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Development of C₈ stationary phases immobilized by γ radiation on zirconized silica for high-performance liquid chromatographic applications

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Abstract

Modification of 10 μm irregular silica with zirconium, followed by poly(methyloctylsiloxane) coating and γ irradiation may overcome limitations presented by silica supports used for preparing reversed phases. Irradiated modified stationary phases showed better chromatographic performance than unmodified silica phases. An increase in efficiency values and excellent peak symmetries for a test mixture of neutral compounds was obtained. The analysis of a basic mixture also presented peaks with good resolution, efficiency and symmetry. These results can be attributed to a less active support as well as poly(methyloctylsiloxane) chain cross-linking, giving stationary phases with better retention capability and columns with high chromatographic performance. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The increasing use of high-performance liquid chromatography (HPLC) has accompanied the development of suitable stationary phases. Porous silica is the most widely used support material in HPLC packings, due to its excellent mechanical strength, large surface area and availability in a wide range of pore sizes and particle diameters [1]. However, its limited chemical stability at extreme pH values and undesirable adsorptive properties toward basic species are negative features of its use in many applications [2,3].

Many different approaches have been employed to

improve these chromatographic supports. Materials like octadecyl-titania [4], polybutadiene (PBD) coated zirconia [5–8], zirconia sorbents having vapor-deposited carbons [9], and octadecyl-zirconia [10,11] have been studied as reversed stationary phases. The oxide of zirconium (zirconia) with coated and thermally fixed PBD is an attractive material with reported applications in bioanalysis [5–8]. Studies with porous zirconia have shown that this support is completely stable over the pH range 0–14 and can be coated with apolar polymeric materials, such as PBD, making a chemically and thermally stable reversed-phase material, with retention properties comparable to conventional silica-based bonded RP materials [12].

Zirconium oxide coated silica has been shown to produce chromatographic matrices which undergo adsorption [13]. The possibility that these materials

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might have greater alkaline stability has stimulated interest in their use as chromatographic supports. Similarly, Gushikem et al. [14–16] studied silica surface modification with zirconium and titanium, using organofunctionalized reagents. These materials were used to extract Cr(VI) for spectrophotometric determinations [14].

In this work, irregular silica was modified with zirconium, followed by coating with a C₈ poly-(methyloctylsiloxane) (PMOS) stationary phase and immobilization by Co-60 γ radiation. As shown by previous studies in this laboratory [17–19], γ radiation is a good immobilization method for liquid phases coated on silica.

2. Experimental

2.1. Instrumentation

HPLC separations were performed on a modular system consisting of the following components: SSI 3XL pneumatic injector with a 10- μ l loop, a Waters 510 pump, a Waters 481 spectrophotometric detector (used at 254 nm; 14 μ l cell volume) and a Hewlett-Packard 3395 integrator. Zirconium quantification on the zirconized silicas were made using a Spectrace 5000 X-ray fluorescence spectrometer. Elemental carbon determinations were made using a Perkin-Elmer 2400 CHN analyzer. The specific surface areas of the zirconized silicas were determined by the conventional BET method [20] using a Micromeritics FlowSorb II 2300 instrument. Infrared spectroscopy was done on a Perkin-Elmer FT-IR 1600 instrument and thermogravimetric analysis with a TA Instruments TGA-2050 instrument.

2.2. Chemicals

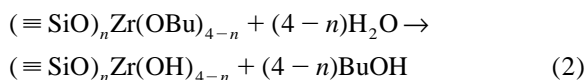
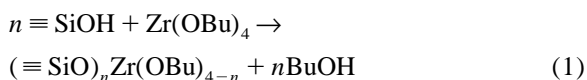
Methanol (Mallinckrodt, HPLC-grade) and water (Milli-Q) were previously filtered to prepare the mobile phases. Dichloromethane, carbon tetrachloride, chloroform and ethanol were Merck (analytical-reagent grade) reagents, toluene was spectroscopic grade from Institute of Chemistry/Unicamp and the nitric acid was from Quimex (analytical-reagent grade). The zirconized silica synthesis reagent (zirconium (IV) butoxide) was from Aldrich

(reagent No. 33394-8, 99.99% pure). The chromatographic test substances (acetone, aniline, benzene, benzonitrile, N,N-dimethylaniline, naphthalene and toluene) were analytical-reagent grade and not further purified. The silica used was Davisil, 10 μ m, nominal pore diameter 6 nm, specific surface area (S_{BET}) of 387 m²/g (Alltech Associates, USA) and the PMOS polymer (average molecular mass of 6200) was obtained from Hüls America (USA).

