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

I. Physical and chemical characterizations

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Abstract

Five different reversed-phase materials for high-performance liquid chromatography were obtained by deposition of poly(methyloctylsiloxane) in HPLC silica particles, followed by immobilization using different processes: thermal treatment (120 or 220 °C for 4 h), irradiation with microwaves (495 W for 15 min), γ radiation (dose of 80 kGy) and self-immobilization. These phases were characterized by gel permeation chromatography, percent carbon, ^{13}C and ^{29}Si solid-state nuclear magnetic resonance spectroscopy, infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy. The results show that the different immobilization processes produce different physical characteristics in the prepared phases. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

At present, reversed-phase high-performance liquid chromatography (RP-HPLC) uses, almost exclusively, columns packed with porous silica supports onto which organic groups are chemically bonded through a siloxane bond. The most used stationary phases have aliphatic chains containing eight or 18 carbon atoms (C_8 and C_{18}). Although there are several different procedures described in the literature for the formation of a chemically bonded layer

on the silica surface, the most used is the silanization reaction starting with bare activated silica and a chloro or alkoxy silane [1–6]. One problem with these stationary phases is related to the non-uniformity of the resulting surface, characterized by the presence of residual silanol groups [7,8], metal impurities [9,10] and ionic sites [11], that alter the retention mechanisms of the different types of solute compounds during a chromatographic separation.

The instability of the silica support in the presence of acidic and basic mobile phases is also a problem because, through slow chemical reactions, the stationary phase can be dissolved in the mobile phase, promoting loss of the silica and, consequently, collapse of the column bed [12,13]. Several methods

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have been employed to improve the stability of the chemically bonded phases; among them are: end capping [14], encapsulation [15,16], introduction of voluminous groups (sterically protected phases) [17], or the promotion of a polymeric immobilized monolayer, obtained by controlled crosslinking on the support surface (horizontal polymerization) [18–21].

In recent years, covering of oxide supports with polymers has been described as an alternative method for the preparation of stationary phases for RP-HPLC [22]. The main advantages of phases with monolayers of polymer, compared to phases consisting of smaller chemically bonded functional groups, are: higher coverage of the active sites of the support and the possibility of greater selectivity of the stationary phase by the appropriate choice of polymer. This selectivity is influenced by the nature and amount of functional groups in the chains of the polymer, the distribution of the polymeric film and the specific surface area and pore structure of the support.

There are several types of polymeric coverings of the support. One of the most common is obtained by a physical adsorption of a pre-synthesized polymer having a well defined chemical composition. Alternatively, a polymer can be introduced by the in situ polymerization of monomers [22]. The principal disadvantage of phases based on adsorbed polymeric coverings are their lower stability, which can be improved with the immobilization of the polymer onto the support. In both cases these polymers can be immobilized by crosslinking induced by peroxide [22–27], thermal treatments [24,28] or γ radiation [22,23,29–31].

With the objective of evaluating different immobilization procedures, we have prepared stationary phases by the deposition of poly(methyloctylsiloxane) (PMOS) in the pores of HPLC silica, followed by immobilization using several different processes: thermal treatment (120 or 220 °C for 4 h), irradiation with microwaves (potency of 495 W for 15 min) or γ radiation (dose of 80 kGy) and self-immobilization [32].

The immobilized phases prepared in this work were characterized by percent carbon, gel permeation chromatography (GPC), ^{13}C and ^{29}Si solid-state cross-polarization magic angle spinning nuclear mag-

netic resonance spectrometry (CP-MAS-NMR), infrared spectroscopy (IR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM).

2. Experimental

2.1. Materials

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

PMOS (product PS-140), number-average molar mass M_n 6200 and weight-average molar mass M_w 16 000, was obtained from Petrarch-Hülls America, while toluene (HPLC-grade) from Merck and hexane (analytical-reagent grade) from Mallinckrodt were used as solvents. Distilled, deionized water (Milli-Q Plus, Millipore) was used throughout.

2.2. Preparation of the stationary phases, $\text{SiO}_2(\text{PMOS})$

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 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.

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 phases by passing hexane at 0.5 ml min^{-1}

flow-rate for 4 h at ambient temperature. The phases were then dried (40 °C for 12 h) and stored in closed containers until needed.

2.3. Characterization of stationary phases

2.3.1. Percent carbon

The percent carbon of the SiO₂(PMOS) phases was obtained through elemental analysis before and after polymer immobilization to evaluate the loading of each stationary phase. These determinations were made with a Model 2400 Perkin-Elmer CHN analyzer.

2.3.2. Gel permeation chromatography of the PMOS extracted from the stationary phases

The different PMOS samples extracted from the stationary phases were analyzed by GPC to determine if there was degradation or crosslinking of the chains during the different immobilization processes, assuming the changes in the GPC curves represent changes in the extracted PMOS after the immobilization process. The GPC was performed in a Waters chromatograph equipped with a model 510 pump, model 410 refraction index detector, model U6K injector and Millennium software for data acquisition. The conditions of the analyses were: coupled Ultrastyrigel 50 and 100 nm (Waters) columns; an injection volume of 150 µl; with both columns and detector at 40 °C. Toluene was used as the mobile phase at a flow-rate of 1.0 ml min⁻¹, while the injected solutions were prepared at concentrations of 0.5% (v/v) in toluene.

