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Development of new urea-functionalized silica stationary phases Characterization and chromatographic performance

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Abstract

New urea-functionalized silica stationary phases were prepared by a single-step surface modification through reaction of LiChrosorb Si100 (5 μm particle size) with a homologous series of alkoxysilanes, synthesized in our laboratory, with the general formula $(CH_3CH_2O)_3Si(CH_2)_3NHC(O)NH(CH_2)_nCH_3$, where $n=4$, 6 and 11. The modified silicas were characterized by elemental analysis of carbon and nitrogen, solid-state ^{29}Si- and ^{13}C-cross polarization magic angle spinning nuclear magnetic resonance and nitrogen adsorption isotherms at 77 K. Chromatographic evaluation of the three urea-functionalized silicas in 150×3.9 mm I.D. HPLC columns was carried out by the separation of a test mixture composed of uracil, acetophenone, benzene, toluene and naphthalene, using acetonitrile–water as mobile phase. These new stationary phases, with embedded polar urea groups, are very promising when compared with amide phases prepared by the conventional two-step modification process. A single-step reaction process silica modification is better for obtaining a well-characterized and homogeneous modified surface.

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

1. Introduction

Designing new functionalized silicas is still a concern for chromatographers who work on the development of new silica-based stationary phases for high-performance liquid chromatography (HPLC). Obviously, silicas modified with C_8 and C_{18} are the most widely used stationary phases for reversed-phase HPLC. A new trend involves the use of modified silicas containing alkyl chains with embedded polar groups as packing materials for separations of basic compounds under reversed-phase conditions [1–4]. These new kinds of functionalized silicas, with polar linking groups, are very promising due to the ability of these groups to interact with unwanted silanol groups on the silica surface. Recent investigations describing stationary phases containing embedded polar groups, have shown the superior performance of these new phases over conventional C_8 and C_{18} phases, when basic compounds are analyzed. In general, these columns exhibit low asymmetry and high efficiency [5].

It is still unclear how these phases work; it was postulated that the polar groups of the alkyl bonded phase can interact with the unwanted silanols on the silica surface. These silanols become less available to interact with polar solute molecules during the chromatographic process, decreasing the peak asymmetry [6]. Another possible advantage is that the phases containing embedded polar groups show different selectivity and are also less retentive, requiring a mobile phase with a lower concentration of organic solvent [7].

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The polar functionalities in modified silicas are often amide or carbamate groups which are stable in almost basic hydro–organic solvents, have strong hydrogen bond capabilities and low reactivities with other chemical compounds [1]. The stationary phases with embedded amide groups are usually prepared by a two-step process. In the first step, the bare silica reacts with an amino organosilane and, in a second step, the aminopropyl silica is acetylated through reaction with acid chlorides in an inert atmosphere [8]. Nevertheless, this conventional procedure suffers from the difficulty of achieving a high yield of acetylated groups as it is well known that the conversion of amine groups to amide in the second modification step is not quantitative [9]. Then, unreacted amino moieties can be found on the silica surface, mixed among the amide groups. The presence of some residual aminopropyl groups, sometimes might be beneficial or cause undesirable interactions during the chromatographic separations.

A single-step process was developed for the preparation of a novel C_8 stationary phase with an embedded polar carbamate group. The carbamate group was first introduced by reaction with octyldimethylclorosilane, in order to obtain a new organochlorosilane. Then, the silica was conventionally modified with this new silane, yielding derivatized silica with a homogenous surface composition [2].

This investigation reports on new urea-functionalized silica stationary phases which were prepared in a similar manner, by a single-step surface modification process through the reaction of bare silica with a homologous series of ureaalkoxysilanes synthesized in our laboratory [10]. The main goal in this study is the physicochemical characterization of the new functionalized silicas and an initial chromatographic evaluation, through the separation of a standard test mixture, in order to evaluate these new kinds of modified silicas as stationary phases with urea embedded groups for reversed-phase HPLC.

