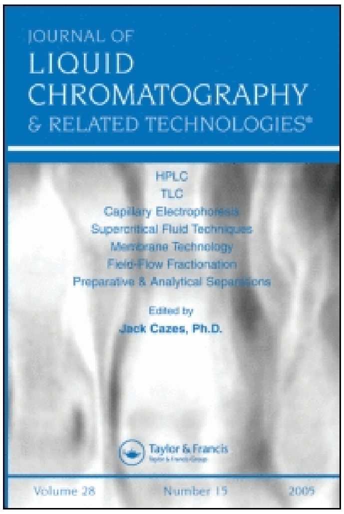

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C-18 STATIONARY PHASES SORBED ON ZIRCONIZED SILICA FOR USE IN HPLC

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ABSTRACT

Silica, with 10 μm diameter particles and 8.7 nm pore size, was zirconized (to 16.2% ZrO_2) by reaction with zirconium butoxide, then functionalized by sorption of PMODS (poly(methyloctadecylsiloxane)) using an evaporation procedure with hexane as solvent. Infrared spectroscopy was used to examine the surface concentration of silanol groups. The thermal stability of the material was verified by thermogravimetric analysis. The C-18 stationary phase was used to pack 150 × 3.9 mm i.d. columns, which exhibited satisfactory efficiencies (25000–30000 plates/m). The values of asymmetry factor, α, k and Rs were within acceptable limits.

INTRODUCTION

Recently, HPLC (high performance liquid chromatography) has become important for analyses of many classes of compounds: proteins, nucleic acids,

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dyes, ionic compounds, synthetic polymers, metallic ions, cations, anions, pharmaceuticals, etc. (1). The increased use of HPLC results mainly from the development of new stationary phases, as well as the improvement of old ones.

Porous silica is the most used support in HPLC (2) because it is available in various particle diameters and pore sizes, is mechanically resistant, and has large surface areas (3). However, one problem in the use of silica as a support is the continued presence of active polar groups-the residual silanols (4). The presence of these groups complicates the chromatographic separations of basic compounds.

Reversed phase column packing material utilizing silica support particles give good results if used with neutral or weakly acid mobile phases. However, bonds to the silica surface are not stable in very acid solutions (due to hydrolysis reactions) and the silica surface itself is soluble at high pH (4). Thus, the useful pH range of non-modified silica is limited to 2–8 (5). Many different approaches have been employed to improve the chemical stability of these supports at extreme pH values. One of these is to substitute the silica support material with another oxide more resisting to attack by acids or bases. Studies with porous zirconia show that it is a material stable over the pH range 0–14, that it can function as an ion exchange material, and it is chemically stable even at elevated temperatures (6–8).

Another approach to improve the chemical stability of the stationary phase is to graft a more stable oxide layer onto the silica surface. Transition metals that can be used in the modification of the silica surface are: titanium, thorium, and zirconium in the form of their oxides (titania, thoria and zirconia, respectively) (9–11). These surfaces are more basic than silica, hence, are more stable in alkaline solutions.

Mello and Jardim (12) modified HPLC silica with zirconium, then functionalized the surface with a gamma radiation immobilized coating of poly(methyloctylsiloxane). Columns prepared from these stationary phases had good efficiency, excellent peak symmetries, and excellent stability (13). Thus, zirconia coated silica was shown to be a support with pH stability similar to that of pure zirconia, which can support stationary phases having similar chromatographic properties to those made from non-modified silica (14).

In the present work 10 μm irregular silica was modified with zirconia and coated with poly(methyloctadecilsiloxane) (PMODS), obtaining a sorbed C-18 phase that was characterized by chemical, physical, and HPLC tests.

EXPERIMENTAL

Materials

Methanol (Omnisolv, Merck), water (Milli-Q, Millipore) were filtered with 0.22 μm Millipore membrane filters. Carbon tetrachloride (Synth) and hexane

(Nuclear) were analytical grade; toluene was spectroscopic grade, purified in the Institute of Chemistry-UNICAMP.

