Poly(alkylmethylsiloxanes) thermally immobilized on silica as stationary phases for high-performance liquid chromatography

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Abstract

Poly(methyloctylsiloxane) (PMOS) and poly(methyloctadecylsiloxane) (PMODS) were sorbed onto porous HPLC silica and thermally immobilized, in the absence of radical initiators, at temperatures in the range of 80 to 180°C. Following extraction of non-immobilized polymer the materials were packed into columns and their chromatographic properties evaluated. The shorter chain (PMOS) stationary phase showed good HPLC characteristics after thermal immobilizations up to 120°C while the longer chain (PMODS) phase gave satisfactory HPLC phases following thermal immobilizations at 80 and 100°C. Stability evaluation for the PMOS and PMODS columns immobilized at 100°C required 250 ml of pH 8.5 mobile phase at 60°C to significantly decrease efficiency, suggesting a long useful life time at neutral pH and ambient temperature. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Most stationary phases in use today are based on C_8 and C_{18} silanes chemically bonded to a silica surface. Bonded phases are in widespread use because of the large number of packing materials available for solving a broad range of different separation problems [1,2]. However, investigations of new packings and optimization of their properties are of continuing interest to chromatographers. Recently, several new stationary phases have been developed with the objective of improving stability and of minimizing undesirable interactions between the solute and the stationary phase. These include sterically protected monomeric phases [3,4], horizon-

tally polymerized phases [5,6], stationary phases with internal functional groups [7] and monolithic phases [8,9].

The coating of silica with a polymer has been investigated in recent years to achieve a more uniform surface coverage [10–13], a subject still being investigated. The preparation of stationary phases for HPLC using polymers, such as polysiloxanes, on silica usually involves an immobilization step involving the cross-linking of free radicals induced by ionizing radiation or by radical initiators (peroxides or azo compounds). Schomburg and others have shown that polysiloxanes immobilized onto silica, by cross-linking induced by peroxides or γ-radiation, provide good reversed-phase packing materials [14–17].

Ohmacht et al. studied the thermal immobilization of poly(methyloctylsiloxane) (PMOS) on HPLC

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silica at high temperatures (200–400°C) in the presence of a catalyst (benzoylperoxide) [18]. The most effective columns contained phases prepared at 400°C. Collins et al. have recently found that thermal immobilization of PMOS on HPLC silica takes place at lower temperatures (80–150°C) in the absence of a cross-linking reagent [19].

In the present work, we have evaluated the physical and chromatographic properties of PMOS (shorter chain) or poly(methyloctadecylsiloxanes) (PMODS) (longer chain) on HPLC silica following thermal immobilization at temperatures from 80 to 180°C.

2. Experimental

2.1. Materials

Filtered methanol (Mallinckrodt, HPLC-grade) and water (Milli-Q) were used to prepare the mobile phases. Dichloromethane, chloroform and hexane (all analytical-reagent grade) were from Mallinckrodt. Most of the compounds used for the chromatography (acetone, acenaphthene, benzene, benzonitrile, phenol, naphthalene, toluene and uracil) were analytical reagent grade and not further purified. *N,N*-dimethylaniline (analytical-reagent grade) was purified by distillation. The silica support used was Davisil-630 (Alltech Associates, USA), having irregular porous 13 μm particles, with an average pore diameter of 8.7 nm and a specific surface area (S_{BET}) of 350 m^2/g. The PMOS and PMODS polymers (average molar masses, M_w, of 14,000 and 28,000, respectively) were obtained from United Chemical Technologies, Inc.

2.2. Preparation of PMOS-loaded silica

The silica was washed with hexane and dried in air at 150°C for 24 h, before being suspended in dichloromethane, followed by sonication for 5 min to displace the air from the pores. An appropriate quantity of a 10% (w/v) solution of PMOS in dichloromethane was then added and the mixture was sonicated for 2 min. The solvent was allowed to evaporate, without stirring, at room temperature. The batch was prepared with a specific mass ($\bar{m}_{PMOS}$) of 1 g$_{PMOS}$/g$_{SiO_2}$.

2.3. Preparation of PMODS-loaded silica

The silica was dried in air at 150°C for 24 h and then an appropriate quantity of a 10% (w/v) solution of PMODS dissolved in hexane was added to give a packing material with $\bar{m}_{PMODS} = 1$ g$_{PMODS}$/g$_{SiO_2}$. The mixture was gently stirred for 3 h at room temperature and the solvent was then allowed to evaporate, without stirring, at room temperature.

