Titanized silicas, modified by C_{18}, as promising stationary phases for high pH separations

César R. Silva, Claudio Airoldi, Kenneth E. Collins, Carol H. Collins*

Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13084-971 Campinas, São Paulo, Brazil

Abstract

To enhance the high pH stability of silica based reversed phases, chemically bonded octadecyl phases were prepared through silanization of titanized silica particles containing approximately 14% titanium oxide on the surface. The present work describes some spectroscopic characterizations using infrared, solid-state ^{13}C and ^{29}Si nuclear magnetic resonance (NMR) and X-ray absorption spectroscopy (XAS). Chromatographic characterizations for the titanized phase as well as for a conventional C_{18} phase, based on the same silica support without titanization, are also described using three different test mixtures containing neutral, polar and basic compounds. After an artificial stability test at pH 10, the titanized phase was again characterized by elemental and X-ray fluorescence analyses to determine the remaining carbon and titanium contents. As an application to real world samples, the separation of some herbicides and highly basic drugs using buffered mobile phases are also shown.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Silica; Titanium oxide; Silanization; Column stability

1. Introduction

Separations of basic compounds on silica based reversed phases are sometimes better at higher pH values because this minimizes tailing caused by ion exchange mechanisms. At pH above 9, the residual silanols are in their ionized form ($\equiv$ Si–O$^-$) and are unable to interact with weak bases in their free form. But it is well known that separations at this pH also reduce column lifetime due to enhanced silica solubilization and consequently, collapse of the chromatographic bed [1].

An alternative approach to suppress peak tailing for bases involves lowering the pH of the mobile phase to a value of about 2, where both ionizable solutes and residual silanols are fully protonated. However, the basic solutes are poorly retained by the stationary phase while many compounds are unstable at low pH. Furthermore, at pH 2, hydrolysis of siloxane bonds is more likely, causing a loss of the organic moieties attached to the silica surface [1].

Attempts made to improve column performance can also affect column stability, because the conditions required lie outside the recommended pH stability range for silica-based phases. For this reason, the synthesis of new reversed phases with improved stability has increased considerably in recent years to overcome the limitations imposed on analyses of highly basic and ionizable compounds, mainly pharmaceuticals.

Various approaches have been taken to extend the high pH stability of silica-based phases, and here, only a few examples will be described. The improvements can be divided into two different groups: those obtained by using newer silylant reagents or even newer silanization procedures and those obtained using phases not based on silica.

Bidentate stationary phases were successfully prepared by Kirkland et al. [2] by modifying the bare silica support with bidentate silanes. Column efficiency and selectivity were equivalent to those of conventional C_{18} stationary phases, and the column stability was improved, especially for high pH separations.

Another example for enhancing the chemical stability of bonded phases was the horizontal polymerization procedure

---

* Corresponding author. Fax: +55 19 3788 3023.
  E-mail address: chc@iqm.unicamp.br (C.H. Collins).

developed by Wirth et al. [3] using mixtures of two different trichlorosilanes (C_{18}/C_1) and carefully humidified silica under dry solvent conditions.

Hybrid phases based on porous organic–inorganic particles have recently been introduced by Neue and coworkers [4]. Column stability studies showed that the hybrid phases have long lifetimes at elevated temperatures under conditions at which conventional bonded silicas fail rapidly. More recently, the use of ethyl-bridged silanes has also been reported for the synthesis of even more stable hybrid silica support particles [5].

Reversed phases based on chemically modified zirconia were developed by Carr and coworkers [6] and have shown good stability with extremely aggressive mobile phases having pH values close to 14. These packings also exhibit good performance at high temperatures, above $60\,^{\circ}C$ [7]. The state of the art in the modification of the metal oxide surface is discussed in details in a recent review [6].

Other reversed stationary phases based on titania, alumina and zirconia have been prepared by a modification process called surface hydrosilylation, developed by Pesek and coworkers [8]. However, the approach suffers from difficulties in preparing these alternative supports in a range of particle and pore sizes appropriated for liquid chromatography.

Another possible approach, developed in our laboratory, is the modification of the silica support particles with titanium alkoxides [9]. The "metalized" silicas can be prepared by well established procedures already used for preparing catalysts, with no loss of the structural integrity of silica, thus maintaining its excellent physical properties and improving the stability of the support in high pH mobile phases [10].

2. Experimental

2.1. Reagents and solvents

Uracil, caffeine, 3,4-dichloroaniline, phenol, propranolol, amitriptyline and phosphoric acid were obtained from Aldrich (Milwaukee, USA) and were used as received. Cyanazine, simazine and atrazine were supplied from Norvatis. Diuron and linuron were from DuPont and Hoechst, respectively. The atrazine metabolites, 2-chloro-4,6-diamino-1,3,5-triazine and 2-hydroxyatrazine were purchased from Chem Service (West Chester, USA). Potassium salts (KH_2PO_4 and K_2HPO_4), potassium hydroxide, benzylamine, toluene, ethylbenzene, butylbenzene, pentylbenzene and o-terphenyl were obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile were HPLC grade and were also purchased from Merck. Deionized water was from a Milli-Q water system from Millipore (Bedford, USA). The mobile phases were prepared volumetrically from individually measured amounts of each component and filtered through a $0.2\,\mu m$ nylon membrane filter and degassed by sonification prior to use.

