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Article

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Evaluation of Polymer Self-Coating on Aluminized Silica Support as Stationary Phase for High-Performance Liquid Chromatography

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The physical adsorption of a polymer, poly(3,3,3-trifluoropropylmethylsiloxane), on aluminized silica particles, without any additional procedure for polymer immobilization, was studied for 180 days to evaluate the new chromatographic support. Morphological, structural, and chromatographic evaluations of the stationary phase were assessed. After the 15th day of adsorption, a sufficient polymer amount adsorbs onto the aluminized silica particles to separate mixtures of compounds in reversed-phase mode. However, after the 60th day, the polymer layer adhesion occurs more strongly, self-coating the aluminized silica particles. The self-coated stationary phases after 60 days showed similar chromatographic profiles with low hydrophobicity and more interactions with polar compounds, characteristics arising from the fluorinated polymer. The aluminized silica support firmly adhered to the polymer layer, resulting in a separation material chemically stable at alkaline conditions, suggesting a surface reactivity like other well-established supports without asymmetrical peaks for polar compounds.

Keywords: fluorinated polymer, polar compounds, HPLC, reversed-phase, mixed oxides

Introduction

For over 50 years, chemically bonded silica-based stationary phases have been the primary separation materials for liquid chromatography, whether as a stationary phase for normal phase liquid chromatography (NP-LC), size-exclusion chromatography (SEC), and hydrophilic interaction liquid chromatography (HILIC) or as a chromatographic support for reverse-phase liquid chromatography (RP-LC), chiral selectors, ion chromatography (IC).[1-8] Morphological characteristics such as high surface area and mesoporous structure with geometry that facilitates access of solutes and ease of surface modification are two of the main factors that make silica an almost ideal chromatographic separation material.[2,9-11]

However, silica-based separation materials have two substantial disadvantages that limit further applications of liquid chromatography, particularly in polar compound separation. The first one is associated with the chemical stability of silica restricted to mobile phase conditions below pH 8 since silica solubilization is accelerated in an alkaline medium.[11-14] Furthermore, silica-based bonded phases undergo hydrolysis below pH 2, cleaving the organic layer from the support surface.[11-13,15] Another disadvantage is associated with the low bonding density with the chemical modification of the silica surface with organosilane reagents, leaving about 50% of the active groups accessible to undesirable interactions with sample solutes.[11,12,15,16] These residual groups affect the chromatographic retention of polar compounds and may lead to the irreversible retention of more basic compounds.

The substitution of silica for metal oxides, such as zirconia and titania, stable in broader pH ranges, was one of the strategies adopted to overcome the disadvantages.[13,15,17-19] However, the poorer chromatographic performance due to the pronounced differences in pore characteristics compared with silica limited more application of these oxides.[5,13,15,17] Alumina was also evaluated as a substitute for silica, but it was more effective as a stationary phase for NP-LC since it has morphological characteristics closer to silica.[13,15,20,21] As chromatographic support, alumina presented very low organic modification densities, restricting more applications in RP-LC.[11,17,20]

Another strategy adopted was coating silica particles with metal oxide layers, especially zirconia, and titania.[22-28] Despite the significant improvement in chemical stability of the stationary phases with these supports, the activity of the

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residual groups was more intense than bare silica support, retaining basic compounds irreversibly.[25,29] Alumina has become a potential candidate for modifying the silica support once its lower surface reactivity than those metal oxides and its morphological structure is closer to silica. Aluminosilicate geopolymers, which consist of an amorphous polycondensed network of Si−O−Al bonds, have been proposed as a stationary phase material for liquid chromatography, presenting high chemical stability in HILIC, NP-LC, and IC modes.[30,31] The geopolymer has shown promising results for hydrophilic analytes with unique selectivity, symmetrical peaks, and high hydrolytic stability under alkaline conditions. These aspects were associated with the presence of alumina in the stationary phase.[30,31] Coating silica with alumina layers, therefore, aims to increase the resistance of the stationary phase in more alkaline conditions without loss of chromatographic performance and increased residual activities. Our group developed aluminized silica particles, synthesizing alumina nanoparticles by the sol-gel process inside reverse surfactant micelles and depositing them on the surface and pores of chromatographic silica.[32,33] Aluminized silica particles showed morphological characteristics similar to silica and significantly increased lifetime in alkaline conditions. However, the reactivity of aluminized silica surfaces and their potential for use as a stationary phase has not yet been studied.

Thus, in this work, we performed an adsorption study of a pre-synthesized polymer onto aluminized silica particles to evaluate the self-coating capacity of the support and its application as a stationary phase for RP-LC. The activity of the surface groups of aluminized silica particles without additional steps for polymer immobilization was evaluated. Also, poly(3,3,3-trifluoropropylmethylsiloxane) (PTFPMS) was chosen for this study because it has less reactivity with the surface of inorganic oxides since it requires more drastic conditions for immobilization in silica than other pre-synthesized polysiloxanes.[34,35] The physicochemical, morphological, and chromatographic characteristics of the PTFPMS self-coating onto aluminized silica particles were evaluated.

