Article

Synthesis and Characterization of a Novel Stationary Phase, Si-Zr/Ti(PMTDS), Based upon Ternary Oxide Support for High Performance Liquid Chromatography

Maura R. Amparo, Fabiana A. Marques and Anizio M. Faria*

Química/FACIP, Universidade Federal de Uberlândia,
R. Vinte, 1600, Bairro Tupã, 38304-402 Ituiutaba-MG, Brazil

Uma nova fase estacionária baseada na imobilização térmica do poli(metiltetradecilsiloxano) (PMTDS) sobre partículas de sílica recoberta com mistura de zircônia e titânia foi preparada e avaliada pela separação cromatográfica de misturas testes neste trabalho. As partículas esféricas foram caracterizadas por análise elementar, MEV, infravermelho e ^{29}Si RMN. As propriedades físico-químicas da fase PMTDS com suporte misto se apresentaram intermediárias às de fases PMTDS baseadas em sílica titanizada e sílica zirconizada. O desempenho cromatográfico da fase Si-Zr/Ti(PMTDS) foi similar ao desempenho de fases contendo apenas um dos óxidos metálicos no suporte, a preparação da fase permitiu a observação do efeito de cada óxido no processo de separação e estabilidade da fase Si-Zr/Ti(PMTDS). A estabilidade hidrolítica da fase Si-Zr/Ti(PMTDS) foi similar a de fases PMTDS com suporte de sílica titanizada, e duas vezes mais estáveis que fases PMTDS com suporte de sílica.

A new stationary phase based on the thermal immobilization of poly(methyltetradecylsiloxane) (PMTDS) on silica particles coated with a mixture of zirconia and titania was prepared and evaluated for the chromatographic separation of test mixtures. The spherical particles were characterized by elemental analysis, SEM, FTIR and ^{29}Si NMR. The physicochemical properties of PMTDS phase supported on Si-Zr/Ti were intermediate between PMTDS phases supported on titanized silica and zirconized silica. The chromatographic performance of Si-Zr/Ti(PMTDS) phase was similar to PMTDS phases based on metal oxide coated silica having only one metal oxide and the preparation of a Si-Zr/Ti(PMTDS) phase allowed evaluation of the effect of each oxide, zirconia and titania, on the separation process and on the stability of the immobilized polymer phase. The hydrolytic stability of Si-Zr/Ti(PMTDS) stationary phase was similar to the Si-Ti(PMTDS) phase, improving the chemical stability of the silica-based PMTDS phase by about 100%.

Keywords: stationary phases, metal oxide coated silica, zirconia, titania, HPLC

Introduction

Chromatographic supports based on silica covered with metallic oxide layers have been efficiently used to prepare stationary phases for reversed phase liquid chromatography (RPLC).[1-8] Among the metal oxides more used for the modification of silica surface, zirconia[3,5,9,10] and titania[4,7,11-13] have received most emphasis. Different methods of preparation of silica covered with zirconia or titania layers has been reported, such as the layer-by-layer self-assembly technique,[2,7,12,14-16] reaction with metal alkoxides at low temperatures,[9-11,13,17-23] sol-gel process,[24,25] etc. These methods are capable of producing silica spheres with multiple layers of metal oxide. Metal oxide coated silica surfaces can be easily functionalized with organic modifiers to generate reversed stationary phases, either by chemically bonding[6,7,11,12] or by coating with pre-synthesized organic polymers.[5,9,10,13] Contrasting with bare zirconia and the bare titania chromatographic supports, the stationary phases chemically bonded or adsorbed/immobilized on mixed oxide supports show performances similar or superior to those achieved with the bare silica-based stationary phases.[7,10,14,15,20,22]

Stationary phases based on metal oxide coated silica supports have as important characteristics the preservation of the morphological properties of silica with an improvement of the chemical stability of silica under alkaline conditions due to the outer metal oxide

*e-mail: anizio@pontal.ufu.br

layers.[5,7,13,26,27] Thus the chromatographic performances of these metal oxide coated silica-based stationary phases are very similar to those obtained with the bare silica-based stationary phases, however with longer lifetimes under alkaline conditions.[21,27]

