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Preparation of a new C_{18} stationary phase containing embedded urea groups for use in high-performance liquid chromatography

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Abstract

A new C_{18} urea stationary phase was prepared by a single-step modification process through the reaction of ProntoSil spherical silica (3 μm, Bischoff) with the trifunctional alkoxysilane $(CH_3CH_2O)_3$–Si–$(CH_2)_3$–NH–C(O)–NH–$(CH_2)_{17}$–CH_3, prepared in our laboratory. The phase was characterized by elemental analysis and solid-state ^{29}Si and ^{13}C nuclear mangnetic resonance spectrometry. Chromatographic evaluations of the new phase in 150×3.9 mm HPLC columns involving the separation of different test mixtures, indicate good performance for both polar and basic mixtures and also show promising results for further applications.

Keywords: Stationary phases, LC; Silica, urea-modified

1. Introduction

Even though high-performance liquid chromatography (HPLC) column technology is somewhat mature, developments of new stationary phases continue [1]. Among the most widely used C_8 and C_{18} reversed phases, new packing materials containing polar linked groups or embedded polar groups are being introduced and becoming increasingly popular for reversed-phase HPLC separations. These new kinds of silica-based stationary phases exhibit some remarkable features such as different selectivities and good efficiencies for polar analytes, stable and reproducible retention times in mobile phases with a lower concentration of organic modifier and also improved peak shapes.

Systematic studies concerned with the chromatographic evaluation and performance of these new phases have shown that, under the same separation conditions and mainly at intermediate pH, columns with conventional C_8 and C_{18} alkyl bonded phases show undesirable peak asymmetry and efficiencies for the analyses of highly basic compounds, when compared with phases containing an embedded polar group [2–4]. Columns with embedded polar groups show different selectivity and retention for basic and polar compounds, when compared with conventional columns bonded with ligands of similar chain length.

The superior performance of these new phases over the conventional C_8 and C_{18} phases can be attributed to the weakening of the interaction between basic analytes and residual silanol groups on the silica surface [5]. However, the mechanism by which embedded polar groups reduces tailing for basic compounds is still unclear. Some mechanisms which have been proposed are the interaction of the polar functionalities with the unwanted silanols

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through hydrogen bonding [5] and an increase in the water concentration on the surface because of the hydrogen bonding ability. As a result, the surface layer should have a higher dielectric constant, weakening the interactions between residual surface silanols and basic analytes [6].

The preparation of these new kinds of functionalized silicas by a two-step modification is a well known process. In the first step, the bare silica is chemically modified with an amino organosilane. In the second step, a reaction of the aminopropyl silica with acid chlorides is carried out [7–10]. A highly deactivated silica-based reversed-phase packing material, containing amide polar groups, was first introduced by Ascah and Feibush [11]. These authors showed that was possible to separate a wide range of organic bases at pH 7, which not be separated on conventional C_{18} bonded phases due to excessive tailing. This conventional approach produces mixed amine–amide phases because the process suffers from the difficulty of achieving a high yield of acetylated groups as the conversion of amine groups to amide in the second modification step is not quantitative [12,13]. A stationary phase, containing embedded amide groups with sterically protective diisopropyl groups in a C_{14} *N*-alkyl chain has also been prepared [14].

Another approach, developed by O'Gara et al. [15], involves a single-step modification process based on the prior preparation of the appropriate organosilane, containing polar functional carbamate groups. With this new monofunctionl organosilane, the silica is chemically modified, yielding derivatized silica with a homogeneous surface composition.

In a previous report, we described the preparation and characterization of a new series of HPLC bonded phases with different terminal *N*-alkyl groups from C_7 to C_{12} containing embedded polar urea groups, using a single-step surface modification process [16]. The initial chromatographic results showed that these phases can be used under reversed-phase conditions to separate some polar test compounds. In this report, a new phase was prepared using a urea–alkoxysilane with a C_{18} *N*-alkyl chain. The main goal of this study is the physicochemical characterization and chromatographic evaluation using different test mixtures, including a test mixture composed of nonpolar, polar and basic compounds, proposed by Neue et al. [17].

2. Experimental

2.1. Chemicals

ProntoSil silica, spherical silica particles, with a mean particle size of 3 μm, mean pore diameter of 21 nm, Brunauer–Emmett–Teller (BET) surface area of 189 ± 5 m^2 g^{-1} and pore volume of 1.0 cm^3 g^{-1}, was kindly supplied from Bischoff Chromatography (Leonberg, Germany). The urea–alkoxysilane has recently been synthesized and characterized, according to a new organic synthesis route [18]. Toluene was purchased from Merck (Darmstadt, Germany). Trimethylchlorosilane and pyridine, from Aldrich (Milwaukee, WI, USA) were used without further purification. Uracil, acetophenone, naphthalene, propranolol, butylparaben, dibutyl phthalate, acenaphthene and amitriptyline were also from Aldrich and were used as received for the test mixtures. Standard Reference Materials SRM 869a and SRM 1647c were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). All other solvents (methanol, acetonitrile and chloroform) were HPLC grade and were purchased from Merck (Rio de Janeiro, Brazil). Deionized water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Synthesis of urea-functionalized silica