2.3.3. Thermogravimetric analysis

The thermal stability of the immobilized and non-immobilized stationary phases was studied using samples of approximately 10 mg, with a heating rate of 10 °C min⁻¹ in an air atmosphere, with a TA model TGA-2050 instrument.

2.3.4. Infrared spectroscopy

The IR spectra were obtained using a Perkin-Elmer model FT-IR 1600 to evaluate the presence of residual silanols and possible alterations caused by the different immobilization procedures.

2.3.5. Nuclear magnetic resonance

The stationary phases were analyzed by solid-state CP-MAS-NMR (¹³C and ²⁹Si) to evaluate the possible interactions between the support and PMOS and the chemical alterations caused by the different immobilization processes.

The ¹³C CP-MAS-NMR spectra were obtained using a Bruker DSX 200 instrument. MAS was carried out with 4 mm double bearing ZrO₂ rotors and spinning rates of 10 000 Hz. The proton 90° pulse length was 7 µs, the contact time and delay time were 3 ms and 3 s, respectively. The line broadening used was 10 Hz.

The ²⁹Si CP-MAS-NMR spectra were collected on a Bruker ASX 300 NMR spectrometer. Representative samples of 200–250 mg were spun at 3500 Hz using 7 mm double bearing ZrO₂ rotors. The spectra were obtained with a cross-polarization contact time of 5 ms. The pulse interval time was 1 s. Typically, 1.5 k FIDs with an acquisition time of 30 ms were accumulated in 1 kilobyte data points and zero-filling to 8 kilobytes prior to Fourier transformation. The line broadening used was 30 Hz and the spectral width for all spectra was about 20 kHz.

2.3.6. Scanning electron microscopy

A morphological study of the immobilized stationary phases was made through micrographs of the particles with ×1500 magnification. The samples were sputter-coated with gold and then examined by a JEOL model JSM-T300 scanning electron microscope at 20 kV.

3. Results and discussion

3.1. Percent carbon, loading and specific mass

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

$$\text{loading} = (m_{\text{PMOS}}/m_{\text{SP}}) \cdot 100$$

where m_{SP} is mass of the stationary phase = $m_{\text{SiO}_2} + m_{\text{PMOS}}$; m_{PMOS} is mass of the PMOS = $(\%C_{\text{SP}} \cdot m_{\text{SP}})/62$; 62 is constant, considering that 62% of the total

Table 1
Percent carbon, percent loading and specific masses of the non-immobilized and immobilized–extracted stationary phases

	Stationary phase					
	Not immobilized	Heated at:		Irradiated with:		Self-immobilized
		220 °C	120 °C	Microwaves	γ Radiation	
Percent carbon	33.7	21.0	14.5	9.5	12.4	5.8
Loading (%)	54	34	23	15	20	9
Specific mass	1.19	0.51	0.30	0.18	0.25	0.10

mass of PMOS is carbon; $\%C_{SP}$ is measured percent carbon of the stationary phase, while the specific mass of polymer on the silica is obtained from:

$$\bar{m} = \text{specific mass} = \%C_{SP} / (62 - \%C_{SP})$$

The non-immobilized stationary phase presented a high loading because this phase was not submitted to an extraction process to remove excess PMOS. Of the immobilized phases, the thermally treated ones presented the higher loadings. The phases irradiated with microwaves and γ radiation presented intermediate loadings and the self-immobilized phase presented the lowest loading. Thus, the different processes of polymer immobilization promoted stationary phases with varied loadings. The most effective process of immobilization was heating for 220 °C for 4 h, from which 63% of the initial polymer was retained, while the self-immobilized stationary phase retained just 17% of the initial polymer.

3.2. Gel permeation chromatography of the PMOS extracted from the stationary phases

The polymers extracted from the immobilized stationary phases were submitted to analysis by GPC and the chromatograms obtained are shown in Fig. 1. All the polymers extracted after the immobilization processes presented the same profile as pure PMOS (without treatment), indicating they did not undergo structural alterations, except for the polymer extracted from the stationary phase heated at 220 °C for 4 h. In this case, significant degradation of the polymer is suggested since a peak of smaller molar

mass is present at 17 min that does not appear in the other chromatograms. This suggests that the only immobilization process that promotes significant breaking of the PMOS chains is the thermal treatment at 220 °C for 4 h.

