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New generation of sterically protected C_{18} stationary phases containing embedded urea groups for use in high-performance liquid chromatography

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Abstract

New monofunctional C_{18} urea stationary phases with sterically protecting dimethyl and diisopropyl groups were prepared by a single step modification process. ProntoSil spherical silica (3 μm) was chemically modified with the monofunctional ethoxysilanes, [(3-octadecylurea)propyl]dimethyl and [(3-octadecylurea)propyl]diisopropyl ethoxysilanes. The phases were characterized by elemental analysis, infrared and solid-state ^{29}Si and ^{13}C NMR spectroscopies. Chromatographic characterizations of the new phases in 50×3.9 mm HPLC columns were performed by the separation of two different test mixtures, containing nonpolar, polar and highly basic compounds.

Keywords: Stationary phases, LC; Urea stationary phases

1. Introduction

The number of new silica-based phases for reversed-phase liquid chromatography has considerably increased in recent years to try to overcome the imposing challenges faced by chromatographers when highly basic and ionizable compounds, mainly pharmaceuticals, are analyzed.

Recent advances are based on new synthetic pathways to produce high purity silica supports without metal impurities, commonly found in the old generation type A silicas. Another trend involves chemical modification of the bare silica with new designed organosilanes, differing from the conventional mono, di or trifunctional octadecyl (C_{18}) and octyl (C_8) chloro- or alkoxysilanes.

Based on this approach, a new family called embedded polar phase has been developed. These phases differ from their alkyl counterparts by the incorporation of polar functional groups after the third methylene unit in the N-alkyl chain of the organosilane. These embedded groups generally contain nitrogen atoms and amide [1–7], carbamate [8–10] and more recently urea groups [11,12] have shown that they can play this important role to minimize the undesirable silanol interactions.

These embedded polar phases were initially prepared by a two-step modification process, where an aminopropyl silica was acylated with acid chlorides to form polar amide groups [1]. Later, another approach was developed by O'Gara et al., involving a modification process based on the prior synthesis of the appropriate organosilane, containing polar functional carbamate groups [8]. Following a similar approach, we have recently prepared new embedded

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polar phases based on urea, through modification of the bare silica with new trifunctional urea silanes with N-alkyl groups varying from C_7 to C_{18} [11,12].

Phases with an incorporated polar group clearly exhibit lower tailing factors for basic compounds, when compared to classical bonded phases, even with phases based on high-purity silica supports. Some mechanisms have been proposed, while some evidence leads to the belief that the surface layer of an embedded polar group phase should have a higher concentration of water due to the hydrogen bonding ability of the polar groups near the silica surface. This virtual water layer suppresses the interaction of the basic analytes with the residual surface silanols and permits separation with mobile phases having almost 100% water [13].

On the other hand, the presence of this water layer seems to contribute to a higher dissolution of the silica support when compared to their alkyl C_8 and C_{18} counterparts. In a systematic column stability evaluation [14], an embedded amide polar stationary phase was less stable and the authors claimed that the underlying silica support was more exposed to dissolution. This result may be predictable, due to the higher water content near the underlying silica surface for the polar embedded phases.

An attempt was made to overcome this limitation by the preparation of a C_{14} diisopropyl amide stationary phase [15]. Nevertheless, the synthetic pathway was by a two-step reaction process and it is known that residual aminopropyl moieties can be found on the silica surface mixed among the amide groups. At pH less than 9, the amino moieties are protonated and negatively charged analytes can be strongly adsorbed or, when eluted, produce poor peak shapes [10].

The present investigation reports the preparation of new monofunctional C_{18} urea stationary phases with sterically protecting dimethyl and diisopropyl groups synthesized by a single step modification process, based on the prior preparation of new monofunctional urea silanes. The principal objective in this study is the physicochemical characterization of the new monofunctional C_{18} urea silica phases and an initial chromatographic evaluation, through the separation of the Neue test mixture, in order to evaluate these new polar embedded phases with urea groups for reversed-phase HPLC.

2. Experimental

2.1. Chemicals

ProntoSil silica, spherical silica particles, with a mean particle size of 3 μm, mean pore diameter of 21 nm, BET surface area of 189 ± 5 m^2 g^{-1} and pore volume of 1.0 cm^3 g^{-1}, was supplied from Bischoff Chromatography (Leonberg, Germany). The monofunctional urea–ethoxysilanes have recently been synthesized and characterized, according to a new organic synthesis route [16]. Toluene was purchased from Merck (Darmstadt, Germany). Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS), from Aldrich (Milwaukee, WI, USA), were used without further purification. Uracil, acetophenone, naphthalene, propranolol, dipropyl and dibutyl phthalate, acenaphthene and amitriptyline were also from Aldrich. Triethylamine (also from Aldrich) was bidistilled over KOH pellets. All other solvents (methanol, acetonitrile and chloroform) were HPLC grade and were purchased from Merck. Deionized water was obtained from a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Preparation of the C_{18} monofunctional urea reversed-phases