Experimental

Reagents and materials

For the preparation, characterization, and applications of the stationary phases, the following materials and reagents were used: nitric acid 65% (Carlo Erba, Milan, Italy); ultrapure water at 18.2 MΩ cm; *n*-butyl acetate 99.5%, isooctane 99%, and ammonium acetate (Dinâmica, Diadema, Brazil); aluminum isopropoxide 99.5%, aniline 99.5%, benzene 99.9%, benzylamine 99%, butylbenzene 99.5%, ethylbenzene 99%, naphthalene 99%, *N*,*N*-dimethylaniline 99.5%, pentylbenzene 99%, *o*-terphenyl 99%, sodium bis-2-ethylhexylsulfosuccinate Na(AOT) 99.9%, toluene 99.9%, triphenylene 98%, and uracil 99% (Sigma-Aldrich, São Paulo, Brazil); caffeine 98% and phenol 99.5% (Vetec, Rio de Janeiro, Brazil); isopropanol (Êxodo Científica, Sumaré, Brazil); toluene, chloroform, acetonitrile, and methanol HPLC-grade (Tedia, Rio de Janeiro, Brazil); poly (3,3,3-trifluoropropylmethylsiloxane) (PTFPMS) (Figure S1, Supplementary Information (SI) section), Mw 4600 g mol^{-1}, 1000 cSt, CAS No. 63148-56-1 (Gelest Inc., Morrisville, USA); Kromasil silica, 5 µm diameter (AkzoNobel, Bohus, Sweden); monoacid and diacid potassium phosphate (JT Baker, Xalostoc, Mexico). Methomyl 98.0%, thiophanate-methyl 98.0%, carboxin 98.0%, terbuthylazine 98.0%, and difenoconazole 95.0% were Pestanal® analytical standards acquired from Sigma-Aldrich.

One-pot synthesis of aluminized silica particles

Reverse micelles were prepared from an anionic surfactant, the sodium bis(2-ethylhexyl)sulfosuccinate (sodium Aerosol OT or Na(AOT)). The synthesis of aluminized silica nanoparticles followed the procedure adopted by Silveira *et al.*[32] Initially, a surfactant solution was prepared by adding 2.44 g of Na(AOT) in 36 mL of isooctane and heating it to 100 °C under magnetic stirring. After 10 min, 1.00 mL of ultrapure water and 0.25 g of aluminum isopropoxide were added to the reaction medium, keeping it under constant agitation at 100 °C for two hours. After that, 0.90 mL of HNO_3 solution at 20 mmol L^{-1} and 1.50 g of Kromasil silica, previously activated at 140 °C for 12 h, were added to the reactional medium. The reaction was maintained for 24 h at 100 °C under constant stirring. The reactional medium was cooled to room temperature (ca. 25 °C), and the aluminized silica was separated by centrifugation at 2500 rpm for 3 min. The aluminized silica particles were sequentially washed to remove unreacted reagents with 2 mL of isooctane, isopropanol, methanol, and water by centrifugation at 2500 rpm for 3 min, discarding the supernatants. The aluminized silica particles were dried at 120 °C for 12 h. The dried particles were transferred to a porcelain crucible and calcined at 500 °C for one hour.

Self-coating of the aluminized silica particles with PTFPMS

The adsorption of PTFPMS on aluminized silica particles was studied for six months (180 days) to evaluate the surface activity of the new chromatographic support. A 10% (m/v) PTFPMS in *n*-butyl acetate solution was prepared. *N*-Butyl acetate was chosen in a preliminary test, in which it showed greater PTFPMS solubility. Aluminized silica, previously activated at 140 °C for 12 h, was added to the PTFPMS solution to obtain a polymer loading of 0.8 $g_{PTFPMS}/g_{support}$. The reaction occurred under magnetic stirring for three hours, sonicating every 30 min to avoid particle aggregates. After this period, stirring was stopped, and the suspension was maintained in a fumed hood at 27 °C until complete evaporation of the solvent (15 days). Portions of 1.0 g of the dried sorbed material, SiAl(PTFPMS), were collected after 15, 30, 60, 90, 120, and 180 days of the initial preparation to assess the self-coating of the aluminized silica particles by the fluorinated polymer. A representative scheme of the preparation of the self-coated SiAl(PTFPMS) stationary phase is shown in Figure S2 (SI section).

The sorbed SiAl(PTFPMS) particles were sequentially washed to remove non-adhered polymer chains with 3 mL of *n*-butyl acetate, water, and methanol by centrifugation at 2500 rpm for 5 min, discarding the supernatants. The sorbed material was dried at 60 °C for one hour.

Column packing of the SiAl(PTFPMS) particles

The balanced-density method[36] was used to pack stainless steel columns with the SiAl(PTFPMS) particles. A suspension prepared by 1.0 g of SiAl(PTFPMS) phase and 20 mL of chloroform was shaken under orbital shaking for 10 min at 30 rpm. Then, the suspension was placed in a RESTEK Pack-in-a-box kit column packing system (Bellefonte, USA), using methanol as the propulsion solvent at a flow rate of 24 mL min^{-1}. The columns 50 mm × 3.9 mm inner diameter (i.d.) were packed with the particles at 45 MPa.

Chromatographic analysis of self-coated SiAl(PTFPMS) columns

The chromatographic performance of the self-coated SiAl(PTFPMS) columns was assessed by the separation of three test mixtures. The first mixture constituted by uracil, benzonitrile, benzene, toluene, and naphthalene evaluated the chromatographic mode and the SiAl(PTFPMS) particles as high-performance liquid chromatography (HPLC) stationary phase. The other two mixtures, proposed by Engelhardt and Jungheim[37] and Tanaka and co-workers,[38] evaluated the chromatographic retention properties of SiAl(PTFPMS) particles. The Engelhardt mixture, composed of uracil, aniline, phenol, *N*,*N*-dimethylaniline, toluene, and ethylbenzene, was used to evaluate the silanophilic activity of the SiAl(PTFPMS) phase and, consequently, the efficacy of the polymer self-coating in protecting the active surface groups of aluminized silica.[37] The silanophilic activity is measured by the asymmetry factor and elution order of the peak of the *N*,*N*-dimethylaniline. The asymmetry factor is a measure of the peak tailing, calculated by the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope at 10% peak height. The Tanaka protocol evaluates the retention properties of reversed stationary phases through six chromatographic parameters, using four test mixtures in four different mobile phase conditions.[38] Table 1 presents the characterization conditions of the original Tanaka protocol.