3.3. Thermogravimetric analysis

The thermograms of bare silica and immobilized and non-immobilized stationary phases are shown in Fig. 2. The curve for bare silica (Fig. 2a) indicates only the loss of adsorbed water in the temperature

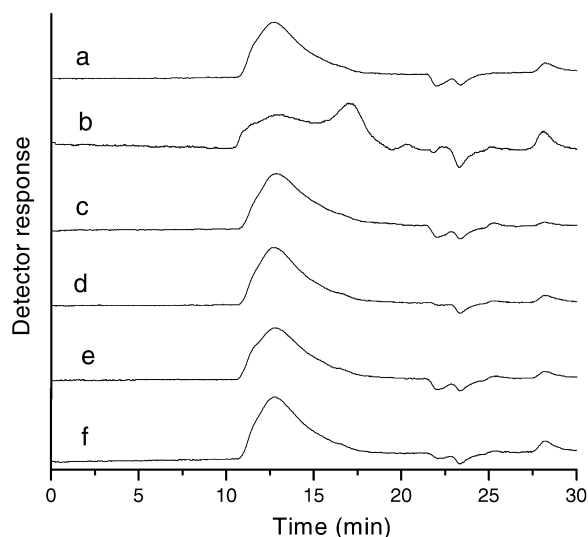


Fig. 1. GPC chromatograms of: (a) pure PMOS; and PMOS extracted from SiO₂(PMOS) stationary phases immobilized by: (b) heat treatment at 220 °C for 4 h; (c) heat treatment at 120 °C for 4 h, (d) microwave irradiation; (e) γ irradiation; and (f) self-immobilization.

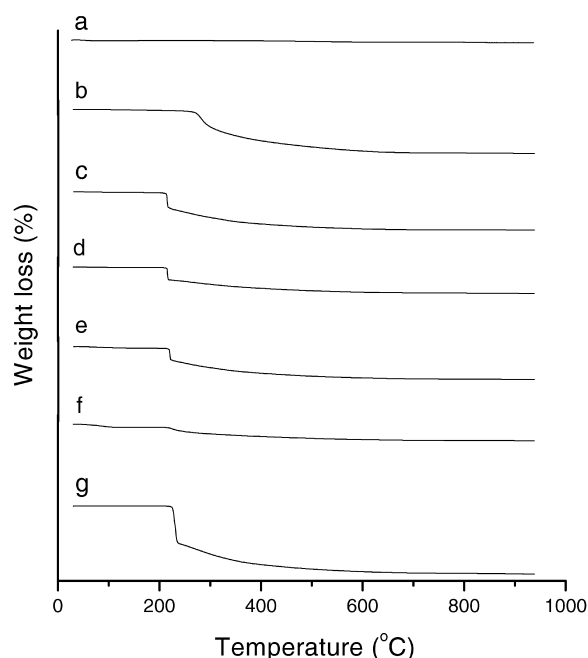


Fig. 2. Thermogravimetric curves of: (a) bare silica; and non-immobilized and immobilized–extracted SiO_2 (PMOS) stationary phases: (b) heat treatment at 220 °C for 4 h; (c) heat treatment at 120 °C for 4 h; (d) microwave irradiation; (e) γ irradiation; (f) self-immobilization; and (g) not immobilized.

range studied. For the stationary phase treated at 220 °C (Fig. 2b), a loss of mass starts near 280 °C, since more volatile compounds were already removed during the thermal treatment. In this case, the resulting PMOS was thermally more stable due to significant structural modification, as also shown by GPC and NMR. The other curves (Fig. 2c–g) show loss of mass near 215 °C, related to breakage of Si–O bonds, with the consequent formation of the volatile cyclic polysiloxanes of low molar mass,

and/or the breakage of the bonds along the aliphatic chains of the polymer and the formation of volatile gaseous products [33,34].

The loss of mass for all stationary phases in the temperature range studied was proportional to the amount of immobilized polymer in the samples (Table 2). Thus, the non-immobilized sample (Fig. 2g) presented the largest loss and the self-immobilized stationary phase the smallest loss (Fig. 2f).

3.4. Nuclear magnetic resonance and infrared spectroscopy

Fig. 3 shows the ^{13}C CP-MAS-NMR spectra of the immobilized and non-immobilized stationary phases. The methyl group directly attached to the siloxane backbone (C–a) causes a signal at 0 ppm, that was greater in the phase treated at 220 °C (Fig. 3a), showing that there was increase in the formation of Si–C bonds with this immobilization process. This stationary phase also showed signals at approximately 180 ppm and between 60 and 80 ppm, probably related with the formation of bonds of the type O=C–OH, CH–OH and CH_2 –OH during the thermal treatment.

The other ^{13}C spectra do not present evidence of the formation of new bonds in the stationary phases. For all stationary phases the carbon atoms of the C_8 chain (C_1 to C_8) show resonances in the range from 15 to 40 ppm.

The formation of the C=O bond in the stationary phase thermally treated at 220 °C was confirmed by the intense band in the infrared spectrum near 1700 cm^{-1} , which does not appear in the spectra of any of the other samples (Fig. 4). The band characteristic of silanols (shoulder at 975 cm^{-1}) was more intense in the spectrum of the self-immobilized sample (Fig.

