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Stability of high-performance liquid chromatography columns packed with poly(methyloctylsiloxane) sorbed and radiation-immobilized onto porous silica and zirconized silica

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Abstract

Reversed-phase packing materials were prepared from HPLC silica and from zirconized HPLC silica support particles having sorbed poly(methyloctylsiloxane) (PMOS) as the stationary phase. Portions of zirconized material were subjected to 80 kGy of ionizing radiation. Columns prepared from these packing materials were subjected to 5000 column volumes each of neutral and alkaline (pH 10) mobile phases, with periodic tests to evaluate chromatographic performance. It was shown that the PMOS stationary phase sorbed onto zirconized silica requires an immobilization treatment (such as gamma irradiation) for long term stability while prior surface zirconization of the silica support surface greatly improves the chromatographic stability of the stationary phase when using alkaline mobile phases. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The use of alternative supports for stationary phases used in high performance liquid chromatography (HPLC) has the purpose of increasing the stability of the stationary phases when used at extreme pH and to decrease the undesirable adsorptive properties of the phase toward basic species [1,2]. Many approaches have been used to produce new chromatographic supports, including materials such as octadecyl coated titania [3], octadecyl coated

zirconia [4,5], polybutadiene (PBD) coated zirconia [6–9] and zirconia sorbents having vapor-deposited carbon [10]. Zirconia (ZrO_2) supports with thermally fixed PBD stationary phase are attractive materials for HPLC with reported applications in bioanalysis [6–9]. Studies with porous zirconia have shown that this support is very stable over the pH range 0–14 and that it can be coated with apolar polymeric material, such as PBD, to produce chemically and thermally stable reversed-phase packing materials, with retention properties comparable to conventional silica RP materials [11]. Stationary phases supported on zirconia have good chromatographic performance when submitted to stability tests, in which large volumes of neutral or alkaline mobile phases are passed through the column [12,13].

The possibility that zirconium oxide coated silica

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might have high alkaline stability has stimulated interest in its possible use as a chromatographic support [14]. Gushikem et al. [15–17] have prepared silica with surfaces modified with zirconium and titanium, using organofunctionalized reagents.

A previous study [18] in this laboratory showed that modification of the surface of 10 μm irregular silica with zirconia, followed by poly(methylsilyloxane) (PMOS) coating and gamma irradiation, gives columns with better chromatographic performance than with unmodified silica supports. The present work reports that columns packed with zirconized silica particles which had been coated with PMOS, then gamma irradiated, have a much higher resistance against attack by aggressive mobile phases. In this work, we carried out stability tests for sorbed and irradiated PMOS stationary phases based on silica and on zirconized silica, under both neutral and alkaline (pH 10) conditions, up to 10 000 column volumes. Gamma radiation immobilization of the PMOS coated phase on the zirconized silica is necessary for long term stability, as suggested by previous studies from this laboratory [19–21].

2. Experimental

2.1. Instrumentation and reagents

Chromatographic tests were performed on a modular system consisting of a Waters 510 pump, a SSI 3XL pneumatic injector with a 10 μl loop, a Waters 481 spectrophotometric detector (used at 254 nm) and a Hewlett-Packard 3395 integrator. The pH of mobile phases was checked on a Digimed DM21 pH meter.

Methanol (Mallinckrodt, HPLC-grade) and water (Milli-Q) were filtered to prepare the mobile phases. High pH mobile phases were prepared with ammonium hydroxide (Merck)/ammonium chloride (Merck) buffer at pH 10. Dichloromethane and carbon tetrachloride were Merck (p.a.) reagents. Davisil silica (10 μm , pore size 8.7 nm, specific surface area (S_{BET}) 387 $\text{m}^2 \text{g}^{-1}$ (Alltech)), zirconium tetrabutoxide (Aldrich) and the poly(methylsilyloxane) (PMOS) polymer (average molecular

mass of 6200) (Hüls America) were used as received.

2.2. Stationary phases and columns

Zirconized silica was prepared by an adaptation of the method of Peixoto et al. [15] by reaction of silica with zirconium (IV) butoxide in toluene, as described in our previous work [18]. The amount of the attached Zr on the surface was 15.3% (1.67 mmol Zr/g silica), as determined by X-ray fluorescence analysis.

Packing materials were prepared from silica and zirconized silica as follows: determined quantities of support were added to solutions of PMOS in dichloromethane (1 g PMOS+support/12 ml CH_2Cl_2) to give packing material with 50% (w/w) of PMOS.

Radiation immobilized packing materials received 80 kGy of absorbed dose in the cobalt-60 irradiation facility of IBRAS-CBO (Campinas, Brazil).