The SiAl(PTFPMS) phase was applied for a pesticide mixture containing: methomyl (LogP 0.60), thiophanate-methyl (LogP 1.40), carboxin (LogP 2.14), terbuthylazine (LogP 3.40), and difenoconazole (LogP 4.40). The analysis was performed at 25 °C using methanol:water (50:50, v/v) as mobile phase at 1.2 mL min^{-1} with detection at 254 nm.

The chromatographic performance of the self-coated phases was evaluated using a Waters Alliance HPLC system (Milford, USA) equipped with an e2695 quaternary pump, an automatic injector, and a Waters 2998 PDA photodiode array detector set at a wavelength range of 200-400 nm with a spectral resolution of 1.2 nm, a sampling rate of 2 points *per* s and a filter time constant at 0.5 s. Data acquisition used Empower 3 Chromatograph Data Software.

Table 1. Chromatographic parameters, retention properties, and mobile phase conditions according to Tanaka test[38]

Test mixture	Chromatographic measurements[a]	Retention properties tested	Mobile phase conditions
Uracil, butylbenzene, pentylbenzene, *o*-terphenyl, and triphenylene	$k_{pentylbenzene}$ $k_{pentylbenzene}/k_{butylbenzene}$ $k_{triphenylene}/k_{o\text{-}terphenyl}$	hydrophobicity methylene selectivity steric/shape selectivity	MeOH:H_2O (80:20, v/v)
Uracil, phenol, and caffeine	$k_{caffeine}/k_{phenol}$	hydrogen bonding capacity	MeOH:H_2O (30:70, v/v)
Uracil, phenol, and benzylamine	$k_{benzylamine}/k_{phenol}$	total ion exchange capacity (pH > 7)	MeOH:20 mmol L^{-1} phosphate buffer at pH 7.6 (30:70, v/v)
Uracil, phenol, and benzylamine	$k_{benzylamine}/k_{phenol}$	ion exchange capacity (pH < 3)	MeOH:20 mmol L^{-1} phosphate buffer at pH 2.7 (30:70, v/v)

[a] Calulated by the equation: $k = (t_r - t_M)/t_M$. t_R: retention time of solute; t_M: dead time measured for the peak of the uracil.

Stability testing

An accelerated column degradation test evaluated the chemical stability of the SiAl(PTFPMS) phase.[39] In this test, a mobile phase consisted of methanol:25 mmol L^{-1} phosphate buffer at pH 10 (60:40, v/v), with the pH adjusted before methanol addition. The flow rates through the columns were 1.0 mL min^{-1} at 45 °C. The column lifetime was periodically monitored by the peak of naphthalene in the chromatogram. For comparison, this test was applied to a commercial C_{18} bonded phase: Kromasil® C_{18}, non-endcapped, with 5 µm particle size, lot No. 2219 packed into a 50 mm × 3.9 mm i.d. column at 45 MPa.

Characterizations of the self-coated SiAl(PTFPMS) particles

The morphological and physicochemical structure of self-coated PTFPMS of aluminized silica particles were studied by N_2 gas adsorption/desorption, elemental analysis (CHN), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD).

Porosity and surface area data of the SiAl(PTFPMS) particles were measured using N_2 adsorption-desorption isotherms at –195.5 °C in an ASAP 2020plus automatic physisorption analyzer from Micromeritics (Norcross, USA). The specific surface area, S_{BET}, was calculated using the Brunauer-Emmet-Teller (BET) method.[40] The total pore volume (V_p) was obtained by converting the amount adsorbed at P/P_0 of 0.9950 to the liquid adsorbate volume.[40] The average pore diameter (d_p) was calculated by the equation $d_p = 4V_p/S_{BET}$. Portions of 5 mg of SiAl(PTFPMS) particles were submitted to elemental analysis, where carbon was determined on a PerkinElmer model 2400 Analyzer (Norwalk, USA). For SEM analysis, about 10 mg of aluminized silica and SiAl(PTFPMS) samples were spread on carbon conductive tape-covered stainless-steel holders and then coated with gold by sputtering. The sample holders were analyzed on a scanning electron microscope with a secondary electron detector (SE) of the Everhart-Thornley type (Tescan Vega3 model, Brno, Czech Republic) operated at 20 kV and observed the surface morphology at different magnifications. Fourier transform infrared (FTIR) spectroscopic measurements of the particles were performed in an Agilent Cary 630 FTIR spectrometer (Danbury, USA) using a single-reflection diamond ATR sampling interface. For each spectrum, an average of 32 scans were performed at a resolution of 4 cm^{-1}, over the 4000-650 cm^{-1} frequency range. Powder X-ray diffraction patterns of SiAl(PTFPMS) sample was obtained in a Bruker D8 Discover diffractometer (Madison, USA) with radiation Cu Kα (40 kV and 30 mA) provided with a graphite monochromator (λ = 1.5418 Å) at 298 K. The relative intensity was registered in a dispersion range (2θ) of 5-40°.

Results and Discussion

In this work, we aim to evaluate the surface activity of aluminized silica particles to better understand their performance as chromatographic support for reversed-phase liquid chromatography. This evaluation involved the study of adsorption of the poly(3,3,3-trifluoropropylmethylsiloxane) over 180 days and the possibility of polymer fixation on the support surface without any physical or chemical induction. The chosen polymer requires a high temperature for immobilization in inorganic oxide particles (> 200 °C), higher than other polysiloxanes evaluated as HPLC stationary phase (< 150 °C).[16,22,26,34,41,42] This condition suggests that the PTFPMS performs fewer interactions with the surface groups of the inorganic oxide supports. So, we understand it was adequate to evaluate the surface activity of the aluminized silica particles. Portions of the sorbed material were packed and applied to chromatographic analyses of a test mixture. The chromatograms of the separation over 180 days of study are in Figure 1.