Table 2

Percentage loss of mass during thermogravimetric analysis from 25 to 935 °C of non-immobilized and immobilized–extracted stationary phases

Loss of mass (%) (25–935 °C)	Stationary phase					
	Not immobilized	Heated at:		Irradiated with:		Self-immobilized
		220 °C	120 °C	Microwaves	γ Radiation	
	36	23	21	14	18	9

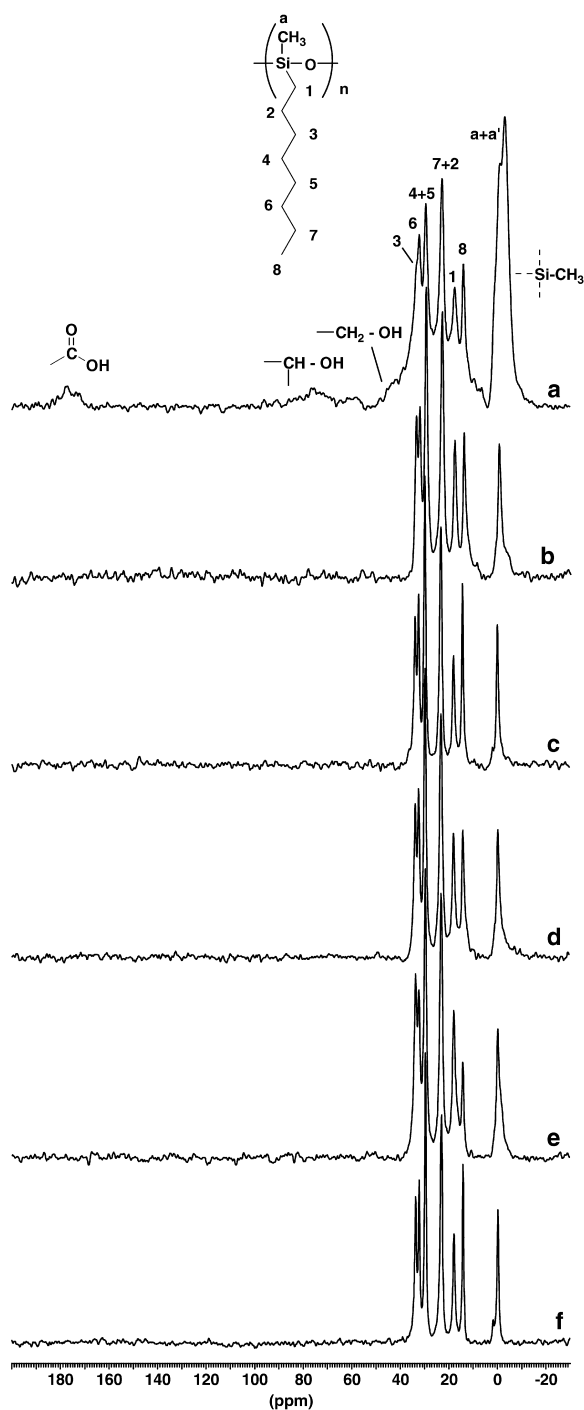


Fig. 3. ^{13}C CP-MAS-NMR spectra of non-immobilized and immobilized-extracted $\text{SiO}_2(\text{PMOS})$ stationary phases: (a) heat treatment at 220°C for 4 h; (b) heat treatment at 120°C for 4 h; (c) microwave irradiation; (d) γ irradiation; (e) self-immobilization; and (f) not immobilized.

4e) while the thermally treated samples show little or no absorption related to silanol (Fig. 4a,b). This shows that the amount of silanols in the stationary phases decreases with the increase of the loading promoted by the different immobilization processes.

The ^{29}Si CP-MAS-NMR spectra of the immobilized and non-immobilized stationary phases are shown in Fig. 5. All stationary phases presented signals at -22 ppm related to resonances of the polysiloxane backbone ($\text{D}^{2''}$). In the immobilized stationary phases, signals of D^1 , D_H^1 , $\text{D}^{2'}$ and M groups can be detected, except for the self-immobilized phase (Fig. 5e). The signals D^1 and D_H^1 may be explained as due the breaking of some $\text{Si}-\text{O}-\text{Si}$ bonds of the siloxane chain by the immobilization processes and the signal in the range of -20 ppm ($\text{D}^{2'}$ groups) can be attributed to the formation of short loops [35]. Some of the PMOS chains are loosely attached or physically adsorbed ($\text{D}^{2''}$ groups) while other are chemically bonded ($\text{D}^{2'}$ groups). The absence of these signals in the self-immobilized phase shows that this process is not efficient for the immobilization of PMOS on Kromasil silica, although such signals have been observed in stationary phases produced by adsorption of PMOS on Davisil silica [32]. This may be related to the presence of a higher number of reactive sites on the irregular Davisil silica, which presents higher amounts of trace metals and a much higher acidity, as compared to high purity, low acidity Kromasil silica.