2.2. Preparation of the octadecyl titanized silica phase (SiTiC$_{18}$)

Spherical Kromasil silica ($5\,\mu m$, $S_{BET} = 310\,m^2\,g^{-1}$) was chemically modified with tetraisopropyl orthotitanate to obtain a titanized silica surface with $5.2 \pm 0.1\,\mu mol$ of Ti m^{-2}, determined by X-ray fluorescence analysis. The titanized silica was then reacted with octadecyltrimethoxysilane to produce a modified surface with $3.45\,\mu mol\,m^{-2}$ of C_{18} groups. The SiTiC$_{18}$ phase was then endcapped with TMCS and HMDS. Non-metalized Kromasil was also reacted with octadecyltrimethoxysilane and then endcapped with TMCS and HMDS and is denoted as SiC$_{18}$. Additional details on the preparation and packing of these phases have been published elsewhere [10].

2.3. Characterizations

Solid-state ^{13}C and ^{29}Si NMR measurements were performed on an INOVA 500 spectrometer (Varian, Palo Alto, USA), using cross polarization and magic angle spinning (CP/MAS). For the ^{29}Si nucleus, a contact time of 5 ms and a pulse repetition time of 3 s were employed and for ^{13}C, a contact time of 3 ms and repetition time of 3 s. Frequencies of 125 and 100 MHz were used for carbon and silicon, respectively.

Diffuse reflectance infrared Fourier transformation (DRIFT) spectra were obtained on a Bomem spectrometer (Hartmann and Braun, Quebec, Canada) using a diffuse reflectance accessory in the range of $4000–400\,cm^{-1}$. For each spectrum, at least 100 scans were performed with a resolution of $4\,cm^{-1}$ at the scan rate of $20\,scans\,min^{-1}$.

The XAS experiments were performed on the XAS beam line of the Laboratorio Nacional de Luz Sincrotron (LNLS-Campinas, Brazil). The XANES spectra were collected in the transmission mode using a Si (1 1 1) channel cut monochromator at the titanium K-edge (4965 eV). The experimental spectra were background corrected and normalized using a polynomial fit. After these operations, the EXAFS spectra were analyzed using WinXAS software version 2.3. Powdered 20 mg samples of pure and titanized Kromasil silicas and of a reference compound (anatase-TiO_2) were suspended in isopropanol and then were deposited on cellulose acetate membranes by filtration under reduced pressure prior to obtaining the transmission spectra.

X-ray fluorescence quantifications were performed on a EDX 700 spectrometer from Shimadzu (Kyoto, Japan). Solid powered 100 mg standards ($n = 6$, containing up to 20% TiO_2) were prepared by dispersing pure TiO_2 (99.7%) in the silica matrix. The standards and the samples were analyzed at the titanium $K\alpha$ emission line (4.3–4.7 keV).

2.4. HPLC experiments

Chromatographic separations were performed using a modular HPLC system from Shimadzu equipped with a LC-

10AD liquid chromatography pump, a SPD-10A UV–vis detector, a CTO-10A column oven and a Rheodyne 8125 injector (Cotati, USA) with a 5 µl loop. Data were acquired and processed using ChromPerfect software from Justice Innovations (Mountain View, USA).

2.4.1. Chromatographic test procedure

The 60 mm × 3.9 mm i.d. HPLC columns packed with the SiC$_{18}$ and SiTiC$_{18}$ phases, respectively, were used in these tests at the optimal flow rate of 0.6 ml min^{-1} with detection at 254 nm using a temperature of 40 °C. The columns were individually downward packed in our lab using 10% (m/v) suspensions of the modified silicas in chloroform at 6000 psi with a Haskel packing pump (Burbank, USA) using methanol as propulsion solvent. A minimum of three columns were packed with each phase. Test procedures were performed on both SiTiC$_{18}$ SiC$_{18}$ columns. Retention factors (k), plate numbers at half peak height (N) and tailing factor (T_F) at 5% of peak height were calculated as recommended [11].

The first chromatographic evaluation was performed with a mixture of uracil (10 mg l^{-1}), used as the marker for the column dead time, butylbenzene (1000 mg l^{-1}), pentylbenzene (1100 mg l^{-1}), *o*-terphenyl (80 mg l^{-1}) and triphenylene (50 mg l^{-1}), using methanol–water (80:20, v/v) as mobile phase. Retention factors, plates per meter, separation factors for butyl and pentylbenzene (methylene selectivity, k_{PB}/k_{BP}, α_{CH_2}) and for *o*-terphenyl and triphenylene (steric selectivity, $\alpha_{T/O}$) were calculated as recommended [12].

The second mixture was composed of uracil (10 mg l^{-1}), caffeine (135 mg l^{-1}) and phenol (270 mg l^{-1}) was chromatographed using methanol–water (30:70, v/v) as mobile phase. The quotient of the retention factor of caffeine to that of phenol (hydrogen bonding capacity, $\alpha_{C/P}$) was calculated [12].

A third test was performed with a mixture of uracil (10 mg l^{-1}), benzylamine (100 mg l^{-1}), and phenol (120 mg l^{-1}), dissolved in a mobile phase composed of methanol–20 mmol l^{-1} KH$_2$PO$_4$/K$_2$HPO$_4$ (30:70, v/v) at pH 7.6. The buffer was prepared by dissolving 1.25 g of K$_2$HPO$_4$ and 0.39 g KH$_2$PO$_4$ in a 500 ml volumetric flask. The pH was adjusted to 7.60 with KOH using a calibrated pH meter before addition of methanol. The ion exchange capacity ($\alpha_{B/P}$) was calculated by the relation between the retention factors for benzylamine and phenol.

