

Stability Evaluations of an Octadecyl Bonded Phase Based upon Titanium-Grafted Silica



The authors describe the preparation of a C18 bonded phase using silica chemically modified with tetraisopropyl orthotitanate. The product was reacted with octadecyltrimethoxysilane to yield a reversed phase. The chromatographic performance of this phase was evaluated for the separation of various test mixtures containing polar, nonpolar, and basic compounds, and its stability at pH 9 and 10 was evaluated and compared with that for an octadecyl bonded phase based upon the same silica support without titanization.

Silica is the most widely used oxide support for high performance liquid chromatography (HPLC) phases, mainly due to its excellent physical properties and its availability in a wide range of particle and pore sizes. In addition, the ease of modifying its surface by reaction of the silanol groups with various chloro or alkoxy silanes has made silica the material of choice for preparing reversed phases (1). However, silica-based phases have two drawbacks: a limited usable pH range, from 2 to 8, and the presence of residual silanols that cause peak tailing on the silica surface (2).

On the other hand, separations of basic compounds are better at higher pH values because a higher pH tends to diminish tailing problems. This occurs because at $\text{pH} > 9$, the residual silanols are in their ionized form ($\equiv\text{Si-O}^-$) and do not readily interact with weak bases whose $\text{p}K_a$ values are lower than 8. But separations at high pH reduce the lifetime of columns having bare silica as support, due to the attack of hydroxide ions on the silica surface. This causes silica dissolution and, consequently, a loss of efficiency (3).

Various approaches have been taken to extend the high-pH stability of silica-based phases, including bidentate phases (4) and hybrid organic-inorganic silica support particles (5). Alternative supports also have been investigated successfully, including

polymer-coated zirconia (6), which shows good stability when used with aggressive mobile phases having pH values close to 14, and alkyl-modified titania, zirconia, and alumina, prepared by a process called hydrosilylation (7).

In our laboratory, reversed phases with potential applications for alkaline mobile phases have been prepared by the immobilization of poly(methyloctylsiloxane) on titanium-grafted silica (8–10). The incorporation of this protective layer of titanium dioxide on the silica surface improves the stability of the silica support at high pH without loss of the structural integrity of the underlying silica, thus maintaining the excellent chromatographic characteristics of silica-based phases.

Following this approach, the present investigation reports the preparation of a chemically bonded stationary phase based upon titanized silica. This phase was evaluated by means of separations of neutral and basic compounds. To evaluate the effects caused by the incorporation of titanium atoms on the silica support, the phase's chromatographic performance and the pH stability at two pH values were compared with that of an octadecyl bonded phase based upon the same silica support without titanization.

Experimental

Chemicals: Kromasil silica batch AT0235

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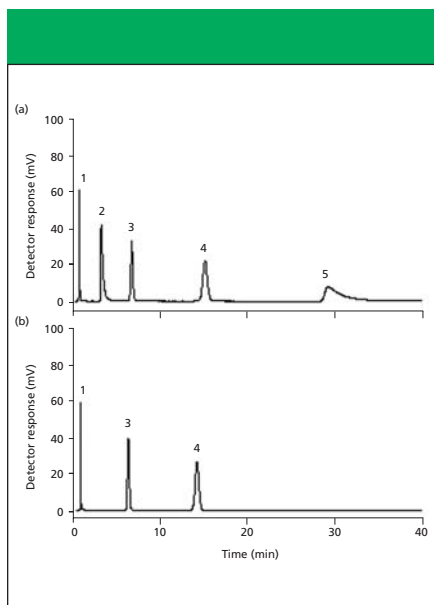


Figure 1: Separation of some components of the Neue test mixture on (a) SiC₁₈ and (b) SiTiC₁₈ phases. Columns: 60 mm × 3.9 mm; mobile phase: 65:35 (v/v) methanol–20 mM phosphate buffer at pH 7.0; flow rate: 0.7 mL/min; detection: UV absorbance at 254 nm; injection volume: 5 μL. Peaks: 1 = uracil, 2 = propranolol, 3 = naphthalene, 4 = acenaphthene, 5 = amitriptyline.

spherical particles with a mean particle size of 5 μm, a mean pore diameter of 10 nm, a BET surface area of 308 ± 5 m²/g, and a pore volume of 0.9 mL/g, was obtained from Akzo Nobel (Bohus, Sweden). Octadecyltrimethoxysilane, trimethylchlorosilane, and hexamethyldisilazane were obtained from Aldrich (Milwaukee, Wisconsin), and tetraisopropyl orthotitanate from Fluka (Buchs, Switzerland). Test mixture solutions were prepared with chemicals of the highest available purity, also purchased from Fluka or Aldrich. Potassium phosphate salts, toluene, chloroform, and the HPLC-grade solvents such as methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, Massachusetts).