The signals of T^3 groups occurring at -65 ppm and T^2 groups at -55 ppm in the heated samples (Fig. 5a,b) increase significantly with the increase of the thermal treatment temperature. These signals appear as a function of the decrease of the Q^2 and Q^3 signals (between -80 and -100 ppm), which correspond to the superficial silanol groups of the silica that are eliminated during the formation of chemical bonds between two silanol groups and between PMOS and the surface of the support. The Q^4 signal was not altered because it is related to the siloxane bonds of the bulk silica structure. Therefore, it can be concluded that a reaction between the polysiloxane backbone and the support material or a reaction within the polysiloxane chain has taken place as a result of the immobilization processes. A schematic model that suggests what might happen in the immobilization processes is shown in Fig. 6.

Summarizing; the thermal treatment at 220°C for

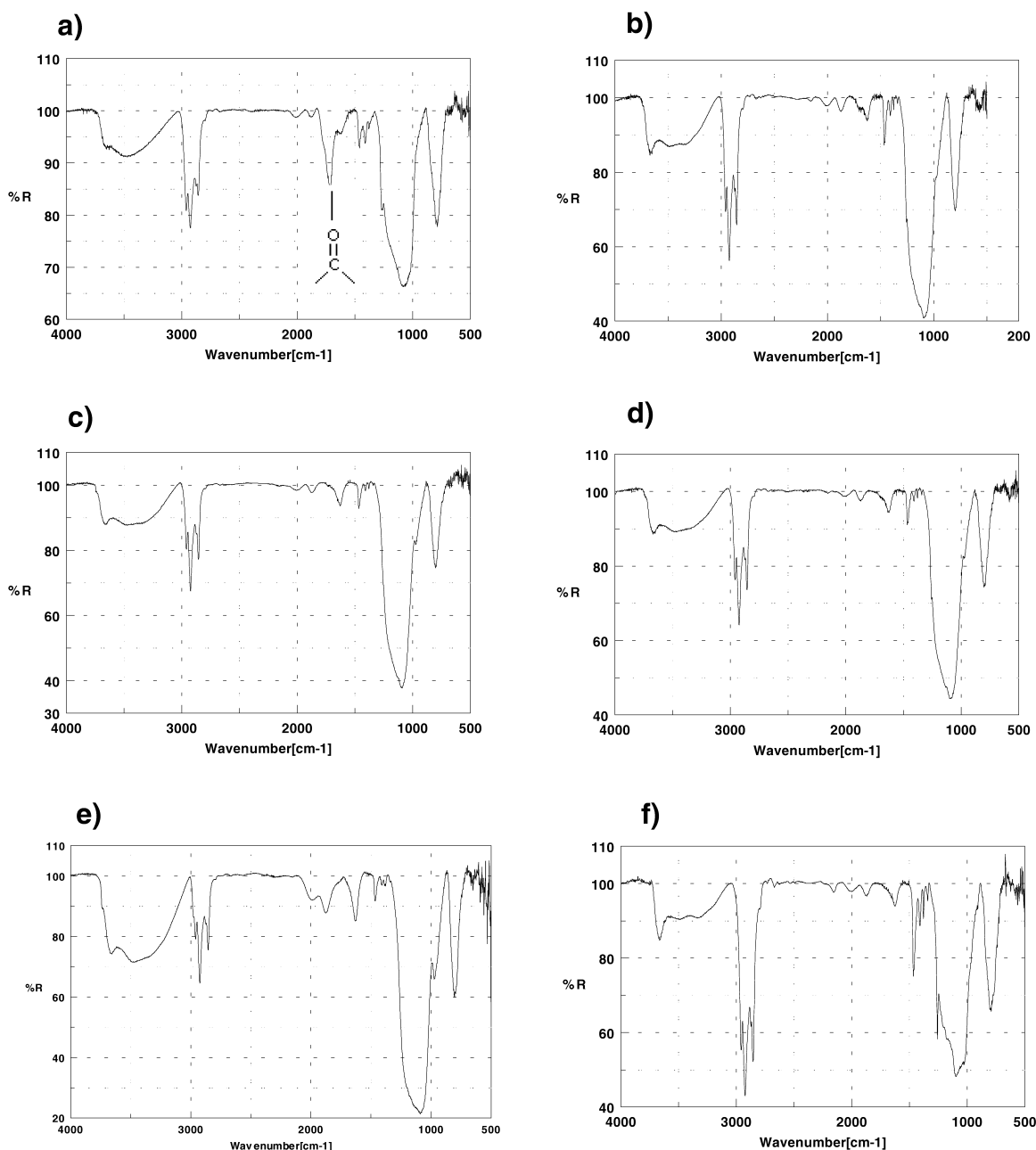


Fig. 4. IR spectra of the non-immobilized and immobilized-extracted SiO_2 (PMOS) stationary phases: (a) heat treatment at 220°C for 4 h; (b) heat treatment at 120°C for 4 h; (c) microwave irradiation; (d) γ irradiation; (e) self-immobilization; and (f) not immobilized.