The silica was Davisil (Alltech), 10 μm, pore size 8.7 nm. PMODS polymer was obtained from United Chemical Technologies Inc., with an average molecular mass of 11000. The zirconized silica synthesis reagent (zirconium [IV] butoxide, 99.99%) was from Aldrich.

The chromatographic test substances (acetone, benzene, benzonitrile, naphthalene, and toluene) were analytical grade and not further purified.

Instrumentation

The chromatography was performed with a modular HPLC system equipped with a Rheodyne 8125–095 injector having a 5 μL loop, a Shimadzu LC 10 AD pump, a UV/VIS spectrophotometric detector Shimadzu SPD 10 AV (used at 254 nm), a Shimadzu CTO 10 AC column oven, Shimadzu FCV 11 AL solvent delivery module, coupled to a Hewlett-Packard 3396 integrator for acquisition of data. Quantification of the zirconium on the silica was made using a Spectrace 5000 X-ray fluorescence spectrometer. Elemental carbon determinations were made using a Perkin Elmer 2400 CHN analyzer. The specific areas and volumes of zirconized silica were determined by the BET method (15) using a Micromeritics ASAP 2010 sorptometer (Norcross, GA, USA). Infrared spectra were obtained using a Perkin Elmer FT-IR 1600 instrument. The thermogravimetric analyses were made with a TA Instruments TGA-2050 instrument. A Digimed model DM 21 pH meter was used for the potentiometric titration analyses.

Column packing was done with a Haskel Model 51769 pump having a pressure range of 7–350 MPa.

Procedures

Synthesis of Zirconized Silica

First, the silica was dried at 150°C, then, this support was modified by reaction with zirconium [IV] butoxide in toluene, following an adaptation of the procedure of Peixoto et al. (16). About 70 g of activated silica were added to a solution containing 45 mL of pure zirconium [IV] butoxide dissolved in 300 mL of anhydrous toluene. This mixture was mechanically agitated and heated at reflux temperature for 12 h under a dry nitrogen atmosphere. The solid obtained was washed (by decantation) with toluene and was then heated at 150°C to eliminate the solvent. The dried material was hydrolysed with a 10^4 mol/L aqueous solution of HNO_3. This solid was then washed with water and ethanol and dried in an oven at 120°C for 5 h.

This process can be represented by equations 1 and 2:

$$n \equiv \mathrm{SiOH} + \mathrm{Zr(OBu)_4} \rightarrow \equiv (\mathrm{SiO})_n\mathrm{Zr(OBu)}_{4-n} + n\ \mathrm{BuOH} \quad (1)$$

$$(\equiv \mathrm{SiO})_n\mathrm{Zr(OBu)}_{4-n} + (4-n)\mathrm{H_2O} \rightarrow (\equiv \mathrm{SiO})_n\mathrm{Zr(OH)}_{4-n} + (4-n)\mathrm{BuOH} \quad (2)$$

where $\equiv$SiOH represents the silanol groups on the inicial silica surface.

The amount of attached ZrO_2 on the surface was determined by X-ray fluorescence analysis.

Preparation of C-18 Stationary Phase on Zirconized Silica

The C-18 stationary phase was prepared by the solvent evaporation method (17–19). Poly(methyloctadecylsiloxane) (PMODS) was dissolved in hexane in the proportion of 1 g PMODS to 12 mL of solvent. The solution was mechanically stirred, and then the support (in the proportion 1 gram support per gram of PMODS) was slowly added. The mixture was stirred for 3 h at room temperature, then, the solvent was evaporated at room temperature.