2. Experimental

2.1. Chemicals

LiChrosorb Si100, irregular silica particles, with mean particle size of 5 μm, mean pore diameter of 15 nm and BET surface area of 223 m^2 g^{-1} was purchased from Merck (Darmstadt, Germany). The ureaalkoxysilanes have been recently synthesized and characterized in our laboratory, according to a new organic synthesis route [10]. Toluene was purchased from Merck. Trimethylchlorosilane and pyridine, from Aldrich (Milwaukee, WI, USA) were used without further purification. Uracil, acetophenone, benzene, toluene and naphthalene are also from Aldrich and were used as received for the test mixture. 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

Three different silicas were prepared using the homologous series of the ureatrialkoxysilanes [(3-pentyl), (3-heptyl), and (3-dodecyl)propyl]ureatriethoxysilane. The derivatized silicas were named C_5, C_7 and C_{12} urea, respectively. First, the silica was washed with deionized water and dried under vacuum for 8 h at 373 K. In each case, 10.0 g of LiChrosorb was suspended in 100 ml of dry toluene and an excess of ureatrialkoxysilane (13.5 μmol per m^2 of bare silica) was added. The suspension was mechanically stirred and refluxed under a nitrogen atmosphere for 72 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 samples were dried under vacuum for 8 h at 353 K prior to an endcapping reaction.

The modified silicas were endcapped using a conventional liquid phase reaction. Briefly, the reactions were performed by refluxing nearly 10 g of each modified silica with a large excess of trimethylchlorosilane (30 ml, 0.28 mol) in 100 ml of dry toluene with 2 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. All materials were dried under vacuum for 8 h prior to characterization or packing.

2.3. Characterization

Carbon, hydrogen and nitrogen percentages for the new stationary phases were determined on a Perkin-Elmer Model 2400 analyzer. At least two determinations were made for each material. Solid-state ^{13}C and ^{29}Si nuclear magnetic resonance (NMR) spectra were performed on a AC300/P spectrometer (Bruker), using cross polarization magic angle spinning (CP-MAS). For the ^{29}Si nucleus, a contact time of 2.5 ms and a pulse repetition time of 2 s were employed and for ^{13}C, a contact time of 3 ms and repetition time of 4 s. The spinner rate was 4 kHz and frequencies of 75.5 and 59.6 MHz for carbon and silicon, respectively, were used. For the end-capped materials, the ^{29}Si-NMR spectra were performed on an INOVA-500 spectrometer (Varian) and analyses were performed at 99.3 MHz with a spinner rate of 6.2 kHz. A contact time of 2.5 ms and repetition time of 2 s were used. All NMR spectra were externally referenced to liquid tetramethylsilane. The BET surface area, average pore diameter and total pore volume of the three different packings were determined from nitrogen isotherms at 77 K, obtained on a Micrometrics Model ASAP 2004 analyzer.

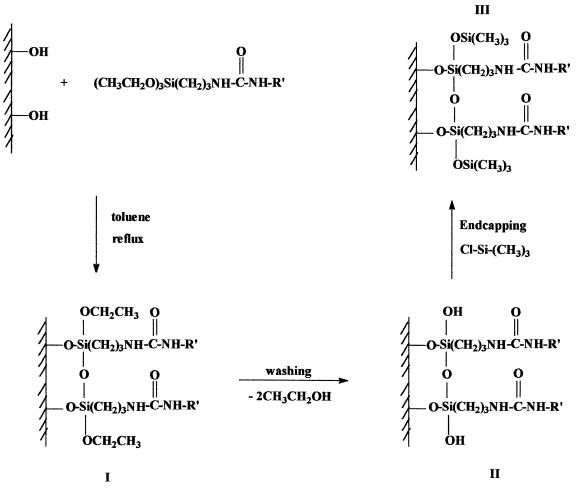

Fig. 1. Preparation of urea-functionalized silicas.

2.4. Column packing

150 mm×3.9 mm I.D. HPLC columns made from 316 stainless steel tubing in our laboratory, had their inner surfaces polished as described in detail elsewhere [11]. The modified silicas were 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 0.20 ml min^{-1}.

2.5. 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 acetonitrile and deionized water. The test mixture used was composed of uracil, acetophenone, benzene, toluene and naphthalene dissolved in mobile phase. The column dead time, t_M, was determined from the retention time for uracil (unretained compound). Plate number, N, retention factor, k, and peak asymmetry at 10% of peak height, A_s, were calculated according to Ref. [12].

3. Results and discussion

3.1. Preparation of urea-functionalized silicas

The preparation of the urea-functionalized silicas is outlined in Fig. 1. First, the three different ureatrialkoxysilanes were covalently attached on 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, the endcapping reaction with trimethylchlorosilane was performed, with the aim of deactivating these new silanol groups (III).