Columns (150 mm \times 3.9 mm I.D.) were made from type 316 stainless-steel tubing. The internal surface was polished using a technique developed in our laboratories. The columns were slurry packed using 10% slurries (w/v) of the stationary phases in carbon tetrachloride. 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, 70:30, v/v) at 0.2 ml min^{-1} prior to testing.

2.3. Stability testing

Three different columns were subjected to this test: column I (silica with sorbed PMOS coating), column II (zirconized silica with sorbed PMOS coating) and column III (zirconized silica with gamma-immobilized PMOS coating). To simulate usual chromatographic practice, the columns were subjected to washing at room temperature at a flow-rate of 1.0 ml min^{-1} to 5000 column volumes (c.v.) with mobile phase A (methanol:water, 70:30 (v/v)) and then to 5000 c.v. with mobile phase B (buffer NH_4^+ (0.03 mol l^{-1})/ NH_3 (0.17 mol l^{-1}), pH 10: MeOH (30:70 (v/v))). During this program, tests

were periodically performed at 0.2 ml min^{-1} using mobile phase A. The test mixture consisted of acetone, benzonitrile, benzene, toluene and naphthalene. Injections of $10 \mu\text{l}$ of appropriate concentrations of these mixtures produced satisfactory chromatographic peaks with UV detection at 254 nm. When mobile phase B was under study, the columns were conditioned with mobile phase A for 20 min at 0.2 ml min^{-1} before injection of the test mixture.

The column dead time, t_M , was determined using methanol as an unretained compound. Column efficiency values (N/L) were determined from peak width at half height. The asymmetry factors (As) were calculated at 10% of the peak height. Retention factors (k) and resolution (R_s) were also determined.

3. Results and discussion

This study involved two steps. First, mobile phase A (neutral pH) was passed through columns I (silica with a sorbed PMOS coating), II (zirconized silica with a sorbed PMOS coating) and III (zirconized silica with a radiation immobilized PMOS coating). As the stability test with neutral mobile phase causes a considerable decrease in resolution for column II, the stability test with mobile phase B (pH 10) was carried out only for columns I and III.

Fig. 1 indicates that the retention factor and resolution for columns I and III change only slightly as functions of column volumes. The efficiency (N/L) of column I was initially 25 000 plates/meter and stabilized at 20 500 plates/meter at 5000 column volumes of mobile phase A, while that for column III started at 32 600 plates/meter and remained at 32 000 plates/meter after 5000 column volumes. On the other hand, the efficiency of column II decreased 50% (25 000 to 12 600 plates/meter), with a consequent reduction of resolution (1.8 to 1.3) and retention factor (1.3 to 1.1) during passage of 5000 column volumes of mobile phase A. The asymmetry factors showed little variation for all columns and were, approximately, 1.5 (column I), 1.9 (column II) and 1.0 (column III).

The significant decreases in efficiency and resolution for column II (Figs. 1a and 1c) suggests a

loss of PMOS (not immobilized in this stationary phase), irreversibly changing the column properties. The small decrease in efficiency for column III at around 500 column volumes (Fig. 1a), with a subsequent return to the initial level (32 000 plates/meter) suggests a rearrangement of the PMOS on the support surface, which is possible because of the high amount of polymer present in this stationary phase. This behavior is also suggested by similar profiles of the curves of efficiency and resolution (Figs. 1a and 1c), against the irreversible decrease in retention (Fig. 1b), for column III.

Fig. 2 shows the results of the stabilization test with mobile phase B. This shows, for column I, that the efficiency decreased 28% (24 000 to 18 000 plates/meter) during this test while the resolution decreased from 1.7 to 1.2. For column III, the efficiency fell only 16% (31 000 to 26 000 plates/meter) and resolution decreased from 3.0 to 2.2 with the same volume of pH 10 mobile phase. The asymmetry factor remained at approximately 1.4 (column I) and 0.9 (column III) throughout the test.

Table 1 compares the chromatographic parameters of columns I and III before and after passing 10 000 column volumes of the mobile phases A (5000 column volumes) and B (5000 column volumes) through the columns. The retentions of the last peak (naphthalene) are similar in both columns, resulting in an analysis time of about 17 min. Column III, packed with PMOS gamma-immobilized on zirconized silica, shows better stability than column I, packed with the same polymer coated on the non-modified silica; the efficiency of column III decreases 20%, while for column I the efficiency fell 28%.

