ELSEVIER

Characterization of a new stationary phase based on microwave immobilized polybutadiene on titanium oxide-modified silica

Lais S.R. Morais, Isabel C.S.F. Jardim*

Laboratório de Pesquisas em Cromatografia Líquida — LABCROM, Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, CEP 13084-971 Campinas, SP, Brazil

Abstract

Titanium oxide-modified silica was prepared by reaction of silica with titanium tetrabutoxide and then was used as support in the preparation of stationary phases with self-immobilized polybutadiene (PBD) and PBD immobilized through microwave radiation. Chromatographic performance of the stationary phases was evaluated in terms of the efficiency (plates/m), asymmetry (A_s), retention factor (k) and resolution (R_s) of two standard sample mixtures, one of then containing the basic compound *N,N*-dimethylaniline. A microwave irradiation of 30 min at 520 W gave the best efficiency (86,500 $N\,m^{-1}$), greater than that of a 6-day self immobilized phase (69,500 $N\,m^{-1}$). Self-immobilized stationary phases prepared with bare silica were also studied for comparison. These resulted in lower chromatographic performance, 43,800 $N\,m^{-1}$, when compared to the self-immobilized phase prepared with titanized silica.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Stationary phase; Titanized silica; Polybutadiene; Microwave radiation

1. Introduction

One of the great attractions of the use of silica as support for reversed phase high-performance liquid chromatography (RP-HPLC) is the ease with its surface can be modified with different groups or polymers by reaction with the silanol groups [1,2]. Residual silanol groups, however, become undesirable in the analysis of basic compounds [3,4] and when the use of acidic or basic mobile phases is required [1,5,6]. Several approaches have been taken to minimize these drawbacks. These include the addition of mobile-phase modifiers, decreasing the concentration of silanols by end-capping [7,8], steric protection of silanols by using bulky groups on the chlorosilane modifiers [9,10] and reducing the activity of unreacted silanols by employing an embedded polar group like an amide, an ester or urea in the alkyl chain [11–15].

The use of polymer-coated silica-based phases has been demonstrated to be very effective in reducing silanophilic effects [16,17]. Immobilizations of organic polymers may be achieved by adsorption and crosslinking of adsorbed chains. Recently, some papers have investigated polymeric coatings on silica with poly(alkylmethysiloxanes) and polybutadiene (PBD). Some of these phases were immobilized by different processes, including thermal treatments [18–20], gamma radiation [21–23], microwave radiation [24] or induced by radical initiators (peroxide). Others were simply self-immobilized, which means the absence of conventional immobilization procedures [25–27].

Metal oxides such as zirconia [28–31], alumina [29,32,33] and titania [29,34–36] are becoming popular chromatographic packings as alternatives to silica, because they are significantly more stable than silica at extreme pH. PBD modified zirconia is stable over the $1 < pH < 14$ range and at high temperatures, up to 200 °C, even during prolonged use [30]. Octadecyl bonded titania is stable up to pH 11 and shows a good ability to separate basic solutes at a high pH [36]. Other support materials of increasing interest are titanium [37–39] and zirconium [40,41] oxide-modified silicas, which combine the favorable characteristics of silica with the chemical stability of zirconium and titanium oxides. Stationary phases based on PMOS immobilized through gamma radiation onto

* Corresponding author. Tel.: +55 19 3788 3061; fax: +55 19 3788 3023.
E-mail address: icsfj@iqm.unicamp.br (I.C.S.F. Jardim).

titanized and zirconized silica showed high stability when using neutral and basic mobile phases [38,41]. In another work, thermal treatment and microwave radiation were shown to be efficient methods to immobilize PMOS onto titanized silica, providing stationary phases with good chromatographic performance [39].

In this work, we evaluated the physical and chromatographic properties of a new stationary phase for RP-HPLC based on polybutadiene immobilized through microwave radiation onto titanium oxide-modified silica. As mentioned earlier, a PBD liquid phase has presented good results when coating zirconia and silica. Titanized silica is a relatively new support that is more stable than bare silica and thus requires more study. The use of microwave radiation as an immobilization technique was first introduced by our group. It is a low cost technique and provides good reversed-phase packing materials, as reported in an earlier work [24].

2. Experimental

2.1. Materials

The silica support used was LiChrospher (100-5) (Merck), having 5 μm porous spherical particles. Titanium tetrabutoxide, Ti(BuO)$_4$, was obtained from Aldrich Chemical Company.

The polybutadiene (PBD) polymer [mean molar mass (*M*), ∼5000; composition, 80% 1,4-*cis/trans* and 20% vinyl] was obtained from Aldrich.

n-Butanol (Vetec, analytical-reagent grade) and nitric acid (Quimex) were used in the preparation of titanized silica. Hexane (Tedia, HPLC-grade) was used as solvent in the preparation of the stationary phase. Filtered methanol (Tedia, HPLC-grade) and water (Milli-Q) were used to prepare the mobile phases. The compounds used for chromatographic testing (acetone, acenaphthene, benzene, benzonitrile, naphthalene, *N,N*-dimethylaniline, phenol, toluene and uracil) were analytical reagent grade and were not further purified.

2.2. Preparation of stationary phase

2.2.1. Modification of silica with titanium oxide (chromatographic support)

This preparation is a modification of one previously used in our laboratory [39]. A solution of 1.1 mol l^{-1} Ti(OBu)$_4$ in anhydrous *n*-butanol was prepared and added to a flask containing humid silica at a ratio of 3 ml of Ti(OBu)$_4$ solution for each gram of silica. This mixture was stirred with a vortex, sonicated for 10 min and then maintained at 40 °C for 6 h. During this time it was removed from the thermostated bath 3 times for stirring with a vortex followed by sonication for 3 min. After 6 h, the suspension was centrifuged for 15 min, the supernatant was discarded and the resulting solid was washed with anhydrous *n*-butanol, again being centrifuged.