The silica was chemically modified using urea–trialkoxysilane{[(3-octadecyl)propyl]ureatriethoxysilane}. First, the silica was washed with deionized water and dried under vacuum for 8 h at 373 K. A 20.0-g amount of ProntoSil was suspended in 200 ml of dry toluene and an excess of urea–trialkoxysilane (0.116 mol) was added. The suspension was mechanically stirred and refluxed under a nitrogen atmosphere for 86 h. The modified silica was washed with toluene, methanol and a water–methanol mixture in order to promote the hydrolysis of the remaining ethoxy groups of the trifunctional organosilane. Subsequently, the sample was dried under vacuum for 8 h at 353 K prior to an endcapping reaction. The silica was named C_{18} urea.

The modified silica was endcapped using a conventional liquid phase reaction. Briefly, the reactions were performed by refluxing nearly 20 g of the modified silica with a large excess of trimethyl-

chlorosilane (60 ml, 0.56 mol) in 200 ml of dry toluene with 4 ml of pyridine. After the mixture was stirred at 395 K, the silica was filtered and purified with repeated washings with toluene, methanol, a water–methanol mixture, water and finally with methanol. The material was dried under vacuum for 8 h prior to characterization or packing.

2.3. Elemental analysis

Carbon, hydrogen and nitrogen percentages for the C_{18} urea phase, before and after endcapping, were determined on a Perkin-Elmer Model 2400 analyzer. At least two determinations were made for each material.

2.4. Solid-state nuclear magnetic resonance (NMR) spectrometry

Solid-state ^{13}C and ^{29}Si NMR measurements were performed on a ASX300 spectrometer (Bruker, Rheinstetten, 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 1.5 s were employed and for ^{13}C, a contact time of 3 ms and repetition time of 2 s. Frequencies of 75.5 and 59.6 MHz for carbon and silicon, respectively, were used. Representative samples of 200–250 mg were spun at 4 kHz using 7 mm double bearing ZrO_2 rotors. Typically, 1.5 k free induction decays (FIDs) with an acquisition time of 35 ms were accumulated in 1 kilobyte (kb) data points and zero-filling to 8 kb prior to Fourier transformation. The line broadening used was 30 Hz and the spectral width for all spectra was about 25 kHz.

2.5. Nitrogen isotherm

The BET surface area, average pore diameter and total pore volume of the bare silica was determined from the nitrogen isotherm at 77 K, obtained on a Micrometrics Model ASAP 2010 analyzer.

2.6. Column packing

HPLC columns of 150mm×3.9 mm were made from 316 stainless steel tubing, and had their inner surfaces polished as described in detail elsewhere [19]. The modified silica was packed using the conventional slurry packing technique. Thus, an amount of 2.20 g of the modified silica was added to 22 ml of chloroform, and the slurry was dispersed for 8 h by mechanical stirring and also sonicated for a further 5 min. Then, the suspension was poured into the reservoir of the packing system, an additional volume of chloroform was added and the system was topped off. The column was downward packed at 41.4 MPa (6000 p.s.i.) using a Haskel packing pump (Burbank, CA, USA) with methanol as propulsion solvent. After packing, a few minutes were allowed for the pressure inside the column to return to atmospheric pressure. The packed column was disconnected from the packing system, the excess of stationary phase on the top of the column was carefully removed and finally the inlet frit and end-fitting were installed and the ends plugged. The columns were conditioned for 4 h with an acetonitrile–water mobile phase at a flow-rate of 0.2 ml min^{-1}.

2.7. Chromatographic evaluation

The chromatographic tests were performed using a modular HPLC system with a Waters 486 tuneable-wavelength absorbance detector, a Waters 510 pump (Milford, MA, USA) and a Rheodyne 7725 injector (Cotati, CA USA). Data were processed using ChromPerfect software (Justice Innovations, Mountain View, CA, USA). All experiments were carried out at 298 K, with detection at 254 nm and an injection volume of 5 μl. All solvents were filtered and degassed before use. The mobile phases were prepared volumetrically from individually measured amounts of each component. Test mixture 1 contained uracil, as marker for column dead volume, acetophenone, benzene, toluene and naphthalene. The second test mixture was chosen to evaluate the ability of the urea groups to reduce tailing for basic compounds and contained a mixture of uracil, naphthalene and acenaphthene as hydrophobic markers, butylparaben and dibutyl phthalate as polar probes, and propranolol and amitriptyline as basic probes, using methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 at pH 7 as mobile phase. The buffer was prepared by adding a solution of the dibasic form of the buffer to a solution of monobasic form. The pH was adjusted to 7.00 before addition of methanol using a calibrated pH meter. Plate number, *N*, retention factor, *k*,

and tailing factor at 5%, t_F, were calculated as previously reported [20].