2.3. Synthesis of zirconized silica

Zirconized silica was synthesized by an adaptation of the method of Peixoto et al. [14] by reaction of silica with zirconium (IV) butoxide in toluene. The silica was dried at 150°C before use. About 70 g of the activated silica were added to a solution containing 45 ml (1.5 mmol Zr/g silica) of pure zirconium (IV) butoxide dissolved in 300 ml of anhydrous toluene. The mixture was heated under reflux for 12 h under a dry nitrogen atmosphere, and the resulting solid was washed by decantation with toluene and then heated at 150°C to eliminate the solvent. This dried material was hydrolysed by immersing it in a 10⁻⁴ M aqueous solution of HNO₃. This solid was washed with water and ethanol and then dried in an oven at 120°C for 5 h.

The following equations can be written to represent the reactions:



where $\equiv \text{SiOH}$ stands for silanol groups on the silica surface.

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.

2.4. Preparation of packing materials

Packing materials were prepared from silica and zirconized silica. The supports were dried in air at 120°C for 24 h. Then, determined quantities of support were added to solutions of PMOS dissolved

in dichloromethane (1 g PMOS+support/12 ml CH₂Cl₂) to give packing material with 50% (w/w) of PMOS.

The mixtures were gently stirred for 3 h at room temperature and then the solvent was allowed to evaporate, without stirring, at room temperature in the fume hood.

2.5. Irradiation

Two portions of packing material of zirconized support were γ irradiated to 80 and 120 kGy, at the cobalt-60 facility of IBRAS-CBO (Campinas, Brazil).

2.6. Column packing

Columns (150×3.9 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 (Haskell 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 prior to testing.

2.7. Chromatographic evaluation

Two test mixtures were used in this study: (1) acetone, benzonitrile, benzene, toluene and naphthalene and (2) aniline and N,N-dimethylaniline. Injections of 10 μ l of appropriate concentrations of

these mixtures produced satisfactory chromatographic peaks with UV detection at 254 nm.

All columns were operated at room temperature. The mobile phases used were methanol–water (70:30, v/v) at 0.2 ml/min for mixture I and methanol–water (60:40, v/v) at 0.5 ml/min for mixture II. The column dead time, t_M , was determined using methanol as an unretained compound. Column efficiency values (N/m) were determined from peak width at half height. The asymmetry factors (A_s) were calculated at 10% of the peak height. Retention factors (k), resolution (R_s) and separation factors (α) were also determined.

3. Results and discussion

3.1. Characterization of silica and zirconized silica

Table 1 shows the results of the characterization tests of unmodified silica and the zirconized silicas. These results show good reproducibility for the three preparations of zirconized silica which show the attachment of 15% (w/w) of zirconium (1.7 mmol Zr/g silica) onto the silica surface. Elemental analysis shows a small amount (1.4%) of carbon on the zirconized silicas due to residual organic material from the butoxide reagent. The specific surface area of silica was also slightly reduced.

The reaction of zirconium with the silica surface occurs at the silanol groups. This promotes a decrease of the intensity of the 975 cm⁻¹ bands characteristic of silanols (Fig. 1). Thermogravimetric analysis (Fig. 2) shows good thermal stability of