These steps were repeated five times and, after the last wash, the butanol residue was evaporated. In the second part of procedure, the dried material was hydrolyzed by addition of 15.0 ml of 10^{-3} mol l^{-1} aqueous HNO$_3$ and centrifuged for 15 min. The solid was washed with 15.0 ml of deionized water, sonicated for 3 min and centrifuged for 15 min and these steps were repeated five times. Titanized silica (SiTi) was then dried in an oven at 120 °C for 12 h. The amount of titanium present on the silica was determined by X-ray fluorescence analysis with a Shimadzu model EDX-700.

2.2.2. Preparation of PBD coatings

Preliminary tests were carried out using the proportions of PBD and SiTi (g$_{PBD}$ to g$_{SiTi}$) of 40, 50 or 60%. To load the PBD into the support pore system, a solution of PBD in hexane (12 ml for each 1 g of PBD) was mixed with a suspension of SiTi in hexane (12 ml for each 1 g of SiTi particles). The suspension was stirred for 3 h. After, the hexane was slowly evaporated, without stirring, at room temperature and the stationary phase remained in storage at room temperature for 6 days. One lot of PBD coating was also prepared on bare silica.

2.2.3. Microwave irradiation of stationary phase

For each experiment, 2 g of the prepared stationary phase were put into a polytetrafluorethylene (PTFE) flask (height, 100 mm; i.d., 50 mm). A household microwave oven (Electrolux — ME27) with a rotating plate operated at a frequency of 2.45 GHz, was used in this immobilization process. The microwave power and time of irradiation were optimized to find the best immobilization conditions. Two procedures for the immobilizations were tested: (i) the phases were continuously irradiated, with no stopping during the immobilization time. This is denominated continuous immobilization, (ii) after each 2 min of microwave irradiation, the flask was withdrawn from the oven, stirred and cooled for 2 min and returned to the oven to continue the irradiation. This is denominated paused immobilization.

A calibration of the microwave oven using the US Environmental Protection Agency (EPA) method [42] was carried out. Household microwave ovens do not have the mode stirrer, which distributes the microwaves in diverse directions inside the oven, therefore the intensity of the radiation is variable and some points are warmer than others [43]. To avoid this variation, during the irradiations, the flask was always placed near the edge of the rotating plate, where there was a greater incidence of the microwaves [24].

2.3. Characterization of support and stationary phases

2.3.1. Percent carbon, surface area, mean pore size and specific pore volume

Determinations of specific surface area (S_{BET}, N$_2$), mean pore diameter (d_p) and specific pore volume (v_p) of silica, titanized silica and the stationary phases were carried out with a Micromeritics Model ASAP-2010 instrument.

Elemental analysis (%C and %H) were obtained for the supports (silica and titanized silica) and stationary phases. Duplicate determinations were made with a Model CHN-2400 Perkin-Elmer Analyzer. These data permitted the determination of the real immobilized PBD content in each prepared stationary phase. From these data, the specific mass of PBD, $\bar{m}_{PDB}$, which represents the mass of polymer per gram of silica, was calculated using the formula: $\bar{m}_{PDB} = \%C/(88.9 - \%C)$, since 88.9% of PBD refers to carbon. With the specific mass data the polymer layer thickness, τ, was calculated, as follows: $\tau = -[(\sqrt{d^2 - Fd^2} - d)/2]$, where the F, the immobilized fraction is, $F = \bar{m}_{PBDimm}/\bar{m}_{PBDfullpores}$, and d is the mean pore diameter. It is assumed for this calculation that the pores of the SiTi have a constant diameter and that the immobilized polymer is characterized by a layer of constant thickness, τ, on the pore walls [19]. The specific pore volume, v_p, for titanized silica is $1.02 \, ml \, g^{-1}$ of SiTi, and the density of PBD is $0.89 \, g \, ml^{-1}$. From the pore volume, the mass of polymer that fills the pore system is $0.91 \, g_{PBD}/g_{SiTi}$.

2.3.2. Hard X-ray absorption spectroscopy (XAS)

The XAS experiments were carried out at the XAS beamline of the National Synchrotron Light Laboratory (LNLS), Campinas, SP, Brazil. The measurements were performed in the energy range of (2.5–24) keV, using a Si(1 1 1) crystal monochromator. The extended X-ray adsorption fine structure (EXAFS) spectra of SiTi and anatase (a-TiO$_2$) and rutile (r-TiO$_2$) reference samples were recorded at the Ti K-edge (4970 eV), at room temperature. Further, more accurate measurements were done in the near absorption edge (XANES) region. The computer program used for the analysis of the X-ray absorption data was the WinXAS package.

2.3.3. Infrared spectroscopy

Infrared spectra were obtained of the PBD, the titanized silica and the stationary phases (immobilized by microwave radiation and self immobilized) using a Perkin-Elmer model FT-IR 1600 instrument.

2.4. Chromatographic evaluation of the stationary phase

2.4.1. Column packing

Columns ($60 \times 3.6 \, mm$ i.d.) were made from type 316 stainless-steel tubing. The internal surface was polished using a technique developed in our laboratory [44]. The columns were downward packed using 10% (w/v) slurries of each stationary phase in chloroform. A constant packing pressure of 34.5 MPa (Haskel packing pump) was used, with methanol as propulsion solvent. Columns were conditioned for 2 h with a mobile phase consisting of methanol:water 60:40 (v/v) at $0.2 \, ml \, min^{-1}$.