4 h is the most efficient process for promoting bond formation (immobilization) of PMOS onto Kromasil silica and self-immobilization is the least effective process. This observation confirms the results seen in the percent carbon determinations (Table 1).

3.5. Scanning electron microscopy

The micrographs of the immobilized stationary phases are shown in Fig. 7. The stationary phase treated at 220°C (Fig. 7a) presented an agglomera-

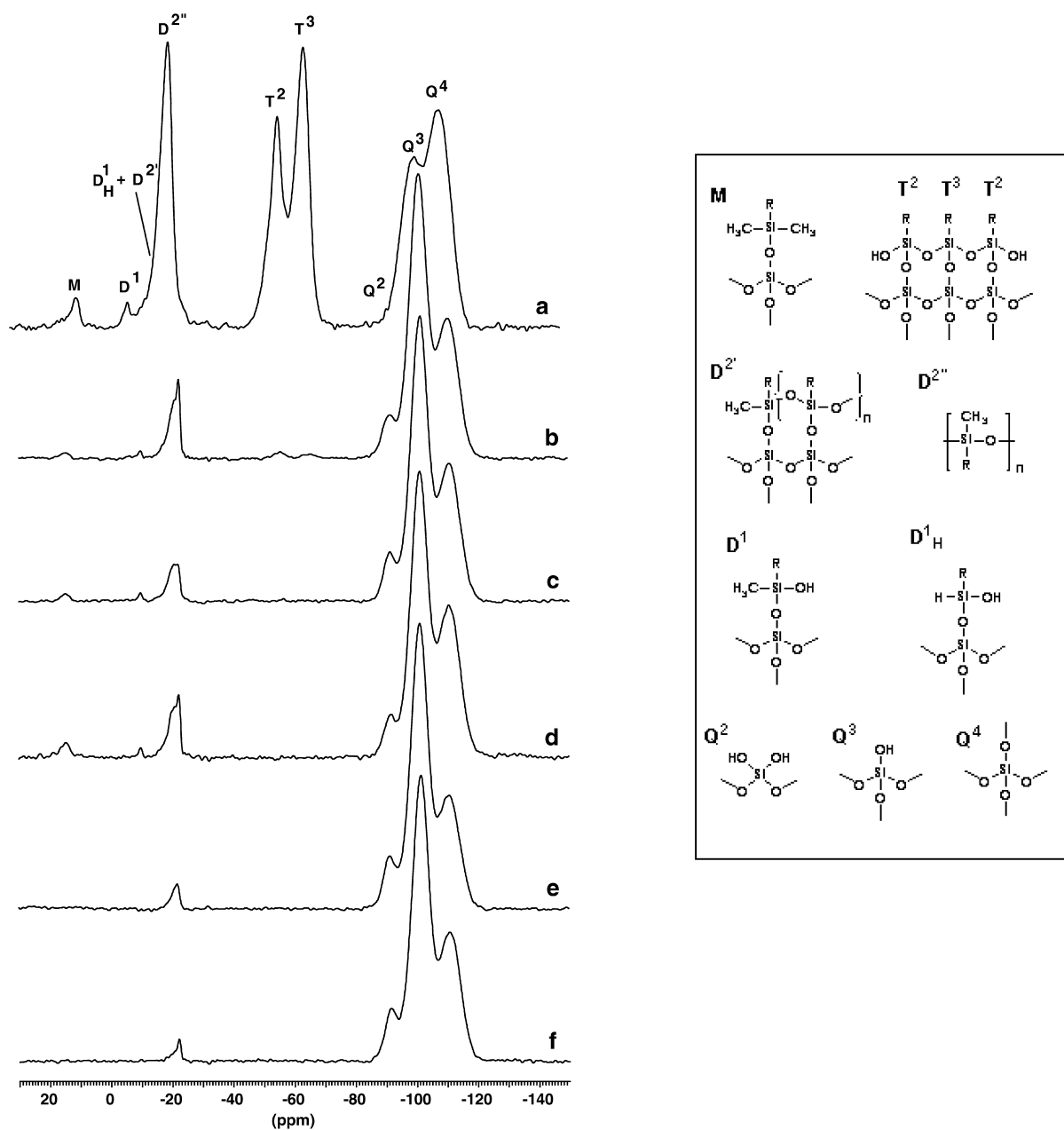


Fig. 5. ^{29}Si CP-MAS-NMR spectra of non-immobilized and immobilized extracted $\text{SiO}_2(\text{PMOS})$ stationary phases: (a) heat treatment at 220°C for 4 h; (b) heat treatment at 120°C for 4 h; (c) microwave irradiation; (d) γ irradiation; (e) self-immobilization; and (f) not immobilized.

tion of particles, due to great amount of polymer immobilized in this phase, even after being submitted to extraction of excess PMOS. The presence

of this polymer external to the silica particles suggests that this stationary phase will probably not present good results in chromatography. The other