Preparation of titanium-grafted silica:

First, the silica was activated under vacuum for 8 h at 100 °C. A sample of 5.0 g of silica was suspended in 100 mL of dry toluene and 37 mmol of tetraisopropyl orthotitanate (corresponding to 24 μmol/m² of silica surface) was added. The mixture was refluxed for 24 h, filtered and washed with toluene, isopropanol, methanol, and finally with a copious amount of deionized water to promote the total hydrolysis of the isopropoxy groups on the titanized silica surface. After

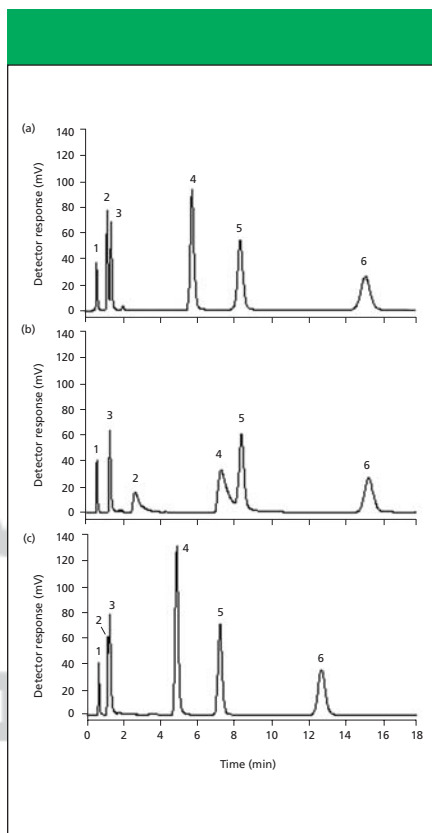


Figure 2: Separation of some components of the Engelhardt test mixture on the (a) SiC₁₈ and (b) SiTiC₁₈ phases. Columns: 60 mm × 3.9 mm; mobile phase: 55:45 (v/v) methanol–water; flow rate: 0.7 mL/min; detection: UV absorbance at 254 nm; injection volume: 5 μL. (c) Chromatogram obtained on SiTiC₁₈, using as mobile phase: 55:45 (v/v) methanol–20 mM phosphate buffer at pH 7.0. Peaks: 1 = uracil, 2 = aniline, 3 = phenol, 4 = *N,N*-dimethylaniline, 5 = toluene, 6 = ethylbenzene.

drying under vacuum for 6 h at 100 °C, the percentage of titanium was determined by X-ray fluorescence.

Preparation of C18 bonded phases: The bonding procedures were performed in the conventional manner for both bare and titanized silicas. 37 mmol of octadecyltrimethoxysilane and an equimolar

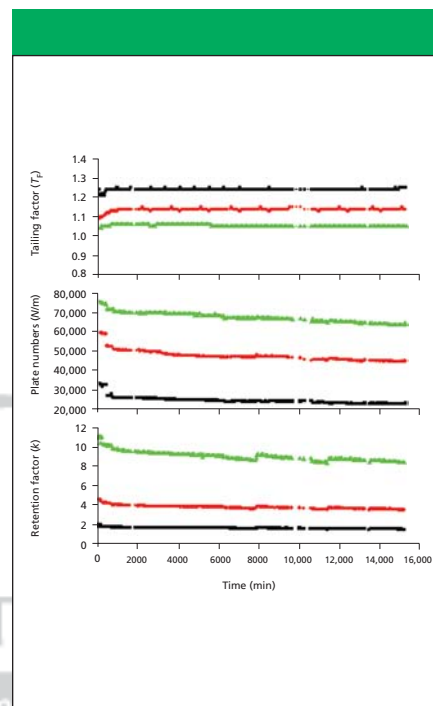


Figure 3: Chromatographic parameters calculated for *N,N*-dimethylaniline (black), naphthalene (red), and acenaphthene (green) during the aging test at pH 9.0 for the SiTiC₁₈ phase. Column: 60 mm × 3.9 mm; mobile phase: 70:30 (v/v) methanol–20 mM K₂HPO₄ solution at pH 9.0; temperature: 25 °C; flow rate: 0.7 mL/min.

