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Article

New Silica Gel-Based Monolithic Column for Nano-Liquid Chromatography, used in the HILIC Mode

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This paper describes the synthesis and chromatographic and morphologic characterization of two monolithic silica nano-columns (50 μm i.d.) prepared by sol-gel processes, using hydrophilic interaction (HILIC) mode separations to evaluate their performance. Two types of monoliths were prepared by varying the precursors (tetraethoxysilane or a tetraethoxysilane–methyltrimethoxysilane mixture) and by changing the type of catalyst (urea and acetic acid or ammonium hydroxide). The monoliths were characterized by scanning electron microscopy, thermogravimetric analysis, infrared spectroscopy and nitrogen adsorption–desorption isotherms. The columns were tested for the separation of several mixtures, with the organically modified silica (ormosil) column successfully separating two challenging mixtures using HILIC conditions.

Introduction

Currently, there is a need for methods and chromatographic techniques to rapidly and efficiently carry out separations. To reduce analysis time, several strategies can be followed. The first is the use of higher temperatures of separation with conventional columns, which causes a reduction in the viscosity of the mobile phases, allowing the use of higher flow rates without the consequent increase in pressure that occurs when applying high flow rates at lower temperatures (1). Another alternative is the use of chromatographic columns with shorter lengths, which also leads to a reduction in retention times, but with lower efficiencies and resolutions (2). However, the use of particles with smaller diameters improves the efficiency of these shorter columns, although the smaller particle sizes result in the need to employ higher pressures (3).

Capillary liquid chromatography (c-LC) or nano liquid chromatography (nano-LC) can be employed when the available sample size, mobile phase and packing materials are limited. c-LC and nano-LC have developed rapidly, especially due to the high demand for new miniaturized separation techniques allowing reduction of solvent consumption and analysis of very small amounts of solute. The techniques of c-LC and nano-LC are based on the miniaturization of high-performance liquid chromatography (HPLC) columns, using fused silica capillaries containing diverse stationary phases. However, packing the stationary phase into capillary columns is not easy, because the internal diameters are very small (4). The numerous practical drawbacks associated with packing and retaining the spherical particles, including the need to use frits for isolation of the stationary phases, especially when using glass or fused silica columns, narrow bore capillaries or separation channels, have progressively led to the development of alternative capillary columns containing *in situ* synthesized stationary phases or monolithic stationary phases.

A monolith is a continuous separation medium that holds the shape of the mold in which it is produced, usually a cylindrical format. A monolithic phase has a solid structure with small pores and channels with larger sizes (of the order of μm) that permit both high permeability and high efficiency when used as a chromatographic column. The channels offer less resistance to passage of a mobile phase than particulate materials used in conventional columns. In addition, diverse materials have been used for the preparation of different monolithic stationary phases due to the ease of incorporation of different groups during preparation (5).

Monolithic columns can offer high permeability, high efficiency and short diffusion paths (6). Depending on the nature of the precursors used for their synthesis, there are two major classes of monoliths: organic polymer-based monoliths (7) and inorganic monoliths such as silica-based types (8). The organic monoliths are used most often in electrophoretic and electrochromatographic separations, when compared with materials based on silica. This is due to the advantages of using organic polymeric materials, such as greater ease and speed of preparation, but there are problems of expansion in the presence of organic solvents. The silica monoliths are stable in the presence of organic mobile phases and the smaller diameter pores are useful for the separations of lower molar mass molecules (9).

Silica-based monolithic columns are synthesized via the sol-gel process that allows independent control of the size of the silica skeleton and through pores (10, 11). Sol-gel processes involve the hydrolysis of a metal alkoxide (precursor), followed by condensation and polycondensation reactions. Hydroxyorganic compounds can be merged with the polysilicate aggregates, producing organically modified silicas (ormosils); further extension of the polymerization process leads to macroscopic gels of ormosils. One of the advantages of these materials is the large flexibility over a wide range of reactant concentrations, which in turn can lead to products with considerably different physical and chromatographic properties. In general, the synthesis of silica-based monoliths is performed by mixing appropriate amounts of a precursor [generally tetraethoxysilane (TEOS), or tetramethoxysilane (TMOS)] as a silica source and polyethylene oxide (PEO) or another polymer in an acidic aqueous solution (12). The function of the PEO is to influence macropore formation, which then influences skeleton diameters. Silica-based monolithic columns prepared by the sol-gel process have polar character, necessitating the use of nonpolar mobile phases when they are used directly for liquid chromatography; alternatively, the silica skeleton is subjected

to a derivatization procedure to convert the monolith to a reversed phase.