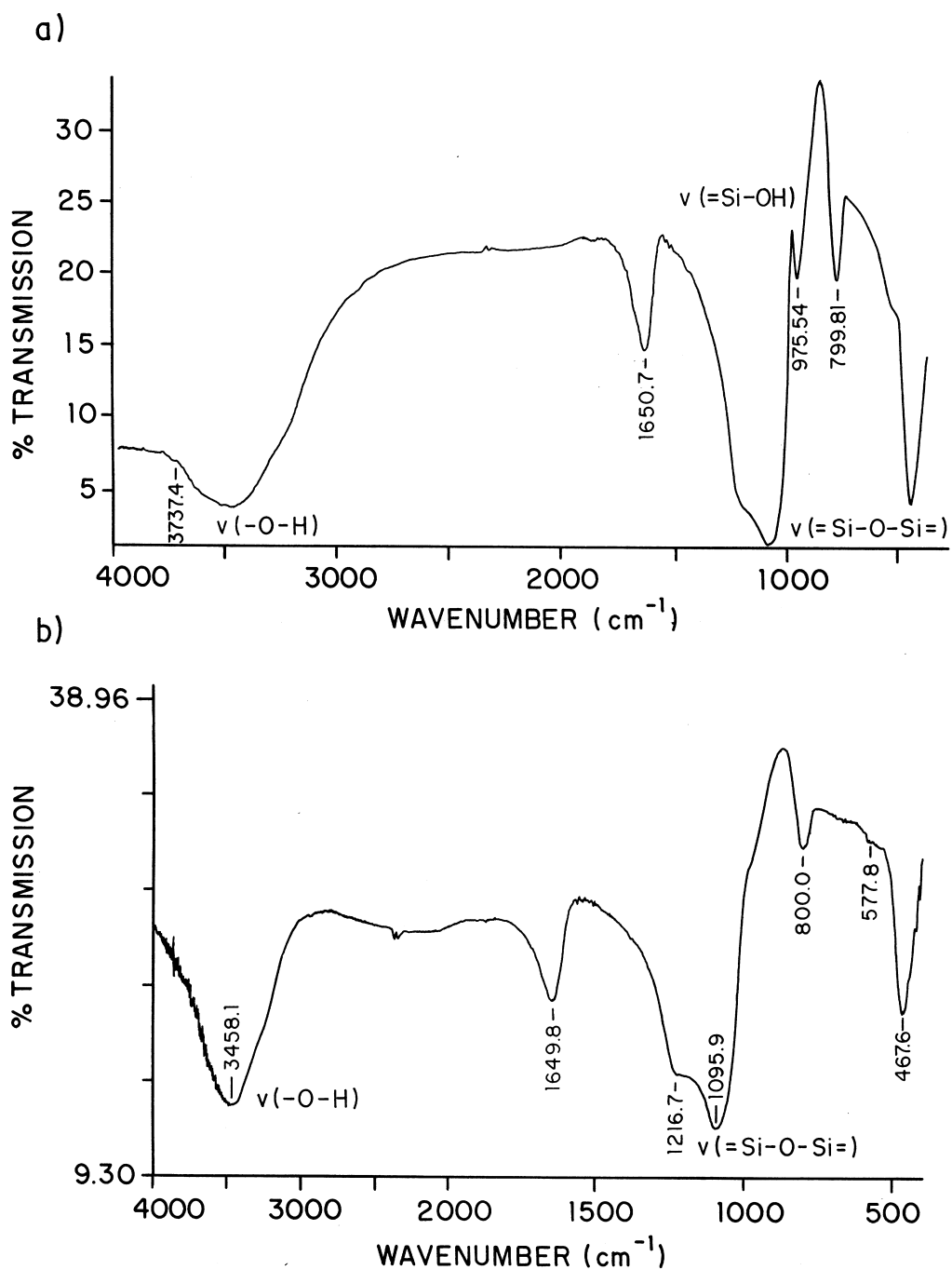
Table 1
Characterization tests results for silica and zirconized silicas

Test	SiO ₂ ^a	SiZr I ^b	SiZr II ^b	SiZr III ^b	Average value ^c
X-ray fluorescence (% Zr)	0.0	15	14	17	15±1.4
X-ray fluorescence (mmol Zr/g silica)	0.0	1.7	1.5	1.8	1.7±0.2
Carbon analysis (% C)	0.19	1.3	1.7	1.1	1.4±0.3
Surface area S_{BET} (m ² /g)	387	367	351	370	363±10

^a SiO₂: non-modified silica.

^b SiZr I, II and III: zirconized silicas, first, second and third preparations, respectively.

^c For zirconized silicas.

Fig. 1. Infrared spectra for (a) SiO_2 and (b) SiZrIII .