As shown in Figure 1, the first portion of SiAl(PTFPMS) evaluated after total solvent evaporation, 15 days (SiAl(PTFPMS)-15), showed separation for all compounds in the standard test mixture. This separation occurred due to the PTFPMS sorbed on the aluminized silica particles since a column packed with aluminized silica particles did not separate the solutes under the same condition, Figure S3 (SI section). Thus, the PTFPMS self-coating of aluminized silica particles occurs after the 15th day. The mobile phase was intentionally used with a high aqueous content to increase the peak resolutions and to evaluate the material chromatographic retentions, despite the compromise in the plate numbers due to the reduction in the mass transfer rate. The chromatographic parameters of the stationary phases are shown in Table S1 (SI section). The separation of compounds in SiAl(PTFPMS) columns occurred in the reversed-phase mode from hydrophobic interactions with the fluorinated polymer layer. However, PTFPMS has differentiated retention for polar compounds since benzonitrile (the most polar compound in the mixture) has a higher retention factor (k > 3.5) than in other polymer-coated reversed phases (k < 1.5).[16,42-45] The separation of the test mixture by SiAl(PTFPMS) particles from the 15th day of sorption is comparable in terms of plate numbers and polymer content (Table S2, SI section) with results obtained for other polysiloxanes sorbed without immobilization

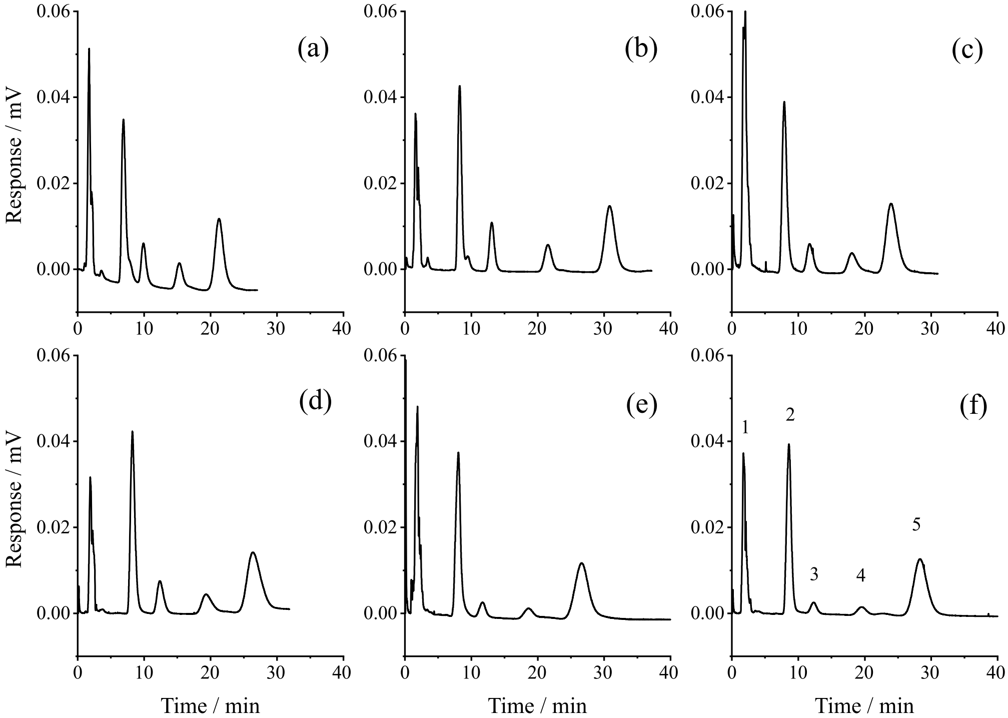

Figure 1. Chromatogram of separation of a test mixture by the SiAl(PTFPMS) particles after (a) 15, (b) 30, (c) 60, (d) 90, (e) 120, and (f) 180 days of adsorption at room temperature. Mobile phase conditions: ACN:H_2O (30:70, v/v); flow rate at 0.3 mL min^{-1}; UV detection at 254 nm; the volume of injection 10 µL; temperature 25 °C. Peak identification: (1) uracil, (2) benzonitrile, (3) benzene, (4) toluene, and (5) naphthalene.

step on silica particles and metalized (zirconia and titania) silica.[27,43,46] Silica and zirconized silica particles promoted the adsorption of a polymeric layer from the sixth day, condition for total solvent evaporation: chloroform and dichloromethane. However, titanized silica particles required 30 days to adsorb a sufficient polymer quantity to separate the same compounds, although the solvent evaporated on the 5th day. In this work, the minimum time for evaporation of *n*-butyl acetate was 15 days. Thus, the surface of aluminized silica presents, at least, similar reactivity to silica and zirconized silica particles well established as chromatographic support since PTFPMS requires a more intense physical immobilization condition than other polysiloxanes.

However, the adhesion of the polymeric layer to the aluminized silica support occurred more strongly from the 60th day onwards. SiAl(PTFPMS) particles evaluated after 15 and 30 days slowly lost retention for the compounds, and they were not considered in further studies.