Another important result is that column III (with the zirconized silica support) had an asymmetry factor for the naphthalene peak of about 1.0 and a resolution for the last pair (toluene–naphthalene) above 2.2 throughout the test. After passing 5000 column volumes of neutral pH mobile phase and 5000 column volumes of an aggressive mobile phase (pH 10), this column still shows symmetric and completely separated peaks (Fig. 3), while column I (silica support) shows less symmetric peaks (As around 1.5) and a reduced resolution of 1.2 after these tests.

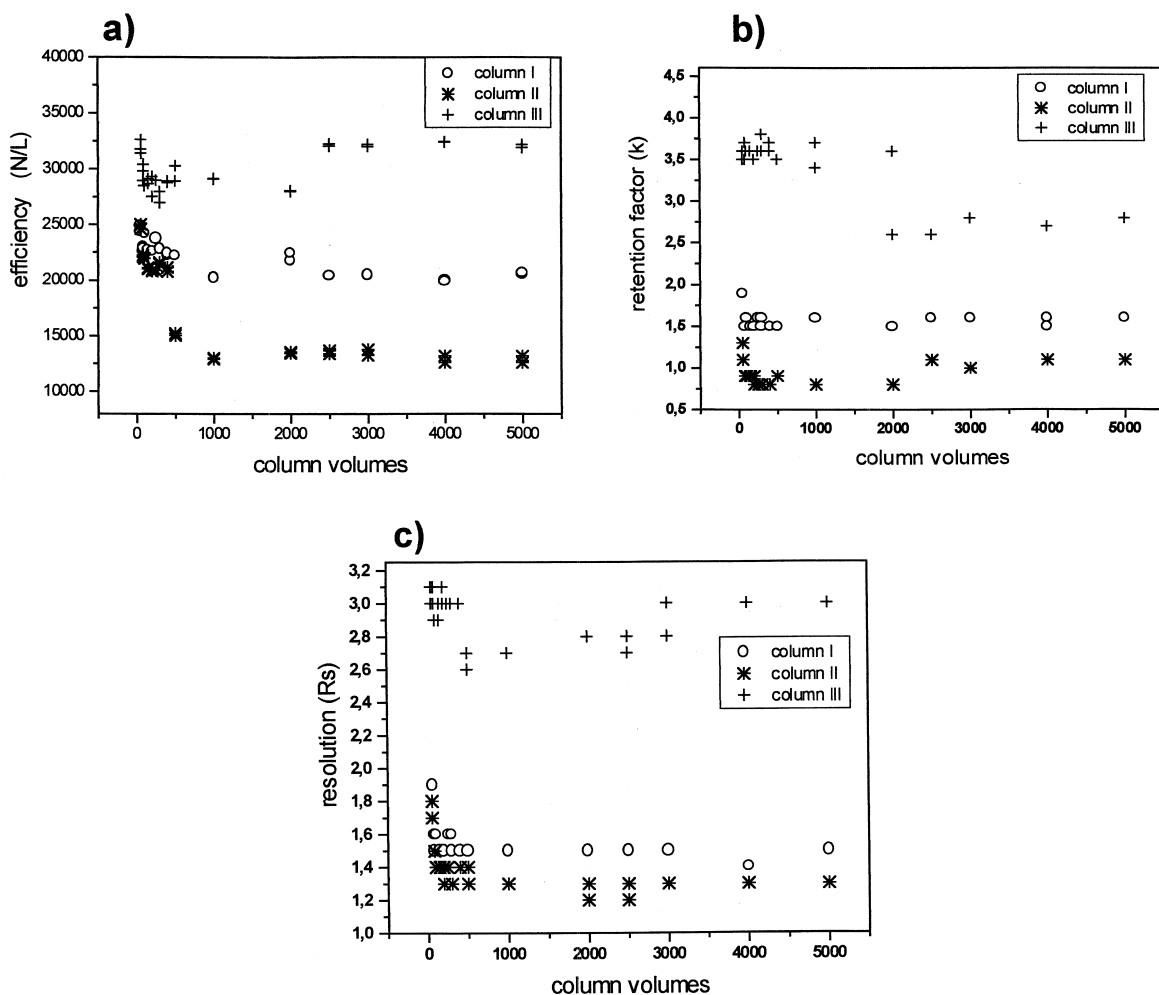


Fig. 1. Column efficiency (a), retention factor (b) and resolution (c) as a function of the volume of mobile phase A passing through the columns. Data for the naphthalene peak (efficiency and retention factor) and for the naphthalene–toluene pair (resolution).

4. Conclusions

The behavior of chromatographic columns in stability tests following washing with large volumes of neutral and alkaline mobile phases has demonstrated that irradiated (80kGy) PMOS stationary phases on zirconized silica give better chromatographic performance than similar stationary phases on unmodified silica.

