Preparation and characterization of a new C$_{18}$ urea phase based on titanized silica

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Abstract

A new stationary phase containing embedded urea groups (−NH−C(O)−NH−) was prepared by a procedure based on the synthesis of a trifunctional C$_{18}$ urea-alkoxysilane, followed by modification of titanized silica and further endcapping to evaluate if the embedded group would minimize the higher retention and tailing for basic compounds seen with C$_{18}$ titanized silica phases. Infrared, ^{13}C and ^{29}Si spectroscopies were employed to characterize the C$_{18}$-urea titanized silica phase. Chromatographic evaluations used hydrophobic, polar and basic compounds to verify the effects of the polar urea groups embedded in the C$_{18}$ urea phase. The chromatographic parameters, especially for the separation of basic compounds, compare favorably with those obtained on a C$_{18}$ titanized silica stationary phase, prepared by silanization of titanized silica with octadecyltrimethoxysilane.
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1. Introduction

The search for supports more stable in basic solutions than silica, for use in high performance liquid chromatography (HPLC), is still of importance in the quest for reversed phases having greater hydrolytic and thermal stability [1,2]. Under aggressive conditions, using high pH mobile phases and elevated temperatures, silica-based reversed stationary phases are known to fail rapidly due to dissolution of the silica support [3].

Accordingly to Buchmeiser [1], the first reports on the preparation of more pH-stable reversed phases by alkyl-silanization of zirconia [4] titania [5] and alumina [6] date from the early 1990s. However, these approaches were somewhat limited due to the difficulty in obtaining these alternative supports in a suitable range of particle and pore sizes. In addition, the surface concentration of the C$_{18}$ ligands on titania was low compared to silica [7].

As alternatives, alumina, titania and zirconia surfaces were modified using triethoxysilane [8]. In a second step, alkenes were immobilized by a hydrosilylation reaction with the reactive Si−H groups on the surface, significantly improving coverage, especially for titania, when compared to the conventional silanization with alkoxysilanes [9].

Reversed phases based on zirconia were successfully developed by Carr and coworkers [2]. To modify the zirconia surface, several approaches were developed, including deposition and immobilization of polybutadiene and polystyrene, and, more recently, derivatization with alkyl chains directly onto a carbon coated zirconia surface [10].

In principle, the dissolution of the silica support can be reduced by the chemical deposition of pH stable metal oxides on the silica surface without sacrificing the porous silica structure, and taking advantage of silica's well-known chromatographic properties. Polymer-coated phases with potential applications using alkaline mobile phases have been prepared by the immobilization of poly(methyloctylsiloxane) on titanium-grafted silica [11–13].

Alternatively, chemically bonded C$_{18}$ phases with improved chemical stability in basic mobile phases have been prepared, based on silanization of titanized silica [14]. However due to the amphoteric properties of the titanized silica support, this phase exhibits higher retention and more tailing

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with highly basic compounds in non-buffered mobile phases [15].

On the other hand, silica-based reversed phases having embedded amide [16], carbamate [17,18], and urea [19,20] polar groups show excellent performance for the separation of basic compounds at neutral pH. More recently, polar embedded phases containing *n*-alkyl thiol functionalities have also been reported [21]. The advantage of these polar embedded phases is provided by the presence of the polar groups, allowing the use of mobile phases having lower percentages of organic solvent without phase collapse [22], improving the retention of polar compounds [23].

The present work describes the preparation of a phase based on titanized silica and having embedded urea polar groups. This new phase, which combines the two different technologies, was then evaluated to verify whether the embedded polar groups could reduce peak tailing for basic compounds.

2. Experimental

2.1. Solvents and chemicals

Amitriptyline, aniline, caffeine, *N,N*-dimethylaniline, phenol, propranolol, pyridine, triphenylene, phosphoric acid and uracil were obtained from Aldrich (Milwaukee, USA) and were used as received. For the synthesis of the silylant agent, octadecylamine (98%, Aldrich, USA) and [(3-isocyanate)propyl]triethoxysilane (99%, United Chemical Technologies, Bristol, USA) were used without further purification. For the preparation of the bonded phases, 5 μm spherical Kromasil silica (Lot AT0235, pore diameter of 10 nm, BET surface area of $308 \pm 5\,\mathrm{m^2\,g^{-1}}$ and pore volume of $0.9\,\mathrm{ml\,g^{-1}}$, from Akzo Nobel, Bohus, Sweden), tetraisopropyl orthotitanate (99%, Fluka, Germany), octadecyltrimethoxysilane (Aldrich), trimethylchlorosilane (TMCS, Aldrich), and hexamethyldisilazane (HMDS, Aldrich) were employed.

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 (MeOH), isopropanol and acetonitrile (ACN) were HPLC grade and were purchased from Tedia (Fairfield, USA). 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 were filtered through a 0.2 μm Nylon membrane filter and degassed by sonication prior to use.

2.2. Equipment

Melting points were determined from Differential Scanning Calorimetry (DSC) performed on a model 2050 Thermal Analyzer from TA Instruments (USA). C, H, N analyses

were performed on a model 2400 analyzer from Perkin-Elmer (Shelton, USA). At least two determinations were made for each sample. Mass spectrometry was performed on an AutoSpec spectrometer from Micromass (USA).

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

Liquid ^{1}H and ^{13}C NMR spectra were obtained on a INOVA 500 spectrometer (Varian, Palo Alto, USA). Frequencies of 500 and 125 MHz were used for hydrogen and carbon nuclei, respectively. A capillary containing deuterated water was used as internal reference for the ^{1}H NMR spectrum and carbon tetrachloride for ^{13}C.

Solid-state ^{13}C and ^{29}Si NMR measurements for the modified silicas, before and after endcapping, were performed on a Bruker AC 300 spectrometer (Germany), 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 were used. The frequencies were 75.5 and 59.6 MHz for carbon and silicon, respectively.