Although a significant number of studies involving metal oxide coated silica-based stationary phases has been published in recent years, the influence of the metal oxide in the retention parameters of solutes still is not clear. Stationary phases based on zirconized silica or titanized silica supports do not seem to have the same retention characteristics.[13,14,19,22] Furthermore, separations with zirconized and titanized silica using more alkaline mobile phases also result in different lifetimes for the stationary phases.[5,26]

In this work we have prepared a new stationary phase based on a ternary oxide support, which consisted of pre-synthesized silica coated with a double layer of titania and zirconia, to compare the influence of metal oxides on retention parameters in chromatographic separations. Comparisons with similar stationary phases containing titanized silica and zirconized silica as chromatographic supports were also performed to clarify the behavior of individual oxides in separations using standard test mixtures. The chemical stability of this stationary phase was also evaluated to determine the effect of both metal oxide layers on the lifetime of metal oxide coated silica-based stationary phases.

Experimental

Reagents

All chemicals were reagent, analytical-reagent or HPLC grade and were used as received unless otherwise specified. Kromasil silica with average particle size of 5 μm, mean pore diameter of 11.1 nm and specific surface area of 343 m^2 g^{-1} was purchased from Akzo Nobel (Bohus, Sweden). Titanium tetrabutoxide and zirconium tetrabutoxide from Sigma-Aldrich (Milwaukee, WI, USA) were used to prepare the chromatographic support. Poly(methyltetradecylsiloxane), PMTDS, was purchased from Petrarch (Piscataway, NJ, USA).

Synthesis of zirconized/titanized-silica (Si-Zr/Ti)

Zirconized/titanized silica was synthesized using a procedure similar to those used for preparation of individual zirconized[3] and titanized silica.[13] Briefly, chromatographic silica, 5.0 g, was added to the solution containing a mixture of zirconium(IV) and titanium(IV) tetrabutoxides, dissolved

in 25 mL of anhydrous toluene. This mixture was placed in a thermostated bath for 5 h at 40 °C. After this period, the solution was centrifuged for 15 min, the supernatant was discarded and the resulting solid washed with anhydrous toluene and again centrifuged. This washing was repeated five times. The material was hydrolyzed with 15 mL of 1×10^{-3} mol L^{-1} aqueous HNO_3, and centrifuged for 15 min. The solid was washed with 15 mL of ultrapurified water, centrifuged for 10 min, and then dried in an oven at 120 °C for 12 h. This procedure was repeated one more time adding the same quantities of zirconium and titanium tetrabutoxides. Small portions of Si-Zr/Ti were submitted to morphological and structural characterizations.

Preparation of PMTDS immobilized stationary phase

Zirconized/titanized silica, 2.00 g, was dried at 140 °C for 12 h and added to a 5% m/v solution of PMTDS in hexane at a specific mass of 0.8 $g_{PMTDS}/g_{Si-Zr/Ti}$. The mixture was slowly stirred for 3 h at room temperature. The hexane was allowed to evaporate, without stirring, at room temperature for 5 days.

The sorbed PMTDS on zirconized/titanized silica was placed in a stainless steel tube (150 mm × 10 mm i.d.), which was fitted with frits and connectors and placed in a tubular oven to induce thermal immobilization. Thermal treatment was performed at 110 °C for 12 h under a low nitrogen flow. After immobilization, the stainless steel tube containing the PMTDS immobilized phase was connected to a Waters 510 pump (Milford, MA, USA) for extraction of non-immobilized PMTDS by passing hexane at 0.5 mL min^{-1} at 50 °C for 4 h.

Column packing

Columns, 60 mm × 3.9 mm i.d., were made from stainless steel tubing. The columns were slurry packed using 5% m/v slurries of stationary phases in chloroform, using a constant packing pressure of 42 MPa with methanol as propulsion solvent, using a Haskell packing pump (Burbank, CA, USA). Columns were conditioned for 1 h with mobile phase at 0.4 mL min^{-1} prior to the chromatographic evaluations.