2.4. Thermal treatments

Portions of the prepared stationary phases were placed in stainless steel tubes (150×10 mm) fitted with frits and connectors and then heated for 4 h in an oven (±1°C) at 80, 100, 120, 150 or 180°C.

2.5. Extraction procedure

After thermal treatment the tubes containing the phase were connected to a Waters 510 pump for extraction of soluble PMOS or PMODS by passing ~150 ml of hexane at 0.6 ml min^{-1} at 25°C.

2.6. 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 [20]. The columns were slurry packed using 10% slurries (w/v) of the stationary phases in chloroform. A constant packing pressure of 34.5 MPa (Haskel packing pump) 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.

2.7. Chromatographic evaluation

Two chromatographic mixtures were used in this study: (1) acetone, benzonitrile, benzene, toluene and naphthalene and (2) uracil, phenol, *N,N*-dimethylaniline, naphthalene and acenaphthene. HPLC separations were performed in two different systems, the first for columns with PMOS stationary phases and

the second for columns with PMODS stationary phases: (1) a Waters 510 pump, a Rheodyne Model 7125 injection valve (5 μl) and a Waters 486 UV–Vis detector (at 254 nm); (2) a Shimadzu LC-10 AD pump, a Rheodyne Model 7125 injection valve (5 μl loop) and a Shimadzu Model SPD-10 AV UV–Vis detector (at 254 nm). The mobile phase was methanol–water (70:30 v/v) at 0.2 ml min^{-1}, the optimal flow-rate as determined by a van Deemter plot. At this flow-rate the pressure was ~2.0 MPa. The column dead time, t_M, was determined using uracil as an unretained compound. Data collection and treatment for both systems was carried out by Chrom Perfect for Windows, version 3.52, and Report Write Plus (Justice Innovations). Chromatographic 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.

2.8. Stability evaluation

Stability evaluation of the phases was carried out using a mobile phase of methanol–0.1 mol l^{-1} NaHCO$_3$ (pH 8.5) (50:50, v/v) at 0.6 ml min^{-1} with the temperature held at 60°C. Samples from mixture 1 were injected directly into the flowing basic mobile phase at 60-min intervals.

2.9. 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 or PMODS, $\bar{m}_{PMOS}$ and $\bar{m}_{PMODS}$, which represents the mass of polymer per gram of silica,

was calculated using the formulas: $\bar{m}_{PMOS} = \%C/(62 - \%C)$ or $\bar{m}_{PMODS} = \%C/(73 - \%C)$.

With the data of specific mass the polymer layer thickness was calculated:

$$F_{imm} = \frac{\bar{m}_{(imm)}}{\bar{m}_{(fillpores)}} = \frac{(8.7)^2 - (8.7 - 2\tau)^2}{(8.7)^2}$$

We consider that the pores of the silica have a constant diameter (8.7 nm) and that the immobilized polymer is characterized by a layer of constant thickness, τ, on the pore walls [19].

Thermal stability tests of the original and polymer-modified silicas were performed by thermogravimetric analysis (TGA) carried out in an argon (inert) atmosphere using a model 2050 TA Instrument. The temperature range was from 25 to 1000°C at a heating rate of 10°C/min.

3. Results and discussion

Previous studies carried out with PMOS on silica point to the formation of a monolayer stationary phase at temperatures of 80 to 120°C [19]. Thermal immobilization at higher temperatures (150–280°C) produced thicker ("bi-layer") stationary phases. In the present work, a similar behavior was observed for stationary phases with a polysiloxane having a longer chain length (PMODS). Table 1 displays the specific mass of each stationary phase after thermal treatment followed by extraction with hexane. The specific pore volume, v_p, for the silica used in this work is 0.75 ml per gram of SiO$_2$ and the density of PMOS and PMODS are 0.91 g ml^{-1} and 0.89 g ml^{-1}, respectively. From these pore volumes, the

Table 1
Specific mass data from the heating/extraction experiments with PMOS or PMODS on silica[a]

Temperature (°C)	Specific mass ($\bar{m} = g_{polymer}/g_{SiO_2}$)		Layer thickness (τ) (nm)	
	PMOS	PMODS	PMOS	PMODS
80	0.281	0.376	1.0	1.4
100	0.269	0.396	0.9	1.5
120	0.303	0.518	1.1	2.3
150	0.603	0.615	2.8	3.1
180	0.529	0.660	2.2	3.8

[a] Average of three determinations.

