mixture 2 are shown in Tables 5–7.

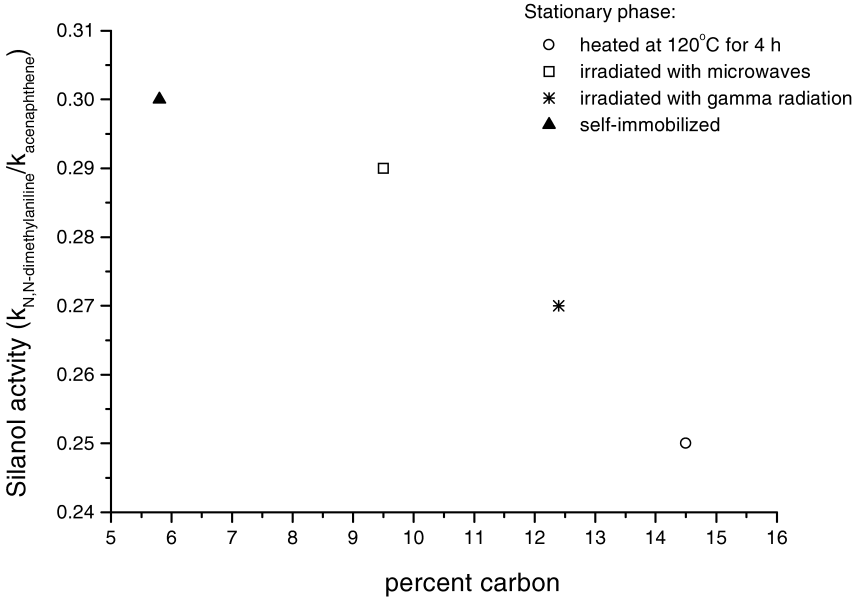

Fig. 2. Silanol activity as a function of percent carbon of the immobilized SiO_2 (PMOS) stationary phases.

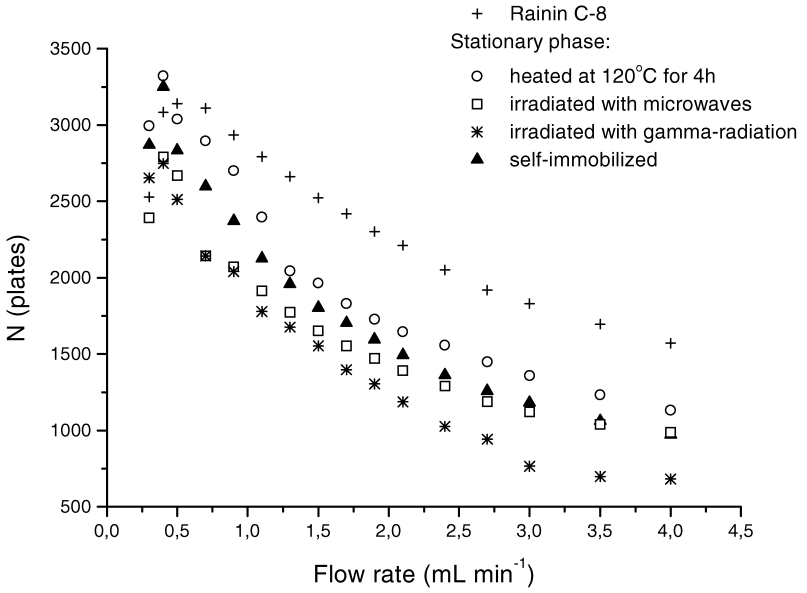

Fig. 3. Curves of efficiency (calculated for acenaphthene) as a function of mobile phase flow rate for columns packed with immobilized SiO_2 (PMOS) stationary phases and a commercial phase (Rainin C_8). Conditions: columns: 50×4 mm, mobile phase methanol–water (7:3, v/v).

Again, it can be noted from the values of the retention factors (Table 5) that the columns present different separations as a function of the degree of polymer coverage resulting from each immobilization process. All the phases provided chromatograms with quite symmetrical peaks (Table 6), since all solutes are neutral. It was not possible to obtain the asymmetry factor for most of the test solutes for the self-immobilized phase, because there was insufficient separation of the peaks. This is also confirmed by the resolution for adjacent peaks (Table 7), where all the columns separate the test solutes, except for the column packed with self-immobilized stationary phase.

The hydrophobicity of the stationary phases was determined by the method of Galusko [46], where $(k_{\text{toluene}}+k_{\text{benzene}})/2$, and the curve of hydrophobicity as a function of percent carbon of the stationary phases is shown in Fig. 5. As expected, the hydrophobicity increases proportional to the increase in the percent carbon of the stationary phases, due to the increase in the quantity of the alkyl chains on the surface of the stationary phase as the percent loading increases. Thus, the stationary phase treated at 120 °C for 4 h showed the largest hydrophobicity while the self-immobilized phase had lowest hydrophobicity.