Reversed-phase liquid chromatography (RPLC) is by far the most popular LC technique. It is characterized by the use of apolar stationary phases and polar mobile phases. One of the limitations of using RPLC is the low retention of polar molecules. In contrast, normal-phase liquid chromatography (NPLC) features polar stationary phases and apolar mobile phases. However, the solubility of polar molecules in non-aqueous apolar mobile phases is quite limited, restricting the applicability of NPLC. Hydrophilic interaction LC (HILIC) was introduced by Alpert in 1990 (13) as an alternative to NPLC. HILIC is characterized by the use of a hydrophilic stationary phase and an aqueous-polar organic mobile phase, typically containing a high concentration of acetonitrile and a small amount of water. When ionizable compounds are separated, it can be necessary to use buffers in the mobile phase, even with a high proportion of organic solvent (14). Similar to NPLC, retention increases with the increased polarity of the analyzed compounds and the stationary phase, and with decreased mobile phase polarity.

In this paper, we describe a synthesis pathway to prepare new silica-based monolithic columns by a sol-gel method for use in the HILIC mode. The monoliths were characterized and used for the separation of several mixtures. The advantages and drawbacks of these types of monolithic stationary phases are discussed.

Experimental

Reagents and chemicals

For the monolithic columns, fused silica capillaries (50 μm i.d.) were obtained from Agilent Technologies (Waldbronn, Germany). TEOS (95%) was purchased from Acros Organics (Morris Plains, USA). Methyltrimethoxysilane (MTMS) and urea were obtained from Sigma Aldrich (São Paulo, Brazil). Carbowax 20M (PEG) was obtained from Analabs (New Haven, USA). Trifluoroacetic acid (TFA) was obtained from Acros (Geel, Belgium). Concentrated aqueous ammonium hydroxide and hydrochloric acid were purchased from J.T. Baker (São Paulo, Brazil).

Acetonitrile (HPLC grade) was obtained from Tedia (Rio de Janeiro, Brazil) and, when used as an LC mobile phase, was filtered through a 0.22-μm filtering membrane before use. Deionized water was from a Milli-Q system from Millipore (Bedford, USA). Naphthalene, toluene, caffeine, benzonitrile and uracil were obtained from Sigma Aldrich (São Paulo, Brazil). Simetryn, simazine, prometon, prometryn and tebutiuron were purchased from Supelco (Bellefonte, USA). Diuron, imidaclorepride and carbofuran were obtained from Dupont (Rio de Janeiro, Brazil). Kaempferol, caffeic acid and gallic acid were obtained from Sigma Aldrich (St. Louis, USA).

The stock solutions of most of these test compounds were prepared at concentrations of 1,000 mg/L in acetonitrile, and solutions of lower concentrations were prepared by serial dilution of the stock solutions. All solutions were stored at 4°C in a refrigerator. The stock solutions of antioxidants kaempferol, gallic acid and caffeic acid were prepared at concentrations of 500 mg/L and diluted to 30 mg/L.

Apparatus

Separations were performed on an Agilent 1200 Series Capillary LC System (Palo Alto, USA) equipped with a 1200 series capillary pump with 20-μL flow sensor operating at 5 μL/min, a G1379A micro vacuum degasser, a 1200 series micro well-plate autosampler including a Rheodyne microinjection valve (injection volume 0.05 μL) and a 1200 Series SL diode-array detector. Data were collected at 80 Hz using ChemStation for LC 3D system software.

Synthesis of pure silica monolith columns (Type A)

The procedure to obtain pure silica monolithic columns (Type A) by the sol-gel route was as follows: a fused silica capillary (0.15 m × 50 μm i.d.) was activated by filling it with a 1.0 mol/L NaOH solution followed by heating at 40°C for 2 h. The excess base was removed by filling the capillary with 0.1 mol/L HCl solution for 30 min, and the activated capillary was subsequently flushed with distilled water and dried at 60°C. Urea (200 mg) and PEG (200 mg) were dissolved in 10 mL of 0.01 mol/L acetic acid under stirring for 30 min. Approximately 500 mg of TEOS were added to the solution. After 30 min under stirring, the activated capillary was filled with the mixture. The capillary was heated at 100°C for 1 h and then at 120°C for 24 h to create the mesopores. The monolithic capillary was washed with deionized water to dissolve urea and remove residues of the Carbowax 20M.

Synthesis of the ormosil monolithic columns (Type B)

Monolithic organically modified silica (ormosil) columns (Type B) were prepared as follows: a fused silica capillary (0.15 m × 50 μm i.d.) was activated as previously described by filling it with a 1 mol/L NaOH solution followed by heating at 40°C for 2 h. The capillary was then filled with 0.1 mol/L HCl solution for 30 min and heated at 60°C for 3 h, flushed with water and dried. MTMS (1.7 mg), TEOS (5.0 mg) and PEG (1.3 mg) were dissolved in deionized water and mixed in a vortex. Immediately afterward, 200 μL of ammonium hydroxide (0.1 mol/L) were added as catalyst, and the mixture was inserted with a syringe inside the pretreated capillary. The capillary was heated at 40°C for 2 h and the newly formed monolith was dried overnight at 120°C. The relative amounts of reagents were selected after a brief preliminary study.