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 carefully dispersing pure TiO_2 (99.5%, Riedel de Häen) in the Kromasil silica matrix. The standards and the samples were analyzed at the titanium Kα emission line (4.3–4.7 keV).

Chromatographic evaluations, using $60\,\mathrm{mm} \times 3.9\,\mathrm{mm}$ columns, were performed using a modular HPLC system from Shimadzu (Kyoto, Japan) 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).

2.3. Preparation of the titanized silica

A sample of 11.0 g of Kromasil silica, activated at 100–120 °C for 6 h under vacuum to remove physically adsorbed water, was reacted with 18 ml of titanium isopropoxide in 150 ml of dry toluene. The mixture was refluxed for 24 h under a dry argon atmosphere, filtered and washed with toluene, isopropanol, methanol and finally with copious amounts of deionized water. After drying under vacuum for 6 h at 100 °C, the percentage of Ti as titanium oxide was determined by X-ray fluorescence, obtaining $11.95 \pm 0.35\%$, which corresponds to a surface coverage of $4.81 \pm 0.15\,\mu\mathrm{mol}$ of Ti $\mathrm{m^{-2}}$.
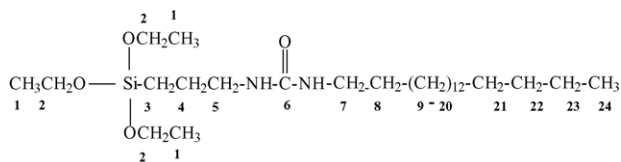
Fig. 1. Proposed chemical structure of the synthesized silylant agent.

2.4. Synthesis of the silylant agent

The synthesis of the silylant agent [(3-urea-octadecyl) propyl]triethoxysilane was based on a proprietary chemical process [24]. Briefly, 40 mmol of the precursor [(3-isocyanate)propyl] triethoxysilane was dissolved in 20 ml of dry toluene. This solution was slowly added to an equimolar octadecylamine solution (40 mmol dissolved in 80 ml of dry toluene) and the reaction mixture were refluxed for 3 h under stirring and in a dry argon atmosphere. At the end of this process, the solvent was removed under reduced pressure to obtain a white solid, which was then washed with dry methanol, dried under reduced pressure and analyzed.

Analyses: melting point 79 °C, elemental analysis for $C_{28}H_{60}N_2O_4Si$ (516 g mol^{-1}), experimental (calculated) percentages, %C, 65.2 ± 1.8 (65.1), %H, 12.1 ± 0.40 (11.4), %N, 5.68 ± 0.80 (5.41). MS, M$^+$ = 516 m/z, IR spectrum, 3330 (ν-N–H), 2915 and 2850 (ν-C–H), 1625 (ν-C=O), 1590 (δ-N–H), 1470 (δ-CH$_2$), 1080 (ν-Si–O) cm^{-1}. ^{13}C NMR spectrum (CCl$_4$), δ = 7 (C3), 14 (C24), 18 (C1), 22.2 (C23), 23.7 (C22), 27 (C21), 29 (C9–C20) 31 (C4), 32 (C8), 40 (C5), 42 (C7), 58 (C2), 96 (CCl$_4$), 158 (C6), according to the structure shown in Fig. 1. ^{1}H NMR spectrum (CCl$_4$), δ = 0.55 (t, 2H), 0.9 (t, 3H), 1.2 (t, 9H), 1.25 (m, 32H), 1.5 (m, 4H), 3.15 (m, 4H), 3.75 (q, 4H), 5.45 (s, 1H), 5.55 (s, 1H).

2.5. Preparation of the C_{18}-urea titanized silica (SiTiC$_{18}$ urea)

The silanization reaction used 37 mmol of freshly synthesized C$_{18}$ urea-alkoxysilane and an equivalent amount of distilled pyridine (3.2 ml) to react with 5.0 g of activated titanized silica, suspended in 100 ml dry toluene. The mixture was refluxed for 48 h under an argon atmosphere and the solid was then filtered, washed with toluene, isopropanol, methanol and deionized water and dried under vacuum at 60 °C for 8 h prior to endcapping, which was carried out by refluxing 4.5 g of product with a large excess of TMCS (15 ml) and HMDS (35 ml) in 100 ml of dry toluene under an argon atmosphere for 48 h.

Another batch of titanized Kromasil, with approximately 14% of titanium oxide on the surface, was reacted with octadecyltrimethoxysilane and then endcapped with TMCS and HMDS to obtain SiTiC$_{18}$ silica (without embedded urea groups) [14].

2.6. Chromatographic experiments

2.6.1. Column packing

In the packing procedure, short HPLC columns of 60 mm × 3.9 mm I.D., made from 316 stainless steel tubing, were used. The columns were individually downward packed using 10% (m/v) suspensions of the modified silicas in chloroform at 40 MPa (6000 psi) with a Haskel packing pump (Burbank, USA) using methanol as propulsion solvent.

2.6.2. Test procedures

To evaluate if the packings were chromatographically adequate and to determine the optimal flow rate, an initial test were performed using a mixture of uracil (10 mg l^{-1}), as marker for column dead volume, acetophenone (200 mg l^{-1}), benzene (1700 mg l^{-1}), toluene (1700 mg l^{-1}) and naphthalene (120 mg l^{-1}) as hydrophobic probes [25], using an acetonitrile-water (60:40, v/v) mobile phase with detection at 254 nm at a temperature of 25 °C.

A second evaluation was performed using buffered and non-buffered mobile phases, for the separation of some compounds from the Engelhardt test mixture [26]. This mixture, containing uracil (12 mg l^{-1}), aniline (100 mg l^{-1}), phenol (220 mg l^{-1}), *N,N*-dimethylaniline (40 mg l^{-1}), toluene and ethylbenzene (1000 mg l^{-1}), was separated using MeOH–water or MeOH–20 mmol l^{-1} phosphate buffer at pH 7.0 (55:45, v/v) as mobile phases. For each separation, retention factors (k), plate numbers (N) and tailing factors (T_F) were calculated as recommended [25].