N_2 adsorption isotherms and elemental analysis

The carbon content and the morphological properties of the SiAl(PTFPMS) particles sorbed over 180 days are in Table 2. The percentage of carbon varied from 8.0 to 11.9% in the aluminized silica particles, corresponding to the amount of polymer sorbed onto the aluminized silica support. These carbon percentages are higher than those obtained by PTFPMS thermally immobilized on bare silica.[34] The adsorption of the polymer on the aluminized silica particles led to a significant reduction (> 80%) of the original specific surface area of the support. The beginning of the adsorption resulted in a more notable reduction in the surface area, which increased continuously throughout the study, probably due to the rearrangement of the polymeric chains, forming thinner polymer layers and clearing the entrance of the pores. After 180 days, the SiAl(PTFPMS) particles showed a specific surface area 2.4 times and a pore volume 2.1 times greater than those recorded for SiAl(PTFPMS)-15 particles. SiAl(PTFPMS) particles maintained uniformly cylindrical pores with a narrow size distribution, as indicated by the profiles of the N_2 adsorption isotherms for the materials in Figure S4 (SI section).

Scanning electron microscopy

In liquid chromatography, the column packing of stationary phase particles is a fundamental factor for better separation performance. The more regular the particle size and shape, the better the packing. Thus, SEM was employed to evaluate the effect of alumina nanoparticle deposition and polymer adsorption on SiAl(PTFPMS) particles, Figure S5 of SI section. Despite the sub-micro fragments registered in the micrographs of the particles after alumina deposition,

Table 2. Morphological properties of aluminized silica and SiAl(PTFPMS) particles

Material	Specific surface area[a] / (m^2 g^{-1})	Total pore volume[b] / (cm^3 g^{-1})	Average pore diameter[c] / nm	Carbon / %
SiAl	423.0	0.97	11.1	–
SiAl(PTFPMS)-15	70.4	0.21	12.1	8.0
SiAl(PTFPMS)-30	50.3	0.14	11.6	9.5
SiAl(PTFPMS)-60	90.6	0.26	11.6	11.1
SiAl(PTFPMS)-90	89.0	0.26	11.6	10.9
SiAl(PTFPMS)-120	117.4	0.33	11.2	11.9
SiAl(PTFPMS)-180	169.6	0.44	10.3	9.1

[a] Determined by the Brunauer-Emmet-Teller (BET) method; [b] determined by the Barrett-Joyner-Halenda (BJH) method; [c] calculated by the equation $d_p = 4S_{BET}/V_p$, where S_{BET}: specific surface area and V_p total pore volume.

the regularity in the particle sizes was not affected. Furthermore, the adsorption of PTFPMS on the aluminized silica particles did not result in agglomerates, maintaining them dispersed from the 60th day of the adsorption study.

Fourier transform infrared spectroscopy

Figure 2 presents the infrared spectra of aluminized silica, PTFPMS, and SiAl(PTFPMS). The presence of PTFPMS on the aluminized silica is supported by the appearance of signals at 2900 and 2970 cm^{-1} that are attributed to the C_{sp3}–H stretching of CH_2 and CH_3 groups. The evidence that the organic coating on the aluminized silica is the PTFPMS polymer is the signals at 1210, 1268, 1372, and 1449 cm^{-1}, which refer to the stretchings of the C–F bonds, characteristic of carbon bonded to three fluorine atoms (trifluoro propyl group), confirming the self-coating of the aluminized silica particles by the fluorinated polymer.[34,35] In addition, the polymeric self-coating of the aluminized silica particles was efficient since a significant reduction in the signals at 1650 and 3460 cm^{-1} is observed in Figure 2b, attributed to hydroxyl groups and adsorbed water molecules on the surface of the aluminized silica support.[32,33]

XRD analysis

X-ray diffraction was used to confirm alumina in SiAl(PTFPMS) particles. The diffractogram, Figure S6 (SI section), registered a half-height enlarged halo centered at 21.8° attributed to the amorphous silica. Another halo at 9.7° is associated with the PTFPMS since its main chain usually cannot be spontaneously arranged into a lattice.[35,47] Alumina is confirmed in the SiAl(PTFPMS) by the peak at 38.4° attributed to the crystalline plane [110] of the α-alumina phase.[48]

Chromatographic characterization of the self-coated SiAl(PTFPMS) phase

The chromatographic retention properties of the self-coated SiAl(PTFPMS) phases were evaluated by separating standard test mixtures proposed by Engelhardt and Jungheim[37] and Kimata *et al.*[38] Besides

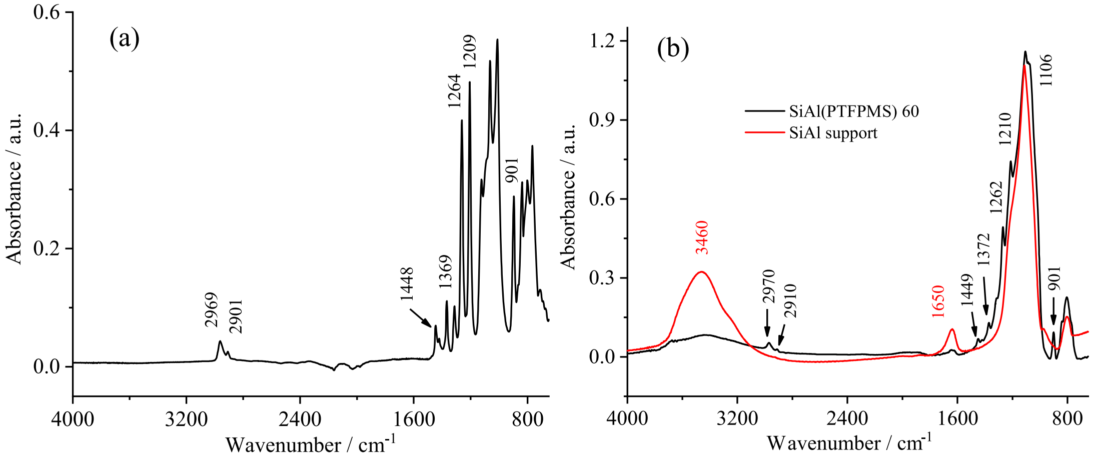

Figure 2. FTIR-ATR spectra of (a) PTFPMS and (b) aluminized silica and self-coated SiAl(PTFPMS) particles after 60 days.