Physical-chemical characterization

These characterizations were carried out on monoliths prepared in a vial using the same mixtures as previously described, except for the scanning electron microscopy (SEM), which was carried out on monoliths formed inside the capillaries (50 μm i.d., L = 0.15 m).

The morphological evaluation of the materials was made by SEM using a Jeol GSM T-300 (Tokyo, Japan). Thermogravimetric analyses of the monoliths were performed under an inert atmosphere (N_2) in a 2050 thermogravimetric analyzer from TA Instruments (New Castle, USA), using the temperature range of 30–1,000°C (heating rate of 10°C/min). The infrared absorption spectra of monoliths A and B in KBr pellets were obtained between 400 and 4,000 cm^{-1}, with a Bomem MB-102 FTIR spectrometer (St-Laurent, Canada). Monoliths A and B were submitted to elemental analysis, where carbon was determined on a Perkin-Elmer model 2400

Analyzer (Norwalk, USA). Porosimetry data of the monolithic stationary phases was measured using nitrogen adsorption-desorption isotherms carried out on an ASAP 2010 volumetric adsorption analyzer from Micromeritics (Norcross, USA). The specific surface area, S_{BET}, was calculated using the standard Brunauer-Emmet-Teller (BET) method. The total pore volume (v_t) was obtained by converting the amount adsorbed, v, at a relative pressure of 0.99 to the volume of liquid adsorbate. The diameter of pores were calculated using the Barret-Joyner-Halenda (BJH) method on the basis of desorption data.

Chromatographic evaluations

The evaluation of the columns containing the monolithic stationary phases was based on the separation of a test mixture containing naphthalene, toluene, caffeine, benzonitrile and uracil, dissolved in acetonitrile–water, 70:30 (v/v). Injection of 0.05 μL of this mixture produced satisfactory chromatographic peaks with detection at 254 nm. The separation was carried out at room temperature with a flow rate of 0.5 μL/min.

Another evaluation of the column packed with the monolithic ormosil stationary phase (type B) was based on the separation of a test mixture containing simetryn, simazine, prometon, prometryn, tebutiuron, diuron, imidaclorepride and carbofuran, dissolved in acetonitrile–water, 70:30 (v/v). Injection of 0.05 μL of this mixture produced satisfactory chromatographic peaks with detection at 220 nm. The separation was carried out at room temperature with a flow rate of 0.5 μL/min.

A further evaluation of the ormosil monolithic stationary phase used a mixture of antioxidants (kaempferol, caffeic acid and gallic acid), also dissolved in acetonitrile–water, 85:25 (v/v). Injection of 0.05 μL of this mixture produced satisfactory chromatographic peaks with detection at 270 nm, using a flow rate of 0.5 μL/min.

For all tests, the column dead time, t_M, was determined from an unretained (or poorly retained) compound. The chromatographic parameters were retention factor (k), efficiency from peak width at half height (N) and asymmetry factor at 10% of the peak height ($As_{10\%}$) for each peak, as well as resolution (Rs) and separation factor (α) for adjacent peaks.

Results

The formation of the silica monolithic column Types A and B involves two major reactions: polycondensation of hydrolyzed precursors of TEOS, in Column A, and of TEOS and MTMS, in Column B, followed by copolymerization of the precondensed precursors. For investigation of the amount of TEOS in the reaction mixture on the resulting monolith of Column A, the ratios of TEOS, PEG and urea were selected based on the literature (15). For Column B, a combination of TEOS and MTMS as precursors and the relative amounts of MTMS, TEOS and catalyst were selected after a brief preliminary experimental study; the selected quantities resulted in gelation and production of an ormosil monolith in less than 24 h, using mild conditions. To obtain the optimum experimental conditions for preparation of monolithic columns, experiments were performed outside the capillary (glass bottle). Two different types of catalysts were evaluated: NH_4OH (basic) and TFA with 5% water (acid). The formation of a gel occurs with both catalysts, use of NH_4OH was chosen due to lower cost and greater availability. Moreover, using the acid catalyst, gelation occurred more rapidly, interfering with the insertion of the sol phase inside the capillaries.

The use of lower amounts of TEOS (in Column A) or of the TEOS–MTMS mixture (in Column B) in the reaction mixture results in no gel formation inside the capillary. Thus, careful adjustment of monomer concentrations in the sol-gel mixture is necessary for obtaining the desired monolithic columns.