2.4.2. Chromatographic evaluation

The chromatographic evaluation of column performance was done with a modular HPLC system equipped with a Waters Model 486 pump, a Rheodyne Model 7725i injection

Table 1
Mean pore diameter (d_p), specific pore volume (v_p) and specific surface area (S_{BET}) of silica and titanized silica

Type of material	d_p (nm)	v_p (ml g^{-1})	S_{BET} (m^2 g^{-1})
Silica	12.8	1.2	412
Titanized silica (SiTi)	10.9	1.0	376

valve (10 µl loop) and a Waters Model 486 UV–vis detector (at 254 nm). Data acquisition used Chrom Perfect for Windows, version 3.52 and Report Write Plus software (Justice Innovations).

Two test mixtures were used for stationary phase evaluation: mixture 1, containing acetone, benzonitrile, benzene, toluene and naphthalene and mixture 2, containing phenol, *N,N*-dimethylaniline, naphthalene and acenaphthene. The column dead time, t_M, was determined using uracil as an unretained compound. Chromatographic performance was evaluated by means of efficiency [plates per meter (N/L)], asymmetry factor (A_s) measured at 10% of the peak height, retention factor (k) and resolution (R_s). The mobile phase was methanol:water (60:40, v/v) at $0.2 \, ml \, min^{-1}$. The optimal flow-rate was determined by a van Deemter plot.

3. Results and discussion

3.1. Modification of silica with titanium oxide (chromatographic support)

Titanium oxide-modified silica has been a material of growing interest in the last two decades because of its use as a catalyst. Therefore, the literature has reported a number of different procedures for the synthesis of this material [45–47]. Many of these procedures involve long and laborious stages, which includes the use of high temperatures, inert atmospheres and systems in reflux. In contrast, our procedure does not require the use of any of these conditions, it is much less drastic and less time consuming. Also, it is very reproducible: in all four titanizations done in this work, the amount of titanium present on silica was the same, 8.9%, as determinated by X-ray fluorescence.

3.2. Characterization of the support and stationary phases

Table 1 shows the specific surface area (S_{BET}), mean pore diameter (d_p) and specific pore volume (v_p) of bare silica and titanized silica. All three measurements decrease with titanium modification. The reduction in the mean pore diameter indicates that the reaction between silica and titanium oxide did not occur homogeneously or that, the titania had preferential access to the larger pores. When reacting with the silanol groups, most of the titania forms a monolayer on the surface of the silica. However, a small amount fills the pores. Another factor that may have contributed to this pore reduc-
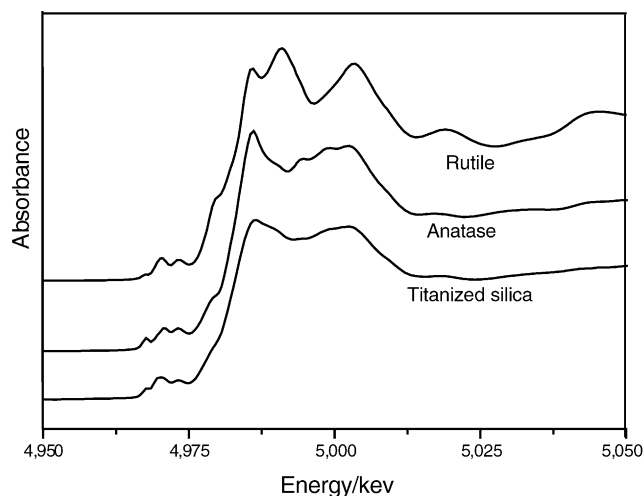
Fig. 1. Ti K-edge XANES spectra of titanium modified silica compared to the standard reference samples, rutile and anatase.

tion is a titania residue that did not react covalently with the silica but was not eliminated with washing.

XAS spectra contains two energy regions: X-ray absorption near edge structure (XANES) of lower energy and EX-AFS of higher energy. The XANES region of the spectra yields information such as structure and bond geometry of TiO_2 layer onto silica [48]. One simple way to obtain the relevant data in the XANES region is to compare the spectra of titanized silica with the spectra of the standard reference samples of titania: rutile (r-TiO_2) and anatase (a-TiO_2). Fig. 1 presents the Ti K-edge XANES spectra of titanized silica compared to r-TiO_2 and a-TiO_2 spectrum. Since the spectrum of titanized silica and anatase are very similar, it is suggested that the titania layer on silica is in a form similar to crystalline anatase. The EXAFS region of the spectra of XAS yields the values of coordination number and Ti–O bond distance on titanized silica and these values were compared with those of a-TiO_2 form reported in the literature [49], as presented in Table 2. Native titania in the anatase crystalline form presents an octahedral geometry with six oxygens coordinated to Ti while the amorphous state and Ti–OH hydroxyls have a five-coordinated Ti species [50,51]. As observed in Table 2, the titanized silica synthesized in this work has coordination number six, endorsing the results obtained in the analysis of XANES spectra that the titania layer on silica is in its anatase crystalline form.

The infrared spectrum of the bare silica and the titanized silica (Fig. 2a and b) present bands in equivalent regions. The band around 3450 cm^{-1} corresponds to the vibrations of the hydroxyl groups with hydrogen bonding to the physically

Table 2
Values of Ti–O coordination number and bond distance in SiTi and in an anatase (a-TiO_2) sample

Type of material	Coordination number	Bond distance (nm)
SiTi	6.0	0.19
a-TiO_2	6.0	0.19

adsorbed water and also to the geminal hydroxyl groups. At 1109 and 975 cm^{-1} appear the bands attributed to the siloxanes and free silanols, respectively. The infrared spectra of PBD (Fig. 2c) presented five main regions that characterize this polymer: absorption at 723 cm^{-1} that is characteristic of the structure *cis*-1,4, the band in 909 cm^{-1} that is attributed to the vinyl structure and the band in ~961 cm^{-1} referring to the *trans*-1,4 double bonds [52]. The three bands around 2900 cm^{-1} are attributed to C–H stretching of the double bonds of PBD and the band at 1440 cm^{-1} is attributed to C–H deformation [53]. Comparing the spectra of titanized silica and PBD separately, it is observed that they present signals in similar regions that overlap in the spectrum of the self-immobilized and immobilized stationary phases (Fig. 2d–f). However, it was possible to verify the incorporation of PBD on the titanized support from the bands around 2900 cm^{-1}, characteristic of PBD and the signals around 1100 cm^{-1} from titanized silica, identified in the spectra of the titanized silica. The spectra of the immobilized phase irradiated for 30 min (Fig. 2f) shows a band at 1723 cm^{-1}, characteristic of ketone formation. This band does not appear in the spectra of an immobilized phase irradiated for 1 min, indicating that long irradiation times in an air atmosphere cause the oxidation of PBD.