Table 2
Chromatographic parameters from thermally immobilized $SiO_2(PMOS)$[a]

Immobilization temperature (°C)	Chromatographic parameters				
	N/L[b]	h[b,c]	A_s[b]	k[b,d]	R_s[e]
80	38,400	1.9	1.3	2.1	1.6
100	41,300	1.8	1.2	2.1	1.7
120	39,500	1.9	1.2	2.2	2.1
150	23,200	3.2	1.3	7.5	0.8
180	4100	18.0	n.d.[f]	5.6	n.d.

[a] Average values from at least three different chromatographic runs with each of two or three different columns.
[b] Calculated for the naphthalene peak.
[c] Calculated: $h = L/(N \cdot d_p)$.
[d] Column dead time was measured with uracil.
[e] Calculated for the toluene–naphthalene pair.
[f] n.d., not determined.

mass of polymer that should fill the pore system of the silica may be calculated:

$$\bar{m}_{PMOS} = 0.75 \text{ ml/g}_{SiO_2} \times 0.91 \text{ g}_{PMOS}/\text{ml}$$
$$= 0.68 \text{ g}_{PMOS}/\text{g}_{SiO_2}$$

and

$$\bar{m}_{PMODS} = 0.75 \text{ ml/g}_{SiO_2} \times 0.89 \text{ g}_{PMODS}/\text{ml}$$
$$= 0.67 \text{ g}_{PMODS}/\text{g}_{SiO_2}$$

Thus we might expect that values of $\bar{m}_{PMOS} = 0.68$ g_{PMOS}/g_{SiO_2} and $\bar{m}_{PMODS} = 0.67$ g_{PMODS}/g_{SiO_2} should fill the pore system of silica.

The results of Table 1 show that thermal treatments of 80 to 120°C for PMOS resulted in phases with the same amount of polymer, while at 150 and 180°C, the amount of polymer was two times or more higher. These quantities may be compared in terms of the average layer thickness of polymer. In the case of PMODS, the temperatures of 80 and 100°C produced similar layer thicknesses of polymer, while the temperature of 120°C produced an increase in the amount of retained polymer, this increase being even higher for temperatures of 150 and 180°C. In a general way, the shorter chain polymer (eight carbon atoms) presents two plateaus: one for low temperature immobilization ($T \leq 120$°C) and one for high temperature immobilization ($T \geq$ 150°C). The longer chain polymer (18 carbon atoms) presents a gradient of layer thickness which appears to increase monotonically with temperature.

Chromatographic evaluations of the phases are presented in Tables 2 and 3 for PMOS and PMODS,

Table 3
Chromatographic parameters from thermally immobilized $SiO_2(PMODS)$[a]

Immobilization temperature (°C)	Chromatographic parameters				
	N/L[b]	h[b,c]	A_s[b]	k[b,d]	R_s[e]
80	34,000	2.2	1.4	1.5	2.6
100	31,300	2.4	1.7	2.2	3.0
120	27,100	2.7	1.2	5.1	3.9
150	1600	45.1	1.9	9.6	1.3
180	4900	15.2	1.3	11.1	2.4

[a] Average values from at least three different chromatographic runs with each of two or three different columns.
[b] Calculated for the naphthalene peak.
[c] Calculated: $h = L/(N \cdot d_p)$.
[d] Column dead time was measured with uracil.
[e] Calculated for the toluene–naphthalene pair.

respectively, and the chromatograms are shown in Figs. 1 and 2. With respect to the chromatographic parameters, the phases treated from 80 to 120°C for PMOS and PMODS present the best results, perhaps due to the monolayer thicknesses of the polymeric layer within the pores of the silica, which permits easier diffusion of solutes into and out of the pore system. The decrease in efficiency of phases immobilized at higher temperatures may be the result of formation of thicker or non-uniform polymer layers or of polymer degradation.

The retention factor, *k*, increases as the immobilization temperature increases in both polysiloxanes. This behavior of *k* parallels the specific mass (Table 1), which increases significantly at 150°C for PMOS and 120°C for PMODS.