The separation of the SRM 869a and SRM 1647c test mixtures were performed using a Merck LiChrograph L-4200A detector with a Merck LiChrograph L-6200A pump (Merck, Darmstadt, Germany). All separations were carried out at 298±1 K, with detection at 254 nm and an injection volume of 10 μl. These two test mixtures were chosen to evaluate the shape selectivity and also to classify the new C_{18} urea phase as a monomeric or polymeric phase.

3. Results and discussion

3.1. Preparation of urea-functionalized silicas

The preparation of the C_{18} urea silica is outlined in the scheme of Fig. 1. First, the urea–trialkoxysilane was covalently attached to the silica surface (I). As described in Fig. 1, the ethoxy groups which can be found on the silica surface are hydrolyzed during the washing procedure with water, resulting in more silanol groups (II). For this reason, an endcapping reaction with trimethylchlorosilane was performed, with the aim of deactivating these new silanol groups (III).

The modification process yielded a modified silica with a ligand surface concentration of 3.22 μmol m^{-2}. The carbon, nitrogen and hydrogen percentages, determined by elemental analysis were 13.07, 2.70 and 1.63%, respectively. The concentration of the organic groups attached to the silica surface was calculated from the carbon percentages and the BET surface area of the bare silica [21]. After the endcapping reaction, elemental analysis for the C_{18} urea phase was again performed and an increase of nearly 0.9% in the carbon content was observed.

As previously stated by Pfleiderer et al. [22], ^{13}C and ^{29}Si CP-MAS-NMR spectrometry is an invaluable tool to investigate the chemical structure of the silyl groups attached to the surface after each step of the modification process. Fig. 2 shows the ^{13}C CP-MAS-NMR spectra of the C_{18} urea silica before and after endcapping. Each spectrum is consistent with the proposed ligand structure, which is inserted in each spectrum, and no chemical changes have occurred in the urea silyl organic groups during the modification processes. Two small signals at 18 and 58 ppm were observed, due to carbons 1 and 2,

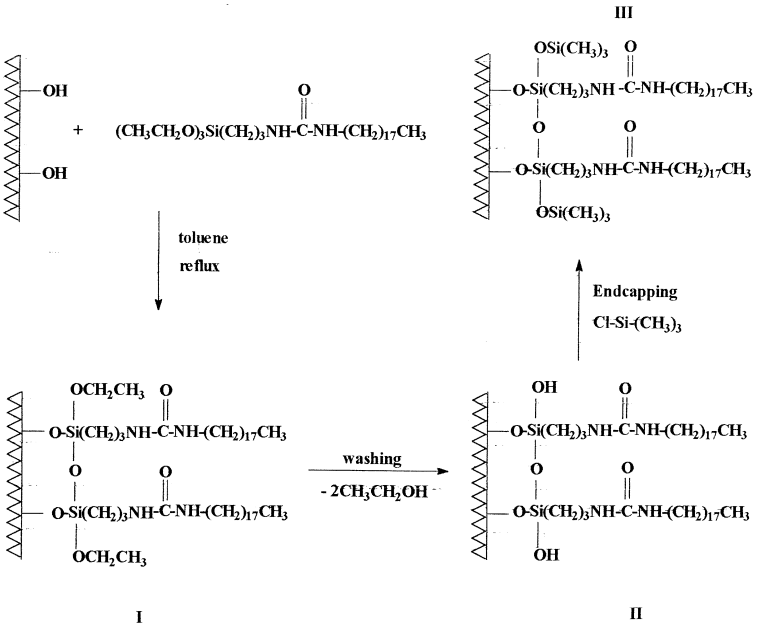

Fig. 1. Preparation of C_{18} urea-functionalized silica.