3.3. Chromatographic evaluation

3.3.1. Optimization of loadings of PBD

The efficiencies (plates m^{-1}) and asymmetry factors (A_s) of non-irradiated stationary phases prepared with 40, 50 and 60% of PBD on titanized silica supports were $69,500 \pm 2900$ and 1.4; $62,800 \pm 1900$ and 0.7 and $64,900 \pm 1700$ and 0.7, respectively. All phases showed good retention factors and resolutions. The stationary phase prepared with a loading of 40% gave the best chromatographic performance.

3.3.2. Evaluation of self-immobilized phases prepared with bare silica and titanized silica

Non-irradiated phases were prepared with bare silica and titanized silica in order to compare their chromatographic performances. The residual percentages of PBD on non-irradiated stationary phases prepared with bare [SiO_2(PBD)] and titanized [SiTi(PBD)] silica supports were 15.0 and 11.5, respectively. The efficiency for SiO_2(PBD) was $43,800 \pm 600$ and for SiTi(PBD) was $69,500 \pm 2900$. Stationary phases based on titanized silica have better performances but smaller quantities of PBD (Fig. 3). This fact can be explained by the following considerations: Bottoli et al. [54] investigated the role of the silanol groups ($\equiv$Si–OH) of the silica surface on the process of polymer sorption that occurs in self-immobilization. The silanol groups of silica were modified with the hydrophobic groups chlorotrimethylsilane or poly(dimethylsiloxane), with posterior self-immobilization of liquid phase PMOS. Chemical, physical and chromatographic tests showed that the incorporation of hydrophobic groups on silica surface before the
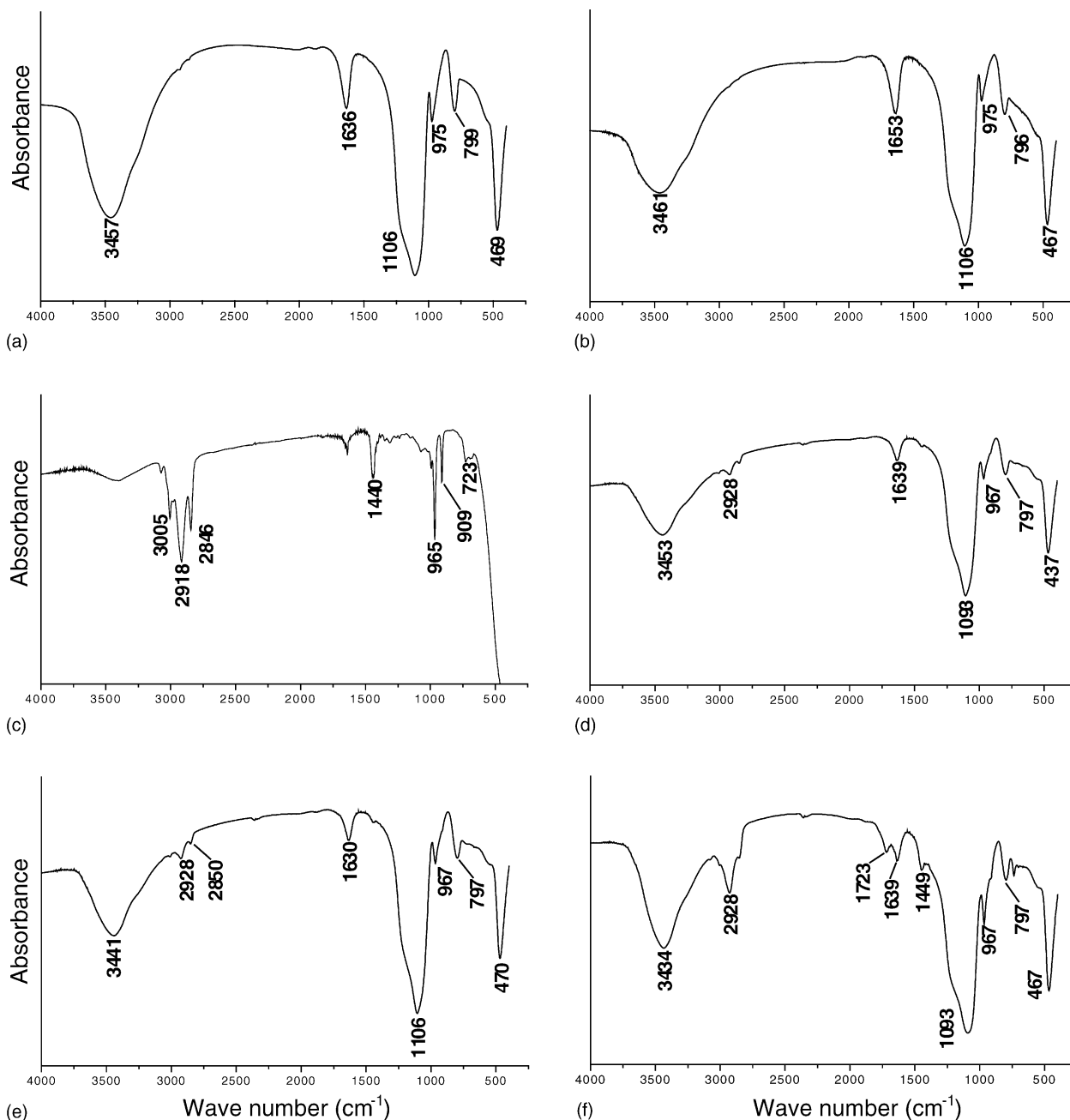
Fig. 2. Infrared spectra of (a) LiChrospher silica; (b) titanized silica; (c) PBD, (d) self-immobilized phase; (e) stationary phase immobilized for 1 min at 452 W and (f) stationary phase immobilized for 30 min at 520 W.