amount of bidistilled triethylamine (5.1 mL) were added to 5.0 g of activated support, suspended in dry toluene, and refluxed for 72 h. The silicas were filtered, washed with toluene, isopropanol, methanol, and deionized water and were dried under vacuum at 70 °C for 8 h. These phases, designated SiC₁₈ and SiTiC₁₈ for the bare and titanium-grafted silicas, respectively, were submitted to elemental analysis to determine the carbon contents. The C18 phases then were endcapped by refluxing 4.5 g of each with a mixture of trimethylchlorosilane (15 mL) and hexamethyldisilazane (35 mL) in 100 mL of dry toluene for 48 h.

Column packing and testing: HPLC columns (60 mm × 3.9 mm) were made

Table 1: Chromatographic parameters* obtained with the C18 phases for the separation of a test mixture of nonpolar compounds

Compound	SiC ₁₈			SiTiC ₁₈		
	<i>k</i>	<i>N/m</i>	<i>T_F</i>	<i>k</i>	<i>N/m</i>	<i>T_F</i>
Acetophenone	1.18	43,200	1.06	1.00	34,600	1.24
Benzene	2.36	59,500	1.03	2.11	57,400	1.21
Toluene	3.58	66,700	1.07	3.27	70,300	1.21
Naphthalene	4.82	69,000	1.03	4.52	78,600	1.07

* Chromatographic conditions: 60 mm × 3.9 mm columns; mobile phase: 60:40 (v/v) acetonitrile–water, optimal flow rate: 0.7 mL/min for the SiC₁₈ and SiTiC₁₈ phases.

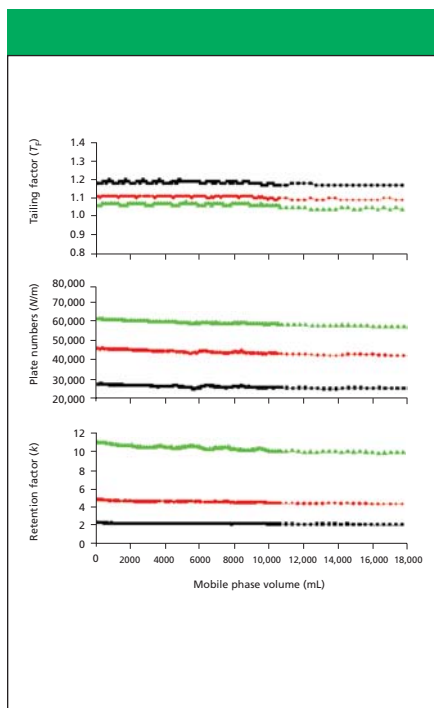


Figure 4: Chromatographic parameters calculated for the separation of *N,N*-dimethylaniline (black), naphthalene (red), and acenaphthene (green) during the aging test at pH 9.0 for the SiC₁₈ phase. Stability and evaluation conditions the same as Figure 3.

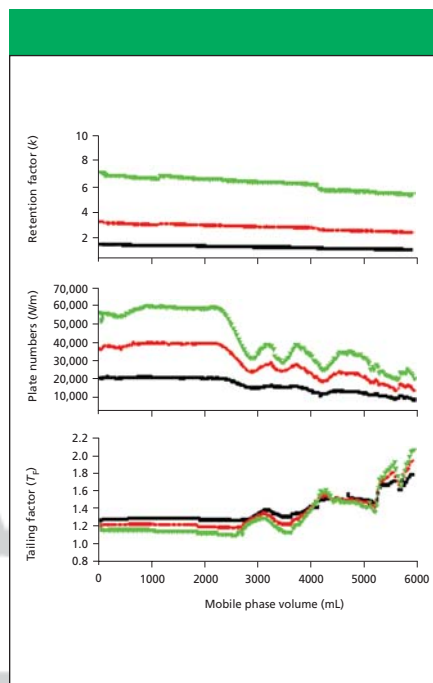


Figure 5: Chromatographic parameters calculated for the separation of *N,N*-dimethylaniline (black), naphthalene (red), and acenaphthene (green) during the aging test at pH 10.0 for the SiTiC₁₈ phase. Column: 60 mm × 3.9 mm; mobile phase: 70:30 (v/v) methanol–50 mM K₂HPO₄ at pH 10.0; temperature: 50 °C; flow rate: 0.7 mL/min.