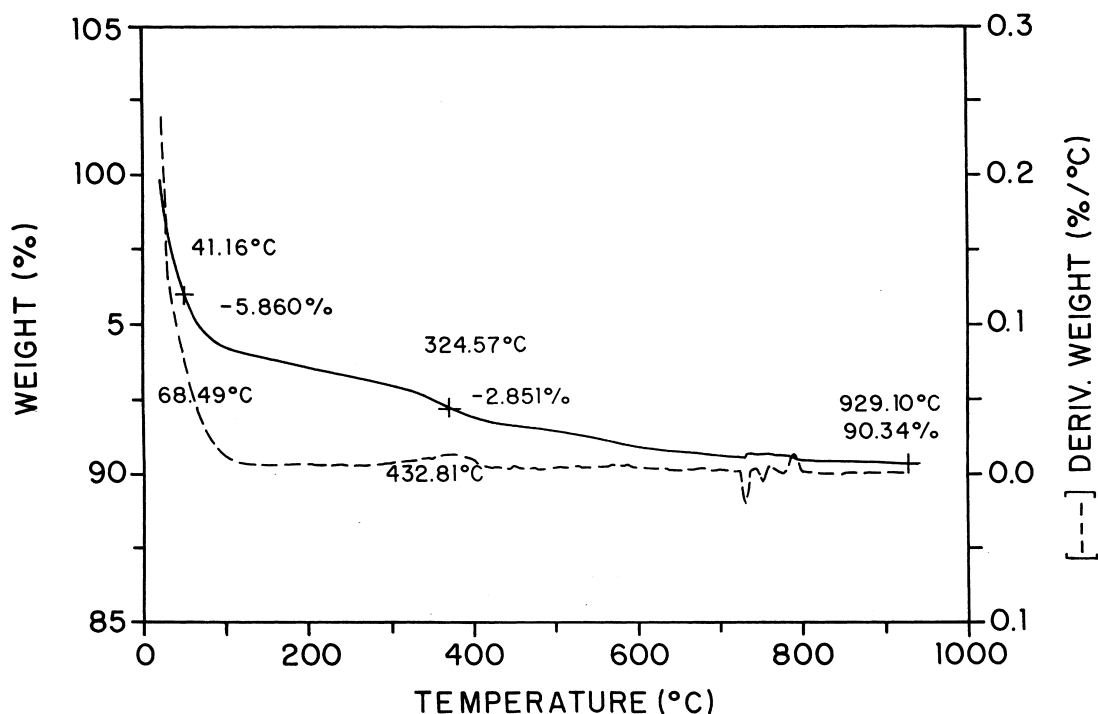


Fig. 2. Thermogram for zirconized silica (SiZrIII).

zirconized silica in the temperature range used for HPLC. The decrease in % (w/w) around 100°C can be attributed to a loss of adsorbed water, since the decrease observed was of 5.860% in mass while for zirconized silica with PMOS coated the TGA shows decrease in mass around 100°C of 1.077%.

3.2. Chromatographic evaluation

As shown in Table 2, columns packed with materials based on PMOS sorbed onto silica or

zirconized silica show similar results for efficiency, resolution and separation factor. The pair of peaks 1–2 (acetone–benzonitrile) and 4–5 (toluene–naphthalene) are not baseline separated, as shown in Fig. 3a and b. Nevertheless, the columns have good efficiencies (about 25 000 plates/m) for 10 μm packing materials. The column packed with material based on PMOS sorbed on zirconized silica has, for naphthalene peak, a shorter retention time, higher asymmetry ($A_s=2.2$) and a lower retention factor ($k=1.3$). These results can be attributed to the lower

Table 2
Chromatographic evaluation of columns for mixture I

Column	t_R^a (min)	N/m^a (m^{-1})	A_s^a	k^a	R_s^b	α^c
I Sorbed on non-modified silica	18.0	24 570	1.5	1.9	1.9	1.6
II Sorbed on zirconized silica	14.4	25 240	2.2	1.3	1.8	1.6
III Irradiated at 80 kGy on zirconized silica	23.9	41 710	1.0	3.5	3.4	1.7
IV Irradiated at 120 kGy on zirconized silica	27.4	25 190	1.1	4.4	2.8	1.7

^a For naphthalene peak.

^b For naphthalene–toluene pair.

^c For toluene–benzene pair.