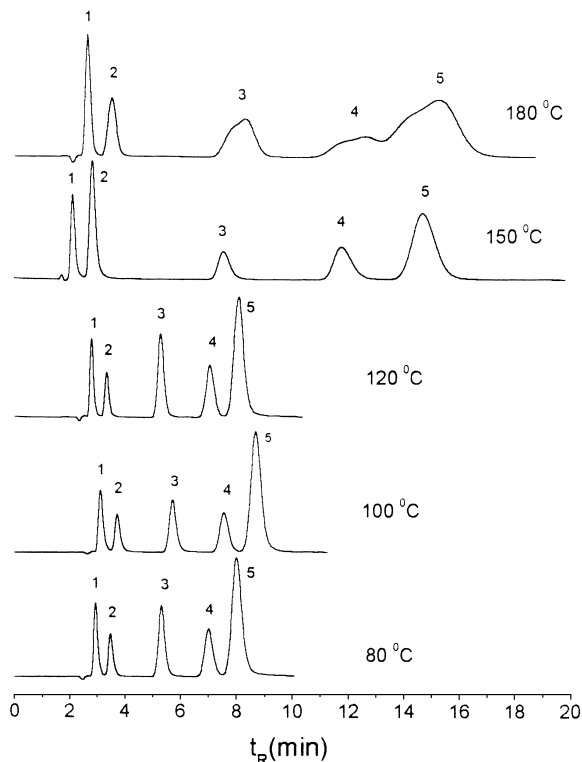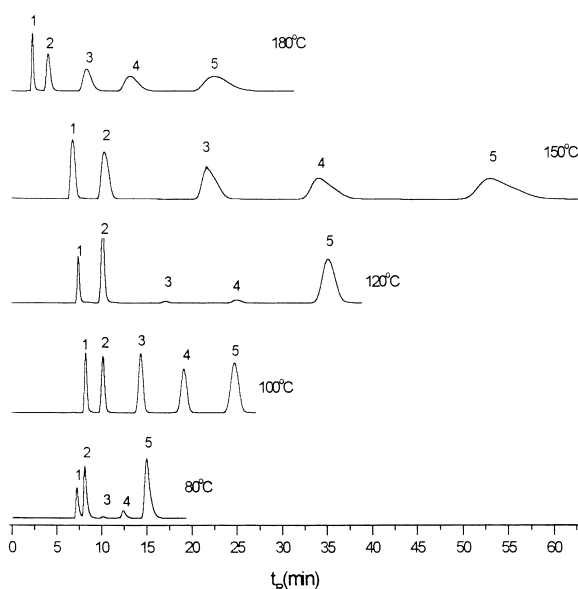To evaluate the behavior of the stationary phases

Fig. 2. Chromatographic behavior of PMODS stationary phases which have been thermally immobilized by a 4-h treatment at different temperatures. Chromatographic mixture and chromatographic conditions as in Fig. 1.

with respect to silanophilic activity with basic and acidic compounds, chromatographic evaluations with the solutes *N,N*-dimethylaniline and phenol were carried out. *N,N*-dimethylaniline is a basic compound which interacts strongly with free silanols to cause peak tailing, resulting in high asymmetry values, while phenol is a weak acid whose presence shows polar interactions or hydrogen bonding capacity [21,22]. Phenol can also interact with the nonpolar and the polar portions of the stationary phase [23]. Table 4 shows that, for PMOS and PMODS, peak asymmetry, as measured with these compounds, increases after thermal immobilization at 150°C (PMOS) and 120°C (PMODS). This suggests that even with a thicker polymer layer, the residual silanol groups are not completely protected or that the higher temperatures generated new silanol groups. Alternatively, the degradation products accompanying immobilization at the higher temperatures in the presence of air might introduce polar groups, which, in turn, would be expected to complicate the formation of simple cross-linkages between the alkyl chains [24].

TGA data were used to evaluate thermal stability

Fig. 1. Chromatographic behavior of PMOS stationary phases which have been thermally immobilized by a 4-h treatment at different temperatures. Chromatographic mixture: 1, acetone; 2, benzonitrile; 3, benzene; 4, toluene; 5, naphthalene. Chromatographic conditions: mobile phase: methanol–water (70:30, v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 5 μl and detector UV at 254 nm.

