

Chromatographic Comparison of Self-Immobilized and Radiation-Immobilized Poly(methyloctylsiloxane) Stationary Phases on Various Silicas

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Dois tipos de fase estacionária, auto-imobilizada e immobilizada por radiação, foram preparadas com poli(metiloctilsiloxano) sobre oito suportes de sílica com tamanho e forma de partículas, e tamanho de poros diferentes. As colunas recheadas com as fases estacionárias preparadas através dos dois procedimentos de imobilização apresentaram eficiências, resoluções e fatores de separação similares, mas as colunas recheadas com a fase estacionária auto-imobilizada tiveram percentagens de carga menores e, portanto, fatores de retenção menores. Os resultados evidenciam diferenças estruturais entre as fases.

Self-immobilized and radiation-immobilized stationary phases were prepared with poly(methyloctylsiloxane) on eight silica supports having different particle sizes, particle shapes and pore sizes. Columns prepared by the two immobilization procedures had similar efficiencies, resolutions and separation factors but columns with self-immobilized stationary phases had lower percent loadings and, thus, lower retention factors. Results show structural differences between the phases.

Keywords: silica, radiation immobilization, stationary phase, poly(methyloctylsiloxane)

Introduction

Silica-based reversed-phase high-performance liquid chromatography (RP-HPLC) has become the method of choice for most liquid chromatographic separations. Much of this popularity can be attributed to the silica itself because of the wide variety of available pore diameters, the uniformity of the pores, the high specific surface areas, the high mechanical strength and chemical reactivity for easy functionalization by reactions with the surface silanol groups.^{1,2} This reactivity, although the key to the success of silica supports, is also a source of its limitations. Silanol groups that are not removed or covered lead to irreversible adsorption of basic solutes and strong peak asymmetry.³

Organic polymers have been sorbed onto, or produced on, the surfaces of HPLC silica to obtain more complete coverage, hence more efficient shielding of residual silanol groups.⁴ The LabCrom group (UNICAMP) has been developing such stationary phases by coating poly(methyloctylsiloxane) (PMOS) onto chromatographic silica particles,⁵⁻⁷ then immobilizing these phase by γ -irradiation⁸⁻¹¹ and by self-immobilization at ambiente temperature.¹²

In the present work we directly compare the physical and chromatographic properties of PMOS phases that have been immobilized by γ -irradiation or by self-immobilization upon the surfaces of various HPLC-silica supports.

Experimental

Reagents and materials

Analytical-reagent grade or HPLC-grade solvents were obtained from Merck (Rio de Janeiro, RJ, Brazil) (methanol, dichloromethane, carbon tetrachloride, acetone, benzonitrile, benzene, toluene and naphthalene) and not further purified. Water was distilled and then purified through a Milli-Q system from Millipore.

Poly(methyloctylsiloxane) (PMOS) polymer (average molar mass of 6 200, viscosity 600 – 1000 cSt, 25 °C) was obtained from Hüls America (Piscataway, NJ, USA).

The silicas tested as chromatographic supports were Davisil, 10 μ m, irregular (Alltech Associates, USA), Sigma, 10 μ m, irregular (Sigma, USA), Lichrosorb Si-100, 10 μ m, irregular and Lichrosorb Si-60, 10, 7 and 5 μ m, irregular (Merck, Germany) and Spherisorb, 8 and 5 μ m, spherical (Phase Separations, UK).

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[#] This paper is dedicated to professors Kenneth and Carol Collins

Preparation of stationary phase and immobilization by gamma irradiation

A known quantity of silica (dried at 150 °C for 24 h) was added to a solution of PMOS in dichloromethane to prepare a PMOS-loaded material, SiO₂(PMOS), having an initial loading of 40% or 50% PMOS. This mixture was slowly agitated at room temperature for three hours and then the solvent was allowed to evaporate, without stirring, at room temperature in the fume hood.

Samples of 5.0 g of prepared stationary phase were sealed in glass ampoules under air. The sealed samples were irradiated to 80 or 120 kGy (irregular silicas)⁸ or 20 kGy (spherical silicas) of absorbed dose, using an industrial Cobalt-60 source (IBRAS-CBO, Campinas, SP, Brazil).

Physical and chemical characterization

The elemental analyses of the immobilized stationary phases were obtained with a Model CHN-2400 Perkin-Elmer analyzer.

Specific surface areas of the various packings were determined by the conventional BET method¹³ using a Model 2300 Micromeritics Flow Sorb II instrument.