4. Conclusions

Taking into consideration the development of stationary phases prepared with monolayers of polymer, it is obvious that the difference between the diffusion rates of the analytes in modern adsorbed/immobilized polymer phases and in chemically bonded phases are of little significance, as confirmed by the present results, where the parameter of importance is the amount of immobilized polymer.

The immobilization of polymers in a porous support is a complex process, influenced by the contribution of the different types of interactions between the support and the polymer and also the solubility of the polymer in the eluents. Not only the chromatographic properties of the composite polymer-support but also the polymer immobilization process are influenced by the properties of the

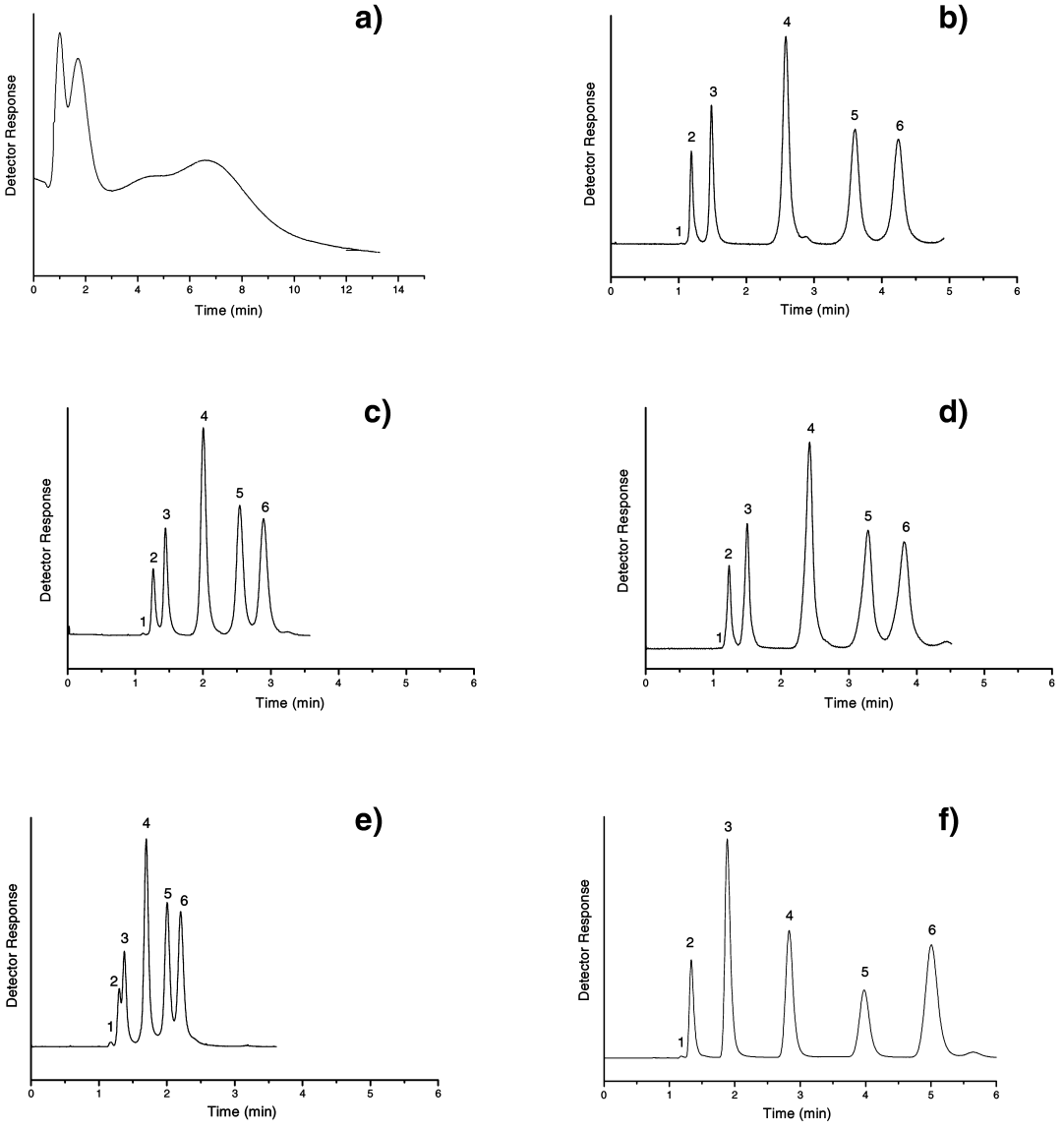

Fig. 4. Chromatograms of SiO_2 (PMOS) stationary phases immobilized by: (a) heat treatment at 220 °C for 4 h; (b) heat treatment at 120 °C for 4 h; (c) microwave irradiation; (d) γ irradiation; (e) self-immobilization; and (f) commercial stationary phase (Rainin C_8). Conditions: columns: 50×4 mm, mobile phase methanol–water (7:3, v/v), flow rate 0.4 ml min^{-1}. Peaks in elution order: 1-uracil, 2-acetone, 3-benzonitrile, 4-benzene, 5-toluene and 6-naphthalene.

support and the processes used to effect the immobilization.