The presence of PEG changes the properties of the reaction media, but is not incorporated into the polysilicate reticulates. The PEG controls the size and volume of macropores in gels because the glycol forms strong hydrogen bonds with the silanols of the growing silicate polymers (16). PEG has potential use as a general additive to improve porosity in any sol-gel formulation for the preparation of monolithic columns.

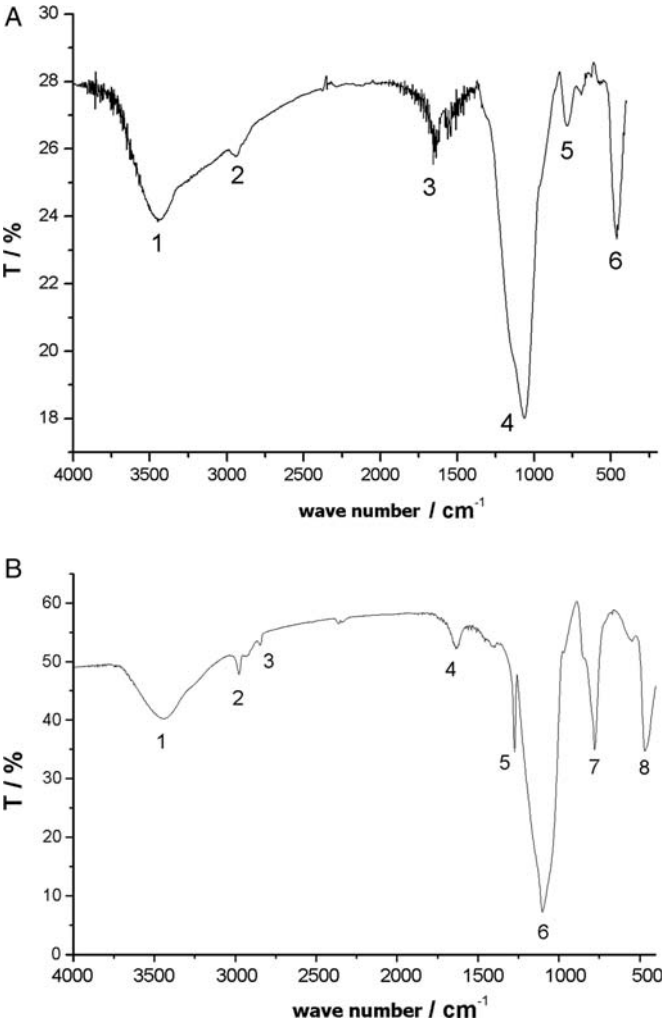

Figure 1. Infrared (KBr pellet) spectra for monolithic silica A and monolithic ormosil B. Band assignment: (1) O-H, (2) C-H, (3) O-H, (4) Si-O-Si, (5) Si-O and (6) Si-O (A); band assignment: (1) O-H; (2) methyl C-H; (3) C-H; (4) -OH and (5) methyl C-H; (6) Si-O and (7) Si-O (B).

Morphological characterization

The infrared (IR) absorption spectra for Monolith A are shown in Figure 1A. The OH bands located at 3,444 cm^{-1} (1 in the figure) and 1,632 cm^{-1} (3) are from unreacted silanol terminations in the polysilicate reticulates. The observed features around 1,062 and 1,058 cm^{-1} (4) indicate Si-O-Si and Si-O-H stretching vibrations, respectively. The bands at 784 cm^{-1} (5) and 456 cm^{-1} (6) result from Si-O vibrations (17).

The IR absorption spectra for monolithic ormosil B are shown in Figure 1B. The IR spectra displays bands at 3,445 cm^{-1} (1) and 1,628 cm^{-1} (4) (axial stretching of O–H bonds on hydroxyl groups from unreacted silanol terminations in the polysilicate reticulates); 2,977 cm^{-1} (2), 2,845 cm^{-1} (3) (methyl C–H stretching) and 1,456 cm^{-1} (5) (C–H bending). The band at 1,273 cm^{-1} (6) is from Si-O stretching. The bands at 779 cm^{-1} (7) and 469 cm^{-1} (8) result from Si-O vibrations (17).

The resulting materials were crushed and the morphologies of the monolithic materials obtained were assessed by SEM studies. Images were also obtained of the insides of the capillaries. Figure 2 shows the SEM of the monolithic columns A (A, B, C) and B (D, E, F) under different magnifications. The material has a silica-gel skeleton that contains macropores with diameters of approximately 5 μm. Methacrylate monoliths possess porous structures and macropore sizes up to 3 μm (18). Monoliths can be compared to a single large particle because they do not contain interparticular voids. However, the porosity influences retention that arises from the abundance of surface groups located in the mesoporous network, as well as column permeability.