Physicochemical characterizations

A structural study of the Si-Zr/Ti support and Si-Zr/Ti(PMTDS) phase was carried out by spectroscopic techniques. FTIR spectra were determined by using a Bomem MB-102 FTIR spectrometer (Hartmann & Braun, St-Laurent, Canada) equipped with a diffuse

reflectance accessory. Solid-state ^{29}Si NMR experiments were performed on an INOVA spectrometer (Varian, Palo Alto, CA, USA) using cross-polarization and magic angle spinning (CP-MAS). A morphological study of the Si-Zr/Ti support and Si-Zr/Ti(PMTDS) phase was made through micrographs of the particles at different magnifications. The samples were sputter-coated with gold and then examined at 20 kV using a JEOL model JSM-6360LV scanning electron microscope (Tokyo, Japan). Elemental analysis was performed with a CHN-2400 PerkinElmer Analyzer (Shelton, CT, USA). The quantities of zirconium and titanium incorporated onto silica were determined using a Shimadzu model XRF-700 spectrometer (Kyoto, Japan) with micro sample holders. The analytical curve was prepared using mechanical mixtures of pure silica and a ZrO_2 or TiO_2 powder, containing from 0 to 21% m/m Zr and from 0 to 15% m/m Ti.

Chromatographic studies

All chromatographic data were collected using a modular HPLC system with a binary ProStar 210 pump controlled by Galaxie® software (Varian). The instrument is equipped with a Rheodyne Model 7125i valve with 20 mL loop and ProStar 325 UV-vis detector. All solutes were detected at 254 nm unless otherwise noted. Experiments were carried out at 25 °C. The mobile phases were prepared volumetrically from individually measured amounts of each solvent. All solvents were filtered and degassed before use. The mobile phase consisted of methanol:water or methanol:phosphate buffer (70:30, v/v) using a flow rate of 0.4 mL min^{-1}. Column dead times were measured from the retention time of the uracil peak.

Stability evaluations

Si-Zr/Ti(PMTDS) stationary phase were submitted to stability testing using methanol:5.0 x 10^{-2} mol L^{-1} KHCO$_3$/K$_2$CO$_3$ (70:30 v/v) at pH 10 as a mobile phase. The pH was adjusted with a calibrated pHmeter before addition of methanol. The flow rates through the columns were 1.0 mL min^{-1} at 50 °C. These conditions were used to accelerate column deterioration, obtaining faster evaluations with considerably less mobile phase and instrument time.[28] The columns were periodically tested for the separation of a test mixture. The column volume, 0.45 mL, was calculated from the retention time of uracil. The hydrolytic stability of Si-Zr/Ti(PMTDS) was compared to the stability of similar PMTDS phases immobilized onto silica, zirconized silica and titanized silica particles.

Results and Discussion

### Characterizations of the Si-Zr/Ti support and the Si-Zr/Ti(PMTDS) phase

One of the main characteristics of metal oxide coated silica is the maintenance the morphological properties of chromatographic silicas, such as their shape and pore structure. SEM images were obtained of particles after preparation of Si-Zr/Ti support and Si-Zr/Ti(PMTDS) phase to view size distribution and shape of these particles, see Figure 1.
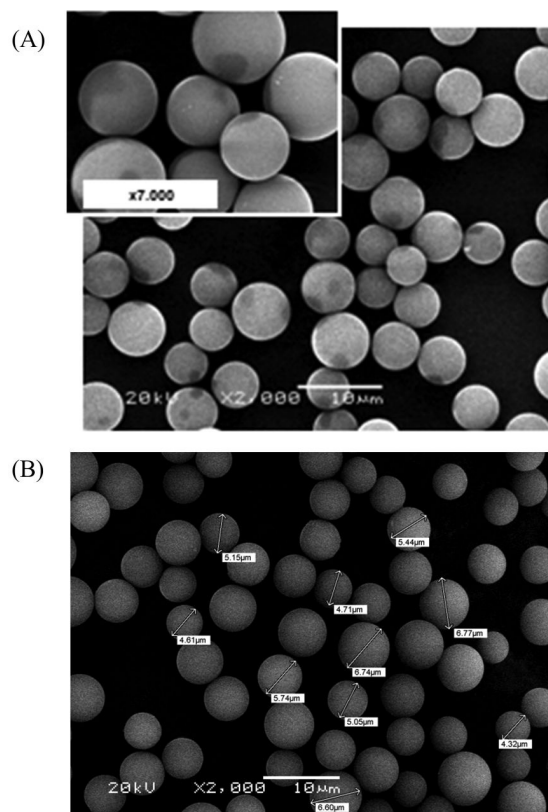
Figure 1. SEM images of (A) Si-Zr/Ti particles and (B) Si-Zr/Ti(PMTDS) stationary phase. Inset image: magnifications at 7000x.