The stationary phase immobilized by thermal treatment at 220 °C for 4 h was not appropriate for chromatographic use because of the large amount of immobilized polymer, which hinders the mass transfer process of the solutes, while the self-immobilized phase was also not appropriate, due to the low quantity of polymeric covering obtained with this immobilization procedure on Kromasil silica.

Table 5
Retention factors for the test solutes of mixture 2 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	k				
	Stationary phase				
	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Acetone	0.14	0.14	0.14	0.10	0.13
Benzonitrile	0.42	0.30	0.39	0.16	0.60
Benzene	1.48	0.80	1.24	0.43	1.40
Toluene	2.47	1.29	2.04	0.70	2.37
Naphthalene	3.09	1.60	2.54	0.86	3.25

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.4 ml min^{-1}.

Table 6
Asymmetry factors for the test solutes of mixture 2 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	A_s				
	Stationary phase				
	Heated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Acetone	1.55	1.63	1.30	–	1.77
Benzonitrile	1.43	1.48	1.17	–	1.62
Benzene	1.15	1.34	0.99	1.26	1.38
Toluene	0.99	1.26	0.86	–	1.24
Naphthalene	1.04	1.20	0.90	–	1.23

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.4 ml min^{-1}.

The phases obtained by the immobilization processes which used thermal treatment at 120 °C or irradiation with microwaves or γ radiation present different chromatographic properties as a function of the different amounts of polymer coating. However, all are suitable for chromatographic purposes. Of

Table 7
Resolution between adjacent peaks for the test solutes of mixture 2 for columns packed with immobilized SiO_2 (PMOS) stationary phases and Rainin C_8

Solutes	R_s				
	Stationary phase				
	Treated at 120 °C	Irradiated with microwaves	Irradiated with γ radiation	Self-immobilized	Rainin C_8
Acetone–benzonitrile	3.26	1.76	2.40	0.72	4.02
Benzonitrile–benzene	8.08	4.32	5.88	2.75	5.17
Benzene–toluene	4.97	3.23	3.81	2.47	4.60
Toluene–naphthalene	2.47	1.77	1.91	1.38	3.21

Column: 50×4 mm; mobile phase: methanol–water (7:3, v/v); flow rate: 0.4 ml min^{-1}.

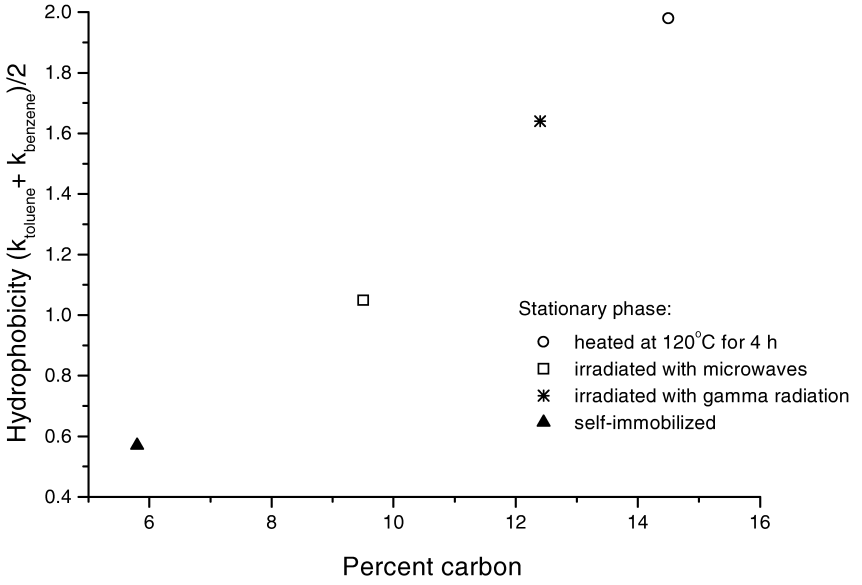

Fig. 5. Hydrophobicity as a function of percent carbon of the immobilized SiO_2 (PMOS) stationary phases.

particular importance is the observation that the columns packed with these immobilized phases present performances similar to that of a commercial phase, with superior performance for the separation of the basic solute (*N*,*N*-dimethylaniline).

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