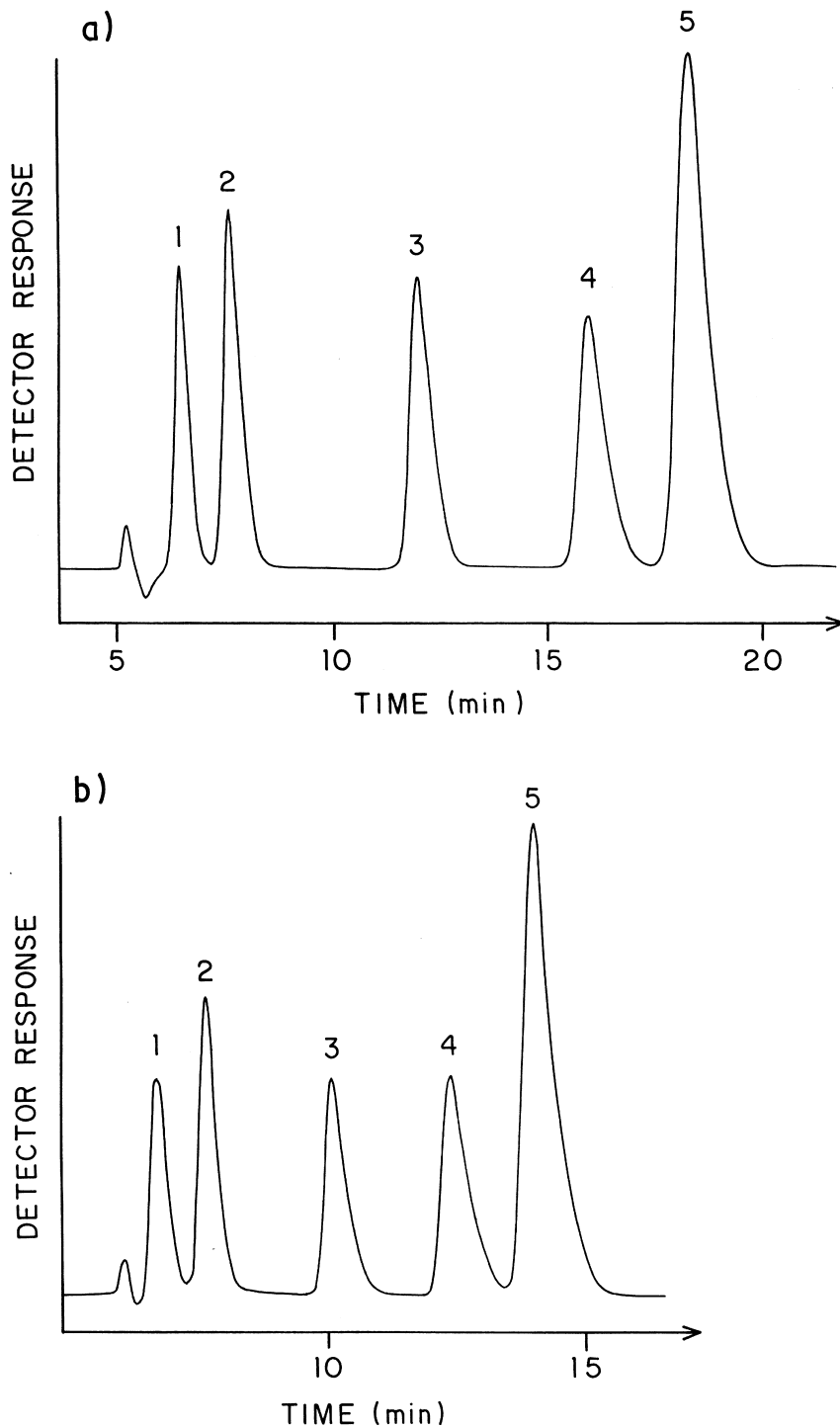


Fig. 3. Chromatograms of test mixture I: 1=acetone, 2=benzonitrile, 3=benzene, 4=toluene and 5=naphthalene, obtained with columns packed with a 50% initial loading of PMOS (a) sorbed on silica, (b) sorbed on zirconized silica and (c) sorbed and γ irradiated at 80 kGy on zirconized silica. Chromatographic conditions: mobile phase: methanol–water (70:30, v/v), flow-rate: 0.2 ml/min, volume of injected sample: 10 μ l, detection: UV, 254 nm.