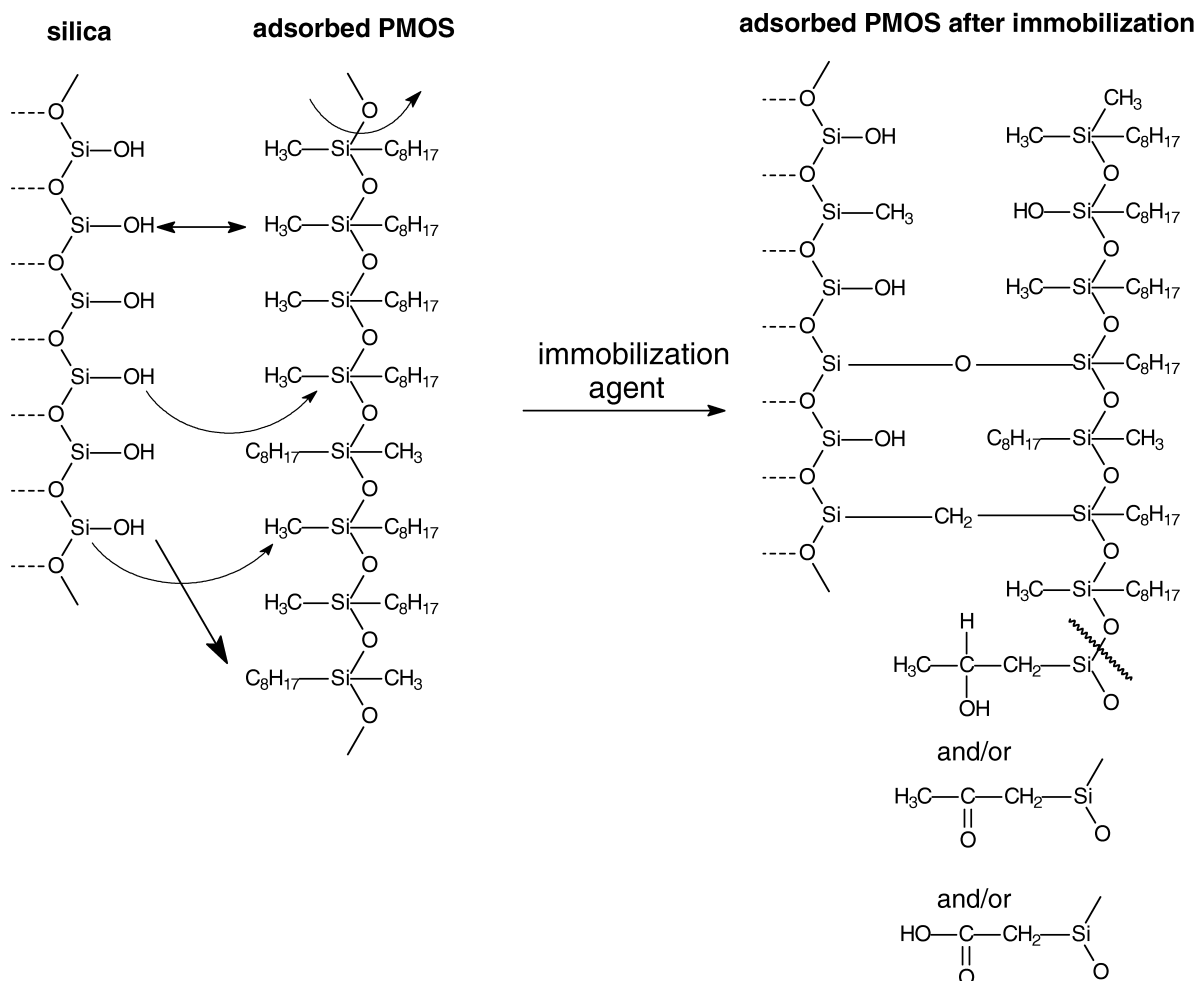


Fig. 6. Schematic model for the immobilization processes.

stationary phases (Fig. 7b–e) show separated, spherical particles, indicating that extraction with hexane was efficient in removing excess superficial PMOS from these phases.

4. Conclusions

The basis for using different immobilization processes is related to their properties and the possible interactions with the polymer and the chromatographic support which they may induce. Gamma

radiation is a high energy electromagnetic radiation that can produce ionization along with the formation of free radicals. Microwave radiation is a type of non-ionizing energy that causes molecular movement by phenomena such as ionic migration and dipolar rotation. Material exposed to microwave radiation undergoes a very fast heating, which can produce free radicals. Thermal treatment normally involves lower energy interactions without production of ions or free radicals. Self-immobilization at ambient temperature [32] may involve interactions of even lower energy. Thus, immobilization does not necessarily involve high energy reactions (ionization and