The preparation involves the conventional chemical modification of the silica by reaction of silanol groups with the appropriate monofunctional urea–ethoxysilanes [(3-octadecylurea)propyl]dimethyl and [(3-octadecylurea)propyl]diisopropyl ethoxysilanes. Firstly, the bare silica was washed with deionized water and activated under vacuum for 8 h at 373 K. In each case, a sample of 5.0 g of ProntoSil was suspended in 100 ml of dry toluene and 23 mmol of the C_{18} urea–ethoxysilane (24 μmol m^{-2} of silica surface) was added. An equimolar amount of bidistilled triethylamine (3.20 ml) was also added to the suspension [17], which was mechanically stirred and refluxed under a nitrogen atmosphere for 86 h. The bonded silicas were isolated by vacuum filtration and purified by repeated washings with toluene, methanol and a water–methanol mixture. Subsequently, the C_{18} dimethyl and diisopropyl urea bonded phases were dried under vacuum for 8 h at 353 K prior to an endcapping reaction.

The C_{18} dimethyl and diisopropyl urea silicas were endcapped using a conventional liquid phase reaction [18]. Briefly, the reactions were performed by refluxing nearly 4.5 g of the modified silica with an equimolar mixture of TMCS (15 ml) and HMDS (35 ml) in 100 ml of dry toluene. The mixture was then stirred at 395 K for 48 h, the silica was filtered and washed with toluene, methanol, a water–methanol mixture, water and finally with methanol. The endcapped materials were dried under vacuum for 8 h prior to characterization or packing.

2.3. Elemental analysis

Carbon, hydrogen and nitrogen percentages for the new monofunctional C_{18} urea phases, 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 NMR spectroscopy

Solid-state ^{13}C and ^{29}Si NMR measurements were performed on an INOVA spectrometer (Varian), 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 3 s. Frequencies of 75.5 and 59.6 MHz for carbon and silicon, respectively, were used.

2.5. Infrared spectroscopy

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

2.6. Column packing

HPLC columns (50×3.9 mm) were made from 316 stainless steel tubing and had their inner surfaces polished as described elsewhere [19]. The modified silicas were individually packed using the conventional slurry packing technique. Thus, an amount of 0.70 g of each modified silica was added to 7 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 (Burbank, CA, USA) packing pump 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 separations

The chromatographic tests were performed using a modular HPLC system with a Waters 510 pump (Waters, Milford, MA, USA), a Rheodyne (Cotati, CA, USA) 7725 injector and a Waters 486 tuneable wavelength absorbance detector. 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 some compounds of the test mixture of Neue et al. [20], such as uracil, naphthalene, dipropyl and dibutyl phthalate, with propranolol and amitriptyline as basic probes, using methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 at pH 7.0 as mobile phase. The buffer was prepared by dissolving 1.68 g of K_2HPO_4 and 1.33 g KH_2PO_4 in a 1 l volumetric flask. The pH was adjusted to 7.00 before addition of methanol using a calibrated pH meter. Plate number per meter, N/m,

retention factor, k, and tailing factor at 5%, T_f, were calculated [21].

3. Results and discussion

3.1. Preparation of the monofunctional C_{18} urea phases

The preparation of the new monofunctional C_{18} urea phases is outlined in the scheme of Fig. 1. In the first step of the chemical modification, the ethoxy group from the monofunctional silane reacts with the surface silanols, yielding the modified phases (I). Triethylamine was used as a basic catalyst to enhance the surface coverage of the silica surface [17,22]. The modified silicas were purified with repeated washings to remove physically adsorbed silane. In a further step, the modified surfaces were endcapped through reaction with an equimolar mixture of TMCS and HMDS. The residual silanols were blocked by substitution by a $-Si(CH_3)_3$ moiety, as represented in (II).

Table 1
Carbon, hydrogen and nitrogen contents and surface coverage (χ) for the new monofunctional urea phases

Urea phase	C (%)	H (%)	N (%)	χ (μmol m^{-2})
C_{18} dimethyl	14.4	2.63	1.75	3.33
C_{18} diisopropyl	10.2	1.66	1.48	1.87

The modification process yielded modified silicas with a ligand surface concentration (χ) of 3.33 and 1.87 μmol m^{-2} for the C_{18} dimethyl and diisopropyl urea phases, respectively, as can be seen in Table 1.