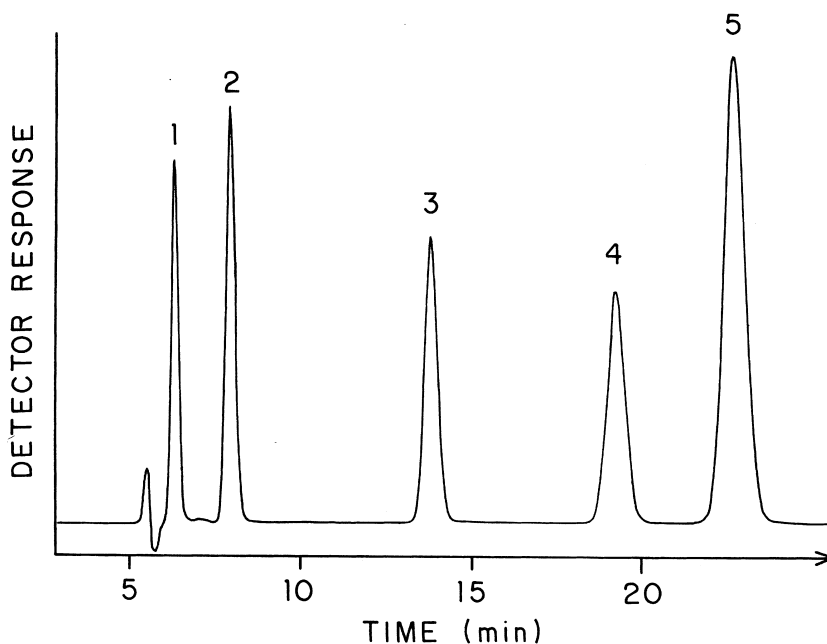


Fig. 3. (continued)

quantity of the stationary phase (PMOS) after packing (11.2% carbon, compared to columns packed with materials based on PMOS sorbed onto silica have 15.5%). Another effect observed was the loss of PMOS during column conditioning and use, resulting in a continual decrease of efficiency, retention factor and resolution.

This behaviour suggests that PMOS is poorly retained on the zirconized silica surface, due to a decrease in the number available silanol groups after the zirconization reaction. To overcome this problem with the zirconized silica support, immobilization of PMOS with γ radiation was evaluated.

Columns packed with irradiated materials showed improvements in all chromatographic parameters (Table 2). The amount of PMOS after packing has the same value as a silica-based column (15.5% for material sorbed and immobilized at 80 kGy and 16.1% after 120 kGy). The efficiency of the column containing material irradiated at 120 kGy is similar to that of the silica-based column (25 190 plates/m) and the column with material irradiated to 80 kGy has even higher efficiency (41 710 plates/m). The retention factors increase and all peaks are baseline separated (Fig. 3c). Finally, the peaks are symmetric,

with asymmetry factors of 1.0 (80 kGy) and 1.1 (120 kGy). These results can be attributed to the cross-linking of the alkyl chains of PMOS, with consequent protection of silanol groups. However there is no evidence of covalent link between PMOS and the support.

Fig. 4 shows the chromatograms obtained with mixture II. The relevant chromatographic parameters are shown in Table 3. The irradiated stationary phases shows better efficiencies, asymmetry factors and resolution than with the non-irradiated material. These results suggest that the support material based on zirconized silica with irradiation stabilization of the PMOS provides better separation of basic solutes than with unmodified silica, due to the modification of the acidic properties of silica.

4. Conclusions

The modification of the surface of 10 μm irregular silica with zirconium, followed by PMOS coating and γ irradiation, was employed in this work with reproducible preparations of zirconized silica (15% zirconium incorporated on the surface). A decrease