Another set of experiments was performed using compounds from the Tanaka test procedure [27]. The methylene selectivity (α_{CH_2}) and steric or shape selectivity ($\alpha_{T/O}$) were calculated from the separation of uracil (10 mg l^{-1}), butylbenzene (1000 mg l^{-1}), pentylbenzene (1100 mg l^{-1}), *o*-terphenyl (80 mg l^{-1}) and triphenylene (50 mg l^{-1}), using MeOH–water (80:20, v/v) as mobile phase. The hydrogen bonding capacity ($\alpha_{C/P}$) was determined from the separation of uracil (10 mg l^{-1}), caffeine (135 mg l^{-1}) and phenol (270 mg l^{-1}) using MeOH–water (30:70, v/v) as mobile phase. The ion exchange capacities ($\alpha_{B/P}$) were calculated for the separation of uracil (10 mg l^{-1}), benzylamine (100 mg l^{-1}), and phenol (120 mg l^{-1}), using a mobile phase composed of MeOH–20 mmol l^{-1} phosphate buffer (30:70, v/v) at pH 7.60 and at pH 2.70.

3. Results and discussion

3.1. Preparation and characterization of C_{18}-urea titanized silica

The preparation of the SiTiC$_{18}$ urea phase is based on a single-step chemical modification of the titanized silica surface with the urea-trialkoxysilane, which was synthesized and characterized before the bonding procedure. The single-step modification has the advantage to ensure a homogeneous

surface composition of the bonded phase, which does not happen when a two-step modification process is employed.

After the titanization procedure and also after the reaction with the urea-trialkoxysilane, a washing procedure with copious amounts of deionized water was used to guarantee the complete hydrolysis of the isopropoxy groups on the titanized silica surface, as well as to remove remaining ethoxy groups after the silanization reaction step with the silylant agent.

After these reactions, the carbon, hydrogen and nitrogen percentages for $SiTiC_{18}$ urea, obtained by elemental analysis, were 19.0 ± 0.1, 3.3 ± 0.1 and $2.0 \pm 0.3\%$, respectively. From the percent carbon, the surface coverage (χ) was calculated, according to the expression of Berendsen et al. [28]:

$$\chi\,(\mu mol\,m^{-2}) = \frac{10^6 \times \%C}{1200 N_C - \%C(M-3)} \times \frac{1}{S_{BET}}$$

where N_C is the number of carbon atoms in the urea-alkoxysilane reagent (22), M is the molar mass ($381\,g\,mol^{-1}$) of the silane ligand attached to the surface, 3 relates to the use of trifunctional alkoxysilane and S_{BET} ($308\,m^2\,g^{-1}$) is the surface area of the bare Kromasil silica. A value of $3.22 \pm 0.22\,\mu mol\,m^{-2}$ was obtained for the $SiTiC_{18}$ urea phase, which is only 6% lower than the value of $3.45 \pm 0.28\,\mu mol\,m^{-2}$ for the C_{18} titanized phase obtained by silanization with octadecyltrimethoxysilane (no embedded urea groups) under similar reaction conditions and using the same silica support [14]. Another important observation is that the concentration of organic groups on the $SiTiC_{18}$ urea surface is the same when compared to the coverage of $3.2\,\mu mol\,m^{-2}$ obtained on a Prontosil silica surface [19], by chemical modification with the same C_{18} urea-trialkoxysilane used to prepare the $SiTiC_{18}$ urea phase.

Taking into account the proposed ligand structure of the silane attached to the titanized silica surface and the absence of any remaining ethoxy groups, the C/N ratio, calculated from the carbon and nitrogen percentages from elemental analysis, is 11.2, which is in agreement to the expected value of 11. However, the slightly higher C/N ratio may indicate the presence of some remaining ethoxy groups bonded to the silicon atom of the silylant agent attached to the titanized silica surface. In the final step, some residual silanols and titanols are endcapped with a mixture containing more reactive silanes, TMCS and HMDS. The carbon content has increased by 0.5% and the C/N ratio is higher, due to the trimethylsilyl groups.

Infrared spectroscopy is a simple and useful technique to check the integrity of the chemical structure of the urea-silane attached to the titanized surface. The DRIFT spectra for the titanized silica before and after derivatization with the urea-trialkoxysilane are shown in Fig. 2. In addition to the bands at 2960 (ν-CH_3), 2920 (ν_s-CH_2), 2850 (ν_a-CH_2) and $1470\,cm^{-1}$ (δ-CH_2) in the spectrum of Fig. 2B, the presence of the urea groups embedded in the alkyl chain is confirmed by the bands at 3348 and 1570, attributed to
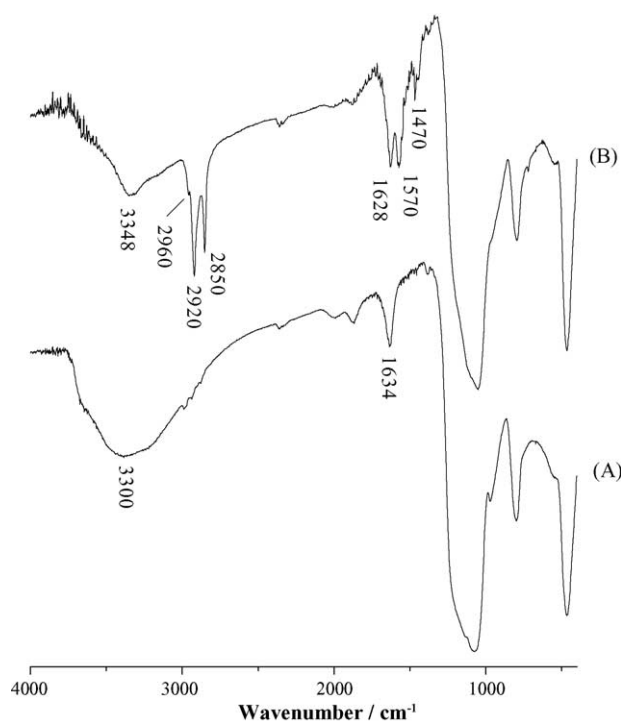

Fig. 2. DRIFT spectra for titanized silica, SiTi (A) and for the $SiTiC_{18}$ urea (B) phase.