Column Packing

Column blanks (150 mm × 3.9 mm i.d.) were made from type 316 stainless-steel tubing having highly polished internal surfaces (20). A slurry of the prepared packing material in carbon tetrachloride, 10% (w/v), was submitted to ultrasonic agitation for 4 min, followed by rotatory agitation for 12 h. A Haskel pump was used to pack the columns at a pressure of 35 MPa. Methanol was the propulsion solvent. Columns were conditioned for 4 h with methanol : water 70 : 30, v/v at 0.2 mL/min prior to testing.

Chromatographic Tests

Columns were tested with the mixture: acetone, benzonitrile, benzene, toluene, and naphthalene. The injection volume was 5 μL and UV detection was at 254 nm. Mobile phases tested were MeOH : H_2O 50 : 50, 60 : 40, and 70 : 30, v/v, which showed (via van Deemter plots) optimum flow rates in the range 0.3 to 0.4 mL/min. All columns were operated at room temperature.

The column dead time (t_M) was obtained using methanol as marker compound. Columns were evaluated utilizing the following parameters: efficiency (N/m), asymmetry factor (As) at 10% peak height, resolution (Rs), retention factor (k), and separation factor (α).

One column was tested for the possible separation of a mixture of herbicides (bentazon, cyanazine, simazine, fluazifop acid, atrazine, diuron, and ametrin). The mobile phase utilized was MeOH : H_2O 60 : 40, v/v, pH $= 4.6$ (with phosphoric acid).

In a stability comparison test of this column with a commercial column (Rainin C-18, 10 μm), each was subjected to the procedure of Fonseca and Collins (21) until column collapse, as indicated by a large drop in column efficiency. This test utilizes the alkaline mobile phase (MeOH : $NaHCO_3$ 0.1 mol/L, 50 : 50, v/v with aqueous NaOH (pH = 10) at 60°C.

Chemical Tests

Potentiometric titration was realized with 0.5 g of silica or zirconized silica or zirconized C-18 coated material suspended in 10 mL of a 10% aqueous solution of NaCl and titrated with 0.01 mol/L NaOH. For titrations of C-18 material, 3 mL of isopropanol were also added. The titration end point was defined as pH 9, utilizing a glass electrode.

Zirconized packing materials recovered from columns after use were submitted for Elemental Carbon Analysis.

Physical Tests

Infrared spectra (4000–500 cm^{-1}) were obtained for silica, PMODS, zirconized silica, and zirconized C-18 (PMODS) packing material. Spectra were obtained with KBr pellets.

Surface areas were measured for silica, zirconized silica, and zirconized C-18 packing material using the BET method with N_2 adsorption.

Specific volumes were determined by nitrogen sorption.

Thermogravimetric analyses were obtained with 6 mg samples at 10°C/min, over the temperature range of 100–900°C.

X-ray fluorescence determinations of ZrO_2 were made by means of an analytical curve obtained with ZrO_2 standard samples.

RESULTS AND DISCUSSION

Characterization of Silica, Zirconized Silica, and Zirconized C-18 Packing Material

Potentiometric titration determinations of residual silanols present on the several surfaces are given in Table 1. These data show that silanol concentration decreases with zirconization and, markedly, with PMODS coverage.

Table 1. Results of Surface Area, Specific Volume and Potentiometric Titration (Silanols) for Silica, Zirconized Silica and Zirconized Silica with PMODS Reversed Phase

	Surface Area (m^2/g)	Specific Volume (mL/g)	Residual Silanols (mmol/g)
Silica	387	0.8	0.500
Zirconized Silica	372	0.6	0.461
Zirconized C-18 Phase*	0.6	0.0007	0.0198

*These values refer to the initially loaded phase.

Infrared spectra were obtained to determine the surface concentration of silanol groups for the three materials. The results for silica, zirconized silica, and zirconized C-18 packing material (Fig. 1) show that there is little, if any, decrease in concentration of silanol groups due to zirconization, as shown by the decrease in the intensity of the 975 cm^{-1} bands (characteristic of silanols), but that there was considerable coverage of these silanols by the PMODS.