The last test was performed using these same compounds dissolved in a methanol–20 mmol l^{-1} phosphate buffered mobile phase at pH 2.7 (30:70, v/v). The buffer was prepared by dissolving 0.99 g of KH$_2$PO$_4$ and 0.28 g of H$_3$PO$_4$ in a 500 ml volumetric flask, with the pH adjusted to 2.70 by addition of HCl. The ion exchange capacity ($\alpha_{B/P}$) at pH 2.7 was calculated [12].

2.4.2. Separation of some herbicides and their metabolites

The separation of a herbicide test mixture containing uracil (2 mg l^{-1}), 2-chloro-4,6-diamino-1,3,5-triazine (1.5 mg l^{-1}), 2-hydroxyatrazine (2 mg l^{-1}), cyanazine (2 mg l^{-1}), simazine (2 mg l^{-1}), atrazine (2 mg l^{-1}), 3,4-dichloroaniline (1 mg l^{-1}), linuron (2 mg l^{-1}) and diuron (2 mg l^{-1}) was performed on both phases (SiC$_{18}$ and SiTiC$_{18}$) using methanol–water and methanol–20 mmol l^{-1} buffer phosphate (50:50, v/v) as mobile phases with detection at 230 nm.

2.4.3. Separation of basic compounds

A test mixture containing uracil (6 mg l^{-1}), propranolol (400 mg l^{-1}) and amitriptyline (100 mg l^{-1}) was separated on the SiTiC$_{18}$ column using methanol–20 mmol l^{-1} phosphate buffer at pH 12 (80:20, v/v). The buffer was prepared by titrating a 20 mmol l^{-1} K$_2$HPO$_4$ solution with 1 mol l^{-1} KOH solution until pH 12.

2.5. Elemental analyses after the artificial stability test at pH 10

The 60 mm × 3.9 mm columns packed with the SiC$_{18}$ and SiTiC$_{18}$ phases were continuously purged at 0.7 ml min^{-1} with methanol–50 mmol l^{-1} K$_2$HPO$_4$ solution at pH 10.0 (70:30, v/v) (not recycled) as mobile phase at 50 °C. After 6 l of the basic mobile phase had passed through the SiTiC$_{18}$ column, it was carefully unpacked and the solid phase was divided into three samples (2 cm from the column inlet, at the middle and 2 cm from the outlet) and each sample was analyzed by elemental analysis and by X-ray fluorescence to determine the carbon and titanium percentages remaining, respectively.

3. Results and discussion

3.1. Preparation and characterization of the C$_{18}$ phases

The preparation of the new C$_{18}$ phase based on titanized silica is outlined in Figs. 1 and 2. In the first step of the chem-
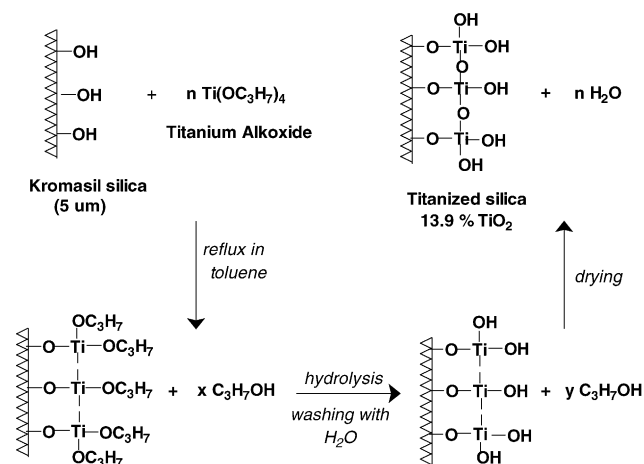

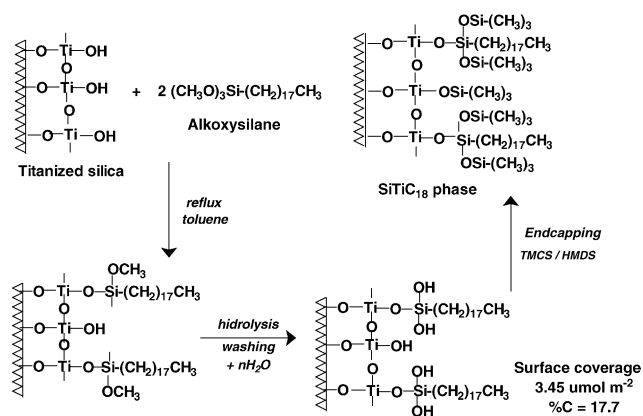Fig. 1. Chemical modification of silica with titanium tetraisopropoxide.

Fig. 2. Modification of the titanized silica with a trimethoxyalkylsilane.

Fig. 3. Solid-state ^{13}C CP/MAS NMR spectra for SiTiC$_{18}$, after the end-capping reaction.

ical modification, the isopropoxy groups from the titanate react with the surface silanols, yielding the titanized silica surface. The washing procedure with water is crucial to hydrolyze the remaining isopropoxy groups, producing new titanol groups, which can condense, allowing new ≡Ti–O–Ti≡ bonds to form during the drying step.