N–H stretching and bending, respectively, and the band at $1628\,cm^{-1}$, which corresponds to the C=O stretching. In the spectrum of Fig. 2A, the absence of methylene and methyl stretching bands is good evidence for the complete hydrolysis of any isopropoxy groups remaining after the titanization reaction.

As previously stated [29], solid-state ^{29}Si CP/MAS NMR spectroscopy is an excellent technique to investigate the different silicon species attached to the chemically modified silica surface after each step of the silanization reactions. The spectra in Fig. 3 were obtained for the $SiTiC_{18}$ urea silica before (A) and after (B) the endcapping reaction. In addition to the well-known resonance for the Q^4 (siloxanes) and Q^3 (free and vicinal silanols) species at −110 and −101 ppm, T^2 and T^3 species were detected at −65 and −55 ppm, respectively. After endcapping, the presence of the M species is confirmed by the signal at +12 ppm.

Quantitative analysis of these silicon species is complicated by the fact that the intensity of each signal in the CP/MAS spectrum depends on the efficiency of cross-polarization and the proton relaxation time [30]. However, comparisons are possible for spectra obtained under the same conditions. In spectrum B, an increase in the relative peak area for the desirable highly crosslinked T^3 species is evident, compared to spectrum A, suggesting the endcapping was successfully performed.

Solid-state ^{13}C CP/MAS spectroscopy was also used to confirm the integrity of the N-alkyl group, covalently attached to the titanized silica surface. Fig. 4 shows the ^{13}C CP/MAS NMR spectra of the C_{18}-urea titanized silica ($SiTiC_{18}$ urea)
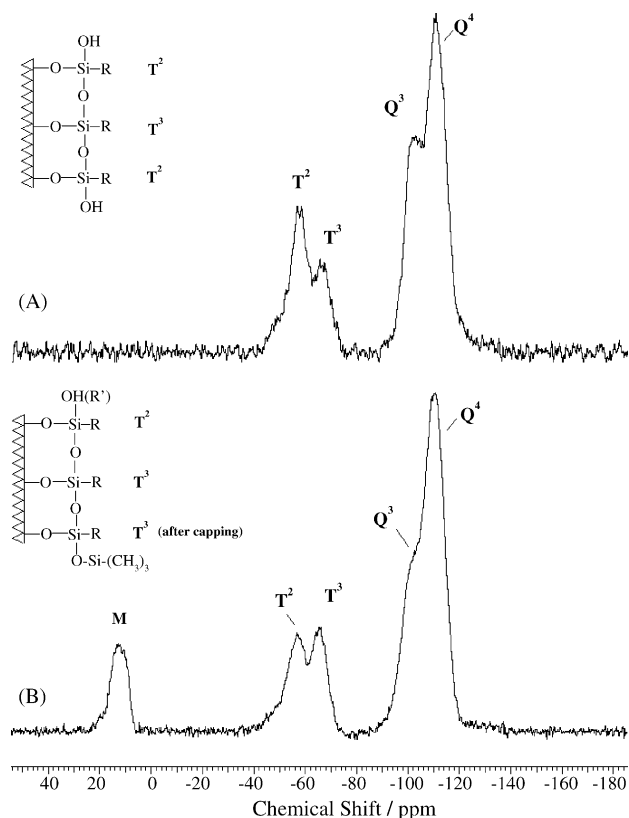
Fig. 3. Solid-state ^{29}Si CP/MAS NMR spectra for SiTiC$_{18}$ urea before (A) and after (B) endcapping.

Fig. 4. Solid-state ^{13}C CP/MAS NMR spectra for SiTiC$_{18}$, before (A) and after (B) the endcapping reaction.

before and after reaction with TMCS and HMDS. Each spectrum is consistent with the proposed structure and no chemical changes have occurred in the urea silyl organic groups during the modification processes, as seen by the presence of the carbonyl of the urea group at 160 ppm. Two small shoulders at 19 and 60 ppm were observed, due to carbons 1 and 2, respectively, of possible remaining ethoxy groups of the trifunctional C$_{18}$ urea-alkoxysilane, consistent with the slightly elevated elemental C/N ratio. After reaction with TMCS and HMDS, a new signal at 2 ppm in spectrum B is observed, due to the presence of the carbons of the trimethylsilyl moieties on the surface.

3.2. Chromatographic tests

The first chromatographic tests with the 60 mm × 3.9 mm column packed with the SiTiC$_{18}$ urea phase were performed to determine the optimal flow rate and to check if the packing was adequate. Uracil, acetophenone, benzene, toluene and naphthalene were well separated using ACN–water (60:40, v/v) as mobile phase. The optimal flow rate was at 0.4 ml min^{-1}, and the plate number (N) and tailing factor (T_F) values for the most retained compound, naphthalene ($k = 4.5$), were 5100 and 1.1, respectively, indicating that the packing was satisfactory. Since the plate numbers value is only 10% lower when the flow rate is increased to 0.6 ml min^{-1}, the remaining chromatographic tests, in-
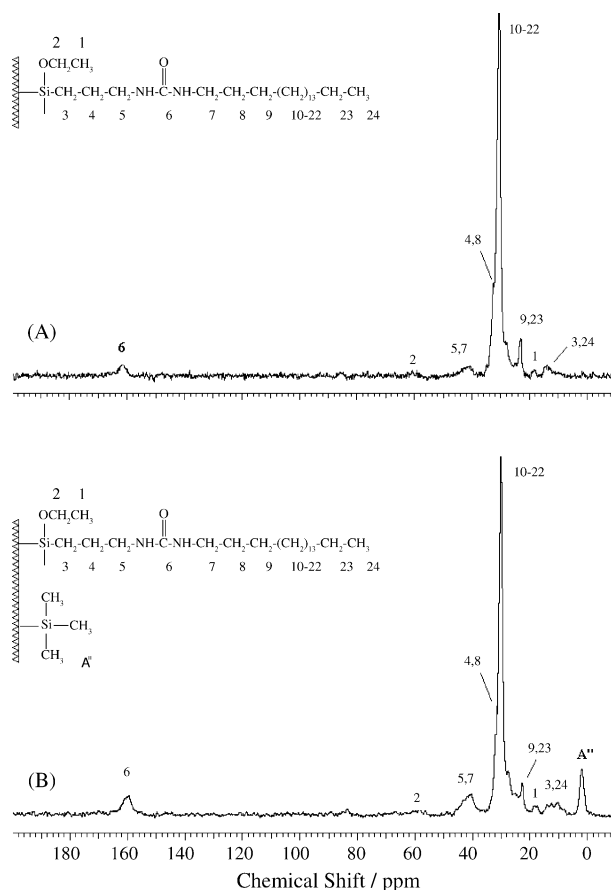
cluding those with the SiTiC$_{18}$ column, were performed at 0.6 ml min^{-1}.