coating with polymer resulted in less reactive silanols, which minimized PMOS and silica interaction. Thus, it was concluded that the silanol groups have a fundamental role in the process of self-immobilization. In the present work, titanized silica was prepared by the reaction of titanium tetrabutoxide with the silanol groups of silica, which means that, in this case, protection of the silanol groups also occurred, leaving them less reactive. After the titanization, it would be logical that titanol groups could replace silanol groups and act as anchors of PBD in self-immobilization. However Delattre and Babonneau [55] used ^{17}O nuclear magnetic resonance to

investigate SiO_2–TiO_2 bonds and noted that no NMR signal assigned to Ti–OH groups was observed in solution or even in the solid state for their SiO_2–TiO_2 system. The explanation for this is the fact that Ti–OH groups have high reactivities and push themselves to condense, resulting in Ti–O–Ti bonds and Ti-based clusters, so the actual amount of free titanols is low. Besides, Ti–OH hydroxyls are in a five-coordinated Ti species and XAS spectra analysis showed that the titania layer on silica was formed mainly of six-coordinated Ti species (indicating Ti–O–Ti bonds) [51]. In the literature, it has been reported that the average silanol concentration of
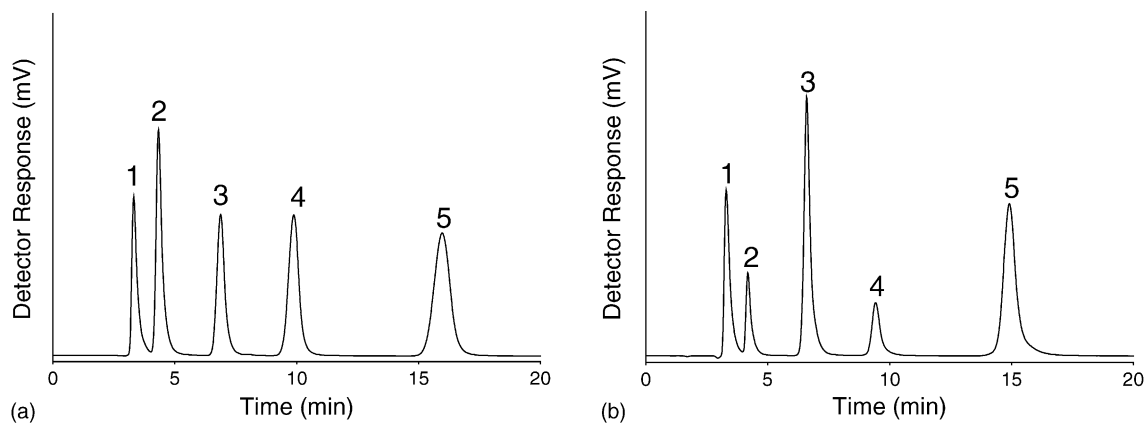
Fig. 3. Chromatographic behavior of stationary phases prepared with PBD self-immobilized onto (a) bare and (b) titanized silica supports. Test mixture 1 = uracil, acetone, 2 = benzonitrile, 3 = benzene, 4 = toluene and 5 = naphthalene. Chromatographic conditions: mobile phase: methanol:water (60:40, v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 10 μl and detector: UV at 254 nm.

a fully hydroxylated silica is about $8.0 \pm 1\,\mu mol\,m^{-2}$ [4]. From this value and the surface area of the LiChrospher silica ($412\,m^2\,g^{-1}$), the theoretical amount of Ti atoms necessary to react with all silanol groups and form a monolayer is 7.0%. However, of the $8\,\mu mol\,m^{-2}$ initial silanol concentration, only 50% can be derivatized [56]. If we consider that only $4.0\,\mu mol\,m^{-2}$ of silanol groups actually reacted with TiO$_2$, then the obtained titanium percent of 8.9% indicates the formation of a double layer of titanium onto silica, which is another evidence of Ti–O–Ti formation.

3.3.3. Evaluation of microwave radiation immobilized phases prepared with titanized silica

Table 3 shows the chromatographic parameters of stationary phases immobilized at a microwave power level of 586 W for different continuous immobilization times. As can be observed, efficiency decreases with the increase of immobilization time. Also, the percent carbon data obtained for these phases showed that the amount of PBD immobilized on titanized silica increases with the increase in immobilization time. The amount of PBD immobilized reaches 24.3%

Table 3
Chromatographic parameters, final percentages of PBD and layer thickness (τ) of stationary phases microwave immobilized at power level 586 W, for different continuous immobilization times

Time (min)	Chromatographic parameters[a]				PBD (%)	Layer thickness (τ) (nm)
	(N m^{-1})[b]	A_s[b]	k[b,c]	R_s[d]		
1	51700 ± 1400	2.0	3.3	5.5	13.5	0.48
2	36000 ± 800	1.6	11	6.5	20.6	0.84
3	21600 ± 400	1.3	11	5.1	22.4	0.95
10	10800 ± 200	0.6	13	3.8	24.3	1.06

[a] Chromatographic conditions: mobile phase: methanol:water 60:40 (v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 10 μl and detector: UV at 254 nm. Values shown are averages of three injections.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene–naphthalene pair.

with an immobilization time of 10 min. In this condition, the thickness of the PBD layer on titanized silica was 1.06 nm and, as reported by Lopes et al. [23,24], the best phases with PBD immobilized by microwave and gamma radiation onto bare silica presented this same layer thickness. In the present case, however, efficiency values decreased with the increase in PBD layer thickness, reaching the lowest value, $10,800\,N\,m^{-1}$. In these tests, the microwave radiation immobilization was continuous, resulting in degradation of the phases.