SEM images indicate that the preparation process of Si-Zr/Ti support did not affect the morphological structure of the silica particles. The Si-Zr/Ti support has maintained the intact spherical particles. The particle size apparently did not suffer any influence of the zirconia/titania double layer, Figure 1A. The thermal treatment to immobilize PMTDS onto Si-Zr/Ti support also did not significantly affect the shape and the size of the particles, Figure 1B. The solvent characteristics of polysiloxanes have maintained stationary phase particles without agglomeration, which is crucial to obtaining a high degree of particle packing in chromatographic columns.

The chemical structures of the Si-Zr/Ti support and Si-Zr/Ti(PMTDS) phase were evaluated by using spectroscopic techniques such as FTIR and ^{29}Si NMR. Figures 2A and 2B show the spectra of silica before and after the metallization process, respectively. The FTIR spectrum of Si-Zr/Ti(PMTDS) phase is shown in Figure 2C.
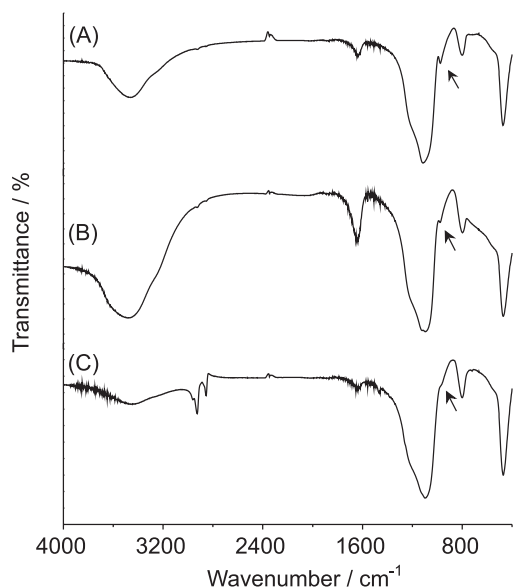
Figure 2. FTIR spectra of (A) bare silica, (B) Si-Zr/Ti support and (C) Si-Zr/Ti(PMTDS) stationary phase.

The principal signals in FTIR spectrum of Si-Zr/Ti support occurred at 3470, 1100 and 980 cm^{-1}. The large band at 3470 cm^{-1} is attributed to the presence of hydroxyl groups from silica, zirconia and titania and also to water adsorbed on support. The intense signal at 1100 cm^{-1} is attributed to stretching of siloxane bonds (Si-O-Si). The small peak at 980 cm^{-1} refers to the vibrations of isolated silanols (Si-OH). The existence of a signal at 980 cm^{-1} in the Si-Zr/Ti spectrum indicates the presence of silanol groups remaining after the process of incorporation of zirconia and titania. The presence of these groups is still observed even after the PMTDS immobilization as a small shoulder, as can be seen in Figure 2C. The signals appearing at 2850 and 2930 cm^{-1} in Si-Zr/Ti(PMTDS) spectrum are assigned to C-H stretching from the CH_2 and the CH_3 groups, respectively, confirming the presence of PMTDS on the support particles. Residual silanol groups, not covered by polymer, retain compounds by adsorption in reversed stationary phases, which may result in order of elution also ruled by adsorption sites. The major problem associated with incomplete covering of active surface groups of the chromatographic support is a strong retention of basic compounds, since these groups are strong adsorption sites.