In the silanization step, the titanols (and also any residual silanols) react with the trimethoxysilane to yield the C$_{18}$ bonded phase, as proposed in Fig. 2. When using a trifunctional silane, repeated washings with water should be performed to promote the hydrolysis of any remaining methoxy groups from the C$_{18}$ silane. These "hydrolyzed silanols" are then reacted with the endcapping reagents to introduce new trimethylsilyl groups on the surface, as is also represented in Fig. 2.

The DRIFT spectrum of the titanized silica shows the absence of methylene and methyl stretching bands, which is good evidence for the complete hydrolysis of any isopropoxy groups remaining after the titanization reaction. By comparing the spectra before and after modification with C$_{18}$ alkoxysilane, new bands at 2960 and 2850 cm^{-1} are attributed to C–H stretching due to the presence of methyl and methylene groups from the C$_{18}$ chain immobilized on the silica surface. The band at 1465 cm^{-1} is attributed to CH$_2$ and CH$_3$ bending. The band at 970 cm^{-1}, which is attributed to free silanol bending, disappeared after the reaction with C$_{18}$ silane.

Fig. 3 shows the ^{13}C CP/MAS NMR spectrum of the SiTiC$_{18}$ after the endcapping reaction. The peak at 2 ppm confirms the presence of the trimethylsilyl groups on the modified surface. The other peaks are consistent with the proposed structure [13]. The peak at 14 ppm is attributed to carbon (1) due to a more rigid environment, while the signal at 12 ppm is due to a carbon (18) with enhanced mobility. These doublet peaks are only observed for long alkyl chains attached at high surface coverages on the silica surface [14]. The shoulder at 50 ppm may be attributed to some residual methoxy groups.

The phase was also investigated by solid-state ^{29}Si CP/MAS spectroscopy. Fig. 4 shows the ^{29}Si CP/MAS NMR

spectra for the titanized phase, before and after endcapping. The species found on the surface are described as Q^n, where n is related to the number of Si–O–Si bonds [15]. In all spectra, the Q^4 (siloxanes) and Q^3 species (free silanols) were detected at −110 and −101 ppm, respectively. Additional peaks at −48, −57 and −65 ppm were also detected and are derived from the trifunctional alkoxysilane used in the silanization reaction [16]. These structural types are denoted as T^1, T^2 and T^3 and are also shown in Fig. 5. After the endcapping reac-
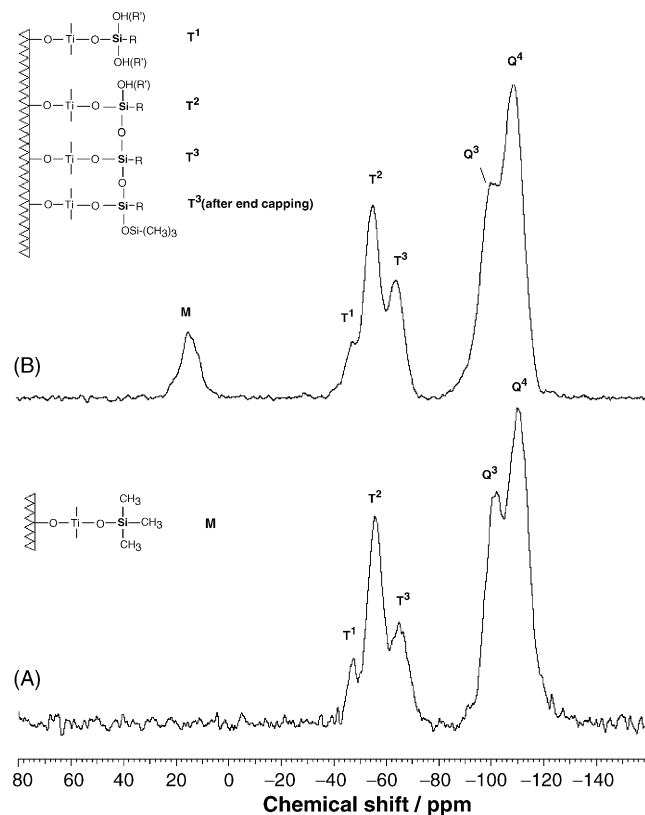

Fig. 4. ^{29}Si CP/MAS NMR spectra for SiTiC$_{18}$ (A) before and (B) after endcapping.

tion, the ^{29}Si CP/MAS spectrum shows a new signal at about +12 ppm (M species), which confirms the presence of the Si(CH$_3$)$_3$ group. Consequently, an increase in the population of the T^2 and T^3 species is also observed.

X-ray absorption spectroscopy (XAS) is a good spectroscopic technique to investigate structure, coordination number and bond lengths of specific chemical bonds. In principle, the XAS spectrum can be divided in two different regions: the X-ray absorption near edge structure (XANES) region, which corresponds to the lower range of the spectrum up to 50 eV above the K-edge absorption of the element under study, and the extended X-ray absorption fine structure (EXAFS) region, which corresponds to the range between 50 and 1000 eV above the K-edge absorption of the element. In the present study, the XAS (XANES and EXAFS) spectra were collected for titanium [17].