The modification process yielded silicas with a ligand surface concentration ranging from 3.58 to 4.60 μmol m^{-2}. Table 1 shows the carbon, nitrogen and hydrogen percentages for C_5, C_7 and C_{12} urea silicas, respectively. The concentration of the organic groups attached to the silica surface was calculated from the carbon percentages [13], taking into account that all ethoxy groups were hydrolyzed after washing (see product II, Fig. 1). This hypothesis is consistent with the observed C/N ratios, also shown in Table 1. As can be seen, a slightly higher coverage of the surface was obtained in the reaction of LiChrosorb with [(3-pentyl)propyl]ureatriethoxysilane. Also in Table 1, the BET surface area, mean pore diameter and the pore volume, calculated from the nitrogen isotherms at 77 K, are shown. Comparing these values with the values obtained for the bare silica (LiChrosorb Si100, BET surface area 223 m^2 g^{-1}, mean d_p=15 nm and V_p=0.83 cm^3 g^{-1}) the surface area did not change very much, but a significant decrease was observed in the pore diameter and pore volume for the modified silicas. It can be concluded that the silanization process also occurred in the silica pores and for this reason, a lower ligand surface concentration was obtained for the silica modified with the ureatrialkoxysilanes with longer alkyl chains. After the endcapping reaction, elemental analyses for C_5, C_7 and C_{12} urea phases were again performed and an increase of nearly 0.3% in the carbon content was observed.

Table 1
Carbon, hydrogen and nitrogen contents and surface coverage (α) for C_5, C_7 and C_{12} urea-functionalized silicas and their structural properties, such as surface area (S), mean pore diameter (d_p) and mean pore volume (V_p), calculated from nitrogen adsorption

	C_5 urea silica	C_7 Urea silica	C_{12} Urea silica
C (%)	9.22	8.93	12.46
H (%)	1.79	1.80	2.31
N (%)	2.20	1.84	1.74
C/N ratio	4.8 (4.5)[a]	5.7 (5.5)[a]	8.3 (8.0)[a]
α (μmol m^{-2})	4.60	3.58	3.60
S (m^2 g^{-1})	195	220	186
d_p (nm)	8.13	8.55	8.88
V_p (cm^3 g^{-1})	0.58	0.69	0.61

[a] The values refer to the theoretical C/N ratio taking into account the organic structure attached on the surface of each modified silica.

The presence of the remaining ethoxy groups on the surface of the modified silicas, before washing, can be confirmed by ^{13}C-CP-MAS solid-state NMR spectroscopy. As stated by Pfleiderer et al., this technique is an invaluable tool to investigate the chemical structure of the silyl groups attached to the surface [14]. Fig. 2 shows the ^{13}C-NMR spectra of all three functionalized silicas (C_5, C_7 and C_{12} urea). 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 groups during the modification process. Only two side bands, indicated with an asterisk, were observed in the ^{13}C-NMR spectrum of C_7 urea silica. Two signals at 20 and 60 ppm were observed for all materials due to carbons 1 and 2, respectively, of the ethoxy groups. For this reason, all silicas were extensively washed with water to promote hydrolysis of these groups, prior to the endcapping process.

The new urea-functionalized silica stationary phases were also investigated by ^{29}Si-CP-MAS-NMR spectroscopy. Fig. 3 shows the ^{29}Si-CP-MAS-NMR spectra of all phases including the bare LiChrosorb silica. The species found on the surface, here are described as Q_n and T_n species, which are related to the number of oxygen (mono-, di-, tri- or tetraoxo) atoms bound to the silicon atom [15,16]. In the spectrum of the bare silica, the Q_4, Q_3 and Q_2 species were detected at −110, −101 and −92 ppm, respectively. In the spectra of C_5, and C_{12} urea modified silicas T_1 at −52 ppm, T_2 around −57 ppm and T_4 species at −67 ppm were also detected, as described before [15]. These structural types are shown in Fig. 4. The presence of the remaining ethoxy groups in T_n species cannot be distinguished