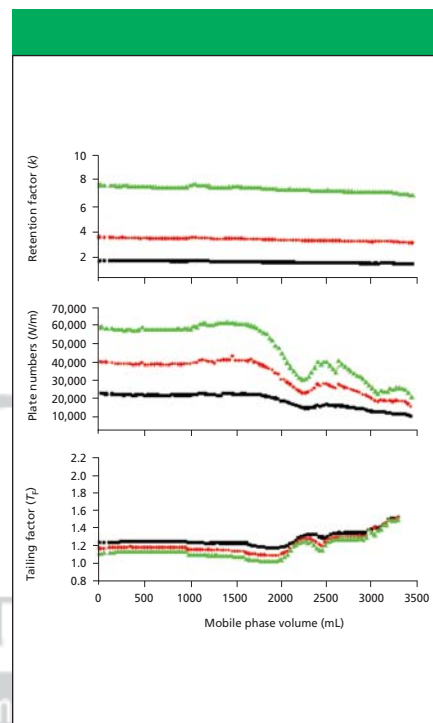


Figure 6: Chromatographic parameters calculated for the separation of *N,N*-dimethylaniline (black), naphthalene (red), and acenaphthene (green) during the aging test at pH 10 and 50 °C for the SiC₁₈ phase. Stability and evaluation conditions the same as Figure 5.

from 316 stainless steel tubing and had their inner surfaces polished as described elsewhere (11). The columns were downward packed individually using 10% (m/v) suspensions of the modified silicas in chloroform at 6000 psi with a Haskel packing pump (Burbank, California) using methanol as propulsion solvent.

Tests were performed using a modular HPLC system with a Waters 510 pump (Milford, Massachusetts), a Rheodyne 7725 injector (Cotati, California), and a Waters 486 detector. Data were processed using ChromPerfect software from Justice Innovations (Mountain View, California). All experiments were carried out at 25 °C, with

detection at 254 nm and an injection volume of 5 µL.

The first chromatographic evaluations used a mixture of uracil (12 mg/L), as marker for column dead volume, acetophenone (200 mg/L), benzene and toluene (1700 mg/L each), and naphthalene (120 mg/L) as hydrophobic probes, with 60:40 (v/v) acetonitrile–water as mobile phase.

A mixture based upon that of Neue (12) was chosen to evaluate the hydrophobic and silanophilic properties of the C₁₈ phases. The mixture contained uracil (10 mg/L), naphthalene (60 mg/L) and acenaphthene (220 mg/L) as hydrophobic probes, and propranolol (420 mg/L) and amitriptyline

(110 mg/L) as basic probes. 65:35 (v/v) methanol–20 mM KH₂PO₄/K₂HPO₄ buffer at pH 7.0 was used as mobile phase.

Another evaluation was performed using buffered and nonbuffered mobile phases, for the separation of some compounds from the Engelhardt test mixture (13). The mixture, containing uracil (12 mg/L), aniline (100 mg/L), phenol (220 mg/L), *N,N*-dimethylaniline (40 mg/L), toluene, and ethylbenzene (1500 mg/L each), was separated using 55:45 (v/v) methanol–water or methanol–20 mM phosphate buffer at pH 7.0 as mobile phases.