The sol-gel process allows independent control of the pore size and it is possible to prepare monolithic silica columns with smaller or larger macropores (19). This makes the monolithic silica columns unique compared to their packed counterparts. The properties of monolithic columns depend on the concentrations of the precursors and of the PEO that controls pore formation. There are several reports in the literature regarding the effects of PEO on the morphology of silica-based materials produced by sol-gel chemistry (20).

In general, the porosity of monolithic silica columns is greater than that of a column packed with particles (21).

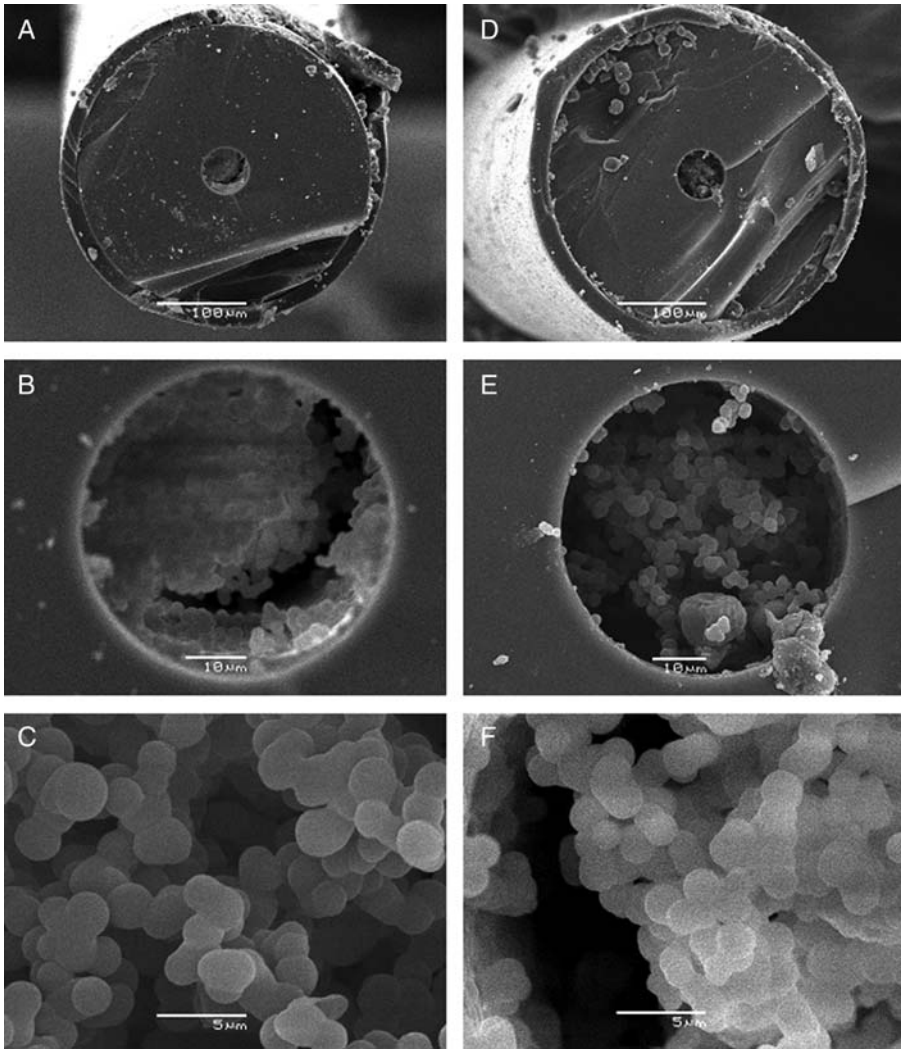

Figure 2. SEM pictures under different magnifications of the structures of monolithic silica column A (A, B, C) and monolithic ormosil column B (D, E, F), prepared by sol-gel processes. Magnifications: 250 times (A) and (D), 2,500 times (B) and (E), 5,000 times (C) and (F).

Conventional monolithic columns have porosities of approximately 80%, while monolithic capillary columns have slightly higher porosities of 90%. For comparative purposes, an analytical column packed with 5 μm C18 silica particles has a porosity of approximately 40%. Thus, the surface areas of monolithic columns are smaller than conventional columns (22).

The BET surface areas were measured by nitrogen isotherms at 77 K. The monolithic materials A and B had BET surface areas of 118 and 130 m^2/g, respectively. To obtain a large surface area, a large number of pores should be incorporated into the material. Values found in the literature range from 24 to 509 m^2/g (23). The pore volumes were also similar, 0.3225 and 0.3333 cm^3/g. The pore diameters obtained were 102 and 109 Å for columns A and B, respectively. The values of pore size found in the literature range from 52 to 301 Å (14). Although preparation of Monolith B does not involve urea, which also acts as a porogen, there are no significant differences in these values.