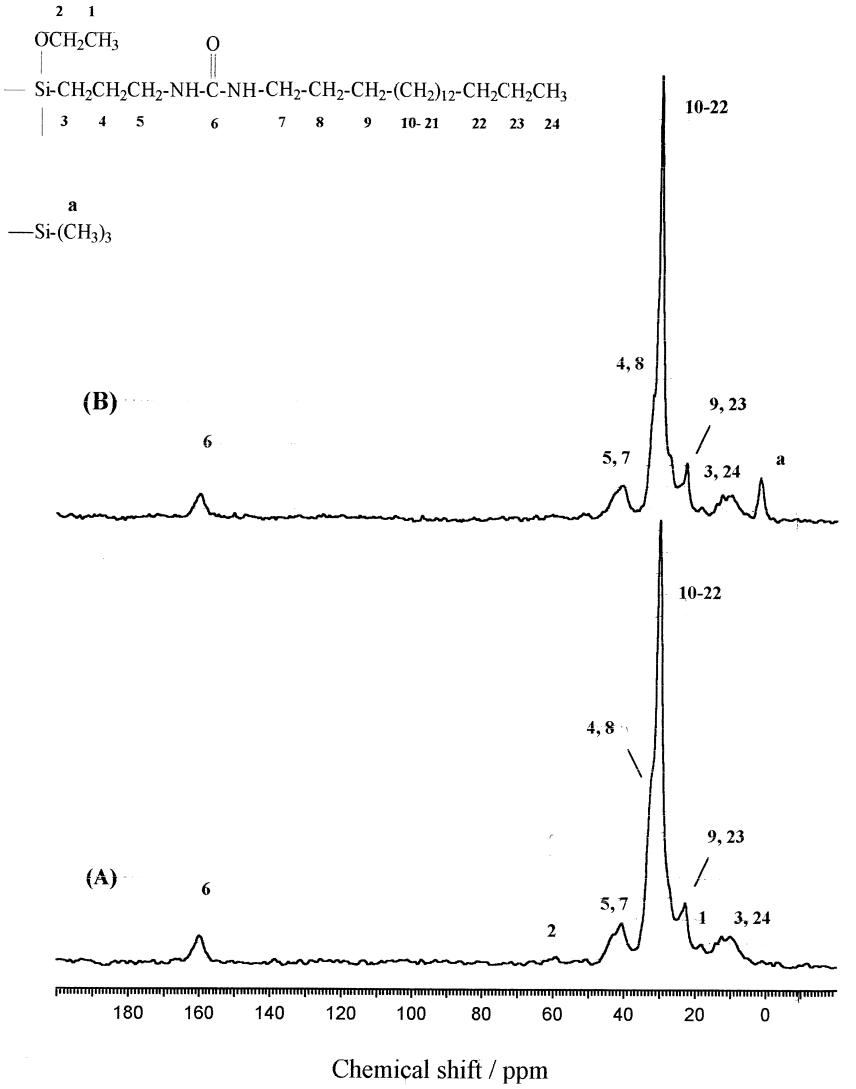

Fig. 2. ^{13}C CP-MAS-NMR spectra for the C_{18} urea-functionalized silica, (A) before and (B) after the endcapping reaction.

respectively, of the remaining ethoxy groups of the trifunctional C_{18} urea–alkoxysilane. For this reason, the C_{18} urea silica was extensively washed with water to promote hydrolysis of these groups, prior to the endcapping process. After the endcapping reaction, a new signal at 1 ppm in the spectrum of Fig. 2B is observed, due to the presence of the $-Si(CH_3)_3$ moieties on the surface (III), as suggested in Fig. 1.

The new C_{18} urea-functionalized silica stationary phase was also investigated by ^{29}Si CP-MAS-NMR spectrometry. Fig. 3 shows the ^{29}Si CP-MAS-NMR spectra before and after endcapping. The species found on the surface, described as the Q^n and T^n species, are related to the number of oxygen (mono-, di-, tri- or tetraoxo) atoms bound to the silicon atom [22–26]. In the spectrum of C_{18} urea silica, the Q^4 and Q^3 species were detected at -110 and -101 ppm, respectively. Additional peaks at -57 ppm and -65 ppm were also detected. These two structural types are denoted as T^2 and T^3 and are also shown in Fig. 3. The presence of the remaining ethoxy groups in T^n species cannot be distinguished from T^n