Infrared spectroscopy was done with a Perkin-Elmer Model 1600 FT-IR spectrophotometer and thermogravimetric analysis with a TA Instruments 2050 TGA.

Solvent extractions were done with a series of three independent extractions (with methanol, benzene and dichloromethane) of six hours each, on each sample, in a Soxhlet extractor using a modification of the method of Sanchez *et al.*¹⁴ After each extraction the solvent was evaporated from the sample and the remaining mass was determined before initiating the next extraction.

Column packing

Columns (125 mm x 3.4 mm i.d.) were made locally from type 316 stainless tubing whose inner surface was highly polished in our laboratory.¹⁵ The columns were slurry packed using 10% (irregular) or 20% (spherical) slurries (m/v) of the stationary phase in carbon tetrachloride. A packing pressure of 38 MPa (Haskel Packing Pump) was used, with methanol as propulsion solvent. Columns were conditioned for four hours with mobile phase (methanol:water, 70:30, v/v) at 0.2 mL min⁻¹ prior to testing.

Chromatographic evaluation

Chromatographic evaluations were performed with a

modular HPLC instrument, equipped with a Waters Model 510 pump, SSI Model 3XL pneumatic injector with a 10 μL loop, a Waters Model 481 spectrophotometric detector (14 μL cell volume) and a Waters Model 740 integrator.

All measurements were carried out at ambient temperature using methanol-water mobile phase at a flow-rate of 0.2 mL min⁻¹, near the optimal flow-rate as determined by a van Deemter plot. The column dead time, t_M , was determined using methanol as an unretained compound.

Two test mixtures were used in this study: (I) acetone, benzonitrile, benzene, toluene and naphthalene and (II) aniline, *o*-, *m*-, *p*-toluidine, and *N,N*-dimethylaniline. In mixture I, a mobile phase of MeOH:H₂O 70:30 (v/v) was used while for mixture II, the mobile phase was MeOH:H₂O 55:45 (v/v). Injections were of 10 μL with UV detection at 254 nm.

Chromatographic performance was evaluated by means of the efficiency (plates/m, N/L), retention factor (*k*), resolution (*R_s*), separation factor (*α*) and asymmetry factor (*A_s*), manually determined from the chromatograms. The asymmetry factor was calculated at 10% of the peak height.¹⁶

Results and Discussion

The infrared spectra of some self-immobilized and γ -immobilized stationary phases (Figure 1) show that the intensity of the signals characteristic of PMOS (2900, 1466 and 1258 cm⁻¹) present a slight increase in intensity in the immobilized stationary phases due to the larger amount of PMOS in the silicas, as a result of cross-linking of the PMOS – as is also indicated by the results of solvent extraction and % carbon.

Table 1 lists physical data (particle shape, particle size, pore size, specific volume and specific surface area) of each of the silicas prior to PMOS loading, the % of PMOS contained in test-column after packings (vertical columns 7 and 8), the % PMOS contained in samples of PMOS loaded silicas following exhaustive extraction by methanol, benzene and dichloromethane (columns 9 and 10). The data for columns 7 to 10 indicate the quantities of immobilized PMOS present in samples from which non-immobilized PMOS has been extracted – either by the packing and testing procedure (columns 7 and 8) or by the 3-solvent extraction procedure (columns 9 and 10). From these data we see the similarities in amounts of immobilized PMOS in the various irregular silicas. As expected, we also see larger amounts of PMOS are immobilized by γ -irradiation⁸ than by self-immobilization.⁷ Columns 11 and