The thermal properties of the sol-gel monolithic materials A and B can be assessed from Figure 3, which shows the TGA curves and the second derivative of these plots. The thermal behavior of silica is primarily characterized by processes of dehydration and dehydroxylation (23). In dehydration, the water molecules weakly adsorbed to the silica surface are eliminated until approximately 150°C, whereas strongly adsorbed water molecules are removed and dehydroxylation occurs between 150 and 600°C. In Figure 3A, one thermal event occurs at approximately 97°C. The total mass loss up to 1,000°C was only 9.5% of the initial mass, showing that Monolith A has very high thermal stability. In Figure 3B, three thermal events are visible. The first starts at 38°C (mass loss of 9.2% of the original material), attributed to the release of water and other low molar mass reaction products sorbed or entrapped inside the pore structure of the monolith. At approximately 192°C, a second thermal event occurs, which can be attributed to loss of the organic groups or unreacted TEOS or MTMS, which have boiling points of 171 and 101°C, respectively. Finally, the last thermal event occurs at 489°C, probably related to dehydroxylation. This analysis indicates that the monolith can be used successfully in the separation of compounds that require the use of temperature programming, which, in practice, uses temperatures up to 100°C, depending on the mobile phase, because the use of higher temperatures may result in bubble formation, hindering the separation of compounds. The total mass loss up to 1,000°C for Monolith B was 27% of the initial mass, also showing a good thermal stability.

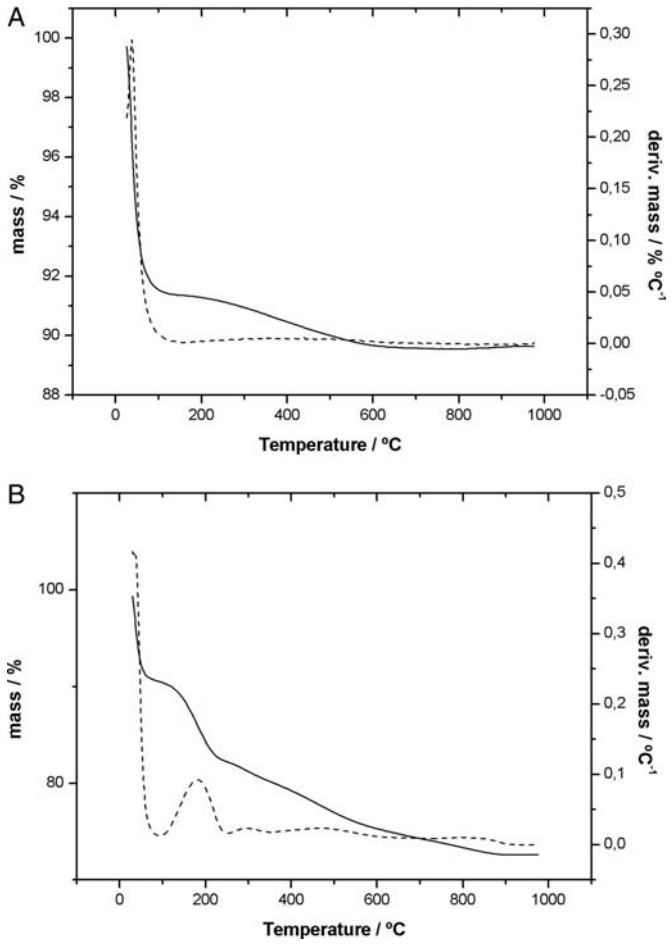

Figure 3. TGA curves (continuous line) and the second derivative of TGA curves (dotted line) for monolithic silica A and monolithic ormosil B.

Chromatographic characterization

In the HILIC mode, adding quantities of acetonitrile above 60% increases the retention and efficiency of some analyses (24). In this work, the retention changes on monolithic columns with the variation of the percent acetonitrile can be confirmed by Figure 4, which shows the effect of acetonitrile concentration on the separation of a mixture containing: naphthalene (1 in the figure), toluene (2), caffeine (3), benzonitrile (4) and uracil (5) (all 1.0 mg/L) using Columns A and B. Larger amounts of acetonitrile improved the separation considerably with both columns. Moreover, a better separation was obtained using the monolithic ormosil Column B.

Table I shows some chromatographic parameters for Columns A and B. The dead times (t_M) of the columns were calculated using the retention time for the least retained compound (naphthalene). Monolithic phase A had efficiencies of 5,300 to 17,000 plates/m and monolithic ormosil B had efficiencies of 16,100 to 25,300 plates/m. The peak asymmetries varied between 0.5 to 1.0 and 0.8 to 1.0 for monolithic phases A and B, respectively. The literature (25) indicates that As_{10} should have values of 0.9 to 1.2. Although less desirable, values up to 1.6 are accepted. Rs values equal to 1.0 are sufficient for quantitative purposes, while Rs values above 1.3 indicate baseline separation of the compounds. The Rs values ranged from 2.2 to 4.7 for monolithic Column A and 2.4 to 14.3 for the column containing monolithic ormosil B. The values of efficiency obtained for both columns were satisfactory; Grafnetter *et al.* (26) reported values of efficiency for a 100-μm monolithic column of 9,090 plates/m. Due to the better performance of the monolithic ormosil column (Type B) further experiments were carried out with it.