The values of surface area and pore volume (Table 1) show small reductions resulting from the zirconization and great reductions after addition of the C-18 phase.

Elemental carbon analyses were made to allow the calculation of the amount of PMODS present in the zirconized reversed phase after packing. The elemental carbon determination (4.7% C), corresponding to 6.5% PMODS, demonstrates that a large fraction of the initial PMODS is lost (by solvent extration) during the packing operation. Similar results were observed by Melo and Jardim (12) and Silva and Collins (11), working with silica modified with zirconium and titanium, respectively, followed by coating with poly(methyloctylsiloxane) (PMOS) stationary phase. In our study, the same explanation given by Melo and Jardim (12), a lower retention of the PMOS on zirconized silica, can be applied. This behavior suggests that PMODS is poorly retained on zirconized silica surface, due to a decrease in the number of available silanol groups on the zirconized surface (Table 1).

Termogravimetric analysis was used to verify the thermal stability of the stationary phase. Only 5% of the PMODS mass is lost at 175°C, which can be attributed to cyclic oligomers present in the polymer, but considerable loss of mass occurs at 414°C.

Fluorescence analysis was used to determine the concentration of zirconium on the silica surface, after modification. This value was 16.2% (1.57 mmol of zirconia per gram of silica) corresponding to a high surface concentration of 4.5 $\mu mol/m^2$.

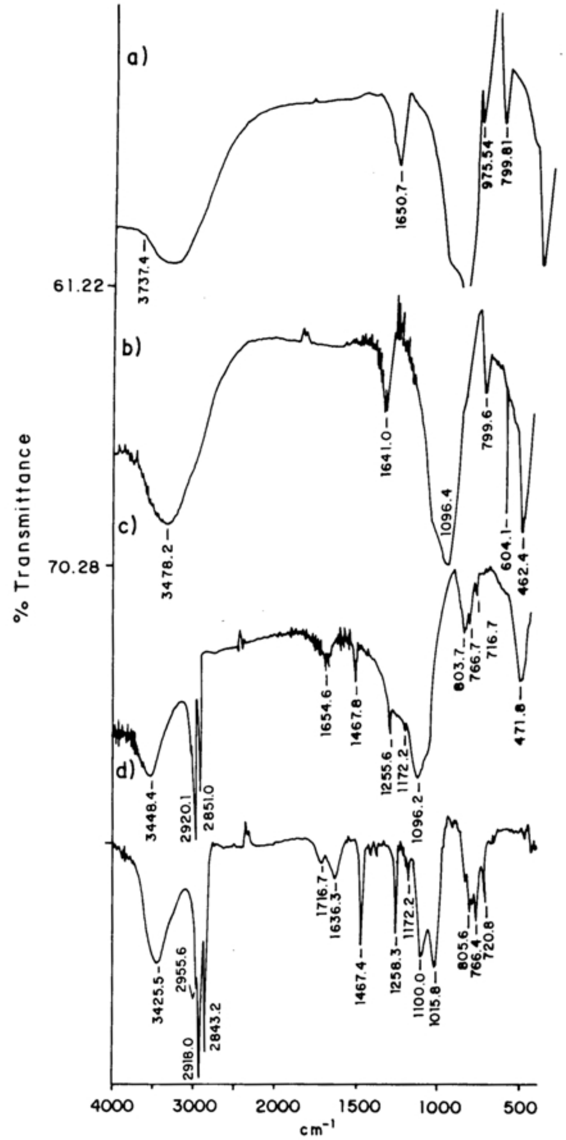

Figure 1. Infrared spectra: (a) silica, (b) zirconized silica, (c) zirconized and PMODS functionalized phase and (d) bulk PMODS.