By analyzing the XANES region at the titanium K-edge absorption at 4970 eV, it is possible to obtain information on the chemical structure of the titanium atom as well as its electronic transitions [18]. By comparing the spectra in the XANES region for titanized silica and for anatase (Fig. 5), it can be concluded that these two spectra are similar although differences in the fine structure are seen suggesting that titanium on the silica surface is not solely deposited as anatase. For example, the fine structure in the pre-edge region of the titanized silica is a little different from that of the TiO$_2$-anatase, which is referred to 1s → 3d electronic transitions (terms A$_1$, A$_2$ and A$_3$). On the other hand, for pure silica, no absorption is observed due to the absence of TiO$_2$ on the surface. The EXAFS region (data not shown) for the titanized silica was analyzed by the WinXas software and the coordination number of titanium atoms was determined as 4.98 ± 0.06 while for the reference compound, anatase, the coordination number is 6. It is well reported in the litera-
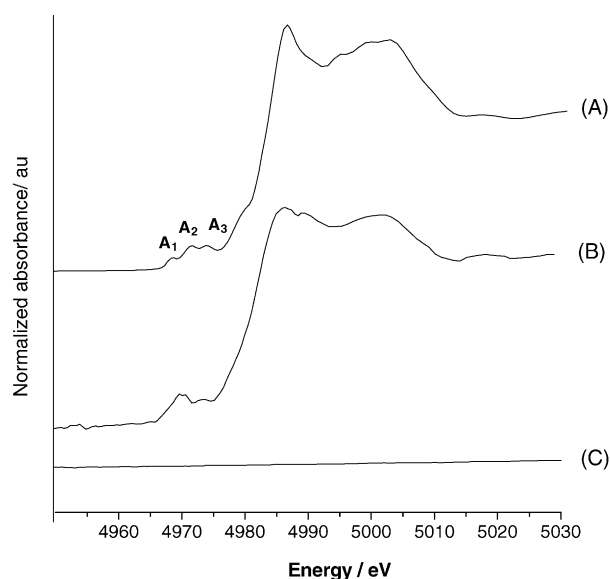
ture that the coordination number of the first atomic layer of titanium bonded to the silica surface is 4 [17].

The value obtained for the titanized silica (∼5) suggests a mixture of coordination numbers 4 and 6, suggesting that the titanium oxide is not just deposited on the silica surface but is also chemically bonded to the silanols, producing tetraedrically coordinated titanium on the titanized silica surface. This observation is in agreement with other studies on the characterization of titanium-grafted silicas by XAS [18].

3.2. Chromatographic evaluations

Short 60 mm × 3.9 mm HPLC columns were chosen because there are some advantages in using columns with small length, such as the lesser quantities of stationary phase required, lesser consumption of mobile phase and considerable time saving for the analysis. Following a previous evaluation of the C$_{18}$ titanized phase [10], using the test mixtures proposed by Neue et al. [19] and Engelhardt et al. [20], the chromatographic characterization is now extended by the evaluation using the popular test procedure of Tanaka and coworkers [12], which is based on the separation of seven compounds, using four different mobile phase compositions.

The first chromatographic evaluation was performed using the test mixture containing hydrophobic probes. Fig. 6 shows the chromatogram obtained, where it is possible to observe that all compounds were separated with good efficiencies and good peak shapes. The shape selectivity ($\alpha_{T/O}$), which gives information about functionality of the silylant reagent, and the methylene selectivity (α_{CH_2}), which gives information about surface area and coverage, were calculated, obtaining a value of 1.2 for both phases. As can be seen in Tables 1 and 2, very similar results were also obtained for the same separation on the SiC$_{18}$ phase, without titanization, because both phases were prepared in a similar manner using the same support and the same trifunctional C$_{18}$ alkoxysilane, and obtaining very

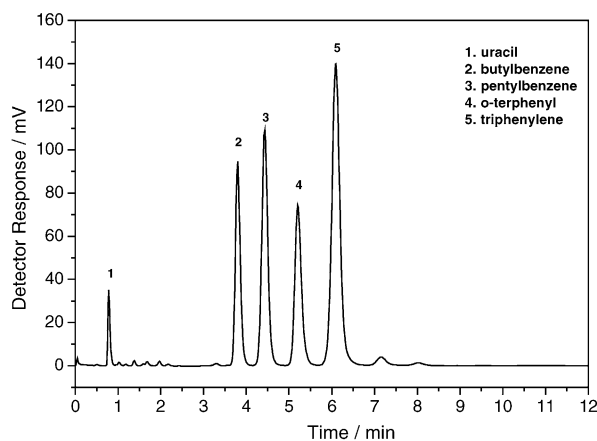Fig. 5. Ti K-edge XANES spectra for (A) TiO$_2$-anatase, (B) titanized silica and (C) for bare Kromasil.

Fig. 6. Separation of uracil (1), butylbenzene (2), pentylbenzene (3), *o*-terphenyl (4) and triphenylene (5) on the SiTiC$_{18}$ phase. Conditions: 60 mm × 3.9 mm i.d. column; mobile phase, methanol–water (80:20, v/v); flow rate, 0.6 ml min^{-1}; injection volume, 5 μl; temperature, 40 °C; and detection, UV at 254 nm.