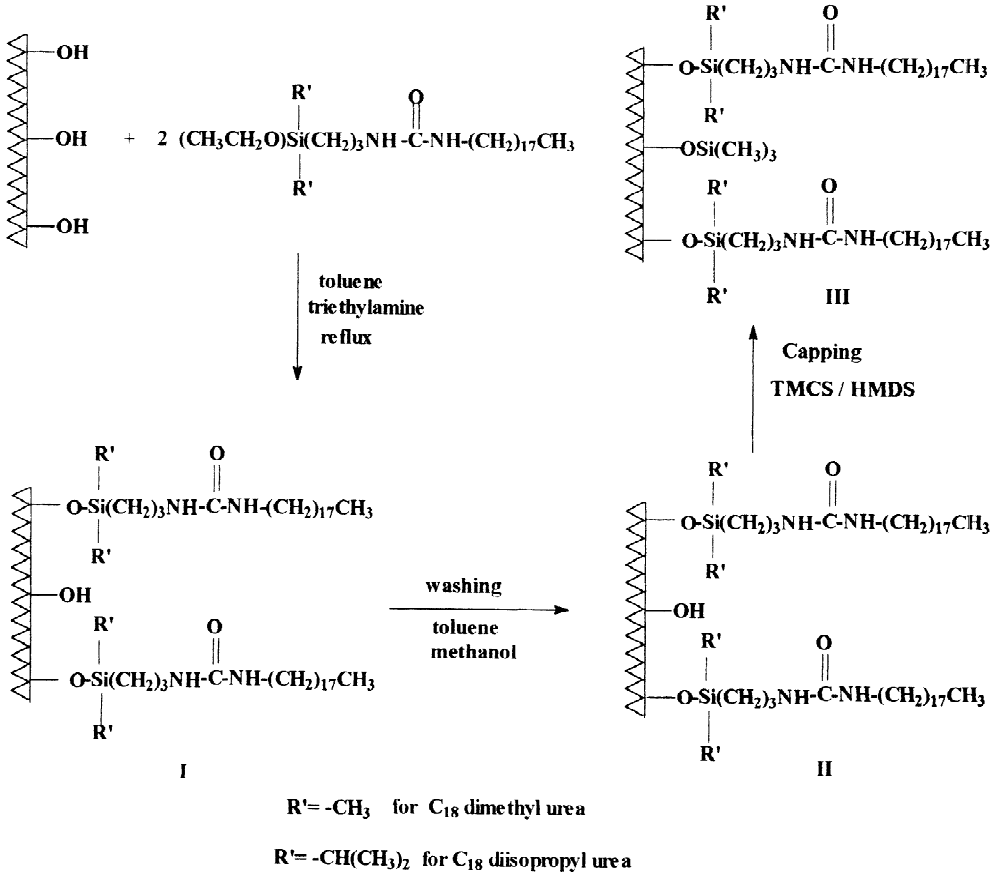

Fig. 1. Preparation of monofunctional C_{18} dimethyl and diisopropyl urea phases.

The concentration of the organic groups attached to the silica surface was calculated using the well-known expression of Berendsen et al. [23,24], which takes into account the carbon percentages determined by elemental analyses and the BET surface area of the bare silica. A predictable lower surface coverage was obtained for the C_{18} diisopropyl urea due to the presence of the diisopropyl groups. This χ value is quite low, when compared to the value of 2.1 μmol m^{-2} obtained for the C_{14} diisopropyl amide stationary phases [15]. This lower surface coverage is typical for dense coverage in the preparation of phases using this kind of sterically protected silanes [25]. Conversely, the χ value for the C_{18} dimethyl urea phase is somewhat higher, when compared to the reported values for the monofunctional C_{18} carbamate phases, using a much more reactive chlorosilane for the chemical modification of the silica surface [9] and also when compared to the surface coverage of 3.22 μmol m^{-2} reported for the polymeric C_{18} urea phase synthesized in our laboratory [12]. After the endcapping reaction, elemental analysis was again performed and an increase of 0.4 and 0.8% in the carbon content for the C_{18} dimethyl and diisopropyl urea phases were observed. The highest increase in the carbon percentage was observed for the C_{18} diisopropyl phase, having the lower surface coverage.

Figs. 2 and 3 show the DRIFT spectra for the C_{18} dimethyl and diisopropyl urea phases, respectively,

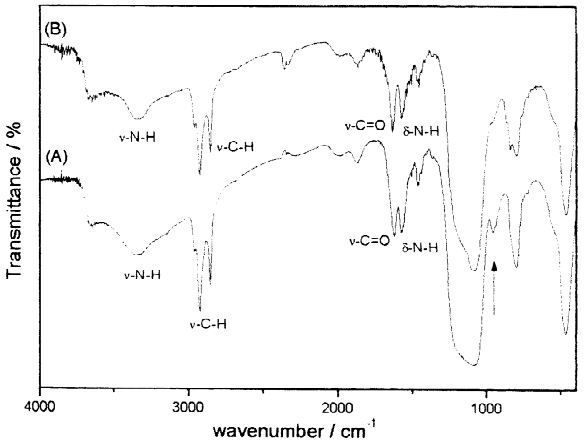

Fig. 2. DRIFT spectra for the C_{18} dimethyl urea phase, (A) before and (B) after the endcapping reaction.

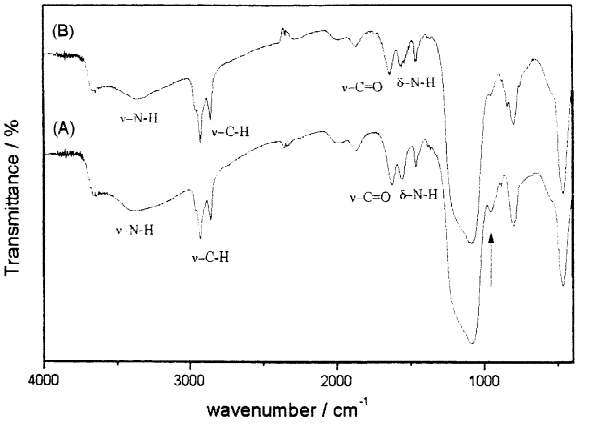

Fig. 3. DRIFT spectra for the C_{18} diisopropyl urea phase, (A) before and (B) after endcapping.