The test mixture used in the second series of experiments was composed of seven compounds from the ten initially suggested by Engelhardt et al. [26], employing MeOH–water (55:45, v/v) as mobile phase. The relation of the retention factors of ethylbenzene and toluene indicates the hydrophobicity of the column, while the retention and peak shape for the weakly basic solutes, aniline and N,N-DMA provide information about acidic silanol activity. The chromatograms obtained on the SiTiC$_{18}$ urea and SiTiC$_{18}$ phases are shown in Fig. 5 and the related chromatographic parameters are listed in Tables 1 and 2.

For both columns, the hydrophobic probes were separated with good efficiency and good peak shapes, while aniline and N,N-DMA show predictable tailing and higher retention, due the enhanced acidity on both titanized silica supports. However, this effect is less pronounced for the SiTiC$_{18}$ urea phase that contains the polar embedded urea groups, as confirmed by the lower relative retention values for the two bases, shown in Table 2.

The polar urea groups partially shield or minimize the effects caused by the residual hydroxyl groups. At least two possible mechanisms might be involved. The first is the presence of a hydrogen bonded layer of water molecules in the
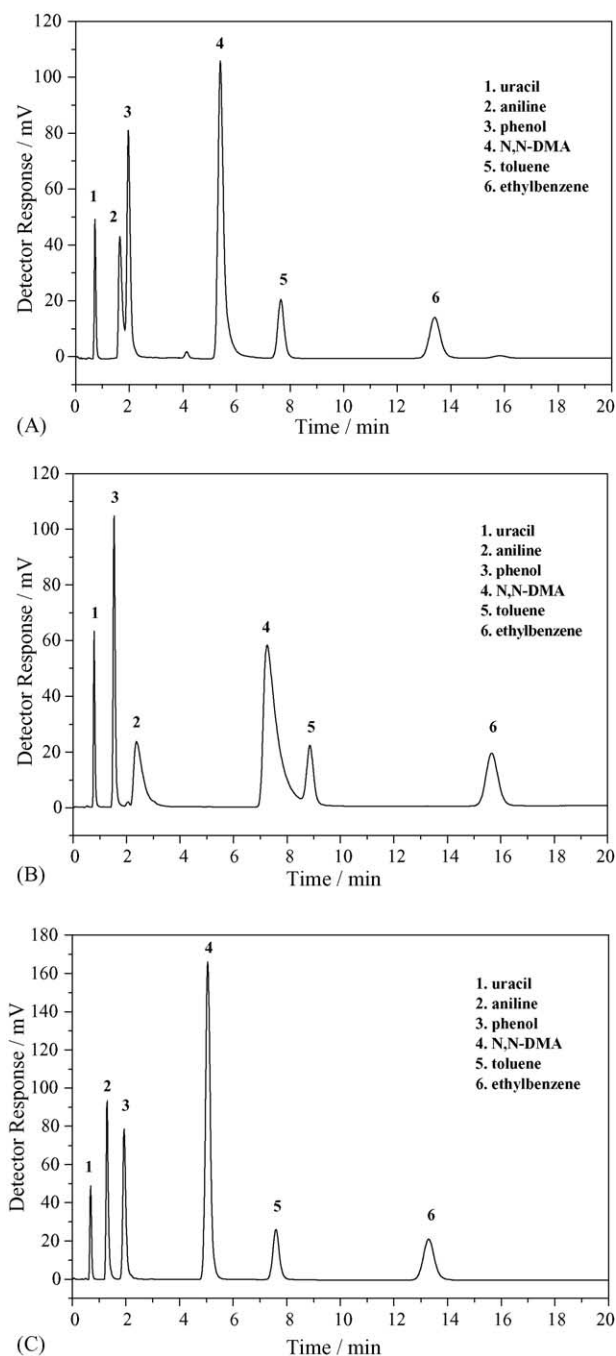
Fig. 5. Separation of some components of the Engelhardt test mixture on the SiTiC$_{18}$ urea (A) and SiTiC$_{18}$ (B) phases. Conditions: 60 mm × 3.9 mm I.D. columns; mobile phase: methanol–water (55:45, v/v) at 0.6 ml min^{-1}; detection: UV at 254 nm; injection volume: 5 μl. (C) shows the chromatogram obtained with SiTiC$_{18}$ urea, using methanol–20 mmol l^{-1} phosphate buffer at pH 7.0 (55:45, v/v) as mobile phase. Peaks: uracil (1), aniline (2), phenol (3), N,N-dimethylaniline (4), toluene (5) and ethylbenzene (6).

underlying surface, making the acidic residual groups less accessible to interact with the basic analytes. The second hypothesis suggests hydrogen bonding of the polar groups to the residual silanols, masking their undesirable effects during the chromatographic process [17].