performing a complete characterization of the retention properties of reversed stationary phases, these protocols were widely used to characterize pre-synthesized polymer immobilized stationary phases, allowing a comparison with the self-coated SiAl(PTFPMS) phase. Despite the protocols measuring similar properties, the simplified Engelhardt mixture evaluated the silanophilic activity of the self-coated SiAl(PTFPMS) phase, i.e., the surface sites of aluminized silica not covered by the polymer layer. Figure 3 shows the chromatogram of the separation of some compounds from the Engelhardt mixture using the self-coated SiAl(PTFPMS) phases after 60 days of adsorption. The separation profiles of the test mixture show close similarity among stationary phases, with a variation in the retention times for hydrophobic compounds (toluene and ethylbenzene) due to the differences in the carbon percentages in stationary phases. The basic solutes (aniline and *N*,*N*-dimethylaniline) eluted as *quasi*-symmetric peaks (As (asymmetry factor) < 1.4, measured at 10% peak height) by the self-coated SiAl(PTFPMS) phases with relationships of the asymmetry of the aniline peak divided by that of the phenol peak lesser than 1.3. The *N*,*N*-dimethylaniline peak eluted before the toluene. These conditions indicate that the self-coated SiAl(PTFPMS) phases have low silanophilic activity.[37] So, the self-coated PTFPMS layer adequately protects the alumina zwitterionic and silica acid sites on the aluminized silica surface.

The chromatographic parameters of the Engelhardt test mixture using the SiAl(PTFPMS) phase were measured and compared with chemically C_{18} bonded to silica and titanized silica supports[49] in Table 3. The chromatographic performance of the compounds in the SiAl(PTFPMS) phase is comparable to those of chemically bonded C_{18} phases when a more aqueous mobile phase is used for the lab-made stationary phase, with 70% *versus* 45% water content. SiAl(PTFPMS) phase presented lesser plate numbers for the compounds but more symmetric peaks for alkaline solutes.

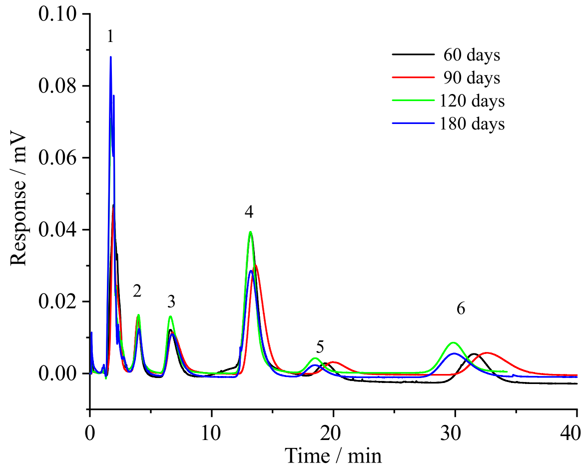

Figure 3. Chromatograms of the separation of some compounds of Engelhardt test mixture by the self-coated SiAl(PTFPMS) phases after 60 days of adsorption. Chromatographic conditions: MeOH:H_2O 30:70 (v/v); 0.3 mL min^{-1} and detection at 254 nm; injection volume of 10 µL; temperature 25 °C. Peak identifications: (1) uracil, (2) phenol, (3) aniline, (4) *N*,*N*-dimethylaniline, (5) toluene, and (6) ethylbenzene.

The Tanaka protocol was used to characterize the retention properties of the self-coated SiAl(PTFPMS) phases based on the measurement of six parameters obtained from the separation of four test mixtures under recommended analysis conditions: hydrophobicity, shape/steric selectivity, hydrogen bonding capacity, ion exchange capacity at pH > 7, and ion exchange capacity at pH < 3.[38] The values of each parameter for the self-coated SiAl(PTFPMS) phases and the chromatograms of the separation of Tanaka mixtures are in Table S3 and Figure S7 (SI section). The retention parameters of the self-coated SiAl(PTFPMS) phases were presented as six-axis radar plots, Figure 4a, which allows a global assessment of the characteristics of the stationary phase and comparison with similar counterparts. The effect of PTFPMS self-coating on aluminized silica particles was evaluated by comparing the parameters measured by the Tanaka protocol with those of a thermally immobilized PTFPMS phase on bare silica published by Maldaner and Jardim,[34] Figure 4b.