before and after the endcapping. Bands at 2960 and 2850 cm^{-1} are attributed to the C–H stretching due to the presence of methyl and methylene groups from the C_{18} N-alkyl chain immobilized on the silica surface. Additional bands at 3330 and 1570 cm^{-1} correspond to N–H stretching and bending, respectively, suggesting the presence of the urea functionality. The band at 1630 cm^{-1}, which is attributed to –C=O stretching, is further evidence for the presence of the embedded polar urea group. After the endcapping reaction, the band at 970 cm^{-1}, which is attributed to free silanol bending, is greatly reduced after the reaction with TMCS/HMDS, showing the success of the endcapping reaction for both phases, as outlined in Fig. 1.

As previously stated by Pfleiderer et al. [26], ^{13}C and ^{29}Si CP–MAS NMR spectroscopy 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. 4 shows the ^{13}C CP-MAS-NMR spectra of the C_{18} dimethyl and diisopropyl urea phases. The presence of the methyl groups of the C_{18} dimethyl urea phase is confirmed by the peak at 1 ppm in the spectrum of Fig. 4A. For the C_{18} diisopropyl urea phase, peaks at 12 and 16 ppm in the spectrum of Fig. 4B are evidence of the protecting diisopropyl groups. Each spectrum is consistent with the proposed ligand structure, inserted in each spectrum, and no chemical changes have occurred in the urea silyl organic groups during the modification processes.

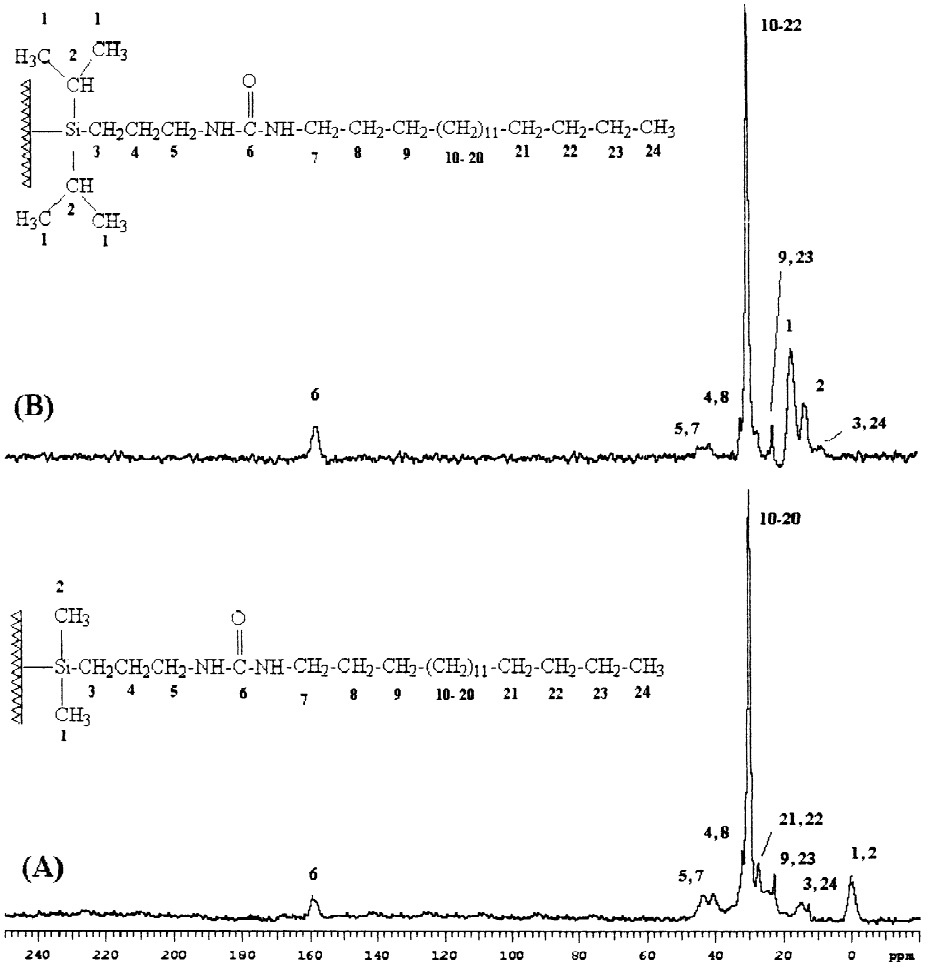

Fig. 4. ^{13}C CP-MAS-NMR spectra for the C_{18} dimethyl and diisopropyl urea phases.