The result of this first test showed that continuous microwave irradiation of stationary phases was causing degradation, when using longer immobilization times. On the other hand, shorter immobilization times did not result in stationary phases with good chromatographic performances. It was necessary to attenuate the intensity of immobilization in order to make possible the use of longer times with high microwave levels. This was done in two different ways: cooling and stirring the phases during the period of irradiation and activating the titanized silica support before stationary phase preparation. In the microwave immobilization, a greater amount of water causes higher energy dissipation, which in turn results in greater quantities of heat. For this reason, in this work most of the stationary phases were prepared with titanized silica which was not dehydrated, which means that water molecules adsorbed onto its surface were kept to promote a maximum absorption of irradiation. With the objective of diminishing radiation absorption by the phase, some were prepared with titanized silica which had been dehydrated.

Table 4 presents the chromatographic parameters of stationary phases immobilized for 30 min at different microwave power levels, with paused immobilization. As can be seen in this table, efficiency values were similar with microwave power levels between 386 and 452 W, with the maximum value, $86,500\,N\,m^{-1}$ at a power of 520 W. When microwave power was increased to 586 W, efficiency values decrease. However, most phases in this evaluation presented good chromatographic performance, better than the continuous immo-

Table 4
Chromatographic parameters, final percentages of PBD and layer thickness of stationary phases microwave immobilized for 30 min at different paused immobilization microwave power levels

Power level (W)	Chromatographic parameters[a]				PBD (%)	Layer thickness (τ) (nm)
	$(N\,m^{-1})$[b]	A_s[b]	k[b,c]	R_s[d]		
386	56700 ± 200	1.0	9.8	8.1	21.8	0.91
452	55000 ± 300	1.1	7.7	7.3	18.2	0.70
520	86500 ± 700	0.8	15.0	10.6	25.3	1.1
586[e]	71900 ± 300	2.1	6.0	7.7	17.3	0.67
586	76700 ± 300	0.9	11.5	9.5	23.4	1.0
653	58000 ± 400	1.3	11.0	7.6	22.4	0.95

[a] Chromatographic conditions: mobile phase: methanol:water (60:40, v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 10 μl and detector: UV at 254 nm. Values shown are averages of three injections.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene–naphthalene pair.
[e] Phase prepared with activated titanized silica.

bilization phases. The phase immobilized at 520 W showed the highest efficiency and also presented the highest retention factor, as can be seen in Fig. 4 and Table 4. Elemental analysis data (%C) of this phase indicated a final percent of immobilized PBD of 25.3%. The higher the percentage of PBD, the higher is the interaction of apolar solutes with the stationary phase and the higher is the retention factor. This greater amount of PBD formed a PBD-layer thickness of 1.1 nm on the support surface. As discussed, in previous works, the best immobilized phases were achieved when the PBD-layer thickness were close to this value. In the earlier tests, using continuous immobilization for 10 min at 586 W, the incorporation of PBD was also high, with the formation of a PBD-layer thickness of 1.06 nm. However, since the irradiation was continuous, the phase was degraded and presented lower efficiencies. So, the cooling and homogenizing of phases during the period of irradiation seems to play an important role in immobilization by microwave radiation. The phase prepared with dehydrated (activated) titanized silica presented retention factors and percent carbon lower than the phase prepared with deactivated titanized silica, indicating that the elimination of water molecules really decreases the absorption of microwave radiation. Moreover, the asymme-

try of naphthalene on the phase prepared with activated SiTi was quite high, as can be seen in Fig. 4.

3.3.4. Evaluation of microwave radiation and self-immobilized phases with a basic compound

Immobilized (power level, 586 W; paused irradiation time, 30 min) and self-immobilized phases were used to analyze test mixture 2, containing the basic compound *N,N*-dimethylaniline. In this test, the chromatographic parameters obtained with these phases were calculated for the basic compound. The efficiency and asymmetry factor were 3900 ± 700 and 3.3 and $31,700 \pm 300$ and 1.7 for non-irradiated and microwave immobilized stationary phases, respectively. The microwave radiation immobilized phase presented chromatographic performances better than the non immobilized phase. As expected, the process of immobilization promoted a better PBD coverage of the chromatographic support, minimizing the interaction of the hydroxyl groups with *N,N*-dimethylaniline. This can be confirmed by comparison of the chromatograms obtained with these two phases (Fig. 5). In the column containing non-irradiated stationary phase the retention time and asymmetry factor of *N,N*-dimethylaniline were higher and its peak position was
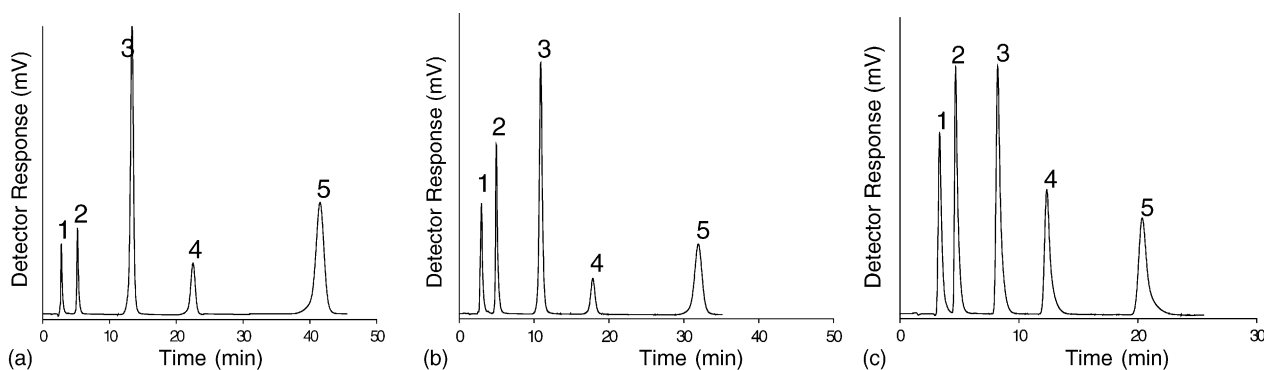