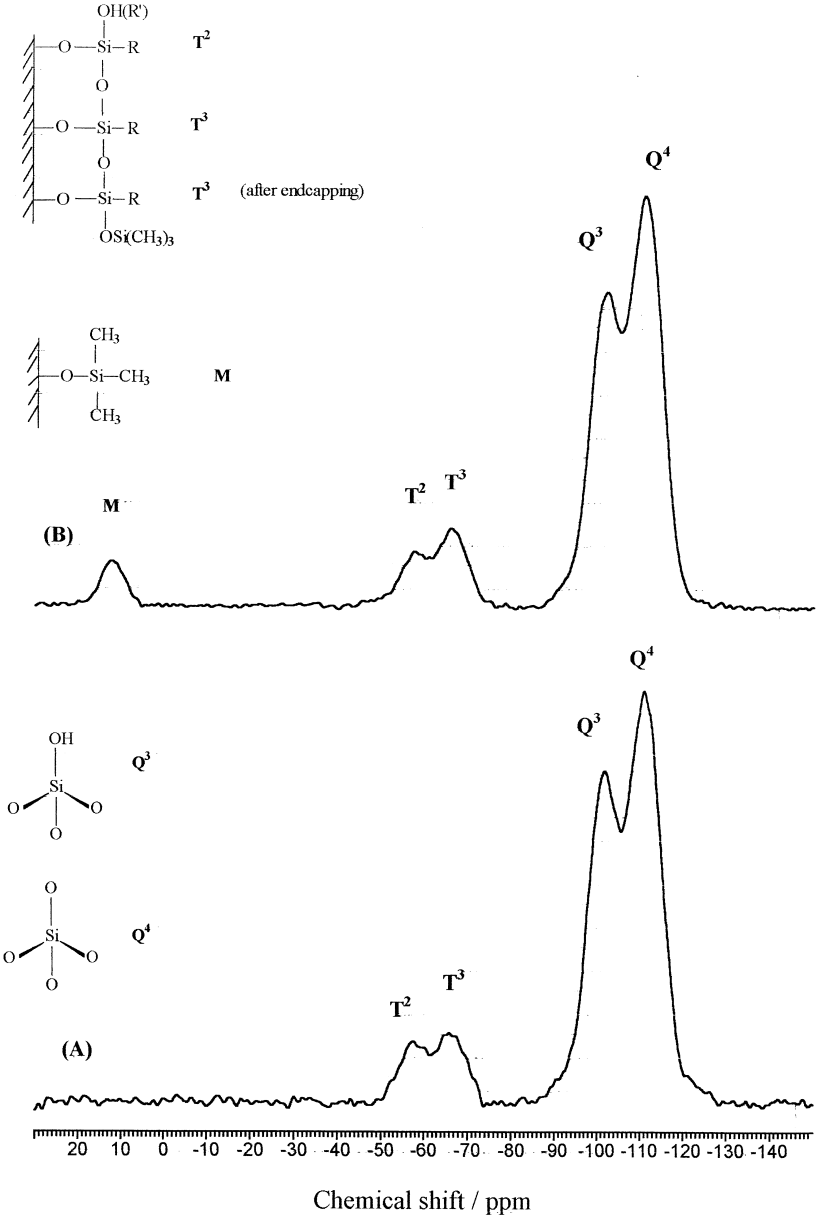

Fig. 3. ^{29}Si CP-MAS-NMR spectra for the C_{18} urea silica, (A) before and (B) after endcapping.

species having hydroxyl groups as neighbors instead of the ethoxy groups, because these species have the same chemical shift [24]. The ^{29}Si CP-MAS-NMR spectrum in Fig. 3B, obtained after endcapping, shows a new signal at about +12 ppm, in addition to the signals of T^2 and T^3 species, indicating the substitution of residual silanols by the $Si(CH_3)_3$ group, as outlined in Fig. 1, and represents the M species.

Comparing the two spectra in Fig. 3, a relative increase in the population of the condensed T^3 species and also a proportional decrease for the T^2

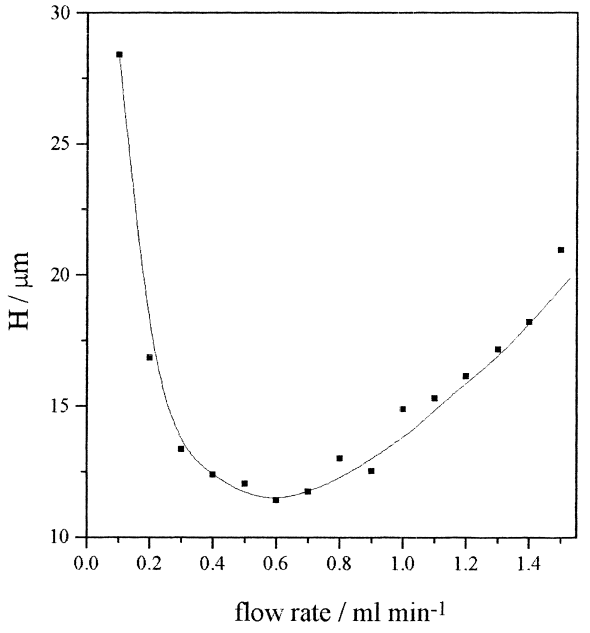

Fig. 4. Plots of *H* (plate height) for naphthalene at different flow-rates for the C_{18} urea column.

species were observed after the endcapping reaction. A relative decrease in the Q^3 species when compared to the Q^4 species, in the spectrum shown in Fig. 3B, suggests that an endcapping reaction has occurred with residual surface silanols. These results suggest the deactivation of the silanols was successfully performed as previously confirmed by the elemental analysis and also by ^{13}C CP-MAS-NMR spectrometry.