The new monofunctional C_{18} urea stationary phases were also investigated by ^{29}Si CP-MAS-NMR spectroscopy. Figs. 5 and 6 show the ^{29}Si CP-MAS-NMR spectra for the C_{18} dimethyl and diisopropyl urea, before and after endcapping. The species found on the surface, described as Q^n where *n* is related to the number of siloxane bridges (mono, di, tri or tetra) in the silicon atom [26–28]. In all spectra, the Q^4 (siloxanes) and Q^3 species (silanols) were detected at −110 and −101 ppm, respectively. In addition to the signals Q^3 and Q^4 of the silica skeleton, a resonance at +12 ppm, which corresponds to M^1 species, is also detected in Figs. 5 and 6. The M^1 species represents the silicon atoms derived from the monofunctional urea silane used as shown in the inserted structure. The presence of the two methyl groups directly bonded to the silicon atom in the M^1 species cannot be distinguished from the M^1 species having diisopropyl groups as neighbors instead of the methyl groups, because the species presents the same chemical shift.

After the endcapping reaction, ^{29}Si CP-MAS-NMR spectroscopy was again performed, where a portion of the residual silanols were blocked by a $-Si(CH_3)_3$ moiety forming a new M^2 species. The new M^2 species, introduced by endcapping, are not distin-

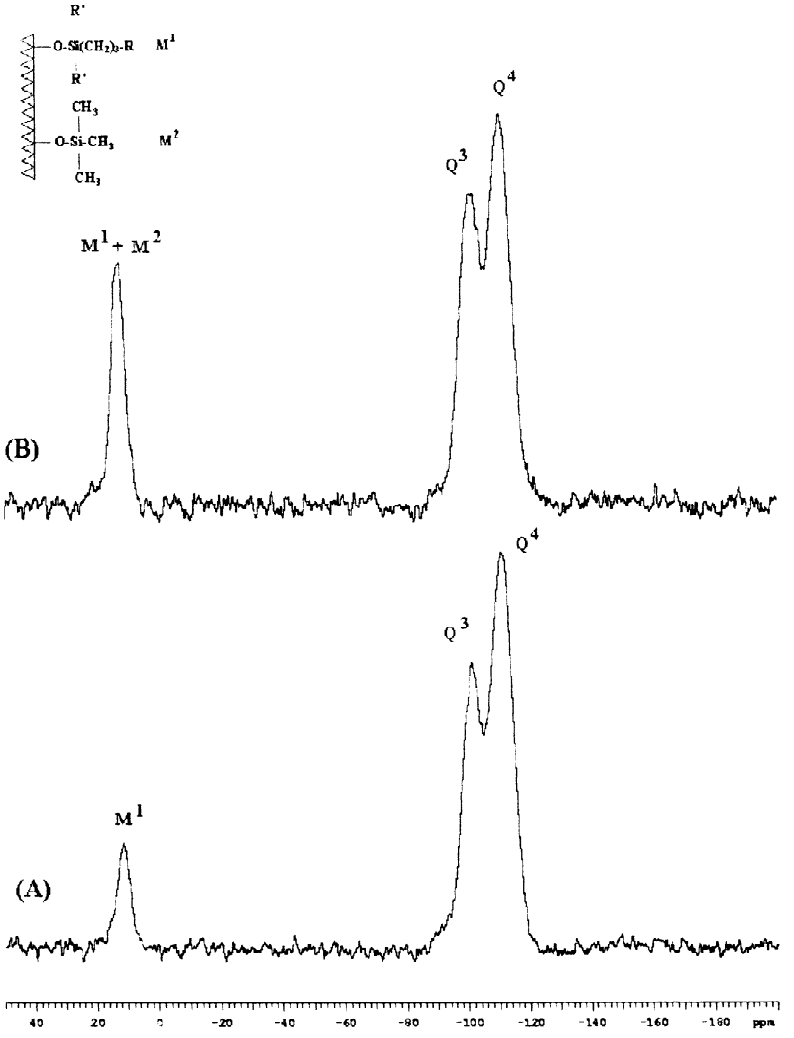

Fig. 5. ^{29}Si CP-MAS-NMR spectra for the C_{18} dimethyl urea silica, (A) before and (B) after endcapping.

guishable from the M^1 species, having the same chemical shift at +12 ppm. All spectra were measured with the same instrument parameters and it is possible to estimate semi-quantitatively the population of these species on the modified silica surfaces. Thus, in the spectrum of Fig. 5B, a relative increase in the intensity for the peak of M species at +12 ppm was observed after the endcapping.

For the spectra of the C_{18} diisopropyl urea phase in Fig. 6, the intensity of the peak at −101 ppm for the Q^3 species is greatly reduced after the endcapping. These results suggest the deactivation of the silanols was successfully performed, as previously indicated by elemental analysis and infrared spectroscopy.