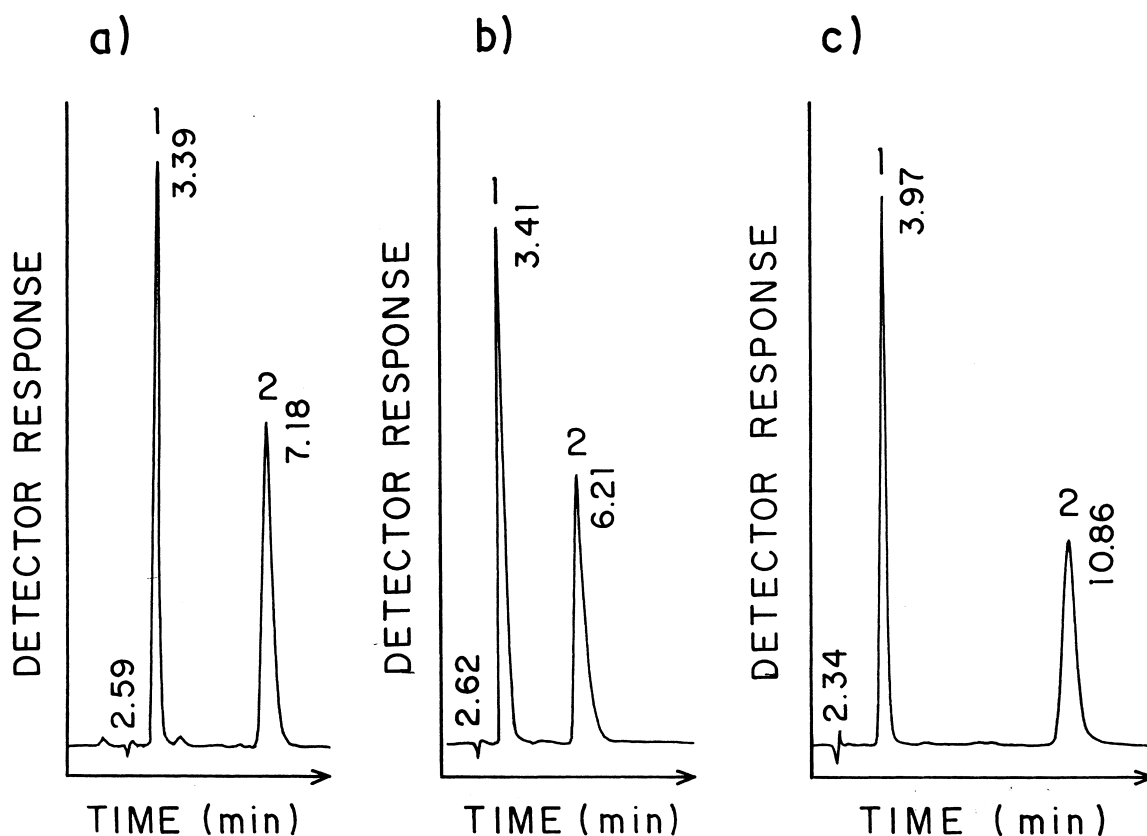


Fig. 4. Chromatograms of test mixture II: 1 = aniline, 2 = N,N-dimethylaniline, obtained with columns packed with a 50% initial loading of PMOS (a) sorbed on silica, (b) sorbed on zirconized silica and (c) sorbed and γ irradiated at 80 kGy on zirconized silica. Chromatographic conditions: mobile phase: methanol–water (60:40, v/v), flow-rate: 0.5 ml/min, volume of injected sample: 10 μ l, detection: UV, 254 nm.

of the surface area and reduction in the number of silanol groups were demonstrated through characterization tests.

Modified and irradiated (80 kGy) stationary phases showed better chromatographic performance than unmodified silica phases. An increase in efficiency and excellent peak symmetries for a neutral

test mixture was observed while analysis of a basic mixture presented peaks with better resolution, efficiency and good symmetry.

These results can be attributed to a reduction of the acidic properties of silica through zirconization, as well as to PMOS chain crosslinking, making a stronger polymeric structure, giving stationary

Table 3
Chromatographic evaluation of columns for mixture II

Column	t_R^a (min)	N/m^a (m^{-1})	A_s^a	k^a	R_s^b	α^b
I Sorbed on non-modified silica	7.16	16 390	1.7	1.8	8.9	5.9
II Sorbed on zirconized silica	6.18	12 210	2.8	1.3	6.3	4.7
III Irradiated at 80 kGy on zirconized silica	10.9	17 490	1.4	3.6	11.6	5.2

^a For N,N-dimethylaniline peak.

^b For N,N-dimethylaniline–aniline pair.

phases with better retention capability and columns with high chromatographic performance.

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