3.2. Chromatographic evaluations

The first chromatographic evaluation was performed using a standard test mixture composed of uracil, acetophenone, benzene, toluene and naphthalene at the optimal flow-rate of 0.6 ml min^{-1}, calculated from the Van Deemter curve, as shown in Fig. 4, using the plate height values, *H*, for naphthalene, and acetonitrile–water (80:20, v/v) as mobile phase. Fig. 5 shows the complete chromatogram obtained and it is observed that the column separates well all components of the test mixture. Plate number per meter, *N*/m, retention factor, *k*, and

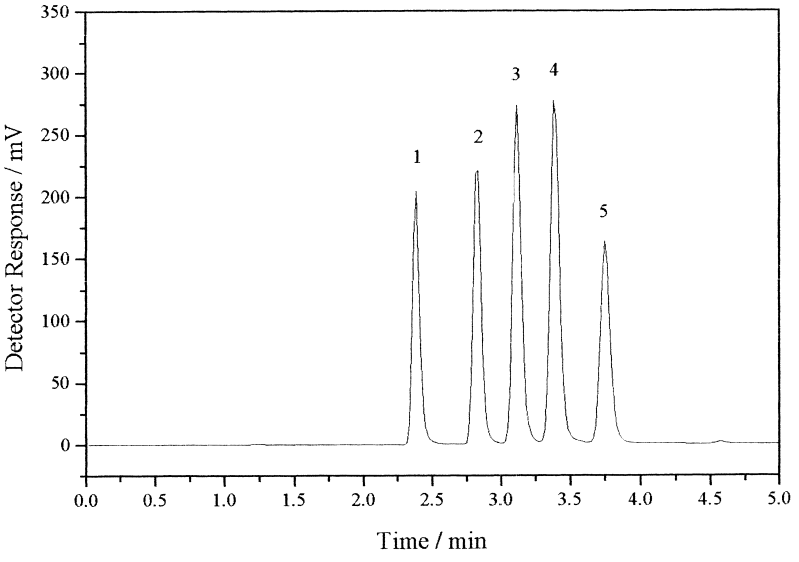

Fig. 5. Chromatogram of the separation of the test mixture composed of uracil (1), acetophenone (2), benzene (3), toluene (4) and naphthalene (5) on the C_{18} urea column. Mobile phase: acetonitrile–water (80:20, v/v) at 0.6 ml min^{-1}, detection: UV at 254 nm and injection volume: 5 μl.

Table 1
Chromatographic parameters obtained with the new C_{18} urea column for the separation of a test mixture of nonpolar compounds

Compound	k	N/m	t_F
Acetophenone	0.16	70 370	1.08
Benzene	0.31	78 992	1.16
Toluene	0.42	81 615	1.24
Naphthalene	0.58	87 653	1.14

Chromatographic conditions: 150×3.9 mm I.D. column packed with C_{18} urea phase; mobile phase:acetonitrile–water (80:20, v/v); optimal flow-rate: 0.6 ml min^{-1}, detection: UV at 254 nm and injection volume: 5 μl.

tailing factor at 5%, t_F, were calculated for each component and the results are summarized in Table 1.

The SRM 869a was chosen to evaluate the shape selectivity of the new C_{18} urea phase. This mixture consists of three polycyclic aromatic hydrocarbons (PAHs): benzo[*a*]pyrene (BaP; planar shape), phenanthro[3,4-*c*]phenanthrene (PhPh; nonplanar shape) and 1,2,3,4,5,6,7,8-tetrabenzonaphthalene (TBN; nonplanar shape). The elution order of these compounds has been shown to correlate with stationary phase shape recognition performance and permits classifying phases into monomeric or polymeric types. A measure of stationary phase selectivity can be also calculated through the selectivity factor based on the $\alpha_{TBN/BaP}$ value. Values for $\alpha_{TBN/BaP} \leq 1$ reflect polymeric phases and values for $\alpha_{TBN/BaP} \geq 1.7$ reflect monomeric phases [27,28]. The separation of the three components is presented in Fig. 6. The elution order PhPh<TBN<BaP and an $\alpha_{TBN/BaP}$ value of 0.8 suggests a polymeric type phase. This result is expected because, in the modification process, a trifunctional urea–alkoxysilane was used. The polymeric characteristics of this phase might be advantageous, enhancing the hydrolytic stability of the silica support presumably because of the multiple bonding.

This column was also evaluated using the SRM 1647c test mixture, composed of 16 PAHs, identified by the US Environmental Protection Agency as priority pollutants [29]. It is known that good separations of these 16 compounds can be obtained with most C_{18} polymeric type phases. The separation of this mixture, shown in Fig. 7, was achieved for almost all components, except for benzo[*ghi*]perylene (15) and indeno[1,2,3-*cd*]pyrene (16). Generally, the separation of these two compounds is not possible for conventional C_{18} stationary phases with $\alpha_{TBN/BaP}$ values near 1.2 (intermediate properties). In our case, even through the separation of SRM 869a mixture indicates a polymeric phase, the complete separation of SRM 1467c was not achieved under normal conditions.