3.2. Chromatographic evaluations

Shorter HPLC columns, packed with the monofunctional C_{18} urea phases, were chosen because there are some advantages in using a column of

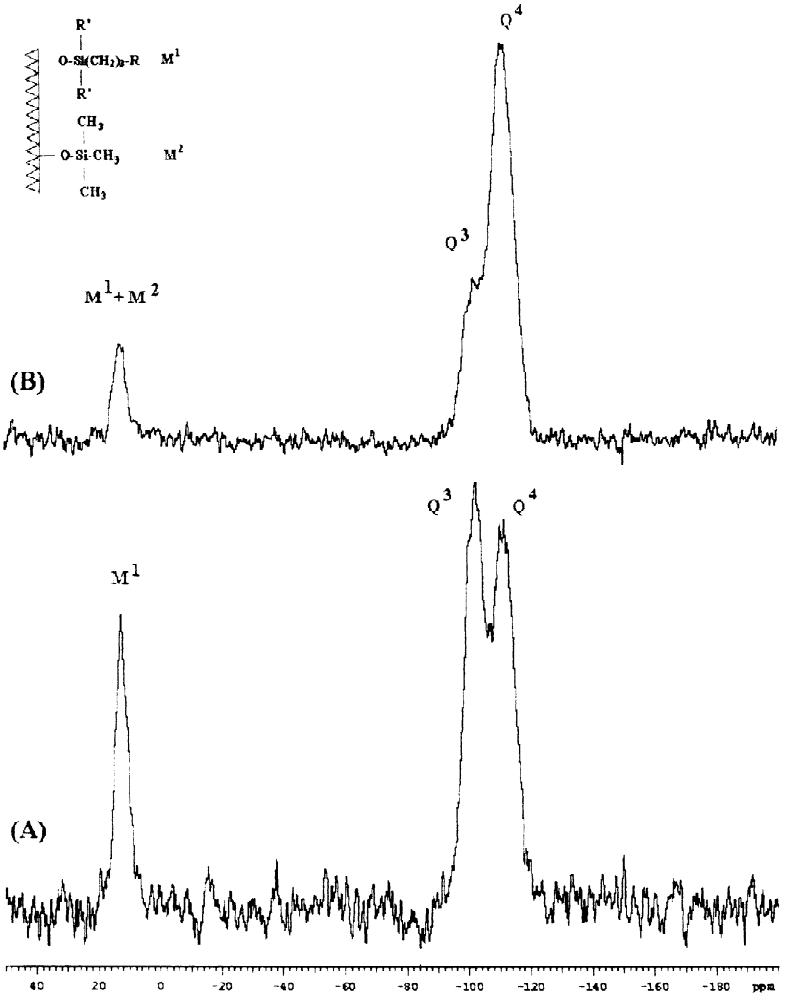

Fig. 6. ^{29}Si CP-MAS-NMR spectra for the C_{18} diisopropyl urea silica, (A) before and (B) after endcapping.

small length, such as the lesser quantity of stationary phase required and a shorter time for analysis.

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 0.8 ml min^{-1}, calculated from the van Deemter plots, shown in Fig. 7 for both phases, using plate height values, *H*, for naphthalene, and acetonitrile–water as mobile phase.

Fig. 8 shows the complete chromatograms obtained and it is observed that both columns separate all components of the test mixture well. To achieve a satisfactory separation of the components of the test mixture with the C_{18} diisopropyl urea phase, the concentration of the organic modifier had to be decreased, indicating a lower hydrophobicity due to the lower surface coverage of 2.1 μmol m^{-2}. Retention factor, *k*, plate number per meter, *N*/m, and tailing factor at 5%, T_f, were calculated for each component and the results are summarized in Table 2. For naphthalene, the *N*/m values were 82,200 and 78,600 and the tailing factors 1.1 and 1.2 for the C_{18} dimethyl and diisopropyl phases, respectively.

The shielding properties of the urea groups, to minimize the undesirable interactions with unwanted surface silanols in the monofunctional C_{18} phases, were evaluated using some compounds of the Neue test mixture, containing uracil as a marker for

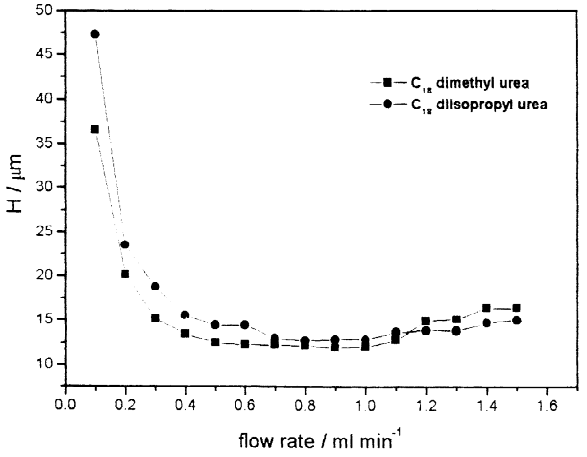

Fig. 7. Plots of *H* (plate height) for naphthalene at different flow-rates for the C_{18} dimethyl and diisopropyl urea columns.

column dead volume, naphthalene as hydrophobic marker, dipropyl and dibutyl phthalate as polar solutes and propranolol and amitriptyline as basic probes. The most interesting probes in this mixture are propranolol and amitriptyline, because these compounds are highly basic ($pK_a > 9$). At neutral pH, the great majority of the residual surface silanols are in their ionized form ($Si–O^-$) and the basic probes are protonated (BH^+). The protonated bases can interact with the deprotonated silanols by ionic interactions, causing peak deformation or even irreversible retention. For this reason, the tailing and the retention factor of these basic probes is a good measure of silanophilic activity [20].