Table 4
Asymmetry factor (10%) for basic and acidic compounds chromatographed on thermally immobilized $SiO_2(PMOS)$ and $SiO_2(PMODS)$[a]

Immobilization temperature (°C)	PMOS		PMODS	
	N,N-dimethylaniline	Phenol	*N,N*-dimethylaniline	Phenol
80	1.3	1.3	1.4	1.6
100	1.3	1.4	1.2	1.3
120	1.2	1.2	1.8	1.3
150	1.6	1.4	n.d.[b]	n.d.
180	n.d.	n.d.	2.5	2.0

[a] Average values from at least three different chromatographic runs with each of two or three different columns.
[b] n.d., not determined.

of the stationary phases. The cumulative (TGA) curves of the samples obtained in an inert atmosphere are shown in Fig. 3. The TGA curve of each sample exhibited one step in the range from 270 to 460°C. Additional information about the temperature dependence of the decomposition rate was observed with the differential (DTG) curves. These sharp steps correspond to degradation of the polysiloxanes. At elevated temperatures in an inert atmosphere linear polysiloxanes degrade by a process that yields a mixture of volatile, low molecular mass products, which have chemical compositions related to the original polymer [24]. The presence of residues after thermal degradation suggests that the Si–C or C–H bonds remain stable at degradation temperatures.

Table 5 shows the values of mass loss during the thermogravimetric analysis for the different stationary phases. Thus, the phases treated at 150 and 180°C had greater TGA mass losses, since the amount of polymer retained after the immobilization by thermal treatment was greater for these phases.

Fig. 4 shows the results of stability evaluations for the two stationary phases (PMOS and PMODS), thermally immobilized at 100°C. These columns initially gave the best chromatographic parameters for both stationary phases. It can be observed, however, that both phases degrade after contact with about 250 ml of alkaline mobile phase at 60°C. It is important to note that these conditions are quite drastic. The $NaHCO_3$ mobile phase is an aggressive reagent [25], since it dissolves the silica more quickly than most other buffered mobile phases. In addition, the relatively high temperature of 60°C greatly enhances the reactivity of this mobile phase [25]. It should be commented here that a typical
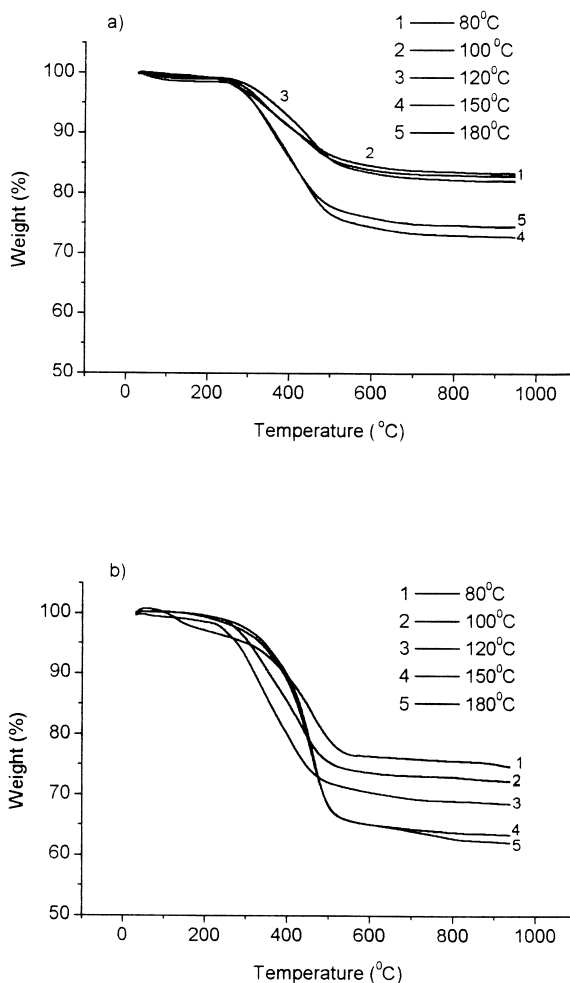

Fig. 3. Cumulative TGA curves for (a) PMOS and (b) PMODS thermally immobilized on silica.

Table 5
Mass loss obtained from TGA curves for thermally immobilized SiO_2(PMOS) and SiO_2(PMODS)

Temperature (°C)	% Mass loss	
	SiO_2(PMOS)	SiO_2(PMODS)
80	17.1	25.2
100	16.5	28.0
120	18.0	31.3
150	27.3	36.7
180	25.5	38.1

commercial phase (Hypersil ODS) degraded in a similar manner after 250 ml of basic mobile phase under similar conditions.