^{29}Si NMR spectra, Figure 3, shows that a significant amount of vicinal and isolated silanol groups, HO-Si-O-Si-OH and Si-OH, remained on the Si-Zr/Ti particles after the incorporation of zirconia and titania. In the spectrum of Figure 3B it can be seen that the PMTDS immobilization occurred on the surface groups of the Si-Zr/Ti support due to the reduction of ratio between the signals of vicinal and isolated silanol groups and siloxane linkages, when compared with the Si-Zr/Ti spectrum, Figure 3A. The PMTDS immobilization did not completely cover the vicinal and isolated silanol groups from the support, confirmed by the intense signal at -110 ppm, corroborating with FTIR results. The signals at -15 and -25 ppm refer to the attachment of PMTDS onto the support. The amount of polymer immobilized onto the support was obtained by elemental analysis. The carbon content in the Si-Zr/Ti(PMTDS) phase was intermediate between those achieved for the Si-Zr(PMTDS) and the Si-Ti(PMTDS) phases, as shown in Table 1. There is also
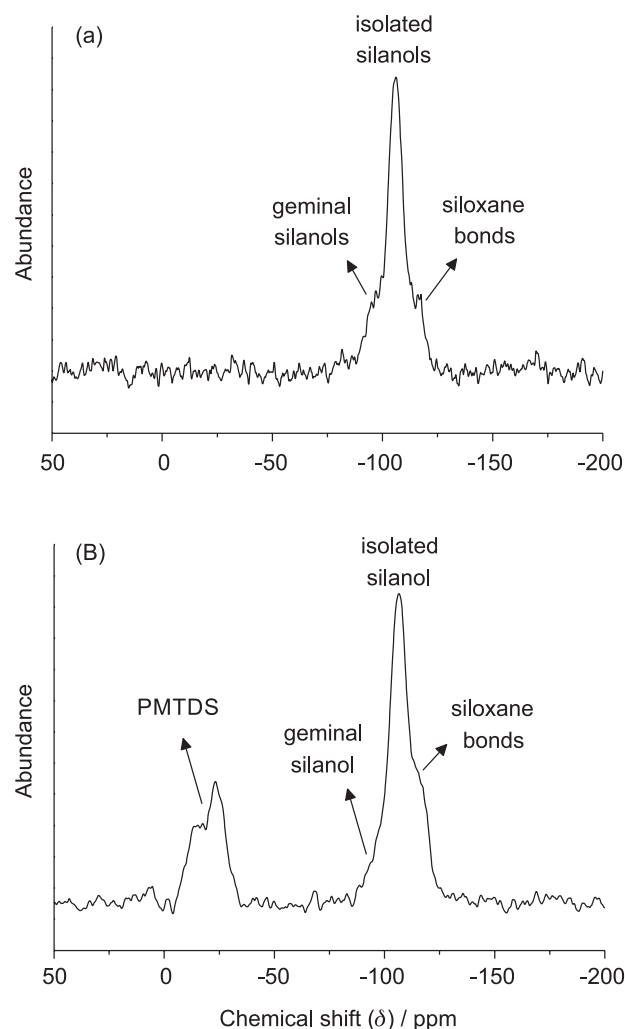
Figure 3. ^{29}Si NMR spectra of (A) Si-Zr/Ti support and (B) Si-Zr/Ti(PMTDS) stationary phase.

a reduction in the amount of either zirconium and titanium incorporated onto the silica surface when compared with metal oxide coated silicas containing only one metal oxide.

Table 1. Physical characterization of stationary phase from PMTDS immobilized onto metal oxide coated silica supports

| Stationary phase	% C	Zr / (μmol m^{-2})	Ti / (μmol m^{-2})	REF.
Si-Zr/Ti(PMTDS)	12.8	3.6	1.9	-
Si-Zr(PMTDS)	11.3	5.4	-	3,19
Si-Ti(PMTDS)	14.1	-	3.3	13
Si(PMTDS)	20.6	-	-	29