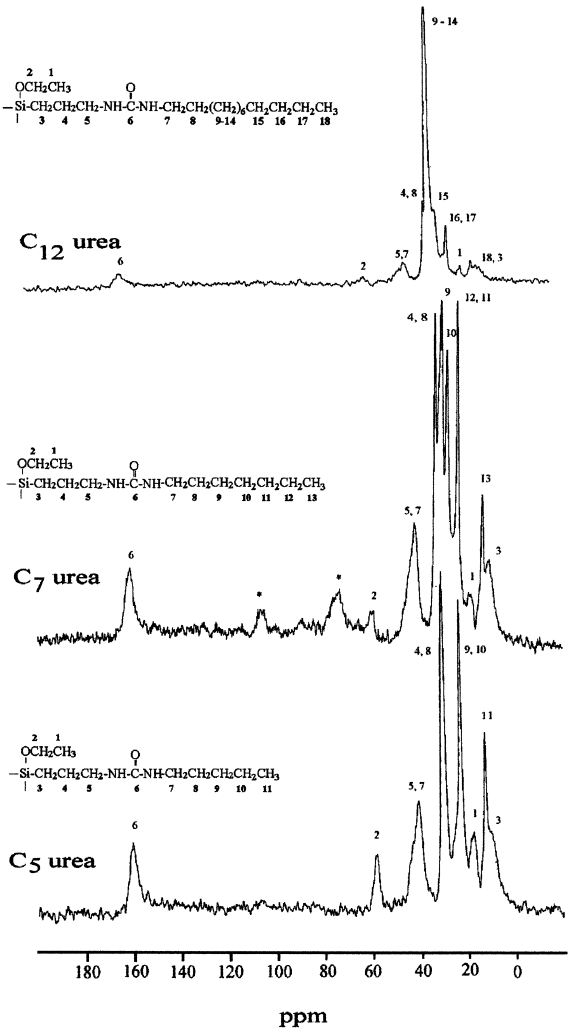

Fig. 2. ^{13}C-CP-MAS-NMR spectra for the urea-functionalized silica, before the endcapping reaction.

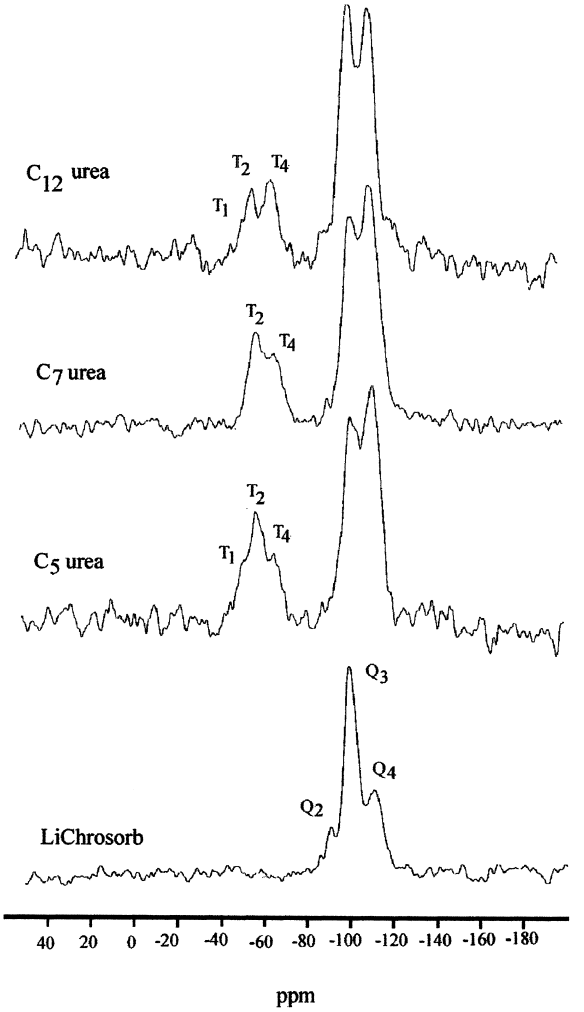

Fig. 3. ^{29}Si-CP-MAS-NMR spectra for bare LiChrosorb silica and also for urea-functionalized silicas, before the endcapping reaction.

from T_n species having hydroxyl groups as neighbors instead of the ethoxy groups, because these species have the same chemical shift. In the spectrum of C_7 urea silica, in Fig. 3, surprisingly, T_1 species were not found, probably resulting from a high degree of crosslinking of the trifunctional species on the silica surface in the modification step. After the endcapping reaction, ^{29}Si-NMR spectroscopy was again performed and a new peak was detected in the spectra of the endcapped materials, as can been seen in Fig. 5. These signals at about +12 ppm, in addition to the signals of T_n species, indicate the substitution of residual silanols by the $Si(CH_3)_3$ group, as outlined in Fig. 1 and represent the M species (Fig. 4).