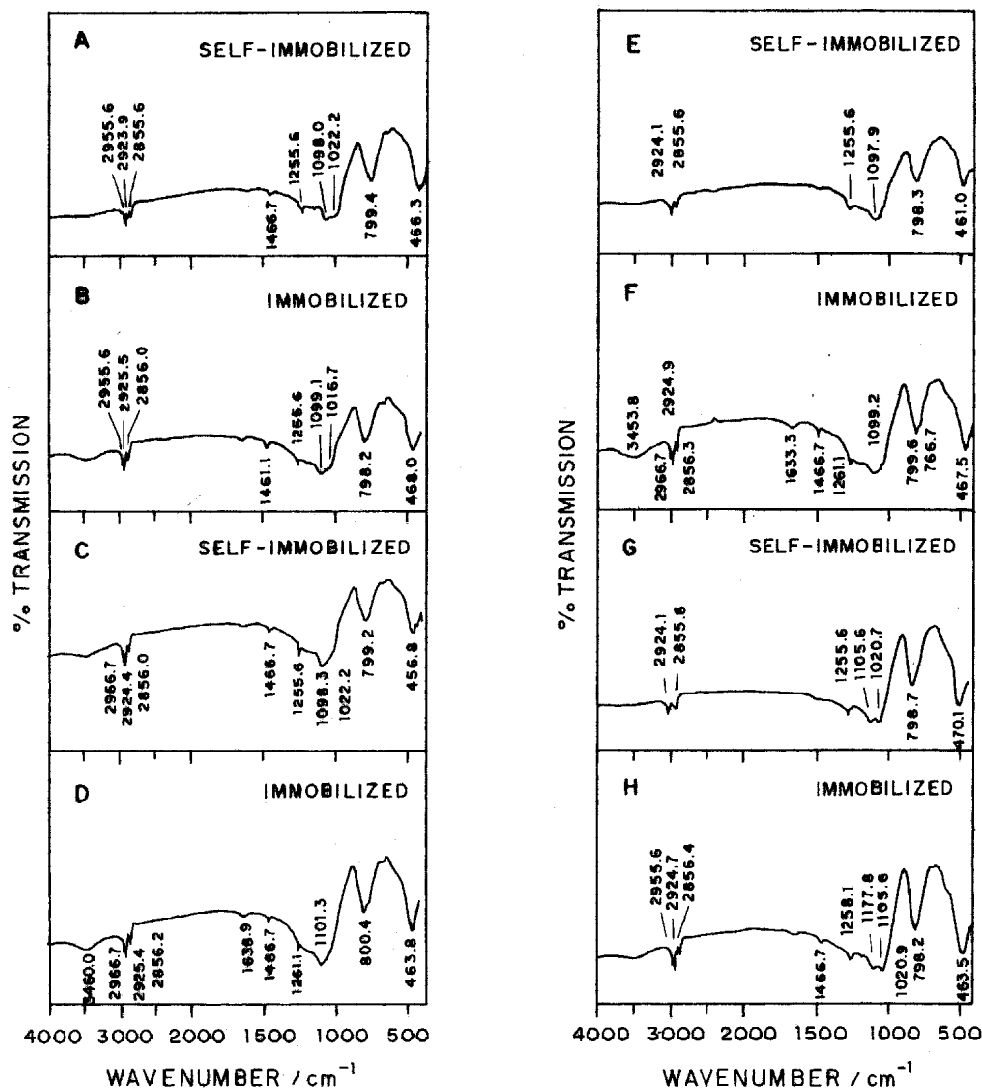


Figure 1. Infrared spectra of self-immobilized and γ -immobilized stationary phases for (A and B) 50% PMOS-Sigma, (C and D) 50% PMOS-Lichrosorb Si-100, (E and F) 50% PMOS-Lichrosorb Si-60, 7 μm , (G and H) 40% PMOS-Spherisorb, 8 μm , respectively.

Table 1. Physical and chemical characteristics of self-immobilized and γ -immobilized stationary phases

1 Stationary phases (% PMOS in initial loading)	2 Particle shape	3 Silica particle size (μm)	4 Silica pore size (nm)	5 Silica specific volume (mL g^{-1})	6 Silica specific surface area ($\text{m}^2 \text{g}^{-1}$)	% PMOS after packing ^a		% PMOS after extractions ^b		% PMOS ratio (self-immob./g-immob.)	
						7 self-immob.	8 γ -immob.	9 self-immob.	10 γ -immob.	11 after P ^c	12 after X ^d
40% PMOS-Davisil	irregular	10	15	1.6	237	18.6	26.3	18.5	25.1	0.71	0.74
50% PMOS-Sigma	irregular	10	6	1.1	393	28.5	35.0	24.4	29.2	0.81	0.83
50% PMOS-Si-100	irregular	10	10	1.25	290	27.3	39.5	26.9	34.1	0.69	0.79
50% PMOS-Si-60	irregular	10	6	0.9	267	-	35.5	19.9	28.5	-	0.70
50% PMOS-Si-60	irregular	7	6	0.9	305	29.0	34.8	20.2	29.4	0.83	0.69
50% PMOS-Si-60	irregular	5	6	0.9	402	30.3	32.9	23.4	26.9	0.92	0.87
40% PMOS-Spher.	spherical	8	8	0.45	149	24.0	24.3	17.0	18.0	0.99	0.94
40% PMOS-Spher.	spherical	5	8	0.45	186	21.8	-	17.9	18.8	-	0.95

^a % PMOS obtained from the % C of used packing material by dividing the % C by the carbon fraction (0.62) of PMOS; ^b % PMOS obtained from the % C of non-used (non-packed) packing material following a series of extractions (methanol, benzene, dichloromethane); ^c after P = after packing; ^d after X = after extractions.