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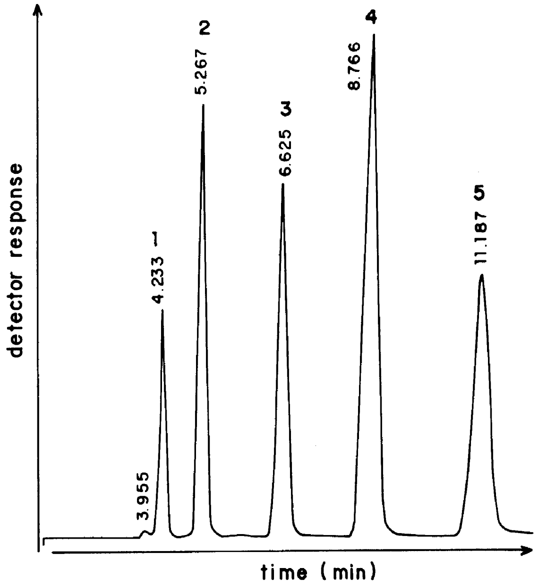

Figure 2. Chromatogram obtained with a column of zirconized and PMODS functionalized stationary phase. Column 150 × 3.9 mm i.d.; mobile phase MeOH : H_2O 70 : 30, v/v; flow rate = 0.4 mL/min; detection UV at 254 nm. Test sample components: 1 = acetone, 2 = benzonitrile, 3 = benzene, 4 = toluene, 5 = naphthalene.

Chromatographic Tests

Columns prepared from packing materials having PMODS stationary phase on zirconized silica had different reversed phase behaviors than columns made on unmodified silica, as shown in Figs. 2 and 3. Columns having PMODS stationary phase on the zirconized support give better separations as indicated by the data summarized in Table 2. Comparing at a flow rate of 0.4 mL/min and a mobile phase of 50 : 50 v/v, we observed that the parameters k and R_s are greater for the zirconized silica based column, probably because the amount of PMODS phase, although low, was higher than for the silica based column.

The good chromatographic parameters are surprising, because, with only 4.7% carbon (6.5% PMODS), the stationary phase gives good efficiency with symmetric peaks. These results are even more promising than those of Melo and Jardim (12).

Fig. 4 shows the herbicide separation using a column packed with the PMODS-functionalized zirconized silica packing material. Although, the

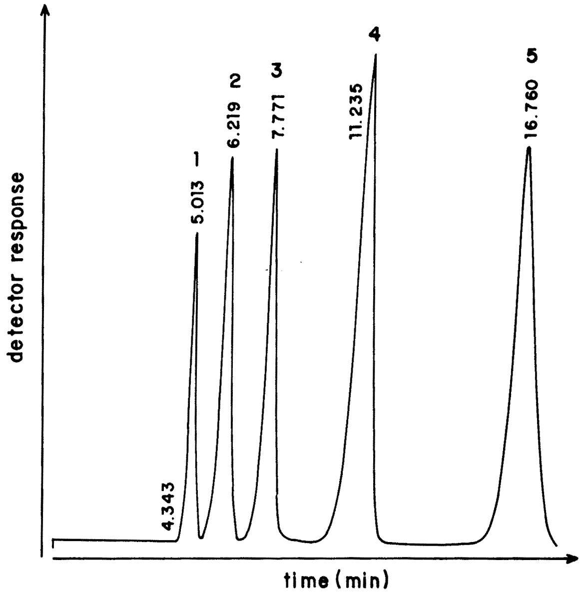

Figure 3. Chromatogram obtained with column having PMODS stationary phase on unmodified silica support. Column 150 × 3.9 mm i.d.; mobile phase MeOH : H_2O 50 : 50, v/v; flow rate = 0.3 mL/min; detection UV at 254 nm. Test sample components: 1 = acetone, 2 = benzonitrile, 3 = benzene, 4 = toluene, 5 = naphthalene.