The self-coated SiAl(PTFPMS) phases showed a chromatographic retention profile similar to the silica-

Table 3. Chromatographic parameters obtained by the Engelhardt test mixture using the SiAl(PTFPMS) phase, compared with chemically bonded C_{18} on silica and titanized silica supports

Compound	Chromatographic parameters			
	k	N / plates	$A_{S\,10\%}$	h
Aniline	3.1;[a] 1.5;[b] 1.9[c]	510;[a] 490;[b] 430[c]	1.1;[a] 2.3;[b] 1.7[c]	19.6;[a] 24.5;[b] 23.3[c]
Phenol	1.5;[a] 1.0;[b] 1.6[c]	1020;[a] 1930;[b] 1860[c]	1.2;[a] 1.2;[b] 1.2[c]	9.8;[a] 6.2;[b] 5.4[c]
N,*N*-Dimethylaniline	7.1;[a] 8.3;[b] 7.7[c]	1650;[a] 2840;[b] 1500[c]	1.4;[a] 1.8;[b] 1.9[c]	6.1;[a] 4.2;[b] 6.7[c]
Toluene	11.0;[a] 11.6;[b] 10.5[c]	3600;[a] 4290;[b] 3510[c]	1.2;[a] 1.2;[b] 1.0	2.8;[a] 2.8;[b] 2.8[c]
Ethylbenzene	18.6;[a] 22.1;[b] 19.0[c]	2010;[a] 3990;[b] 3200[c]	1.0;[a] 1.2;[b] 0.9	5.0;[a] 3.0;[b] 3.1[c]

[a]SiAl(PTFPMS) after 180 days, mobile phase MeOH:H_2O (30:70, v/v); [b]bonded C_{18} TiO_2/SiO_2,[49] mobile phase MeOH:H_2O (55:45, v/v); [c]Kromasil® bonded C_{18}/SiO_2, mobile phase MeOH:H_2O (55:45, v/v). k: retention factor; N: plate numbers; $A_{S\,10\%}$: asymmetry factor measured at 10% of peak height; h: reduced plate height.

based thermally immobilized PTFPMS phase, as seen in Figure 4. The main characteristics observed refer to the low hydrophobicity of the polymer layer (k_{PB}) compared to chemically bonded alkylsilane phases.[50] The separation of the Tanaka mixture by the SiAl(PTFPMS) phase did not occur under the conditions recommended by the test (Figure S7, SI section), unlike the silica counterpart, which separated the compounds with poor resolution. The complete separation of the Tanaka test mixture I by the SiAl(PTFPMS) phase; however, occurs with highly aqueous mobile phases (> 60%). On the other hand, the self-coated SiAl(PTFPMS) phases showed relatively high values for shape selectivity ($\alpha_{T/O}$), polar interactions ($\alpha_{C/P}$), and ionic interactions ($\alpha_{B/P}$) at pH 7.6 and pH 2.7, similar to the thermally immobilized silica-based counterpart and chemically bonded fluorinated phases.[34,50] These results confirm the polar character of the stationary phase due to the self-coating PTFPMS of the aluminized silica particles and that the influence of this support on the separation is relatively small. Thus, the self-coating PTFPMS of the silica particles effectively occurred since the retention properties of the stationary phase are intimately related to the polymer layer characteristics.

Chemical stability of the self-coated SiAl(PTFPMS) phase

One of the main advantages of aluminized silica is its improved chemical stability compared with bare silica, making it capable of resisting more alkaline mobile phase conditions. The chemical stability of the self-coated SiAl(PTFPMS) phase after 60 days of adsorption was evaluated under fast degradation conditions to determine its lifetime with less solvent and equipment time.[39] A commercial bonded C_{18} phase passed the same test for comparison. Figure 5 shows the degradation profile of the two stationary phases, monitoring the reduced plate height for a test solute, naphthalene. The column death occurred by a sharp rise in the reduced plate height for the naphthalene peak, indicating the loss of its separation capacity. The self-coated SiAl(PTFPMS) phase after 60 days did not show a significant rise in the number of plates during the stability study, limited to 1000 column volumes. However, the commercial chemically bonded C_{18} phase lost its ability to separate at ca. 300 column volumes. This result shows the effect of the aluminized silica on the resistance of the chromatographic support to mobile phase degradation conditions and the adhesion of the polymer layer since the reduced plate heights are associated with the hydrophobic interactions between naphthalene and the polymer.

Separation of a pesticide mixture

The applicability of the self-coated material for 60 days was evaluated in a mixture of five pesticides with different polarities, LogP 0.60 to 4.40, methomyl, methyl thiophanate, carboxin, terbuthylazine, and difenoconazole. Figure 6 shows the chromatogram of pesticide separation by the self-coated SiAl(PTFPMS) phase and by a commercially bonded C_{18} phase (in the inside frame). The self-coated SiAl(PTFPMS) phase separated all compounds in less than six minutes using a mobile phase with 60% water. The commercial bonded C_{18} one did not separate the more polar pesticides from the mixture and required twice as long to elute the more hydrophobic pesticides. This result corroborates those obtained in the retention properties of the self-coated SiAl(PTFPMS) phases, which present lower hydrophobicity than conventional reversed phases and more interaction with polar compounds. Pesticide peaks eluted symmetrically (As < 1.3) and showed a

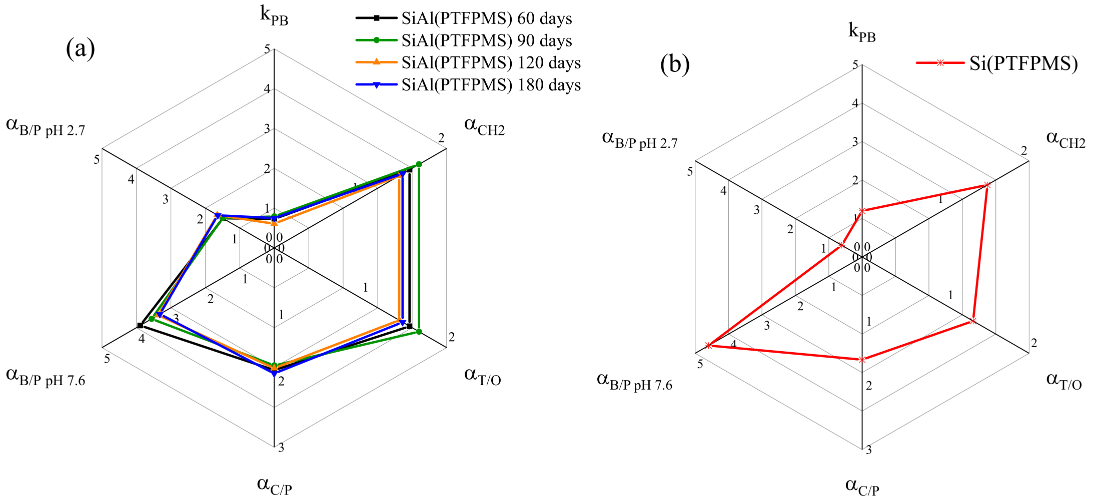

Figure 4. The radar plot for the (a) self-coated SiAl(PTFPMS) phases after 60 days and (b) Si(PTFPMS) phase thermally immobilized at 200 °C for 12 h, as published by Maldaner and Jardim.[34]