12 of Table 1 give estimates of the relative quantities of PMOS immobilized by the two immobilization procedures.

Thermogravimetric analyses (Figure 2) show good thermal stability of self-immobilized and γ -immobilized stationary phases in the temperature range used for HPLC,

with decomposition of the stationary phase only starting at about 400 °C for all phases except self-immobilization 50% PMOS-Lichrosorb Si-60, 10 μm (~ 370 °C) and γ -immobilized 40% PMOS-Davisil (~ 290 °C).

Table 2 lists the efficiencies (N/L) obtained from

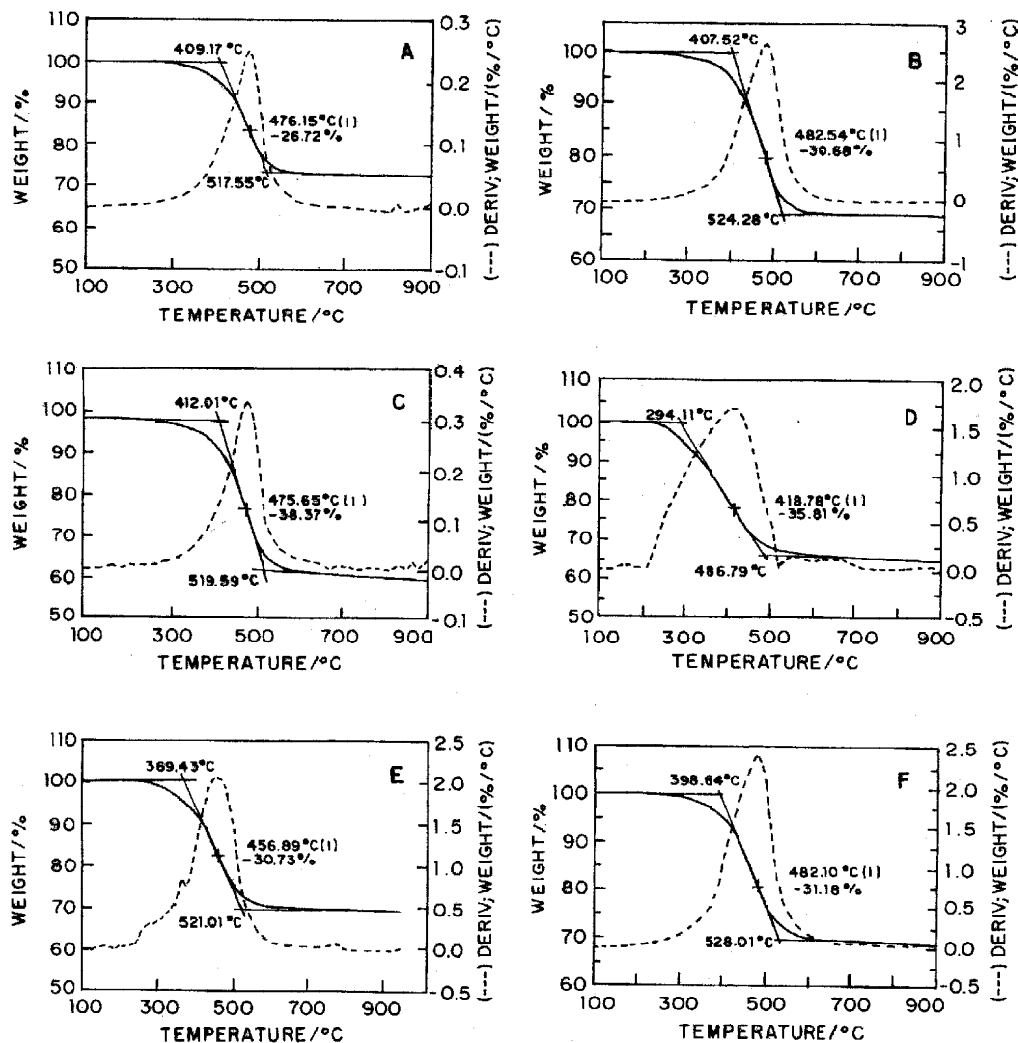


Figure 2. Thermograms of self-immobilized and γ -immobilized stationary phases for (A and B) 40% PMOS-Davisil, (C and D) 50% PMOS-Lichrosorb Si-60, 10 μm , (E and F) 40% PMOS-Spherisorb, 5 μm , respectively.