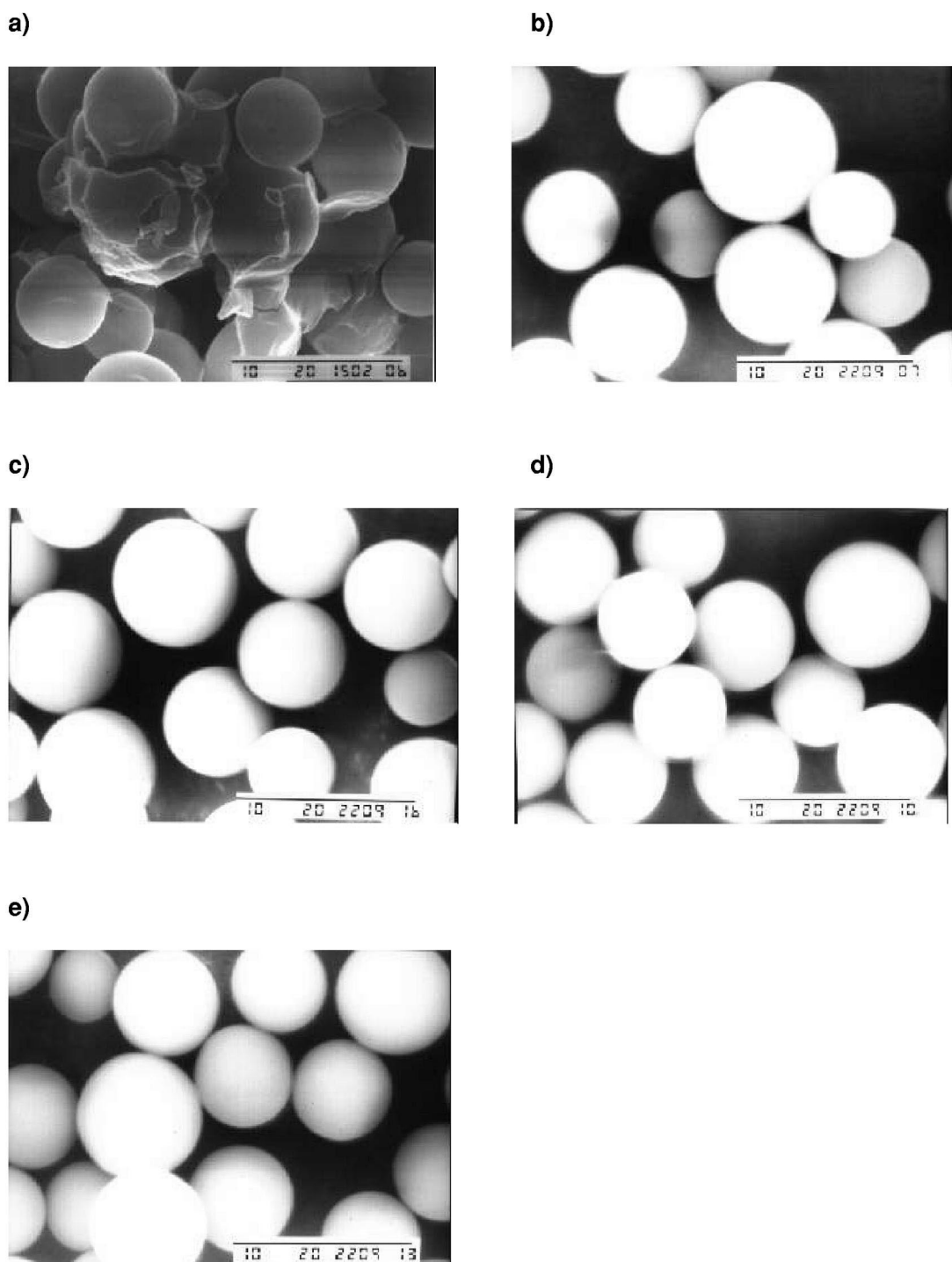


Fig. 7. Micrographs of non-immobilized and immobilized–extracted SiO_2 (PMOS) stationary phases: (a) heat treatment at 220 °C for 4 h; (b) heat treatment at 120 °C for 4 h, (c) microwave irradiation; (d) γ irradiation; and (e) self-immobilization.

free radicals), slow immobilization at low temperatures apparently can give similar results.

The percent carbon determinations show that the thermally treated stationary phases retained the largest quantity of polymer. The phases irradiated with microwaves or γ radiation had intermediate loadings and the self-immobilized phase presented the lowest loading. The stationary phase treated at 220 °C is more thermally stable than the other immobilized phases because the PMOS chains were chemically altered during the immobilization process, as confirmed by GPC and NMR spectra. The ^{29}Si CP-MAS-NMR spectra of the immobilized samples showed signals characteristic of bonded polymer, except for the self-immobilized sample, which did not present these signals. The large amount of immobilized PMOS produced by the treatment at 220 °C promoted agglomeration of the particles and this phase possibly will not present good performance as a packing material. All the other phases show separate spherical particles in SEM, suggesting that they may show good chromatographic performances.

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