Table 1
Chromatographic parameters obtained for the separation of the compounds of the Tanaka test mixture on the $SiTiC_{18}$ and SiC_{18} phases using different mobile phase compositions

Compound	$SiTiC_{18}$			SiC_{18}		
	k	N/m	T_F	k	N/m	T_F
Butylbenzene[a]	3.87	88,200	1.16	4.27	71,400	1.14
Pentylbenzene[a]	4.68	79,800	1.09	5.27	70,500	1.06
o-Terphenyl[a]	5.67	89,900	1.16	6.23	73,600	1.12
Triphenylene[a]	6.81	85,800	1.06	7.49	70,400	1.03
Caffeine[b]	1.69	39,100	1.38	2.08	42,700	1.37
Phenol	3.04[b]	58,700[b]	1.40[b]	3.78[b]	67,700[b]	1.10[b]
	3.12[c]	59,900[c]	1.40[c]	3.70[c]	65,300[c]	1.23[c]
	3.29[d]	47,400[d]	1.59[d]	3.74[de]	59,900[de]	1.20[de]
Benzylamine	11.08[c]	22,000[c]	1.77[c]	0.29[c]	13,100[c]	1.49[c]
	nd[d]	nd[d]	nd[d]	3.74[de]	23,200[de]	1.89[de]

Conditions: 60 mm × 3.9 mm column; flow rate, 0.6 ml min^{-1}; temperature, 40 °C, detection, UV at 254 nm; injection volume, 5 μl.
[a] Methanol–water (80:20, v/v).
[b] Methanol–water (30:70, v/v).
[c] Methanol–20 mmol l^{-1} H_3PO_4/KH_2PO_4 buffer (30:70, v/v) at pH 2.70.
[d] Methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer (30:70, v/v) at pH 7.60.
[e] This value was obtained from separate injections.

similar surface coverages of 3.54 and 3.45 for the SiC_{18} and $SiTiC_{18}$ phases, respectively.

The hydrogen bonding capacity ($\alpha_{C/P}$) is a good measure of the number of available silanols and the degree of end-capping. It was calculated from the separation of caffeine and phenol, obtaining a value of 0.56 for the $SiTiC_{18}$ phase, which is again very similar to the value of 0.58 obtained for the SiC_{18} phase without titanization.

On the other hand, the ion exchange capacities in neutral and acidic media are strikingly different for the $SiTiC_{18}$ phase when compared to the SiC_{18} phase (Tables 1 and 2). The latter has, at pH 2.7, an $\alpha_{B/P}$ value of 0.13, and at pH 7.6, a value of 1.0. For the $SiTiC_{18}$ phase, benzylamine is not eluted at pH 7.6, and at pH 2.7, the higher $\alpha_{B/P}$ of 3.55 is a good indication that the residual silanols or titanols are deprotonated in this acidic medium, causing a higher retention for benzylamine, which is totally protonated. A similar behavior has also been observed for the reversed phase based on polybutadiene-coated zirconia (at pH 2.7, $\alpha_{B/P}$ = 7.88) [21]. In the present case, the increase in the acidity of the residual groups is certainly caused by the presence of the titanium layer on the silica surface.

As an example of real world samples, some triazine herbicides, including their metabolites 2-chloro-4,6-diamino-1,3,5-triazine and 2-hydroxyatrazine, and two urea herbicides, diuron and linuron, and their metabolite, 3,4-

Table 2
Values of surface coverage (χ), methylene and steric selectivities, hydrogen bonding and ion exchange capacities at pH 7.6 and 2.7 for the $SiTiC_{18}$ and SiC_{18} phases

	χ (μmol m^{-2})	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ at pH 7.6	$\alpha_{B/P}$ at pH 2.7
$SiTiC_{18}$	3.45	1.20	1.20	0.56	nc	3.55
SiC_{18}	3.54	1.20	1.20	0.58	1.00	0.13

dichloroaniline, were separated. Fig. 7 shows the separations obtained with both columns. For the $SiTiC_{18}$ phase, a totally different selectivity is observed due to the higher retention of the basic triazine compounds and their two metabolites as well was for 3,4-dichloroaniline. However, when the separation was performed using a phosphate buffered mobile phase at pH 7, tailing and retention for the basic compounds were suppressed and the separation is comparable to that of obtained on the SiC_{18} without titanization.

Due to the higher ion exchange capacity of the titanized phase, the separation of highly basic pharmaceutical compounds, such as propranolol and amitriptyline, whose pK_a values are higher than nine, was only possible when using high pH mobile phases. Fig. 8 shows the separation of these two compounds using methanol–20 mmol l^{-1} phosphate buffer at pH 12 (80:20, v/v) as mobile phase. At pH 12, both compounds are in their free form and do not interact with the residual groups that are totally deprotonated, minimizing tailing as well as the retention time.

3.3. Column stability at pH 10

Previous studies have shown that phosphate mobile phases having pH above 9 and the use of elevated temperatures can increase the silica dissolution rate, dramatically reducing column lifetime [22]. Thus, although these conditions are not routinely used for silica-based phases, they were chosen to provide rapid evaluations [23].