Table 2. Comparisons of column efficiencies^a

Stationary phase	d_p (μm)	N/L		$(N/L)_5^b$	
		Self-immob.	γ -immob.	Self-immob.	γ -immob.
Davisil	10	24 000	26 400	62 400	68 640
Sigma	10	34 400	36 400	68 800	72 800
Si-100	10	33 600	36 400	67 200	72 800
Si-60	10	35 200	43 600	70 400	87 200
Si-60	7	41 200	37 600	57 700	52 640
Si-60	5	50 400	66 400	50 400	66 400
Spherisorb	8	58 400	39 600	93 440	63 400
Spherisorb	5	74 400	70 400	74 400	70 400
			mean	68 090	69 280
				$68\ 685 \pm 595$	

^a efficiencies were obtained from the naphthalene peak of test mixture I; ^b $(N/L)_5$ = plates per meter normalized to 5 μm particle size by the factor $d_p/5$. For example: for the case of 10 μm particles, $(N/L)_5 = (N/L)_{10} \times 10/5$.

columns packed with the stationary phases. The efficiencies normalized to $5\ \mu\text{m}$ particles, $(N/L)_s$, show that column efficiencies are, on the average, no better for γ -immobilized PMOS phases than for self-immobilized phases. Nevertheless the overall average efficiency of almost 70 000 plates per meter compares well with commercial phases.

Table 3 shows that, for the test solutes of mixture I, the A_s , R_s and α values do not differ greatly between the different silicas or for the two immobilization procedures. The k values, however, show consistent differences for the two immobilization procedures: the γ -immobilized stationary phases, with their greater % PMOS values, have consistently higher k , probably due to the thickness of the PMOS coating, so there is a decrease in the velocity of mass transfer and, consequently, an increase in the retention factor (Figure 3). This seems to imply that the self-immobilized stationary phases, with separation efficiencies similar to those of the γ -immobilized phases, can perform separations faster, which could be a decided advantage.

Table 4 shows the results obtained using test mixture II, part of the mixture proposed by Engelhardt and co-workers^{17,18} as basic probes, with aniline as a weak base, *N,N*-dimethylaniline (*N,N*-DMA) as a strong base, and the isomeric *o*-, *m*- and *p*-toluidines as probes for silanophilic interactions. Methanol:water (55:45, v/v), without addition of buffer or salt solution, was used as the mobile phase. A column can be considered "good" for the analysis of basic compounds, according to the Engelhardt criteria^{17,18} if the isomeric toluidines coelute or have value below 1.3. The findings obtained from self-immobilized stationary phases were discussed in previous work.⁷

Table 4 reveals that, although the separation factors (α) for the toluidines may be quite similar for both the self-immobilized and γ -immobilized phases, the asymmetry factors for *N,N*-DMA tend to be consistently different, thus pointing again to the possibility that the actual interaction surfaces of the stationary phases which are immobilized in different ways have fundamentally different structures, as