When the separation is performed using a buffered mobile phase, the interactions between the weak bases and residual groups are greatly reduced and better peak shapes and efficiencies are obtained, as shown in the chromatogram of Fig. 5C. This behavior is observed due to the pH buffer, where aniline (pK_a = 4.87) and N,N dimethylaniline (pK_a = 5.07) are in their free form, excluding the possible ionic interactions with residual groups. In addition to this, phosphate anions can interact with the Lewis acid sites on the titanized silica, avoiding this kind of interaction with both bases.

The retention and tailing factors for phenol are significantly higher on the titanized urea phase, due to a higher number of possible hydrogen bonds between the analyte and the polar group during the chromatographic separation. Such behavior has already been observed for other phenolic compounds on phases containing embedded carbamate groups [31] and this enhanced selectivity is one of the features that differentiate packings with a polar group [32].

The Tanaka characterization protocol is a well-established approach that is recommended to obtain information on some properties as well as to establish the repeatability and reproducibility of commercially available reversed phases [33]. The most relevant properties, which are measured by the chromatographic parameters for the separation of seven compounds using four different mobile phase compositions, are shape and methylene selectivities, hydrogen bonding and ion-exchange capacities in acidic and neutral media.

The chromatogram of Fig. 6 shows the separation of two homologous alkyl benzenes and the non-planar and planar polyaromatic hydrocarbons, where it was observed that pentylbenzene was not totally separated from o-terphenyl. For an easier comparison, the chromatographic parameters, obtained for the separation on both SiTiC$_{18}$ urea and SiTiC$_{18}$ phases, are summarized in Table 3. A methylene selectivity (α_{CH_2}) of 1.31 and a shape selectivity ($\alpha_{T/O}$) of 1.54 were obtained for the urea embedded phase. The $\alpha_{T/O}$ value of 1.21 for the SiTiC$_{18}$ phase is lower. Generally, the $\alpha_{T/O}$ value is higher for "polymeric" phases, i.e. those prepared by silanization with di- or trifunctional silanes, as well as for phases with longer alkyl chain lengths. In this case, both phases were prepared using a trifunctional alkoxysilane. The alkyl chain length of the urea-silyl is slightly longer than that of the octadecyl-silyl group, but the presence of polar urea group may also play a significant role in improving shape selectivity as a consequence of the π interactions between the polar group (i.e. carbonyl function) and the π–π active moieties on the less hindered planar triphenylene. This phenomenon was also observed for embedded polar phases with amide and carbamate groups [34] and, more recently, polar endcapped phases with amine groups [33] as well as for alkyl thiol and naphthalimide stationary phases [35].

The hydrogen bonding capacity ($\alpha_{C/P}$) is a good measure to discriminate endcapped and non-endcapped phases, as a result of estimation of the number of accessible silanols. It was calculated from the separation of caffeine and phenol, shown in Fig. 7, obtaining a value of 0.20 for the SiTiC$_{18}$ urea

Table 1

Chromatographic parameters obtained for the separation of some compounds of the Engelhardt test mixture composed of nonpolar and basic analytes, on *both* C_{18} titanized phases

Compounds	SiTiC$_{18}$ urea phase[a,b]			SiTiC$_{18}$ phase[a]		
	k	N	T_F	k	N	T_F
Aniline	1.29[c] (0.90)	735 (1245)	1.85 (1.39)	2.04	245	2.45
Phenol	1.74 (1.84)	1260 (1410)	1.31 (1.33)	0.96	1740	1.23
N,N-DMA	6.50 (6.43)	3490 (3910)	1.69 (1.19)	8.31	1110	2.83
Toluene	9.63 (10.2)	4795 (4670)	1.15 (1.12)	10.4	5230	1.07
Ethylbenzene	17.6 (18.6)	4980 (4820)	1.10 (1.12)	19.1	5530	1.11

[a] Chromatographic conditions: 60 mm × 3.9 mm I.D. columns, mobile phase: MeOH–H$_2$O (55:45, v/v), flow rate: 0.6 ml min^{-1}.

[b] The values in parenthesis are those obtained using a MeOH–20 mmol l^{-1} phosphate buffer at pH 7.0 (55:45, v/v) as mobile phase.

[c] This value was obtained from a separate injection.

Table 2

Relative retentions for aniline ($\alpha_{A/T}$), phenol ($\alpha_{P/T}$), *N,N*-dimethylaniline ($\alpha_{DMA/T}$) and ethylbenzene ($\alpha_{E/T}$) for separations on the SiTiC$_{18}$ urea and SiTiC$_{18}$ columns

	$\alpha_{A/T}$	$\alpha_{P/T}$	$\alpha_{DMA/T}$	$\alpha_{E/T}$
SiTiC$_{18}$ urea[a]	0.13	0.18	0.68	1.81
SiTiC$_{18}$[a]	0.20	0.09	0.80	1.83
SiTiC$_{18}$ urea[b]	0.09	0.18	0.63	1.81

[a] Chromatographic conditions: 60 mm × 3.9 mm I.D. columns, mobile phase: MeOH–H$_2$O (55:45, v/v), flow rate: 0.6 ml min^{-1}.

[b] Same conditions except mobile phase: MeOH–20 mmol l^{-1} phosphate buffer at pH 7.0 (55:45, v/v).

phase, which is much lower than the value of 0.56 obtained for the SiTiC$_{18}$ phase, due to the presence of the embedded polar urea groups.