The repeatability of the fabrication method of monolithic Column B was evaluated by preparing two distinct batches of

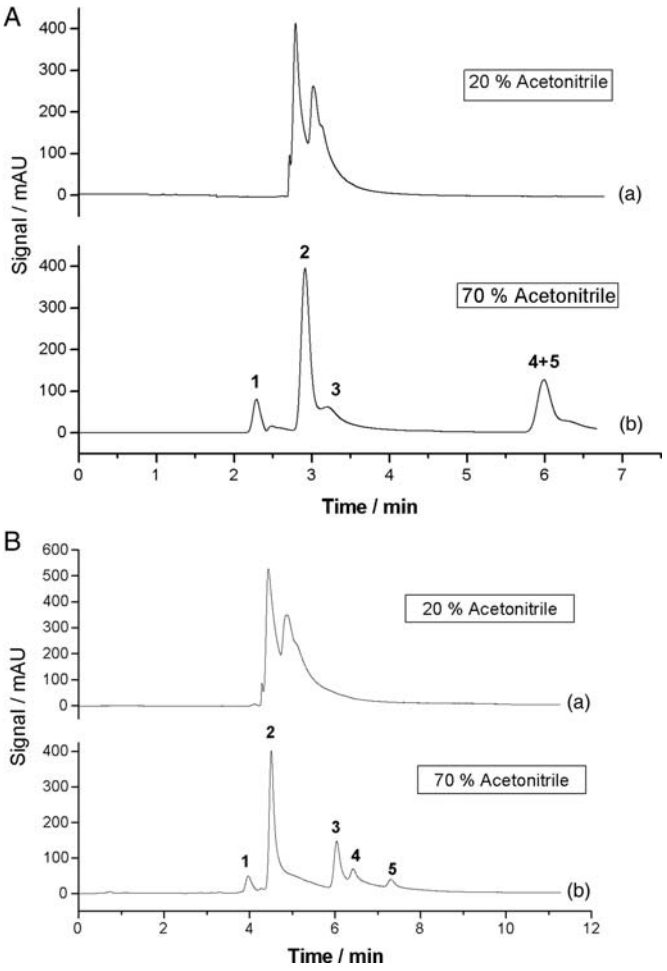

Figure 4. Separation of naphthalene (1), toluene (2), caffeine (3), benzonitrile (4) and uracil (5) using the monolithic silica column A (A); the same compounds using the monolithic ormosil column B (B). Mobile phase: acetonitrile–water, 20:80 (v/v) (A) and 70:30 (v/v) (B). Injection volume: 0.05 μL, flow rate: 5 μL/min, detection: UV at 254 nm.

Table I
Chromatographic Parameters for the Monolithic Stationary Phases using ACN–H_2O, 70:30 (v/v)

Column	Compounds	Efficiency (N/m)	$As_{10\%}$	Rs*
A	Naphtalene	17,000	1.0	—
	Toluene	14,700	0.9	4.7
	Caffeine	5,300	0.5	2.2
	Benzonitrile	NS†	NS	NS
	Uracil	NS	NS	NS
B	Naphtalene	16,100	0.9	—
	Toluene	21,800	0.8	2.4
	Caffeine	20,700	1.0	14.3
	Benzonitrile	18,200	1.0	3.5
	Uracil	25,300	0.9	9.2

*Resolution calculated between adjacent peaks.
†NS: not separated.

columns (Figure 5). The filling of the capillary with the sol phase was evaluated with the use of a microscope. If the chromatographic bed would not be homogeneous (presence of bubbles), the column was discarded. Good repeatability was observed for the chromatographic behavior between different batches. This result is satisfactory, taking into consideration that this degree of repeatability is at least similar to those obtained for commercially available stationary phases.