Column aging studies: The column aging tests were performed with an HPLC system from Shimadzu Corp. (Kyoto, Japan) equipped with an LC-10AD pump, a SPD-10A UV-vis detector, a CTO-10AS column oven, a SIL-10AD auto injector, and an SCL-10A system controller. Data were acquired and processed using the CLASS VP program. In one test, the columns packed with SiC₁₈, and SiTiC₁₈ phases were purged continuously at 0.7 mL/min with 70:30 (v/v) methanol–20 mM K₂HPO₄ solution at pH 9.0 (not recycled) as mobile phase at 25 °C. The columns were tested periodically by injecting uracil (10 mg/L), *N,N*-dimethylaniline

Table II: Chromatographic parameters* obtained for the separation of some compounds of the Neue test mixture composed of nonpolar and basic analytes, on the C₁₈ phases

Compound	SiC ₁₈			SiTiC ₁₈		
	<i>k</i>	<i>N/m</i>	<i>T_F</i>	<i>k</i>	<i>N/m</i>	<i>T_F</i>
Propranolol	1.85	16,500	2.86	nc	nc	nc
Naphthalene	9.68	66,400	1.05	7.96	73,150	1.03
Acenaphthene	21.8	69,600	1.06	19.4	76,500	1.01
Amitriptyline	43.8	24,400	5.23	nc	nc	nc

nc = not calculated

*Chromatographic conditions: 60 mm × 3.9 mm columns, mobile phase: 65:35 (v/v) methanol–20 mM phosphate buffer at pH 7.0; flow rate: 0.7 mL/min.

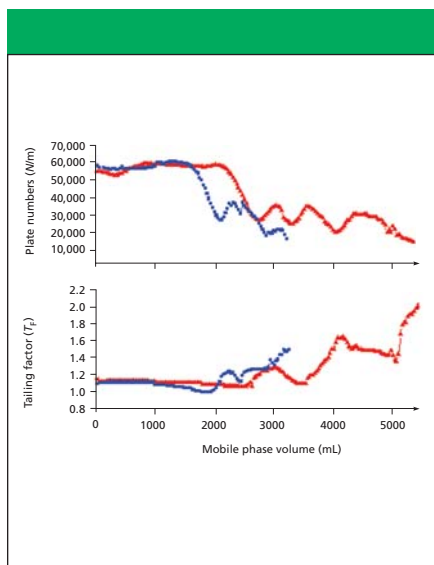


Figure 7: Comparison of the chromatographic parameters, plate numbers, and tailing factors obtained for acenaphthene during the artificial aging test at pH 10 and 50 °C on the SiTiC₁₈ (red) and SiC₁₈ (blue) phases. Stability and evaluation conditions the same as Figures 5 and 6.

(30 mg/L), naphthalene (60 mg/L), and acenaphthene (200 mg/L) at the same flow rate, using the same pH 9 mobile phase. Using this same test mixture, a more aggressive test also was performed with both the SiC₁₈ and the SiTiC₁₈ phases by passing 70:30 (v/v) methanol–50 mM K₂HPO₄ solution at pH 10.0 (not recycled) as mobile phase at 50 °C. This protocol was adapted from a rapid method for column stability evaluation recently developed in our laboratory (14).

The pH of the aqueous phosphate solutions was adjusted with 1 M KOH solution before the addition of methanol by using a calibrated pH meter. Although phosphate solutions do not buffer at pH 9–10, nonetheless, these solutions considerably increase the silica dissolution rate (15). Plates per meter (*N/m*), retention factor (*k*), and tailing factor at 5% (*T_F*) were calculated and monitored as a function of the volume of mobile phase that passed through the columns to detect any change or degradation (16).

Results and Discussion

Preparation of the SiTiC₁₈ phase: In the first step of the preparation of the titanized silica phase, the isopropoxy groups from the titanate react with the surface silanols, yielding the titanium-grafted surface. During washing, the remaining isopropoxy groups are hydrolyzed, forming titanol groups,