These results can be attributed to a reduction of the acidic properties of silica through zirconization, as well as to PMOS chain cross-linking which gives a stronger polymeric structure, resulting in a station-

ary phase with better retention capability and columns with good chromatographic performance.

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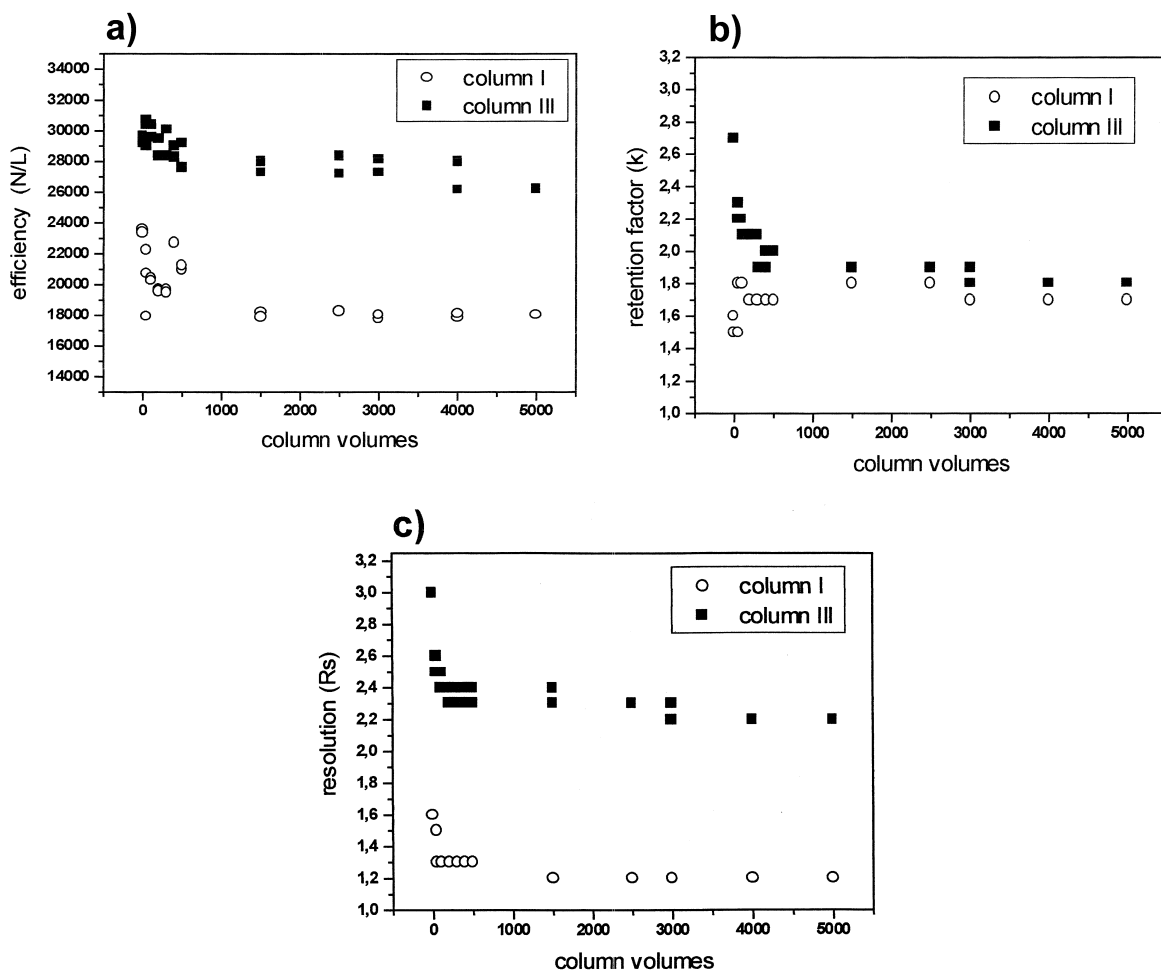


Fig. 2. Column efficiency (a), retention factor (b) and resolution (c) as a function of the volume of mobile phase B (pH 10) passing through the columns. Data for the naphthalene peak (efficiency and retention factor) and for the naphthalene–toluene pair (resolution).

Table 1

Chromatographic performance data for columns I and III before and after treatment with 5000 column volumes of neutral mobile phase and 5000 column volumes of alkaline (pH 10) mobile phase

Column	Before treatment				After both treatments				Loss in efficiency (%)
	N/L^a	k^a	Rs^b	As^a	N/L^a	k^a	Rs^b	As^a	
I ^c	25 000	1.9	1.9	1.6	18 000	1.7	1.2	1.4	28
III ^d	32 600	3.6	3.1	1.0	26 000	1.8	2.2	0.8	20

^a For naphthalene peak.