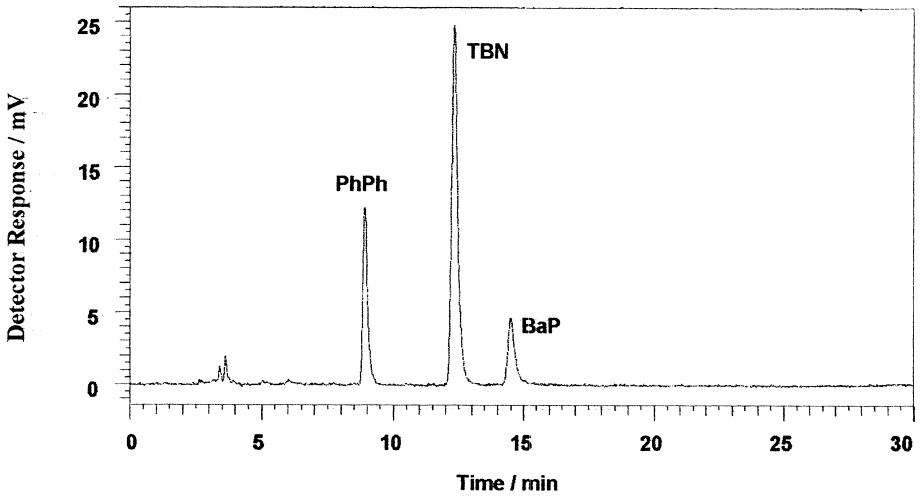

Fig. 6. Chromatogram of the separation of the test mixture SRM 869a from NIST, composed of phenanthro[3,4-*c*]phenanthrene (PhPh), 1,2,3,4,5,6,7,8-tetrabenzonaphthalene (TBN) and benzo[*a*]pyrene (BaP) on the 250×4.6 mm I.D. C_{18} urea column. Mobile phase: acetonitrile–water (85:15, v/v) at 1.0 ml min^{-1}, detection: UV at 254 nm, injection volume: 10 μl and temperature: 25±1°C.

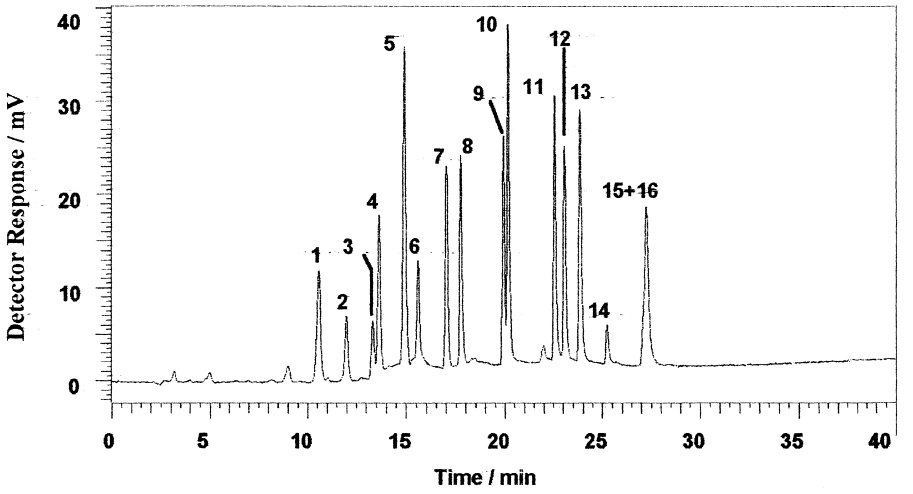

Fig. 7. Chromatogram of the separation of the test mixture SRM 1647c (NIST) composed of naphthalene (1), acenaphthylene (2), acenaphthene (3), fluorene (4), phenanthrene (5), anthracene (6), fluoranthene (7), pyrene (8), benzo[*a*]anthracene (9), chrysene (10), benzo[*b*]fluoranthene (11), benzo[*k*]fluoranthene (12), benzo[*a*]pyrene (13), dibenz[*a*,*h*]anthracene (14), benzo[*ghi*]perylene (15) and indeno[1,2,3-*cd*]pyrene (16), on the 250×4.6 mm I.D. C_{18} urea column. Conditions: gradient elution program: 5 min hold at acetonitrile–water (50:50, v/v), 15 min linear gradient to 100% acetonitrile, and further 15 min hold at 100% acetonitrile at 1.0 ml min^{-1}, detection: UV at 254 nm, injection volume: 10 μl and temperature: 25±1°C.

The ability of the urea groups to minimize the undesirable interactions with unwanted silanol groups on the silica surface was evaluated using a test mixture containing nonpolar, polar and high basic compounds. The test mixture, proposed by Neue et al. [17], is composed of uracil as a marker for column dead volume, naphthalene and acenaphthene as hydrophobic markers, butylparaben and dibutyl phthalate as polar probes and propranolol and amitriptyline as basic probes.

The most interesting probes in this mixture are propranolol and amitriptyline, because these compounds are highly basic (pK_a>9) and often exhibit tailing under reversed-phase conditions due to interaction with residual surface silanols. Fig. 8 shows the chromatogram obtained where almost all components were well separated, except acenaphthene and amitriptyline.

The chromatographic parameters were calculated for each compound and the results are shown in Table 2. The *N*/m value for naphthalene was 96 000 and tailing factors of 1.9 and 1.8 were observed for propranolol and amitriptyline, respectively. The tailing factors values are lower when compared with the values observed for these same basic compounds under the same separating conditions using a classical C_{18} reversed phase [2].