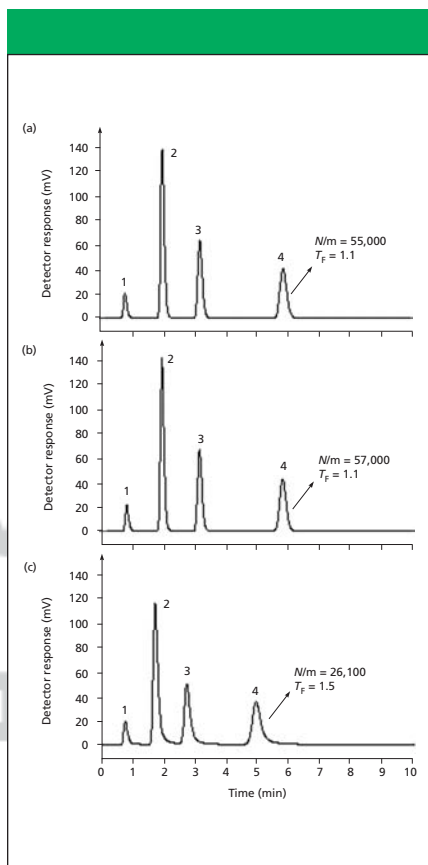


Figure 8: Chromatograms obtained for the SiTiC₁₈ phase during the artificial aging test at pH 10 and 50 °C. (a) Initial, (b) after the passage of 2100 mL of phosphate mobile phase at pH 10, and (c) after the passage of 5000 mL of the mobile phase. Chromatographic conditions are the same as Figure 5. Peaks: 1 = uracil, 2 = *N,N*-dimethylaniline, 3 = naphthalene, 4 = acenaphthene.

which can condense, forming new ≡Ti–O–Ti≡ bonds upon drying. In the second step, the titanols and the remaining silanols react with the trimethoxysilane to yield the C₁₈ bonded phase. Triethylamine is used to enhance the reactivity of the alkoxy silane and thus improve the surface coverage on both surfaces (17,18). The bonded phase is washed extensively to remove physically adsorbed silane and also to promote the hydrolysis of any remaining methoxy groups. In a last step, residual silanols and titanols are blocked by the substitution of a –Si(CH₃)₃ moiety through reaction with the mixture of trimethylchlorosilane and hexamethyldisilazane (19).

According to X-ray fluorescence analysis, the modification process of the first step yielded a titanized silica with mass percentage of 8.4% of titanium, which represents a surface concentration of 5.6 μmol/m² of Ti. Following the silanization reaction with the trimethoxysilane, the concentration of

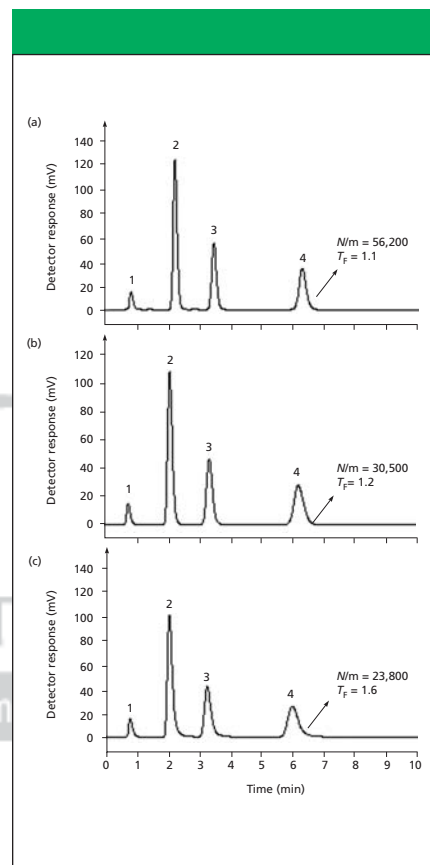


Figure 9: Chromatograms obtained for the SiC₁₈ phase during the artificial aging test at pH 10 and 50 °C. (a) Initial, (b) after the passage of 2100 mL of phosphate mobile phase at pH 10, and (c) after the passage of 3000 mL of the mobile phase. Chromatographic conditions are the same as Figure 5. Peaks: 1 = uracil, 2 = *N,N*-dimethylaniline, 3 = naphthalene, 4 = acenaphthene.

the octadecyl groups was calculated from the carbon percentages (20), obtaining similar surface coverages of 3.54 and 3.45 μmol/m² for bare and titanized silicas, respectively, indicating the titanols on the silica surface react with methoxy groups from the C₁₈ silane in a manner similar to the silanols. The high surface coverage obtained for both C₁₈ phases is due to the use of a trifunctional organosilane in the bonding procedure. The carbon percentages for both silicas were increased by approximately 0.4% after endcapping as a result of the presence of the trimethylsilyl groups.