^b For naphthalene–toluene pair.

^c PMOS sorbed on non-modified silica.

^d PMOS on zirconized silica, irradiated to 80kGy.

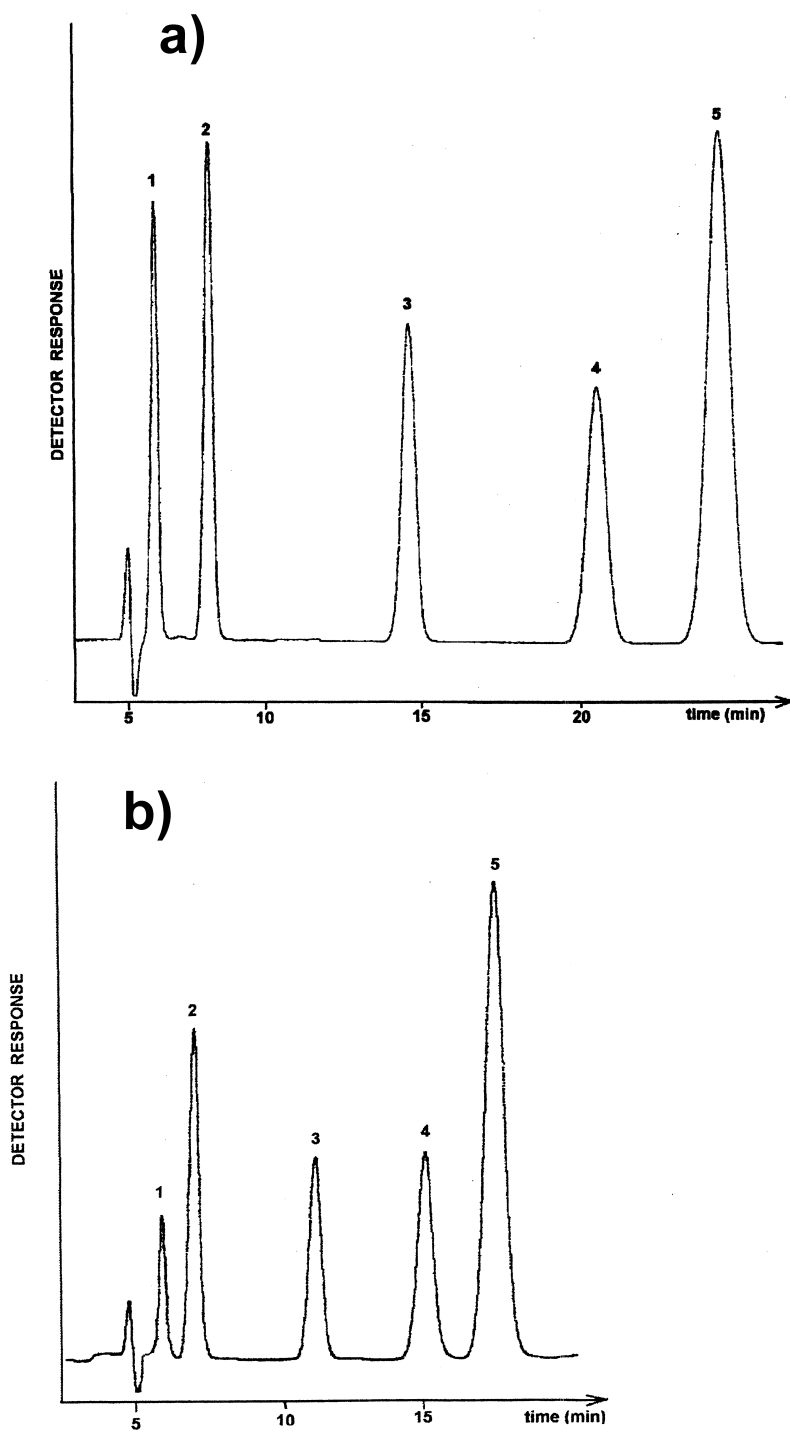


Fig. 3. Chromatograms of a mixture of acetone (1), benzonitrile (2), benzene (3), toluene (4) and naphthalene (5), obtained with column III (zirconized silica with radiation immobilized PMOS coating) (a) before initiating the stability test and (b) after completing the stability test with 5000 column volumes each of neutral and alkaline (pH 10) mobile phases.

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