For all modified and endcapped silicas, peak deconvolution was performed assuming that peaks exhibit gaussian shapes and the area for each T_n species was calculated for each modified silica. The results are summarized in Table 2 and, as can been seen, a relative increase in the percentages of the condensed species T_2 and T_4 was achieved for all silicas after the endcapping reactions. Another important fact is that the endcapping reaction was more

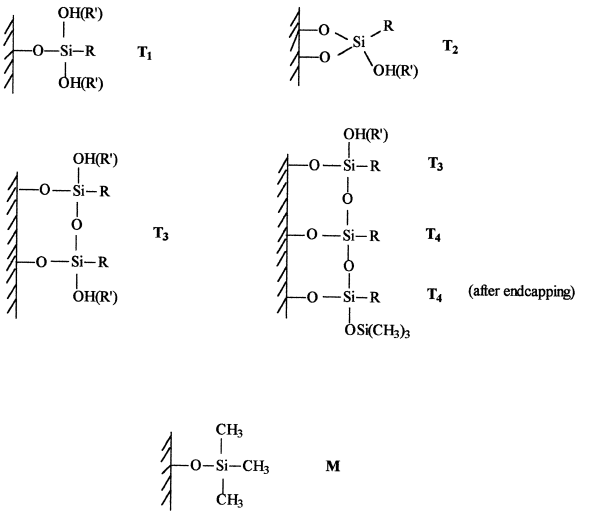

Fig. 4. Proposed structures for T_n and M species found by ^{29}Si-NMR spectroscopy on the modified silica surfaces.

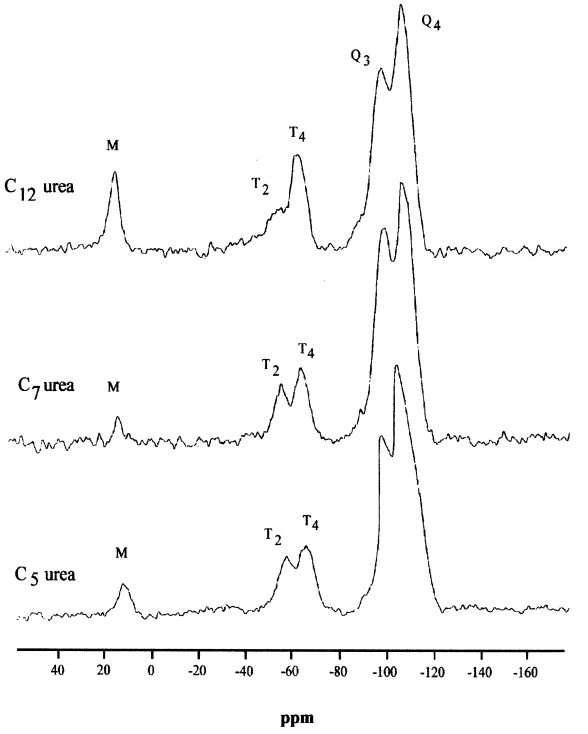

Fig. 5. ^{29}Si-CP-MAS-NMR spectra for the urea-functionalized silica stationary phases, after the endcapping reaction.

Table 2
Relative percentages of the species T_n found on the modified silica surfaces by ^{29}Si-NMR spectroscopy before (B) and after (A) the endcapping reaction

Material	T_n	Relative amount (%)	
		B	A
C_5 Urea silica	T_1	15	–
	T_2	64	53
	T_4	20	47
C_7 Urea silica	T_1	–	–
	T_2	67	41
	T_4	33	59
C_{12} Urea silica	T_1	25	–
	T_2	37	40
	T_4	38	60

effective for the C_{12} urea silica, when the peak area for M species at +12 ppm is compared to the other modified silicas. This behavior may be expected due to the fact that the low percentages of T_2 and T_4 for this derivatized silica, in Table 2, suggest a large amount of residual silanols on the surface before the endcapping reaction. In this same way, for C_7 urea silica, the absence of the T_1 species can suggest a high amount of the condensed species after the modification with the ureatrialkoxysilane and, consequently, only a small amount of residual silanols is available to react with trimethylchlorosilane.