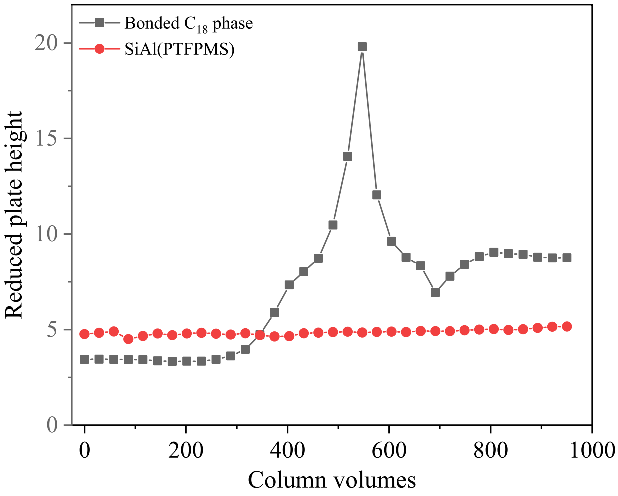

Figure 5. Changes in reduced plate height for the (●) self-coated Si(PTFPMS) phase after 60 days of adsorption and a (■) commercial chemically bonded C_{18} phase, after passing the mobile phase methanol: 25 mmol L^{-1} phosphate solution at pH 10.0 (60:40, v/v); injection volume of 10 µL; temperature 45 °C; detection at 254 nm. Solute tested: naphthalene.

column efficiency of 60,000 plates m^{-1} for carboxin and difenoconazole, indicating good separation performance due to effective coating of the chromatographic support by the PTFPMS layer. The chromatographic parameters obtained by the pesticide mixture separation in Figure 6 using the SiAl(PTFPMS) and commercial bonded C_{18} columns are shown in Table S4 (SI section). This result can be extended to the self-coated SiAl(PTFPMS) phases sorbed at 90, 120, and 180 days.

Conclusions

The adsorption of PTFPMS, without any physical immobilization procedure, on aluminized silica particles occurred practically instantaneously after solvent evaporation. After 60 days, stronger polymer adsorption on the support surface resulted in the self-coated stationary phase. The superficial reactivity of the aluminized silica particles, through acidic and zwitterionic groups from the mixed oxide, favor this strong adsorption of pre-synthesized polymers. The self-coated SiAl(PTFPMS) phases presented selectivities associated with the polymeric layer and a negligible effect of aluminized silica on compound retentions. On the other hand, the aluminized silica support provides chemical stability for the stationary phase, improving its lifetime in the alkaline mobile phase. So, aluminized silica is a potential chromatographic support for reversed stationary phases in liquid chromatography.

The SiAl(PTFPMS) phases present more retention for polar compounds, with typical interactions in the reversed-phase LC mode, eluting acidic and basic compounds as symmetrical peaks without any additive in the mobile phase. The chromatographic performance of the SiAl(PTFPMS) phase is comparable to those conventional bonded reversed-phase columns with substantially lower retention for non-polar compounds, requiring aqueous mobile phases. These characteristics, associated with PTFPMS, are suitable for analytes with different polarities, as observed for a pesticide mixture.

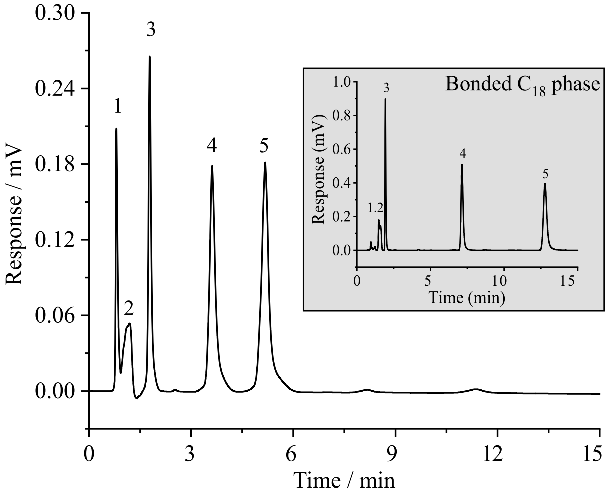

Figure 6. Chromatogram of separation of pesticide mixture by the self-coated SiAl(PTFPMS) after 60 days of adsorption and by the commercial bonded C_{18} phase (inside the frame). Column dimensions: 150 mm × 4.6 mm i.d. Analysis conditions: mobile phase ACN:H_2O (40:60, v/v); flow rate of 1.2 mL min^{-1}, and UV detection at 230 nm; injection volume of 10 µL; temperature 25 °C. Peak identifications: (1) methomyl, (2) methyl thiophanate, (3) carboxin, (4) terbuthylazine, and (5) difenoconazole.

Supplementary Information

Supplementary information (additional physicochemical characterizations and chromatograms) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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