Chromatographic evaluations: The first chromatographic evaluation, performed using a test mixture composed of uracil, acetophenone, benzene, toluene, and naphthalene, was used to determine the optimal flow rate of 0.7 mL/min for SiC₁₈ and SiTiC₁₈ phases. Using this optimal flow rate, the chromatographic parameters were determined (Table I). The lower retention

Table III: Chromatographic parameters obtained for the separation of some compounds of the Engelhardt test mixture composed of nonpolar, polar, and basic analytes on the C18 phases

Compound	SiC ₁₈ *			SiTiC ₁₈ *			SiTiC ₁₈ †		
	<i>k</i>	<i>N/m</i>	<i>T_F</i>	<i>k</i>	<i>N/m</i>	<i>T_F</i>	<i>k</i>	<i>N/m</i>	<i>T_F</i>
Aniline	0.84	23,400	1.57	3.01	5680	3.40	0.73	14,300	2.26
Phenol	1.13	27,450	1.34	1.03	22,500	1.77	0.87	20,550	1.30
<i>N,N</i> -DMA	7.59	65,500	1.22	9.78	18,300	3.51	6.04	65,950	1.15
Toluene	11.4	67,500	1.06	11.4	73,400	1.12	9.39	80,800	1.12
Ethylbenzene	21.4	66,700	1.04	21.3	79,300	1.12	17.2	82,000	1.07

* Chromatographic conditions: 60 mm × 3.9 mm columns; mobile phase: 55:45 (v/v) methanol–water; flow rate: 0.7 mL/min.
† Same conditions, except using 55:45 (v/v) methanol–20 mM phosphate buffer at pH 7.0 as mobile phase.

factor for the compounds on the SiTiC₁₈ phase is an indication of a somewhat lower hydrophobicity due to the slightly lower surface coverage obtained.

Both phases also were evaluated with some compounds of the Neue test mixture (12). The most interesting probes in this mixture are propranolol and amitriptyline ($pK_a > 9$), because these compounds are highly basic. At neutral pH, some residual surface silanols and titanols are in their ionized form ($\equiv\text{M-O}^-$), and the basic probes are protonated (BH^+). These protonated bases can interact with the ionized silanols or titanols, causing peak deformation or even irreversible retention (12). Figure 1 shows the chromatograms obtained with these phases. Under these same conditions, propranolol and amitriptyline were not eluted from the SiTiC₁₈ column, indicating irreversible adsorption. On the other hand, the hydrophobic probes were well separated with good peak shapes. This can be seen clearly from the parameters in Table II.

The striking differences between the two chromatograms of Figure 1 are caused by the incorporation of titanium atoms on the silica support. The residual titanols are more acidic than the silanols, enhancing the ionic interaction with the protonated basic solutes.

Some of the compounds of the Engelhardt test mixture (13) also were used to evaluate the phases. Figure 2 shows the chromatograms and Table III lists the chromatographic parameters obtained for the separation using unbuffered and buffered methanol–water mobile phases. The higher retention and tailing factors observed for the amines on the SiTiC₁₈ phase is an indication of higher anion exchange interactions on the titanized support.

Figure 2 also shows the chromatogram obtained when the mobile phase was buffered at pH 7. Tailing is reduced greatly

due to the suppression caused by the potassium ions in the mobile phase. In addition to this, phosphate ions can block the Lewis acid sites on the titanized silica, avoiding the interaction with both weak bases, aniline ($pK_a = 4.87$) and *N,N*-dimethylaniline ($pK_a = 5.07$), which are in their free form in the mobile phase buffered at pH 7.0. The parameters with these new separation conditions were calculated and are presented in Table III.

The higher *k* values for ethylbenzene and toluene are a good indication of the high surface coverage obtained with both phases. The methylene selectivity (α_{CH_2}) also was calculated, taking into account the relation between the *k* values for ethylbenzene and toluene. Values of 1.87 and 1.84 were observed for SiC₁₈ and SiTiC₁₈ phases, respectively. The small differences in these values could be related to the small differences in the surface coverages of these phases.

Column aging studies at pH 9 and 10:

Previous studies have indicated that aqueous mobile phases having pH greater than 9 and containing phosphate anions accelerate silica dissolution and degrade a bonded phase more rapidly (15). Thus, mobile phases containing phosphate solutions were chosen for the aging tests.