Table 2. Chromatographic Evaluation of Columns Prepared with PMODS Functionalized Silica and PMODS Functionalized Zirconized Silica (10 μm)

Column	Flow Rate (mL/min)	Mobile Phase MeOH:H_2O (v/v)	t_R (min)[a]	N/m (m^{-1})[a]	As (%)[a]	k[a]	α[b]	Rs[c]
Silica + PMODS	0.4	50 : 50	12.7	15600	0.9	2.8	1.8	5.2
Silica + PMODS	0.3	50 : 50	16.8	17000	0.9	2.9	1.8	4.8
Zirc. Silica + PMODS	0.3	70 : 30	11.2	31400	1.1	1.9	1.7	3.0
Zirc. Silica + PMODS	0.4	50 : 50	33.9	25100	1.1	10.7	2.0	8.5
Zirc. Silica + PMODS	0.4	60 : 40	14.5	22750	0.9	4.0	1.7	5.5
Zirc. Silica + PMODS	0.4	70 : 30	7.9	25300	1.2	1.7	1.5	3.3

[a]For naphthalene peak; [b]For toluene-benzene pair; [c]For toluene-naphthalene pair.

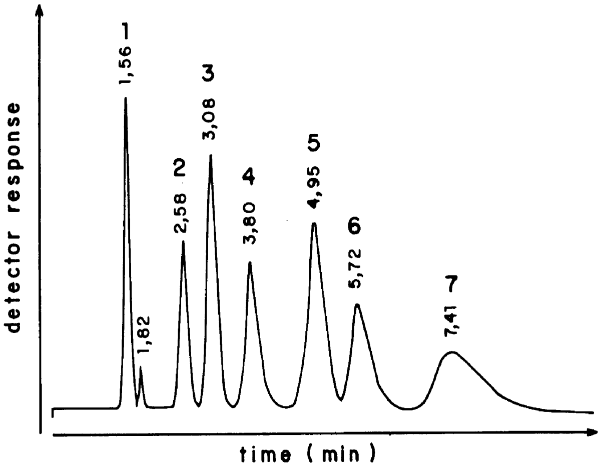

Figure 4. Chromatogram obtained with a column of zirconized and PMODS functionalized stationary phase. Column 150 × 3.9 mm i.d.; mobile phase MeOH : H_2O 60 : 40, v/v, pH = 4.6 (phosphoric acid), flow rate = 0.8 mL/min, detection at 230 nm. Herbicide mixture: 1 = bentazon, 2 = cyanazine, 3 = simazine, 4 = fluazifop acid, 5 = atrazine, 6 = diuron, 7 = ametrin.

efficiency was not high for this column, the chromatogram clearly shows good separation of all peaks even though these compounds have different chemical characteristics: bentazon and fluazifop acid are acidic, cyanazine, simazine, atrazine, and ametrin are basic and diuron is a neutral compound.

The stability tests showed that a column packed with PMODS functionalized zirconized silica survived with good efficiency, after treatment with 650 mL of an aggressive alkaline mobile phase, while a conventional C-18 bonded-phase column (packed with Rainin C-18 material) failed following treatment with 200 mL of this aggressive mobile phase (21).

CONCLUSION

Zirconium modified silica surfaces can be prepared with a surface concentration of 4.5 μmol/m^2 of zirconium by reaction of a solution of zirconium butoxide with HPLC silica. This zirconized silica can be functionalized with C-18 stationary phase by sorption of PMODS into the pore system. Although, only low carbon loadings (4.7% C) were obtained, infrared spectra showed almost no silanol group activity and the reversed phase chromatographic properties were quite acceptable.

Preliminary chromatography tests, with a mixture of herbicide compounds having widely differing chemical characteristics (acidic, basic, and neutral) show that zirconized silica support particles functionalized with PMODS is a promising type of packing material having reasonable separation efficiency (25000–30000 plates/m for 10 μm particles).

The stability of the new packing material against attack by an aggressive basic mobile phase was shown to be very good, significantly better than that of a good conventional C-18 bonded-phase material.

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