As previously reported [26], the retention factor does not change throughout the stability evaluation; the alkaline mobile phase apparently dissolves the silica support but not the polymeric layer, which continues to interact chromatographically with the test solutes. As the retention factor is related to solute interaction with the organic phase, stability is better evaluated by observing the variation in efficiency.

Table 6 gives %C data for the PMOS and PMODS phases sampled both at the beginning and at the end of the stability evaluation. After the stability evaluation, there was more carbon-containing material at the top of the column, in relation to the rest of the column. The reason for this is that the mass of silica has decreased at the top of the column so that the residual polymer represents a larger fraction of the column mass than is initially present.

4. Conclusions

Thermal treatment in the absence of any cross-linking catalyst is a reasonable procedure for immobilization of polysiloxanes (PMOS and PMODS) on silica surfaces. The resulting stationary phases present a more uniform surface coverage of ~4 μmol (organic) per square metre. For both polymers, immobilization temperatures in the range 80 to 100°C can be recommended since chromatographic parameters such as efficiency (N), asymmetry (A_s) and retention factor (k) compare well with those of commercial columns of similar type (C_8 or C_{18}). At
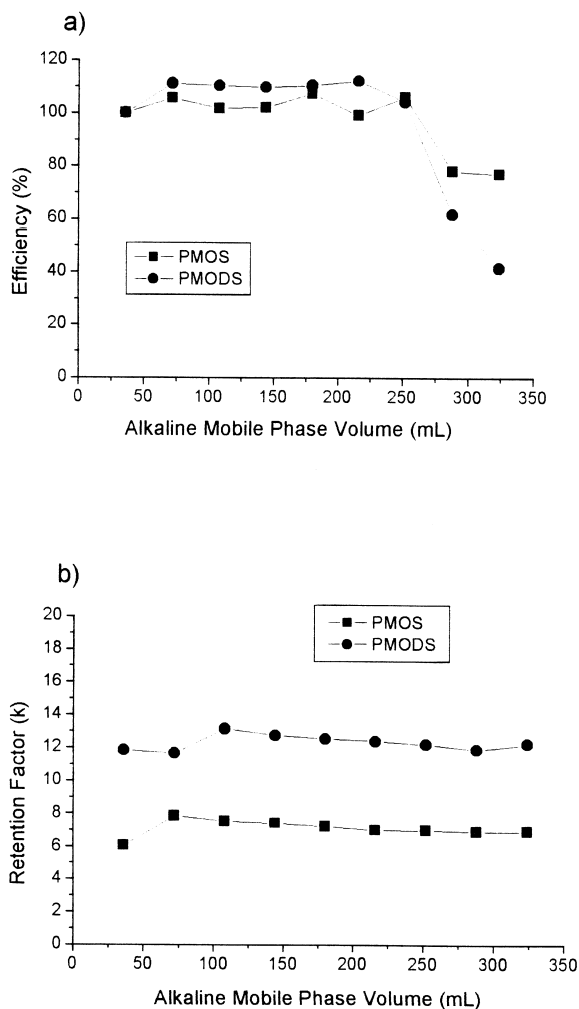

Fig. 4. Stability evaluation of PMOS or PMODS stationary phases in terms of (a) relative efficiency and (b) retention factor. Test conditions: mobile phase: methanol–0.1 mol l^{-1} NaHCO$_3$ (pH 8.5) (50:50, v/v), flow-rate: 0.6 ml min^{-1}, temperature: 60°C. Chromatographic conditions: same as evaluation conditions. Sample: same as Fig. 1.

Table 6
Carbon analyses of stationary phases before and after stability evaluation for polysiloxane phases immobilized at 100°C

Source of sample	% Carbon	
	SiO_2(PMOS)	SiO_2(PMODS)
Unused packing	13.5	20.7
Top of column	15.2	20.5
Middle of column	12.7	16.7
Bottom of column	12.4	16.4

immobilization temperatures of 150°C and higher, the efficiency decreases drastically, probably due to the presence of oxygen (air) during the thermal treatment. The degradation of these polysiloxanes becomes significant at temperatures above 120°C. It is probable that higher temperature polysiloxane degradation produces immobilized polymer-containing silanol groups, resulting in increased asymmetries for basic compounds.

Acknowledgements

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