The aging test using a methanol–20 mM K₂HPO₄ solution at pH 9.0 as mobile phase was performed to compare the chemical stability of the siloxane bonds between the bonded octadecyl phase and the titanized silica support with that of a conventional phase. It is important to point out that the experimental conditions used in this test were chosen to simulate routine chromatography separations that commonly use pH 9 for the separation of real-world basic samples. Both hydrophobic and basic solutes were used in the evaluation of chromatographic performance. Naphtha-

lene and acenaphthene were used as neutral probes to measure the loss of the organic bonded phase from the surfaces, because their retention is affected extensively by the concentration of the organic groups on the supports. *N,N*-dimethylaniline was chosen as a basic probe, although its interaction with the residual groups is greatly reduced at pH 9, where the residual titanols and silanols are charged negatively and the base is not protonated.

Figures 3 and 4 illustrate the chromatographic parameters obtained during the aging test with the columns packed with the SiTiC₁₈ and SiC₁₈ phases, respectively. Small reductions of approximately 15% and 14% in the retention factor and in the *N/m* values, respectively, were observed for the most retained compound, acenaphthene, after the passage of 21,000 column volumes of the phosphate mobile phase at pH 9 on the SiTiC₁₈ column, when the test was stopped arbitrarily. Similar results were obtained on the column packed with the SiC₁₈ phase under the same experimental conditions, as shown in Figure 4. Thus, there is some evidence for a small loss of bonded C₁₈ ligands from both support surfaces.

From the aging test performed at pH 9, it can be concluded that the C18 titanized phase has roughly the same chemical stability as the conventional C18 phase under the experimental conditions employed. These results are in agreement with those obtained by Kirkland and colleagues (15) for different commercial C18 silicas, using a similar test at pH 9 and ambient temperature.

The possible advantage in preparing C18 phases using titanized instead of bare silica supports was explored by the artificial aging test using pH 10 at a temperature of 50 °C. These conditions are not used routinely for real-world samples. However it can be advantageous because it provides rapid evaluations, saving considerable time and minimizing mobile-phase consumption. The experimental conditions are more aggressive than the conventional test performed at pH 9 because they use a higher phosphate concentration, a higher temperature, and also higher pH.

Figures 5 and 6 show the chromatographic parameters obtained for three analytes during the artificial test at pH 10 for the SiTiC₁₈ and SiC₁₈ phases, respectively. Here, the retention factors did not change significantly during the aging procedure, suggesting that retention factor is not as useful as other parameters for evaluating stationary phase stabilities. Column failure was

characterized in both phases by a rapid decrease in N/m values, together with an increase in tailing factors for all compounds. This behavior is more pronounced for the most hydrophobic and retained compound, acenaphthene. For this reason, the chromatographic parameters for acenaphthene are summarized in Figure 7.

It was observed that the SiC₁₈ column began to fail after the passage of 2110 mL of mobile phase, while for the SiTiC₁₈, this failure started only after the passage of 2770 mL of mobile phase. Fluctuations in N/m values and tailing factors after the first column failure were observed in both cases and can be interpreted as a result of silica dissolution and, consequently, rearrangement of the chromatographic bed.

To better illustrate, Figures 8 and 9 show the chromatograms obtained during the artificial aging test at high pH for the SiTiC₁₈ and SiC₁₈ columns, respectively. After the passage of 3000 mL of the mobile phase, the SiC₁₈ phase is degraded totally, while reasonable separations still can be performed up to 5000 mL for the SiTiC₁₈ phase.

From the stability study performed at pH 10, it can be concluded that the titanized silica surface significantly postponed silica dissolution, allowing a longer column lifetime for the SiTiC₁₈ phase in this aggressive artificial aging test.

Conclusions

A C18 stationary phase based upon a titanized silica support was prepared through reaction with octadecyltrimethoxysilane. The chemical modification was similar to that for bare silica, as similar surface coverages were obtained for both phases. Neutral compounds were well separated on the C18 titanized phase with good peak shapes and high efficiency.

When compared with a conventional C18 phase prepared by a similar reaction, the results indicate that the presence of titanium atoms on the silica surface does not affect the surface coverage. However, the overall chromatographic performance for basic compounds is reduced due to a great number of interactions at neutral pH with the highly anionic titanols.

An artificial stability test performed at pH 10 and 50 °C shows that the SiTiC₁₈ is significantly more stable than the conventional C18 phase for high pH separations.

Acknowledgments

The authors would like to thank Fundacao de Amparo a Pesquisa do Estado de Sao

Paulo (Brazil) (FAPESP) and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (Brazil) (CNPq) for financial support and fellowships.

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