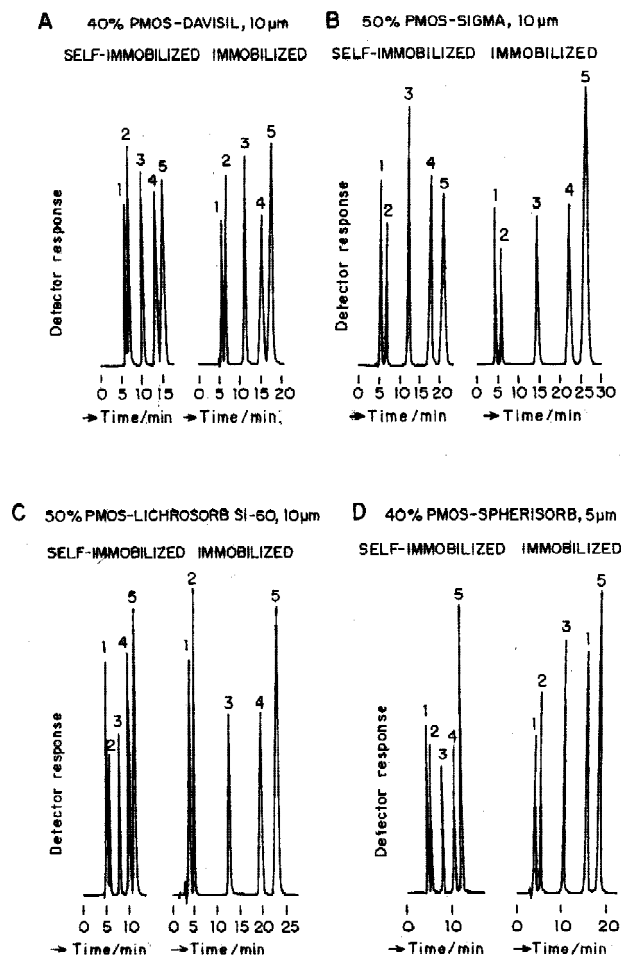


Figure 3. Chromatograms of test mixture I: 1 = acetone, 2 = benzonitrile, 3 = benzene, 4 = toluene and 5 = naphthalene, obtained with columns packed with: (A) 40% PMOS-Davisil self-immobilized and γ -immobilized, (B) 50% PMOS-Sigma self-immobilized and γ -immobilized, (C) 50% PMOS-Lichrosorb Si-60, $10\ \mu\text{m}$, self-immobilized and γ -immobilized, (D) 40% PMOS-Spherisorb, $5\ \mu\text{m}$, self-immobilized and γ -immobilized. Chromatographic conditions: mobile phase: methanol:water (70:30, v/v), flow-rate: $0.2\ \text{mL min}^{-1}$, volume of injected sample: $10\ \mu\text{L}$, detection: UV, 254 nm.

opposed to, for example, simply having somewhat more complete coverage or a thicker layer of PMOS having the same surface structure.

Table 3. Chromatographic parameters obtained using test mixture I

Stationary Phase (% Initial Loading)	d_p (μm)	A_s^a		k^a		R_s^b		α^b	
		Self-immob.	γ -immob.	Self-immob.	γ -immob.	Self-immob.	γ -immob.	Self-immob.	γ -immob.
40% PMOS-Davisil	10	1.4	1.3	1.8	2.5	1.8	1.9	1.2	1.2
50% PMOS-Sigma	10	1.1	1.2	3.6	6.6	2.7	2.7	1.2	1.2
50% PMOS-Si-100	10	1.0	1.1	3.2	6.3	2.4	2.6	1.2	1.2
50% PMOS-Si-60	10	1.2	1.2	1.5	6.2	2.0	3.0	1.2	1.2
50% PMOS-Si-60	7	1.3	0.9	3.1	6.4	2.7	2.7	1.2	1.2
50% PMOS-Si-60	5	1.2	0.9	4.8	6.4	3.2	3.6	1.2	1.2
40% PMOS-Spher.	8	1.0	0.7	4.1	6.5	3.4	3.0	1.2	1.2
40% PMOS-Spher.	5	1.3	0.9	2.1	4.7	3.3	4.0	1.2	1.2

^a calculated for the naphthalene peak; ^b calculated for the toluene-naphthalene pair.

Table 4. Chromatographic parameters obtained using test mixture II

Stationary Phase (% Initial Loading)	dp (μm)	Chromatogr. parameter	Test solute							
			Aniline		N,N-DMA		p-/m-t		m-/o-t	
			S-I	γ -I	S-I	γ -I	S-I	γ -I	S-I	γ -I
40% PMOS-Davisil	10	As	2.5	2.2	3.3	1.9				
		α					1.3	1.4	1.0	1.0
50% PMOS-Sigma	10	As	1.3	a	1.2	a				
		α					1.2	a	1.0	a
50% PMOS-Lich.Si-100	10	As	1.8		1.9	1.1				
		α					1.2	1.1	1.0	2.1
50% PMOS-Lich.Si-60	10	As	1.5	b	1.3	b				
		α					1.0	b	1.0	b
50% PMOS-Lich.Si-60	7	As	1.6		1.4	3.6				
		α					1.2	1.1	1.5	1.4
50% PMOS-Lich.Si-60	5	As	2.2	2.3	2.1	1.4				
		α					1.0	1.0	1.4	1.5

p-/m-t = p-/m-toluidine pair; m-/o-t = m-/o-toluidine pair; a = result not reproducible; b = problems of absorption of the compounds ; S-I = self-immobilized ; γ -I = γ -immobilized.

Conclusions

Although self-immobilized and γ -immobilized PMOS phases have similar chromatographic properties, the γ -immobilized phases show somewhat higher efficiencies and the self-immobilized phases, having lower carbon contents, can give more rapid separations. Both types of immobilized phase may be used at moderately elevated pH.

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