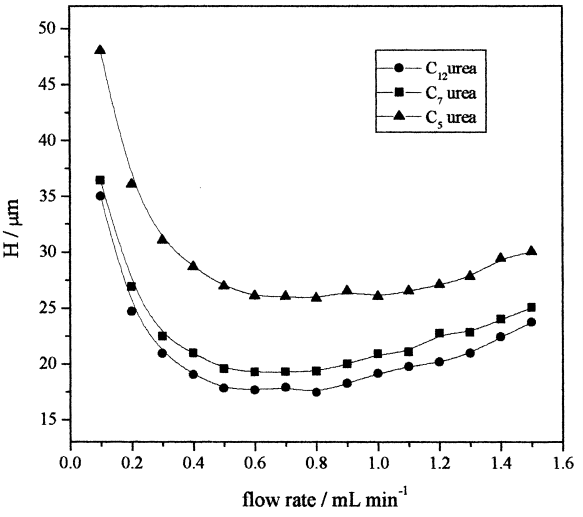

Fig. 6. Plots of *H* (plate height) at different flow-rates for the C_5, C_7 and C_{12} urea columns.

3.2. Chromatographic evaluation

The chromatographic evaluations were performed with packed columns with C_5, C_7 and C_{12} urea phases 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 Van Deemter curves, as shown in Fig. 6, using the plate height values, *H*, for naphthalene and acetonitrile–water at 50:50. 60:40 and 70:30 (v/v) as mobile phase for the C_5, C_7 and C_{12} urea silicas, respectively. Fig. 7 shows the complete chromatograms obtained for each column and it is observed that the columns have reasonably good *N*/m (plates/m) values and separate well all components of the test mixture. *N*/m values for naphthalene ranged from 57 000 for C_{12} urea silica to 38 000 for C_5 urea silica column with peak asymmetry values of about 1.4 for all columns tested, as can been seen in Table 3.

The difference between the *N*/m values for the C_5 urea silica column and the *N*/m values for the C_7 and C_{12} urea silica columns suggest that the C_5 column was not as successfully packed, when compared to the other two columns.