Fig. 9 shows the chromatograms obtained where all components were well separated with good peak shapes and high efficiency on the C_{18} dimethyl urea phase. The same performance was not achieved on the C_{18} diisopropyl urea phase. Under the same conditions, naphthalene co-eluted with dipropyl phthalate and much higher tailings were also observed mainly for propranolol and amitriptyline. This can be clearly seen from the calculated chromatographic parameters shown in Table 3. By comparing the *k* values, all compounds are less retained in the C_{18} diisopropyl urea phase. In order to obtain a better separation, the concentration of methanol in the mobile phase, buffered at pH 7, was adjusted to 55% (v/v). The chromatogram obtained can be seen in Fig. 10. Tailing is still observed and, surprisingly,

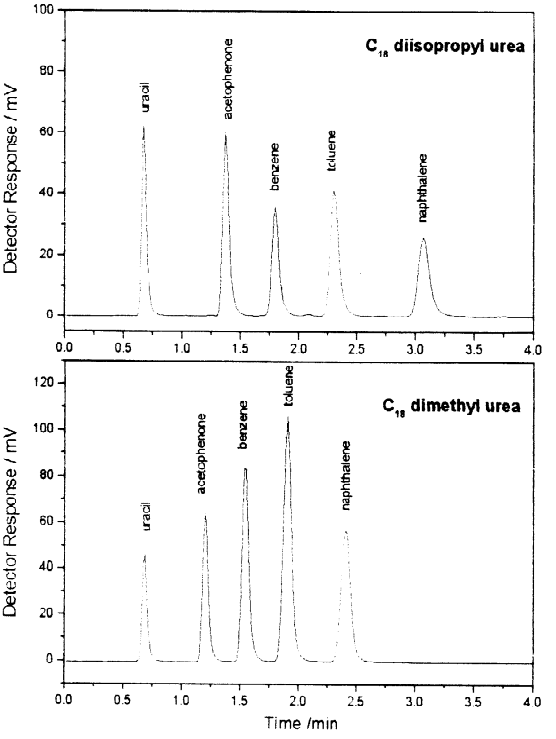

Fig. 8. Chromatogram showing the separation of the test mixture composed of nonpolar compounds on the C_{18} dimethyl and diisopropyl urea phases in 50×3.9 mm I.D. columns. Mobile phase, acetonitrile–water at flow-rate of 0.8 ml min^{-1}; detection, UV at 254 nm; injection volume, 5 μl. For the separation on the C_{18} dimethyl urea phase, the mobile phase composition was acetonitrile–water (60:40, v/v) and for the C_{18} diisopropyl urea phase, it was 50:50, v/v.

Table 2
Chromatographic parameters obtained with the new monofunctional urea phases for the separation of a test mixture of nonpolar compounds

Compound	C_{18} dimethyl[a]			C_{18} diisopropyl[b]		
	k	*N*/m	T_f	*k*	*N*/m	T_f
Acetophenone	0.76	55,955	1.19	1.04	58,788	1.36
Benzene	1.26	61,692	1.28	1.68	65,830	1.22
Toluene	1.79	76,212	1.15	2.44	73,389	1.25
Naphthalene	2.53	82,230	1.20	3.58	77,183	1.20

[a] Chromatographic conditions: 50×3.9 mm I.D. columns; mobile phase, acetonitrile–water (60:40, v/v); optimal flow-rate, 0.8 ml min^{-1}; detection, UV at 254 nm; injection volume, 5 μl.

[b] The same separation conditions using acetonitrile–water (50:50, v/v) as mobile phase.

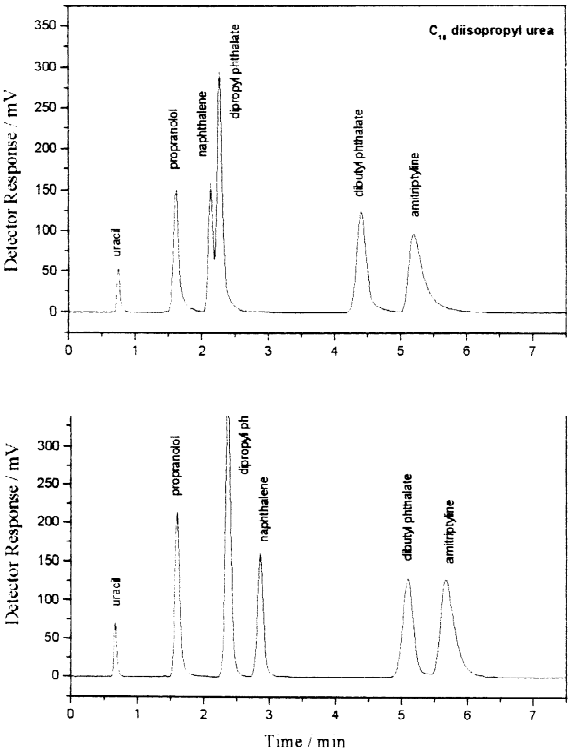

Fig. 9. Separation of the Neue test mixture on the C_{18} dimethyl and diisopropyl urea 50×3.9 mm I.D. columns. Mobile phase, methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (65:35 v/v), at 0.8 ml min^{-1}; detection, UV at 254 nm; injection volume, 5 μl.

amitriptyline eluted before dibutyl phthalate. The chromatographic parameters with these new separation conditions were calculated and are also presented in Table 3.