In our previous publication [10], it was possible to verify that the titanium oxide layer on the surface improved the stability of the titanized phase in this accelerated aging test, which was performed at pH 10 with a temperature of 50 °C. Column failure started to be observed at approximately 2400 column volumes of the pH 10 mobile phase for the SiC_{18} phase without titanization, while for the titanized phase, col-
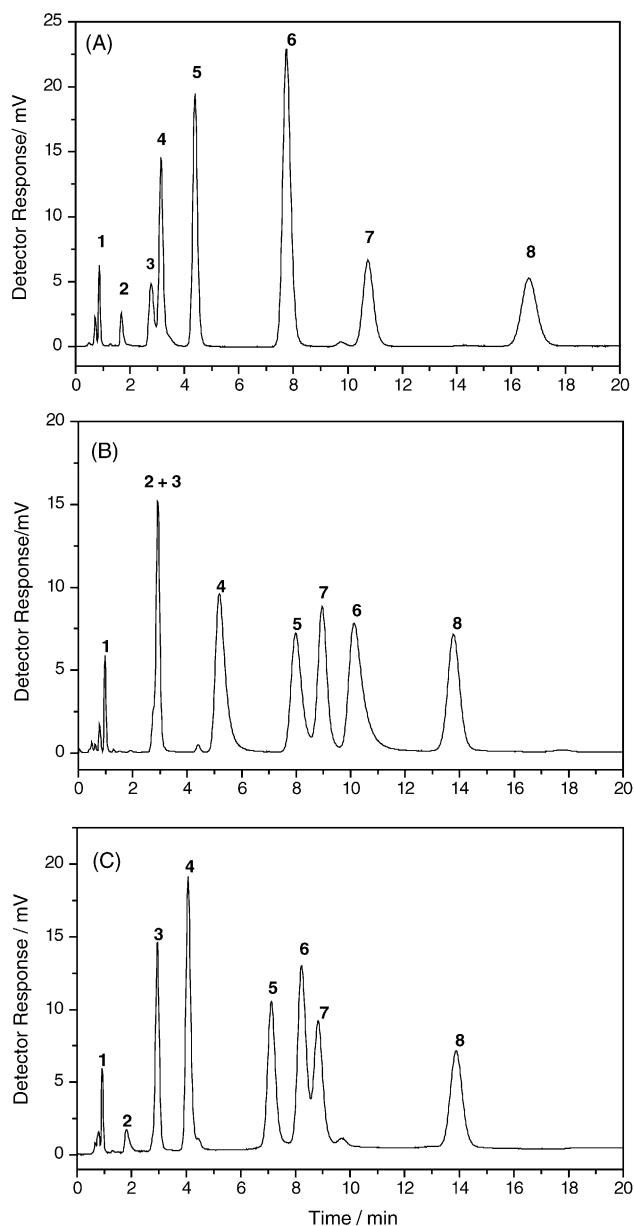
Fig. 7. Chromatograms of the separation of 2-chloro-4,6-diamino-1,3,5-triazine (1), 2-hydroxyatrazine (2), cyanazine (3), simazine (4), atrazine (5), 3,4-dichloroaniline (6), diuron (7) and linuron (8) performed on the SiC_{18} (A) and on the $SiTiC_{18}$ (B) phases. Conditions: 60 mm × 3.9 mm columns; mobile phase, methanol–water (50:50, v/v); flow rate, 0.6 ml min^{-1}; injection volume, 5 μl; temperature, 27 °C; detection, UV at 230 nm. (C) Same separation on the $SiTiC_{18}$ phase using MeOH–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 at pH 7.0 (50:50, v/v) as mobile phase.

umn failure started to be observed only after 3300 column volumes. A drop of 50% in plate numbers are observed at nearly 3000 and 4000 column volumes for the SiC_{18} and $SiTiC_{18}$ phases, respectively.

After approximately 10,000 column volumes, the $SiTiC_{18}$ column was unpacked and subjected to X-ray determination of the remaining titanium and elemental analysis for the C_{18} chains. Table 3 gives the carbon and titanium percentages for the $SiTiC_{18}$ phase before and after the aging procedure
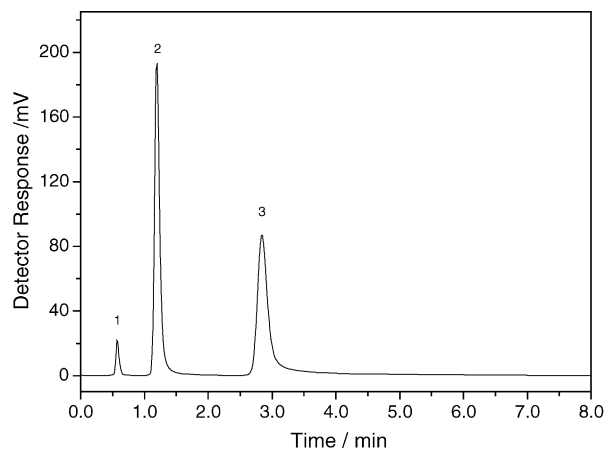

Fig. 8. Separation of uracil (1), propranolol (2) and amitriptyline (3) on the $SiTiC_{18}$ phase. Conditions: 60 mm × 3.9 mm i.d. column, mobile phase, 20 mmol l^{-1} methanol–phosphate buffer at pH 12 (80:20, v/v); flow rate, 0.7 ml min^{-1}; injection volume, 5 μl; temperature, 27 °C; detection, UV at 254 nm.

at pH 10. From the carbon contents, it can be observed that percentages are at least 2% higher at the column inlet, when compared to the initial value. For the titanium content, determined by X-ray fluorescence, the titanium percentage is also significantly higher at the column inlet.