Fig. 4. Chromatograms obtained for stationary phases microwave immobilized for 30 min at different paused immobilization microwave power levels of (a) 520; (b) 586 W on non-activated support and (c) 586 W on activated support. Test mixture 1 = uracil, acetone, 2 = benzonitrile, 3 = benzene, 4 = toluene and 5 = naphthalene. Chromatographic conditions: as in Fig. 3.
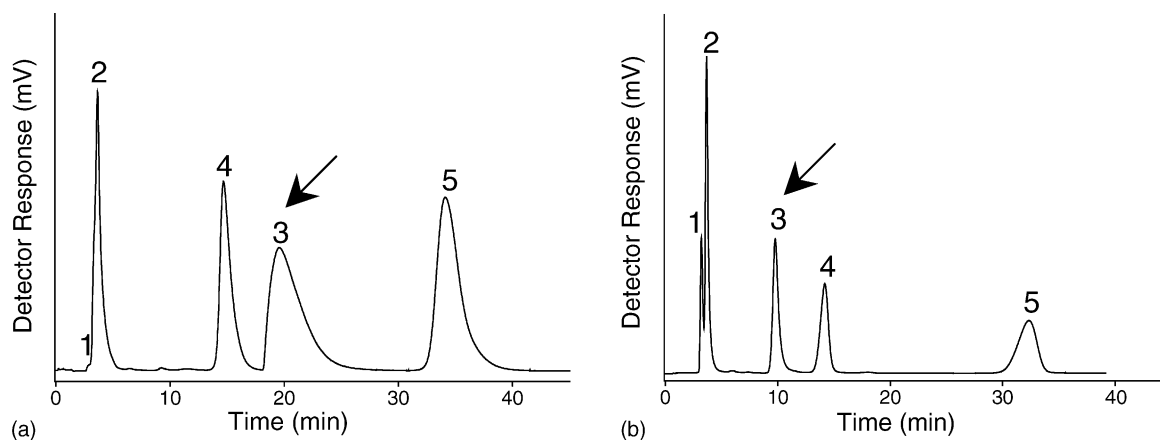
Fig. 5. Chromatograms obtained with test mixture 2 with different stationary phases. (a) self-immobilized; (b) paused microwave radiation immobilized for 30 min at power level 586 W. Test mixture 2:1 = uracil, 2 = phenol, 3 = *N,N*-dimethylaniline, 4 = naphthalene and 5 = acenaphthene. Chromatographic conditions: as in Fig. 3.

transposed, eluting after the naphthalene peak, because of the interaction of this compound with hydroxyl groups, which were more exposed in this phase. As can be observed in Fig. 5, this interaction with hydroxyl groups resulted in peak tailing of *N,N*-dimethylaniline, and an asymmetry factor of 3.3.

In the immobilized phase the coverage of the support was greater and there were fewer acidic hydroxyl groups to adsorb *N,N*-dimethylaniline, which resulted in a lower retention time and greater efficiency and symmetry of this peak.

4. Conclusions

The evaluation of self-immobilized phases prepared with bare silica and titanized silica showed that the incorporation of titanium onto silica provides an increase in the chromatographic performance of the stationary phases, even without microwave immobilization of the PBD stationary phase.

The optimization of the best immobilization condition showed that short immobilization times were not sufficient while long, continuous times caused the degradation of the phase. With the inclusion of cooling and stirring the phases during the irradiation, it was possible to increase the immobilization time and promote a better coating without degradation. A phase that was microwave immobilized at 520 W for 30 min showed the best chromatographic performance.

The asymmetry factor of *N,N*-dimethylaniline was 3.3 on a self-immobilized phase and 1.7 on a microwave immobilized phase. This result shows that the immobilization of PDB by microwave radiation promoted better surface protection, reducing the interactions of the basic solute with hydroxyl groups.

Microwave radiation is a versatile and low cost technique for immobilization that presents potential for the preparation of stationary phases. Investigations of its use are continuing.

References

[1] J. Nawrocki, C. Dunlap, A. McCormick, P.W. Carr, J. Chromatogr. A 1028 (2004) 1.
[2] M.R. Buchmeiser, J. Chromatogr. A 918 (2001) 233.
[3] D.V. McCalley, LC GC 17 (1999) 440.
[4] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[5] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
[6] J.J. Kirkland, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 691 (1995) 3.
[7] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, K. Kimata, N. Tanaka, J. Chromatogr. 481 (1989) 147.
[8] Y. Soda, J. Chromatogr. A 757 (1997) 3.
[9] A.B. Scholten, H.A. Claessens, J.W. de Haan, C.A. Cramers, J. Chromatogr. A 759 (1997) 37.
[10] M.J.J. Hetem, J.W. de Haan, H.A. Claessens, L.J.M. van de Ven, C.A. Cramers, J.N. Kinkel, Anal. Chem. 62 (1990) 2288.
[11] T. Czajkwoska, M. Jaroniec, J. Chromatogr. A 762 (1997) 147.
[12] C.P. Jaroniec, R.K. Gilpin, M. Jaroniec, J. Chromatogr. A 797 (1998) 103.
[13] C.R. Silva, S. Bachmann, R.R. Schefer, K. Albert, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 948 (2002) 85.
[14] C.R. Silva, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 987 (2003) 127.
[15] C.R. Silva, C.H. Collins, I.C.S.F. Jardim, C. Airoldi, J. Chromatogr. A 1030 (2004) 157.
[16] G. Schomburg, Trends Anal. Chem. 10 (1991) 163.
[17] M. Petro, D. Berek, Chromatographia 37 (1993) 549.