The ion exchange capacity ($\alpha_{B/P}$) at pH 2.70 is useful to estimate the acidic properties of the silanol groups and in some cases, the metal impurity contents of the silica support. For the SiTiC$_{18}$ urea phase, the separation of benzylamine and phenol at this pH is illustrated in Fig. 8. The $\alpha_{B/P}$ of 1.32 is a consequence of the higher acidity of residual groups on the titanized silica surface, a result that is similar to reversed
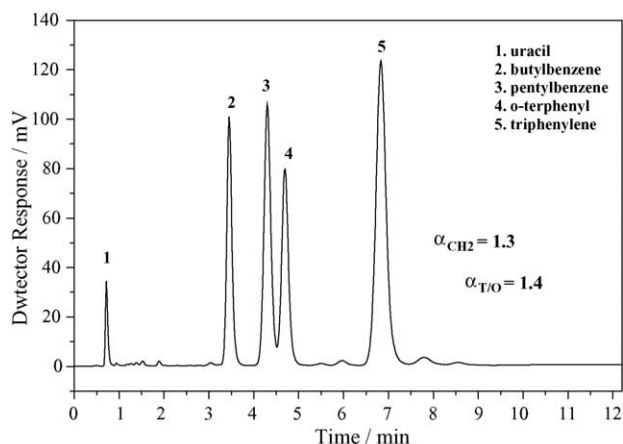

Fig. 6. Separation of uracil (1), butylbenzene (2), pentylbenzene (3), *o*-terphenyl (4) and triphenylene (5) on the SiTiC$_{18}$ urea phase. Conditions: 60 mm × 3.9 mm I.D. column; mobile phase: methanol–water (80:20, v/v); flow rate: 0.6 ml min^{-1}; injection volume: 5 μl; temperature: 40 °C; detection: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

Table 3

Chromatographic parameters obtained for the separation of compounds of the Tanaka test mixture on the SiTiC$_{18}$ urea and SiTiC$_{18}$ phases using different mobile phase compositions

Compounds	SiTiC$_{18}$ urea phase			SiTiC$_{18}$ phase		
	k	N	T_F	k	N	T_F
Butylbenzene[a]	3.79	4460	1.19	3.87	5290	1.16
Pentylbenzene[a]	4.97	3710	1.14	4.68	4790	1.09
o-Terphenyl[a]	5.51	4625	1.04	5.67	5390	1.16
Triphenylene[a]	8.49	4300	1.10	6.81	5150	1.06
Caffeine[b]	1.13	1620	1.25	1.69	2350	1.38
Phenol	5.64[b]	2320[b]	1.22[b]	3.04[b]	3520[b]	1.40[b]
	5.17[c]	3070[c]	1.29[c]	3.12[c]	3600[c]	1.40[c]
	5.05[d]	2270[d]	1.26[d]	3.29[d]	2840[d]	1.59[d]
Benzylamine	6.82[c]	875[c]	2.15[c]	11.1[c]	620[c]	3.77[c]
	nd[d,e]	nd[d,e]	nd[d,e]	nd[d,e]	nd[d,e]	nd[d,e]

Conditions: 60 mm × 3.9 mm I.D. column, flow rate: 0.6 ml min^{-1}, temperature: 40 °C, detection: UV at 254 nm, injection volume: 5 μl.

[a] MeOH–H$_2$O (80:20, v/v).

[b] MeOH–H$_2$O (30:70, v/v).

[c] Methanol–20 mmol l^{-1} H$_3$PO$_4$/KH$_2$PO$_4$ buffer (30:70, v/v) at pH 2.70.

[d] Methanol–20 mmol l^{-1} KH$_2$PO$_4$/K$_2$HPO$_4$ buffer (30:70, v/v) at pH 7.60.
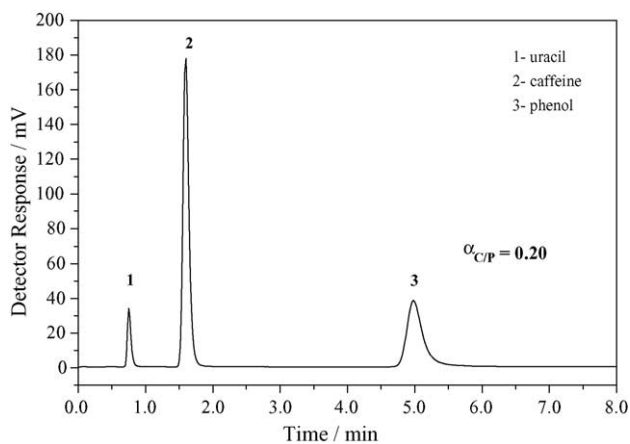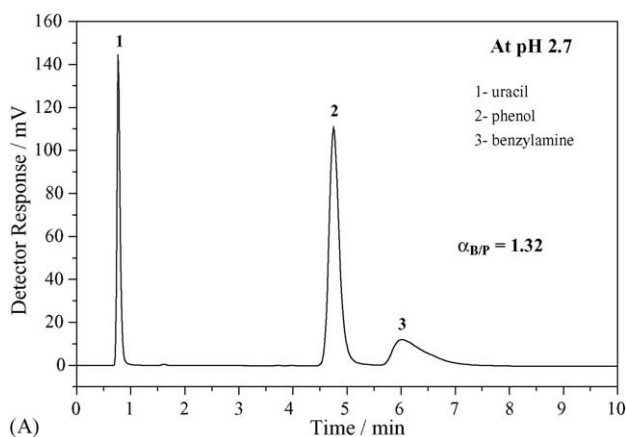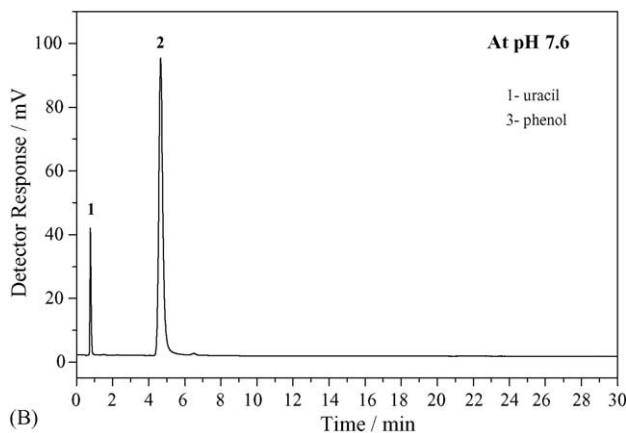
[e] Not seen in the chromatogram.