These results suggest that during the artificial aging procedure, the titanium oxide layer as well the chemically bonded C_{18} silanes were not leached from the column. In principle, column failure is exclusively attributed to dissolution of the silica support, causing increase in the percentages of carbon and titanium at the column inlet. These results are in agreement with the chromatographic behavior observed during the aging, since the retention factors did not change significantly [10]. Another important observation is that, when the frit was removed a void was observed at the column inlet. Thus, the chromatographic column collapse observed here is very similar to that already reported by Kirkland at al. [24], who also observed higher carbon percentages at the column inlet when performing stability tests at pH 9 and 10 for several commercial chemically-bonded silica phases.

A comparison of these aging tests with those for "highly-stable" commercial phases (i.e., ethyl-bridged hybrid [5], bidentate silica phases [2] and polymer-coated zirconia phases [6] is quite difficult because the test conditions, such as type and buffer concentration, pH, flow rate and temperature change significantly from one author to another. Even the methods of determination of the column volume of mo-

Table 3
Carbon and titanium percentages determined before and after the artificial aging test at pH 10 and temperature of 50 °C

	Initial C %	Initial Ti %	After test C %	After test Ti %
	17.73	7.02		
Inlet			19.70	8.67
Middle			16.75	6.99
Outlet			16.20	6.60

bile phase that had passed though the column vary. However, drastic conditions similar to those used here caused column degradation of a 5 μm Hypersil ODS phase and of a 4 μm NovaPak-C18 phase after approximately 350 column volumes [23].

4. Conclusions

The trifunctional C_{18} stationary phase, based on a titanized silica support, was characterized by NMR, FTIR and X-ray absorption spectroscopies, showing that the chemical modifications were successfully carried out.

From the Tanaka test procedure [12], it can be concluded that for neutral compounds, the overall chromatographic performance of the C_{18} titanized phase was very similar to that of the conventional C_{18} phase prepared by a similar reaction.

However, for basic compounds, the efficiency of the titanized phase is greatly reduced due to interactions with the highly anionic residual titanols and silanols. Use of a buffered or high pH mobile phase reduces these effects, as observed in the separation of the s-triazine herbicides as well as basic drugs.

The results obtained from elemental analyses, after an artificial aging test at pH 10 and 50 °C, showed that column failure can be attributed to the dissolution of silica. Knowing that the titanium oxide layer can delay this dissolution, we look forward to further improvements in stability by increasing the amount of titanium oxide on the surface, presumably without affecting the chromatographic properties of the silica support.

Acknowledgments

References

[1] J.J. Kirkland, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 797 (1998) 111.
[2] J.J. Kirkland, J.B. Adams Jr., M.A. van Straten, H.A. Claessens, Anal. Chem. 70 (1998) 4344.
[3] M.J. Wirth, R.W.P. Fairbank, H.O. Fatumbi, Science 275 (1997) 44.
[4] Y.F. Cheng, T.H. Walter, Z. Lu, P. Iraneta, B.A. Alden, C. Genderau, U.D. Neue, J.M. Grassi, J.L. Carmody, J.E. O'Gara, R.P. Fisk, LC–GC 18 (2000) 1162.
[5] K.D. Wyndhan, J.E. O'Gara, T.H. Walter, K.H. Glose, N.L. Lawrence, B.A. Alden, G.S. Izzo, C.J. Hudalla, C.P. Iraneta, Anal. Chem. 75 (2004) 6781.
[6] J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, J. Chromatogr. A 1028 (2004) 31.
[7] J.D. Thompson, P.W. Carr, Anal. Chem. 74 (2002) 4150.
[8] A. Ellwanger, M.T. Matyska, K. Albert, J.J. Pesek, Chromatographia 49 (1999) 424.
[9] D.A. Fonseca, K.E. Collins, C.H. Collins, J. Chromatogr. A 1030 (2004) 209.
[10] C.R. Silva, C. Airoldi, K.E. Collins, C.H. Collins, LC–GC 22 (2004) 632.
[11] U.D. Neue, HPLC Columns, Theory, Technology and Practice, Wiley-VCH, New York, 1997.
[12] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[13] K. Albert, E. Bayer, J. Chromatogr. 544 (1991) 345.
[14] M. Pursch, L.C. Sander, K. Albert, Anal. Chem. 68 (1996) 4107.
[15] S. Bachmann, L.F.C. Melo, R.B. Silva, T.A. Anazawa, I.C.S.F. Jardim, K.E. Collins, C.H. Collins, K. Albert, Chem. Mater. 13 (2001) 1874.
[16] C. Hellriegel, U. Skogsberg, K. Albert, M. Lämmerhofer, N.M. Maier, W. Lindner, J. Am. Chem. Soc. 126 (2004) 3809.
[17] W.B. Kim, S.H. Choi, J.S. Lee, J. Phys. Chem. B 104 (2000) 8670.
[18] K. Asakura, J. Inukai, Y. Iwasawa, J. Phys. Chem. 96 (1992) 829.
[19] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[20] H. Engelhardt, M. Arangio, T. Lobert, LC–GC 15 (1997) 856.
[21] M.R. Euerby, P. Petersson, J. Chromatogr. A 994 (2003) 13.
[22] H.A. Claessens, M.A. van Straten, J.J. Kirkland, J. Chromatogr. A 728 (1996) 259.
[23] D.A. Fonseca, H.R. Gutierrez, K.E. Collins, C.H. Collins, J. Chromatogr. A 1030 (2004) 149.
[24] J.J. Kirkland, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 691 (1995) 3.