[18] G. Schomburg, J. Köhler, H. Figge, A. Deege, U. Bien-Vogelsang, Chromatographia 18 (1984) 265.

[19] C.B.G. Bottoli, Z.F. Chaudhry, D.A. Fonseca, K.E. Collins, C.H. Collins, J. Chromatogr. A 948 (2002) 121.

[20] C.B.G. Bottoli, C.R.M. Vigna, G. Fischer, K. Albert, K.E. Collins, C.H. Collins, J. Chromatogr. A 1030 (2004) 217.

[21] U. Bien-Vogelsang, A. Deege, H. Figge, J. Köhler, G. Schomburg, Chromatographia 19 (1984) 170.

[22] I.C.S.F. Jardim, K.E. Collins, T.A. Anazawa, J. Chromatogr. A 849 (1999) 299.

[23] N.P. Lopes, K.E. Collins, I.C.S.F. Jardim, J. Chromatogr. A 987 (2003) 77.

[24] N.P. Lopes, K.E. Collins, I.C.S.F. Jardim, J. Chromatogr. A 1030 (2004) 225.

[25] T.A. Anazawa, I.C.S.F. Jardim, J. Liq. Chromatogr. 17 (1994) 1265.

[26] C.B.G. Bottoli, K.E. Collins, C.H. Collins, J. Chromatogr. A 987 (2003) 87.

[27] K.E. Collins, C.B.G. Bottoli, C.R.M. Vigna, S. Bachmann, K. Albert, C.H. Collins, J. Chromatogr. A 1029 (2004) 43.

[28] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2193.

[29] A. Kurganov, U. Trüdinger, T. Isaeva, K. Unger, Chromatographia 42 (1996) 217.

[30] C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A. Wang, A.M. Weber-Main, LC GC 18 (2000) 515.

[31] C.J. Dunlap, C.V. McNeff, D. Stoll, P.W. Carr, Anal. Chem. 73 (2001) 599.

[32] Y. Mao, B.M. Fung, J. Chromatogr. A 790 (1997) 9.

[33] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 952 (2002) 1.

[34] K. Tani, Y. Suzuki, J. Chromatogr. A 722 (1996) 129.

[35] J.J. Pesek, M.T. Matyska, J. Ramakrishnan, Chromatographia 44 (1997) 538.

[36] C. Stella, S. Rudaz, J.-L. Veuthey, A. Tchapla, Chromatographia 53 (2001) S-113.

[37] R.B. Silva, C.H. Collins, J. Chromatogr. A 845 (1999) 417.

[38] R.B. Silva, Y. Gushikem, C.H. Collins, J. Sep. Sci. 24 (2001) 49.

[39] D.A. Fonseca, K.E. Collins, C.H. Collins, J. Chromatogr. A 1030 (2004) 209.

[40] L.F.C. Melo, I.C.S.F. Jardim, J. Chromatogr. A 845 (1999) 423.

[41] L.F.C. Melo, C.H. Collins, K.E. Collins, I.C.S.F. Jardim, J. Chromatogr. A 869 (2000) 129.

[42] H.M. Kingston, L.B. Jassie, Introduction to Microwave Sample Preparation, ACS Professional Reference Book, Washington, DC, 1988.

[43] E.R. Pereira Filho, J.J.R. Rohwedder, M.A.Z. Arruda, Analyst 123 (1998) 1023.

[44] K.E. Collins, A.C. Franchon, I.C.S.F. Jardim, E. Radovanovic, M.C. Gonçalves, LC GC 18 (2000) 106.

[45] A. Fernandez, J. Leyrer, A.R. González-Elipe, G. Munuera, H. Knözinger, J. Catal. 112 (1988) 489.

[46] L.T. Kubota, Y. Gushiken, S. Castro, J.C. Moreira, Colloids Surf. 57 (1991) 11.

[47] J. Retuert, R. Quijada, V.M. Fuenzalida, J. Mater. Chem. 10 (2000) 2818.

[48] N. Watanabe, A.Y. Ramos, M.C.M. Alves, H. Tolentino, O.L. Alves, L.C. Barbosa, J. Mater. Res. 15 (2000) 793.

[49] M.F. Ruiz-López, A. Muñoz-Paez, J. Phys: Condens. Matter 3 (1991) 8981.

[50] E.R. Leite, V.R. Mastelaro, S.M. Zanetti, E. Longo, Mater. Res. 2 (1999) 93.

[51] W.B. Kim, S.H. Choi, J.S. Lee, J. Phys. Chem. B 104 (2000) 8670.

[52] R.R. Hampton, Anal. Chem. 21 (1949) 923.

[53] D. Nava, T.R. Parada, E. González, N. Boscán, C. Cruz, Spectrochim. Acta A 52 (1996) 1201.

[54] C.B.G. Bottoli, C.R. Silva, K.E. Collins, C.H. Collins, J. Liq. Chromatogr. Rel. Technol. 27 (2004) 407.

[55] L. Delattre, F. Babonneau, Chem. Mater. 9 (1997) 2385.

[56] A. Berthod, J. Chromatogr. 549 (1991) 1.