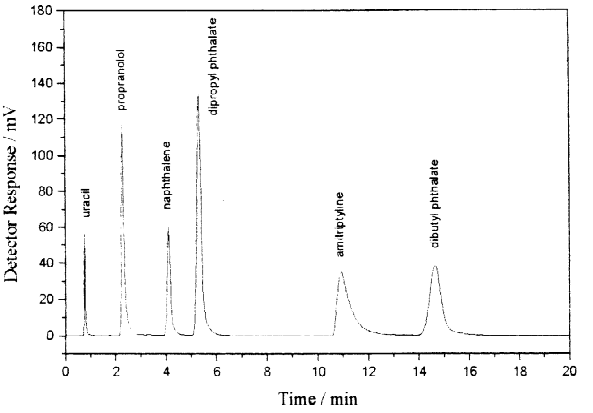

Fig. 10. Separation of the Neue test mixture on the C_{18} diisopropyl column, using the same conditions as Fig. 9 except for mobile phase: methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (55:45 v/v).

According to these results we may conclude that the C_{18} diisopropyl urea phase is not as useful as the dimethyl urea phase for the separation of polar and basic compounds, mainly at neutral pH. One possible explanation for the observed decrease in the retention of almost all compounds and the less than desirable tailing for the basic compounds is the lower surface coverage obtained for this phase with the sterically protecting diisopropyl groups.

The main goal of the use of the sterically protected diisopropyl C_{18} urea phase was to enhance the hydrolytic stability of the silica support, which may be reduced by the presence of the embedded polar groups. However, from the results presented, the use

Table 3
Chromatographic parameters obtained for the separation of some compounds of the Neue test mixture composed of nonpolar, polar and basic analytes on the C_{18} dimethyl and diisopropyl urea columns

Compound	C_{18} dimethyl urea[a]			C_{18} diisopropyl urea[a]		
	k	N/m	T_f	k	N/m	T_f
Propranolol	1.48	48,641	1.47	1.18 (2.05)[b]	34,998 (42,735)	1.44 (1.99)
Dipropylphthalate	2.68	79,711	1.12	2.07 (6.05)	60,193 (76,157)	1.41 (1.39)
Naphthalene	3.44	94,989	1.07	1.88 (4.45)	67,359 (83,279)	nc[c] (1.35)
Dibutylphthalate	7.05	91,341	1.02	4.95 (18.3)	72,474 (80,691)	1.32 (1.31)
Amitriptyline	8.03	66,810	1.59	6.03 (13.5)	48,624 (43,083)	2.12 (2.67)

[a] Chromatographic conditions: 50×3.9 mm I.D. columns; mobile phase, methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (65:35, v/v); optimal flow-rate, 0.8 ml min^{-1}; detection, 254 nm; injection volume, 5 μl.

[b] The values in parentheses refer to the parameters obtained using methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (55:45, v/v) as mobile phase.

[c] nc, Not calculated.

of this new kind of urea phase may be disadvantageous due to the dependence of retention and peak shape, especially for basic compounds, on the surface coverage of the stationary phase. The inherent ability of the urea groups to minimize tailing for basic compounds seems to be affected by the lower concentration of these organic groups on the silica surface.

On the other hand, promising results were obtained for the C_{18} dimethyl urea phase, especially when compared with the polymeric C_{18} urea phase, based on the same silica support [12]. The tailing factors for amitriptyline and propranolol were lower for the new monofunctional C_{18} dimethyl urea phase. One possible explanation for this better performance is the advantage of using monofunctional silanes in the modification process, avoiding extra ethoxy groups which may produce more residual silanols on the silica surface during hydrolysis. Another explanation is the higher surface concentration obtained for the C_{18} dimethyl urea phase. The tailing factor observed for amitriptyline with the C_{18} dimethyl urea phase was also lower, when compared with the value of 2.1 in the Symmetry C_{18} bonded phase, which is based on high purity silica, under identical separating conditions [13].

The stability of such phases is a subject of ongoing investigation in order to evaluate the possible advantages or disadvantages of using monofunctional instead of trifunctional urea–ethoxysilanes to prepare these new phases, containing embedded urea groups.

4. Conclusions

New monofunctional C_{18} urea stationary phases with sterically protecting dimethyl and diisopropyl groups, containing polar urea groups embedded into a N-C_{18} alkyl chain were successfully prepared by a single step modification process. The Neue test mixture, composed of nonpolar, polar and basic compounds, was well separated on the C_{18} dimethyl urea phase with good peak shapes and high efficiency, indicating the potential application of this monofunctional phase in the separation of basic compounds at neutral pH.

For the monofunctional C_{18} urea with sterically protecting diisopropyl groups, the compounds were not well separated and higher tailing factor values were observed, mainly for the basic compounds. These results when compared to those obtained on the C_{18} dimethyl urea phase, may be attributed to a lower surface coverage which affects the overall chromatographic performance. In conclusion, the use of dimethyl C_{18} urea silane is advantageous over the diisopropyl analog for the preparation of such polar urea phases.

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