Fig. 7. Separation of uracil (1), caffeine (2) and phenol (3) using a non-buffered mobile phase on the SiTiC$_{18}$ urea phase. Conditions: 60 mm × 3.9 mm I.D. column; mobile phase: methanol–water (30:70, v/v); flow rate: 0.6 ml min^{-1}; injection volume: 5 μl; temperature: 40 °C; detection: UV at 254 nm.
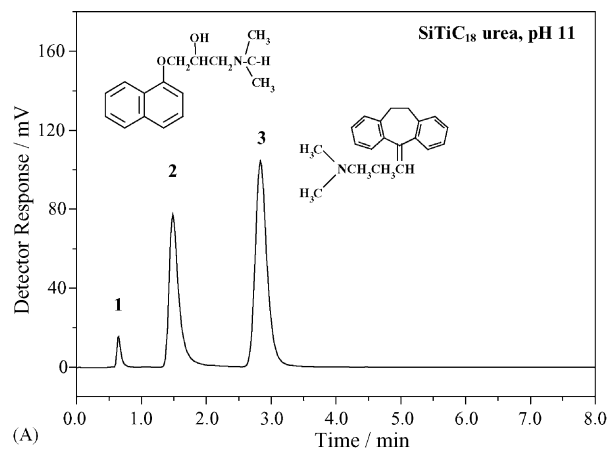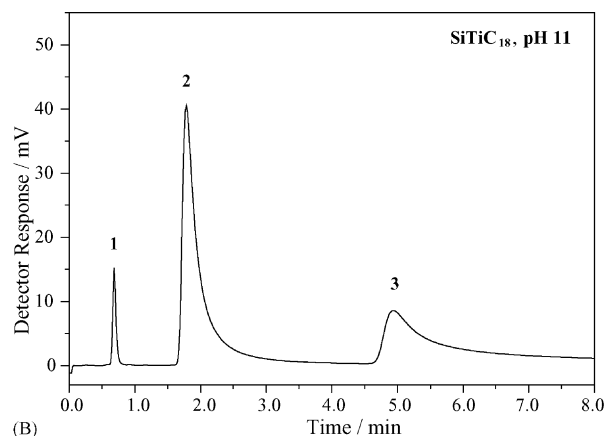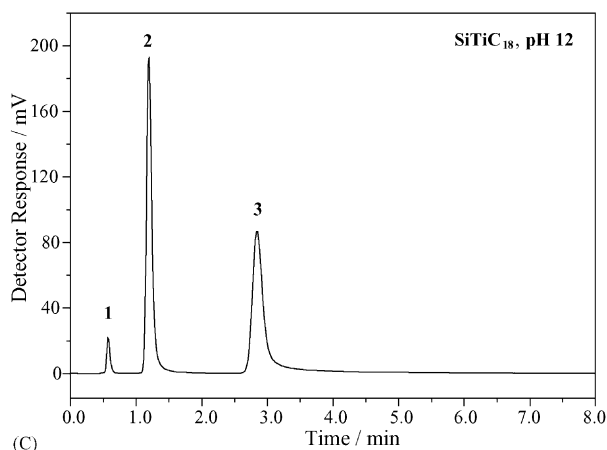

Fig. 8. Separation of uracil (1), benzylamine (2) and phenol (3) at pH 2.70 (A) and pH 7.60 (B) on the SiTiC$_{18}$ urea phase. Conditions: 60 mm × 3.9 mm I.D. column; mobile phase: methanol–buffer phosphate (30:70, v/v); flow rate: 0.6 ml min^{-1}; injection volume: 5 μl; temperature: 40 °C; detection: UV at 254 nm.

Fig. 9. Separation of uracil (1), propranolol (2) and amitriptyline (3) on the SiTiC$_{18}$ urea column (A) at pH 11 and on the SiTiC$_{18}$ phase at pH 11 (B) and pH 12 (C). Conditions: 60 mm × 3.9 mm I.D. column; mobile phase: 20 mmol l^{-1} methanol–phosphate buffer (80:20, v/v), flow rate: 0.6 ml min^{-1}; injection volume: 5 μl; temperature: 25 °C; detection: UV at 254 nm.

phases prepared using type A silicas, containing measurable amounts of metal impurities, such as iron and aluminum, as well as for phases based on zirconia. The $\alpha_{B/P}$ of 3.56 for the SiTiC$_{18}$ phase is significantly higher when compared to the value for the SiTiC$_{18}$ urea phase.

On the other hand, the separation at pH 7.6 maximizes the ion exchange capacity, where the great majority of the

residual groups are in their ionized form and benzylamine ($pK_a = 9.3$) is partially protonated. As a result, benzylamine is not eluted on either phase at this pH, indicating that the presence of the polar urea groups minimizes but does not eliminate these effects.

As an application, the separation of two highly basic drugs, propranolol and amitriptyline on the $SiTiC_{18}$ urea column is shown in Fig. 9A using a phosphate buffer at pH 11. On the other hand, for the $SiTiC_{18}$ phase, without the embedded polar urea group, the separation at pH 11 shows tailing peaks as can be seen in Fig. 9B. These effects were reduced when using a buffered phosphate mobile phase at pH 12.

4. Conclusions

A new C_{18} urea stationary phase was successfully prepared by silanization of titanized silica with a urea-trialkoxysilane, according to characterizations by solid state infrared and nuclear magnetic resonance spectroscopies. The surface coverage was quite similar to that for the C_{18} (no embedded group) counterpart, based on the same titanized support.

From the chromatographic evaluation, based on the test proposed by Engelhardt, it can be concluded that the chromatographic performance for neutral compounds is similar to that for the C_{18} titanized phase, while the retention factors for the weakly basic compounds are lower, although, some tailing is still observed when using non-buffered mobile phases.

The presence of the embedded polar urea groups on the titanized silica phase did not solve the irreversible retention of benzylamine at pH 7.6, where ion-exchange interactions are maximized. Nevertheless, some improvements for the $SiTiC_{18}$ urea phase are observed for separations in an acid medium as well for the separation of some weaker aromatic amines.

Acknowledgments

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