Chromatographic separations

The Si-Zr/Ti(PMTDS) stationary phase was chromatographically evaluated by separation of two test mixtures. The first mixture, consisting of apolar compounds, was separated by Si-Zr/Ti(PMTDS) phase to evaluate reversed phase elution. The chromatogram of Figure 4 indicates that the elution order of the compounds is predominantly by a reversed phase separation mechanism, i.e., the separation occurs due to interactions of compounds with the polymeric layer of PMTDS phase. The retention parameters of compounds in the Si-Zr/Ti(PMTDS) phase presented intermediate values when compared to the stationary phases with PMTDS thermally immobilized on zirconized silica (Figure 4A) and titanized silica (Figure 4C). These results are well-suited to the amount of immobilized polymer on the support surfaces.
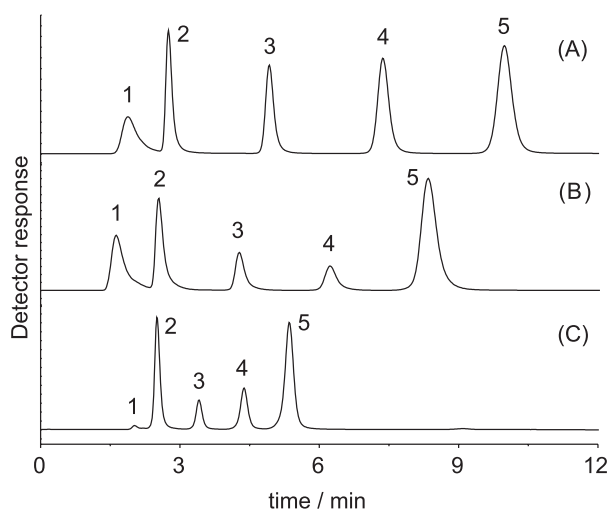
Figure 4. Chromatograms showing the separation of a test mixture on a PMTDS thermally immobilized onto (A) zirconized silica, (B) zirconized/titanized silica and (C) titanized silica. Mobile phase: methanol:water (70:30, v/v); flow rate: 0.4 mL min^{-1}; injection volume: 20 mL; UV detection: 254 nm and room temperature. Peak identifications: (1) uracil, (2) benzonitrile, (3) benzene, (4) toluene and (5) naphthalene.

The mixed mode performance in the separation, i.e., the contribution of residual active groups from Si-Zr/Ti support was evaluated by the separation of a test mixture adapted from the Engelhardt and Jungheim test mixture.[30] This mixture, containing an acidic compound, phenol and a basic one, N,N-dimethylaniline, was analyzed by Si-Zr/Ti(PMTDS) phase and the retention properties were indicative of contributions of some residual groups in the chromatographic separation. The chromatogram of separation of this test mixture is presented in Figure 5.
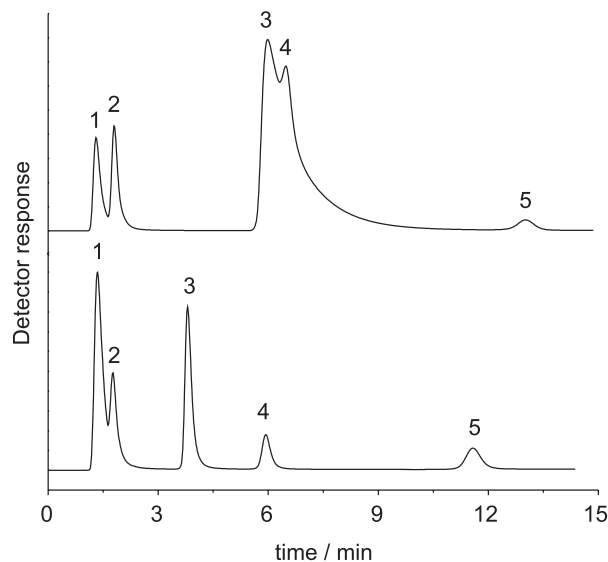
Figure 5. Chromatograms showing the separation of a test mixture on Si-Zr/Ti(PMTDS) stationary phase. Mobile phase: (A) methanol:water (70:30, v/v) and (B) methanol: 5 x 10^{-3} mol L^{-1} KH$_2$PO$_4$/K$_2$HPO$_4$ at pH 7.00; flow rate: 0.4 mL min^{-1}; injection volume: 20 mL; UV detection: 254 nm and room temperature. Peak identifications: (1) uracil, (2) phenol, (3) N,N-dimethylaniline, (4) toluene and (5) naphthalene.