It can be speculated that the phase containing embedded urea groups is less retentive, as indicated by comparing the retention factors for naphthalene and acenaphthene in the new C_{18} urea phase, with the values of 5.82 and 13.6, respectively, reported for a monomeric embedded polar group bonded phase, *N*-octadecyl carbamate, with the same carbon length and a surface coverage of 3.24 μmol m^{-2} [2]. A different elution order was also observed for acenaphthene and amitriptyline in the C_{18} urea phase. On the carbamate phases, amitriptyline is eluted first, followed by acenaphthene. The high retention of amitriptyline is probably because of ion-exchange interactions with surface silanols, indicating that residual silanol groups are still present in our C_{18} urea phase. However this effect is less pronounced when compared to that observed for classical C_{18} columns, due to the influence of the embedded polar urea groups [2].

On the other hand, the tailing factors observed for the basic probes with the C_{18} urea column are higher, when compared to the tailing factors of these same analytes in commercially available carbamate

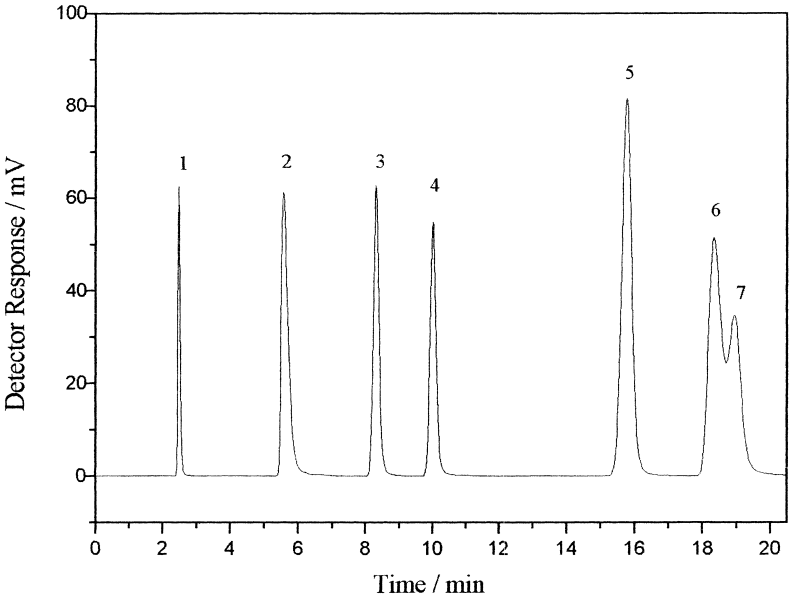

Fig. 8. Chromatogram of the separation of the test mixture composed of uracil (1), propranolol (2), butylparaben (3), naphthalene (4), dibutyl phtalate (5), acenaphthene (6) and amitriptyline (7) on the C_{18} urea phase. Mobile phase: methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7 (65:35, v/v) at 0.6 ml min^{-1}, detection: UV at 254 nm and injection volume: 5 μl.

[2,15] and amide phases [5,14]. In further studies, efforts will be taken to improve the bonding chemistry of the silica by increasing the surface coverage, and also designing a new, more reactive, monofunctional urea–organosilane.

4. Conclusions

A new stationary phase, containing urea polar groups embedded into a *N*-C_{18} alkyl chain, was prepared by a single-step modification process and showed promise for separating nonpolar, polar and basic ionizable compounds with good peak shapes and column efficiencies. Using a test mixture composed of nonpolar, polar and basic compounds, it was possible to verify the influence of the polar urea groups in interacting with the unwanted silanol groups on the silica surface, minimizing peak tailing for propranolol and amitriptyline. The separation of the test mixture SRM 869a reflected a polymeric type phase. This polymeric characteristic enabled the C_{18} urea column to separate almost all compounds of SRM 1467c. The methodology of preparing an organosilane with the desired substituents and then bonding it to the silica surface is advantageous over the two-step modification process. The absence of the second reaction step allows obtaining a homogeneous composition attached to the silica surface.

Table 2
Chromatographic parameters obtained with the new C_{18} urea column for the separation of a test mixture composed of nonpolar, polar and basic analytes

Compound	k	N/m	t_F
Propranolol	1.25	24 532	1.95
Butylparaben	2.35	79 550	1.15
Naphthalene	3.04	96 617	1.14
Dibutyl phtalate	5.35	87 852	1.06
Acenaphthene	6.39	51 492	1.04
Amitriptyline	6.64	64 517	1.81

Chromatographic conditions: 150×3.9 mm I.D. column packed with C_{18} urea phase, mobile phase: methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 at pH 7 (65:35, v/v); optimal flow-rate: 0.6 ml min^{-1}, detection: UV at 254 nm and injection volume: 5 μl.

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