The different selectivities of each packed column

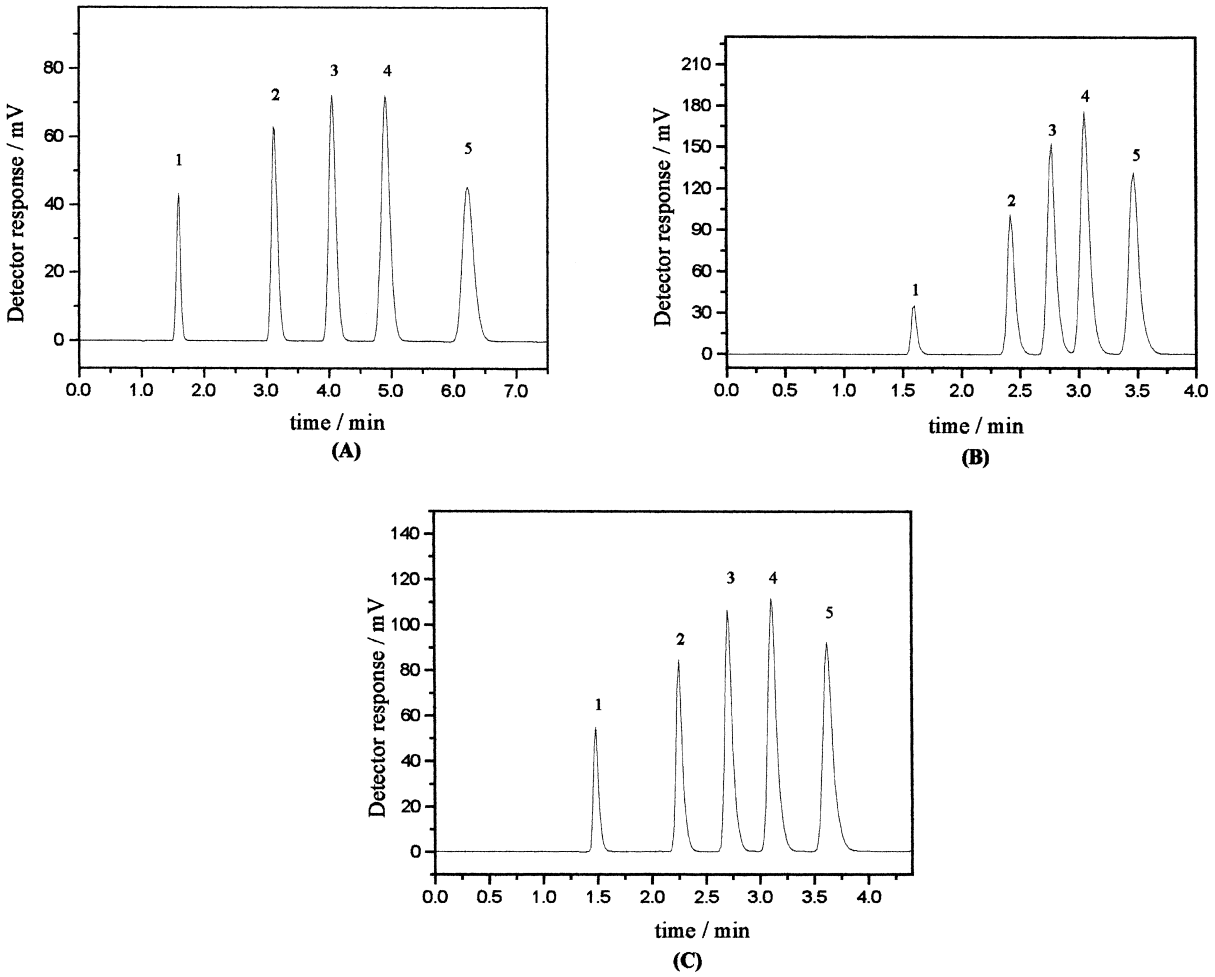

Fig. 7. Chromatograms of the separation of the standard test mixture composed of uracil (1), acetophenone (2), benzene (3), toluene (4) and naphthalene (5) on columns containing C_5 (A), C_7 (B) and C_{12} (C) urea phases. Flow-rate: 0.8 ml min^{-1}, detection at 254 nm and injection volume of 5 μl, using different acetonitrile–water mobile phase compositions of 50:50, 60:40 and 70:30 (v/v) for C_5, C_7 and C_{12} urea phases, respectively.

Table 3
Chromatographic parameters obtained with the columns packed with C_5, C_7 and C_{12} urea stationary phases[a]

Compound	C_5 Urea phase			C_7 Urea phase			C_{12} Urea phase		
	k	N/m	A_s	k	N/m	A_s	k	N/m	A_s
Acetophenone	0.95	35 400	1.5	0.52	47 700	1.4	0.52	49 800	1.5
Benzene	1.5	37 800	1.3	0.74	48 000	1.2	0.83	49 200	1.3
Toluene	2.1	39 000	1.3	0.92	50 100	1.3	1.1	54 000	1.4
Naphthalene	2.9	38 000	1.4	1.2	51 600	1.3	1.5	57 300	1.5

[a] Chromatographic conditions: 150×3.9 mm I.D. column packed with C_5 C_7 and C_{12} urea phases, mobile phase: acetonitrile–water at 50:50 60:40 and 70:30 (v/v) for C_5 C_7 and C_{12} urea phases, respectively; flow-rate: 0.8 ml min^{-1}, detection: UV at 254 nm and injection volume: 5 μl. A_s=Peak asymmetry factor.

are clearly observed since the concentration of the organic modifier in the mobile phase composition was increased to achieve a satisfactory separation of the components of the test mixture. For example, a mobile phase composition of acetonitrile–water (70:30) was used for C_{12} urea while, for C_5 and C_7 urea phases, mobile phase compositions of 50:50 and 60:40 (v/v) were used, respectively. Another important fact is that the use of different ureaalkoxysilanes with variable alkyl chains attached allows designing stationary phases with different hydrophobicity for special separations.

The stability of these new phases is still under investigation as well as the efficacy of the polar urea groups in interacting with residual silanols on the silica surface. However, if amide and carbamate groups show superior performance in the separation of basic compounds, it can be speculated that the urea groups will also provide these same effects.

The results from the reversed separation of the components of the standard test mixture show reasonable efficiency, taking into account the type of silica used for the modification process [17] (LiChrosorb Si100, irregular shape, particle size of 5 μm) and also encourage us to perform further evaluations, separating basic organic compounds.

4. Conclusion

New stationary phases, containing urea polar groups embedded into the alkyl chain, were prepared by a single-step modification process. Initial chromatographic tests show that these phases can be used under reserved conditions to separate some polar test compounds. This methodology of preparing the organosilane, with the desired substituents, and then bonding it to the silica surface is advantageous over the two-step modification process, which is commonly used in the preparation of amide-functionalized phases. The absence of the second reaction step allows obtaining a homogeneous composition attached on to the silica surface.

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