The separation of the test mixture by the Si-Zr/Ti(PMTDS) phase resulted in a strong retention for the N,N-dimethyl-aniline peak, causing a long tailing peak and coelution with the toluene peak. Furthermore, the phenol peak has low retention on the phase and elutes shortly after the dead time marker peak of uracil. These facts suggest some acidity to the Si-Zr/Ti(PMTDS) phase as well as a contribution from adsorption in the separation process. However, the retention of basic compounds by the adsorption mode can be minimized by controlling the pH of the mobile phase. The use of a buffer solution at pH 7.00 in the aqueous component of the mobile phase reduces the interactions with support surface and allows the basic compounds to elute with symmetrical peaks, as shown in the chromatogram of Figure 5B. To reduce such surface interactions, the Lewis acid sites on zirconia and on titania were blocked by adsorbing a hard Lewis base such as phosphate.

Table 2. Chromatographic performance of PMTDS phases supported on the different metal oxide coated silicas

Stationary phase	Chromatographic parameter						
	N/m[a]	$A_{s\,10\%}$[a]	$A_{s\,10\%}$[b]	$A_{s\,10\%}$[b,e]	k[a,c]	R_s[d]	REF
Si-Ti(PMTDS)	67,700	1.0	2.0	0.9	1.5	3.1	13
Si-Zr(PMTDS)	80,600	1.1	4.0	1.2	3.3	4.9	19
Si-Zr/Ti(PMTDS)	70,600	1.2	> 5.0	1.6	4.1	4.0	-

[a]Calculated for the naphthalene peak; [b]calculated for the N,N-dimethylaniline peak; [c]t_M measured from retention time for the uracil peak; [d]calculated for the toluene-naphthalene pair; [e]mobile phase buffered at pH 7.00 (phosphate, 5.0×10^{-2} mol L^{-1}).

The chromatographic performance of Si-Zr/Ti(PMTDS) phase is summarized in Table 2, which shows similar performance, in terms of column efficiency, when compared with PMTDS phases supported on Si-Zr or Si-Ti particles. The strong retentions for basic compounds were also observed in Si-Zr(PMTDS) phases, showing that the influence of zirconia in the separation process is more significant than that of titania. This fact may be related to the metal oxide content, as there is more zirconium than titanium, incorporated into the support. A higher reactivity of zirconium oxide could also contribute.

The stationary phase based on ternary oxide support, Si-Zr/Ti(PMTDS), allows to evaluate the influence of both titanium and zirconium oxides in the retention parameters of reversed stationary phases. The phase Si-Zr/Ti(PMTDS) provides a larger retention of nonpolar substances that the Si-Ti(PMTDS) phase, as can be seen in Figure 4 and Table 2. The reactivity of titanized silica surface is smaller than the zirconized silica one. This fact results in a smaller amount of immobilized PMTDS on the metalized silica support and, consequently, in a lower hydrophobicity of its stationary phase. Additionally, Si-Zr/Ti(PMTDS) phase presented symmetrical peaks for basic compounds using buffered mobile phases at pH 7.00, while the Si-Zr(PMTDS) phase shows similar behavior using buffered mobile phase at pH 10.00. Therefore, the Si-Zr/Ti(PMTDS) phase has advantages such as the higher peak capacity and the lower residual retention for basic compounds compared to the Si-Ti(PMTDS) and the Si-Zr(PMTDS) phases, respectively, presenting a similar chromatographic performance.

Stability studies

The chemical stability of Si-Zr/Ti(PMTDS) phase was evaluated by monitoring the column efficiency for acenaphthene and N,N-dimethylaniline, apolar and basic compounds, respectively, after continuous passing of alkaline mobile phase. The mobile phase composition used for the stability test was MeOH:5.0×10^{-2} mol L^{-1} KHCO$_3$/K$_2$CO$_3$ at pH 10.00 (70:30, v/v) and the flow rate

was 1.0 mL min^{-1} with the temperature controlled at 50 °C. Figure 6 shows the degradation profile of Si-Zr/Ti(PMTDS) phase with the performance loss for the acenaphthene and the N,N-dimethylaniline peaks.
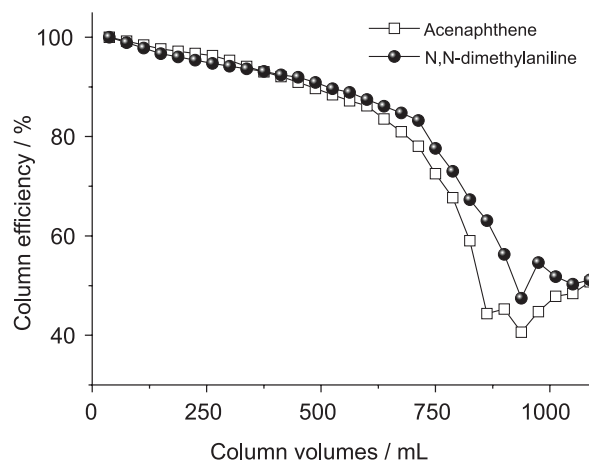
Figure 6. Variation of column efficiencies of the Si-Zr/Ti(PMTDS) stationary phase. Chromatographic conditions: mobile phase methanol:5.0×10^{-2} mol L^{-1} KHCO$_3$/K$_2$CO$_3$ at pH 10. Test probes: acenaphthene and N,N-dimethylaniline.

The sharp drop in column efficiency in Figure 6 is associated with the dissolution of significant quantities of chromatographic support in these conditions, making it unsuitable for chromatographic applications, after passage of approximately 930 column volumes. It is also observed that the behavior of column efficiency did not depend on the monitored compounds, either apolar or basic. The resistance of Si-Zr/Ti(PMTDS) phase in alkaline mobile phase is similar to that of Si-Ti(PMTDS) phase but lower than that observed with the Si-Zr(PMTDS) phase, as can be seen in Figure 7. This fact indicates that, in terms of chemical stability, the behavior of Si-Zr/Ti(PMTDS) phase seems to reflect a more significant influence of titania. The gain of chemical stability due to the presence of titania and zirconia onto the support particles becomes evident when compared to the silica-based PMTDS phase as the stability of Si-Zr/Ti(PMTDS) phase was about two times higher.
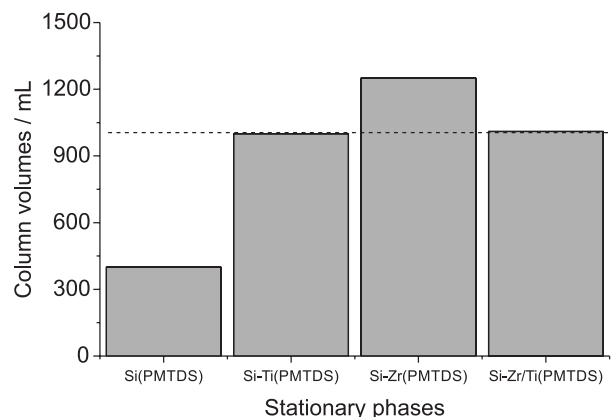
Figure 7. Hydrolytic stability of laboratory-made PMTDS stationary phases. Column volumes (calculated from mobile phase retention determined using uracil) that causes a 50% decrease in the column efficiency measured with acenaphthene while passing mobile phase MeOH:5.0×10^{-2} mol L^{-1} KHCO$_3$/K$_2$CO$_3$ at pH 10, using a temperature at 50 °C.

Conclusions

The thermal immobilization of PMTDS onto silica coated with two layers of titania/zirconia is presented as a simple and easy way of preparing a HPLC stationary phase. The principal characteristic of the Si-Zr/Ti particles is the maintenance of morphological properties of chromatographic silica, such as its spherical shape and particle size. However, the immobilization of PMTDS does not completely cover the active sites of the zirconized/titanized silica surface, resulting in separations driven by both partition and adsorption mechanisms. The high acidity of Si-Zr/Ti(PMTDS) phase promotes a strong retention of basic compounds, also common for zirconized silica-based PMTDS phases. The hydrolytic stability of the Si-Zr/Ti(PMTDS) phase in alkaline conditions is similar to that of a titanized silica-based PMTDS phase and about two times higher than for a bare silica-based PMTDS phases. The presence of zirconia and titania in the Si-Zr/Ti(PMTDS) phase influences different properties. The zirconia affects the surface reactivity of the stationary phase and its retention parameters for the polar and basic compounds while the titania influences the chemical stability of the Si-Zr/Ti(PMTDS) phase. This new stationary phase can be used to separate any type of polar and nonpolar compounds and, especially, basic substances when are used buffered mobile phases.

Acknowledgements

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