To show the potential of the lab-made monolithic ormosil columns in HILIC separations, more challenging mixtures were tested by comparing the separation of a mixture of herbicides in Column B using different compositions of mobile phase. Figure 6 shows the resolution (Rs) between two pairs of compounds, prometryn and ametrine, and imidacloprid and terbutiuron. Resolution values greater than 1 are acceptable, and the compounds are completely separated when Rs is greater than 1.3. Five mixtures of organic solvents and water were tested: 90:10, 70:30, 60:40, 40:60 and 30:70 v/v. The experiments were conducted at room temperature and at a flow rate of 0.5 μL/min. As expected, changes in the constitution of the

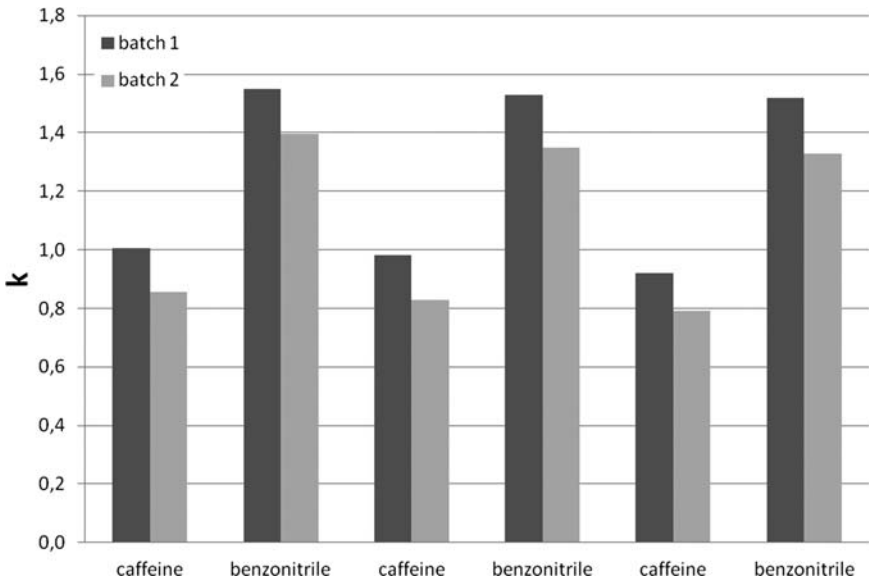

Figure 5. Evaluation of repeatability in the preparation of monolithic ormosil column B.

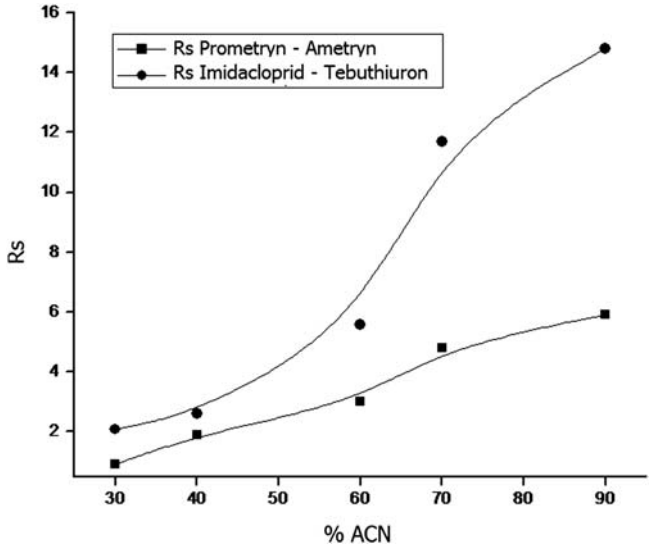

Figure 6. Variation of the Rs values versus the organic solvent percentage (%ACN) using monolithic ormosil column B.

mobile phase influence the separation. Ibrahim *et al.* (24) found the same behavior in separation of naphthalene, phthalic acid and cytosine using a monolithic silica column.

Figure 7 shows the effect of the amount of acetonitrile used in the mobile phase composition on the separation of several herbicides [(1) prometryn, (2) ametryne, (3) simetrine, (4) atrazine, (5) carbofuran, (6) simazine, (7) tebuthiuron and (8) imidacloprid] on Column B. As expected in the HILIC mode, the elution order of the analyte is less polar to more polar. Prometryn has a methyl group so it is less polar than ametrine and elutes first. Atrazine has one $-CH_2$ group more than simazine, so it elutes before simazine. The retention mechanism in HILIC (with high concentrations of acetonitrile) is based on the partition of analyte between the water layer associated with the polar surface of the stationary phase and mobile phase with lower polarity (27).

Table II illustrates the chromatographic parameters obtained for Column B with the mixture of herbicides. The performance of monolithic column B showed efficiencies that ranged from 7,300 to 79,400 plates/m. The peak asymmetries vary from 0.85 to 2.85. The resolutions are acceptable, indicating good separation between the compounds.

Figure 8 shows a chromatographic separation of an antioxidant mixture containing kaempferol (1), caffeic acid (2) and gallic acid (3) using nano-LC in the HILIC mode. Antioxidants are compounds that prolong the shelf-life of foods by protecting against deterioration caused by oxidation (28). These compounds are not separated well using RPLC with isocratic elution or without use of acidic mobile phases (29). Using the HILIC mode and Column B, a group of three antioxidants was easily separated.

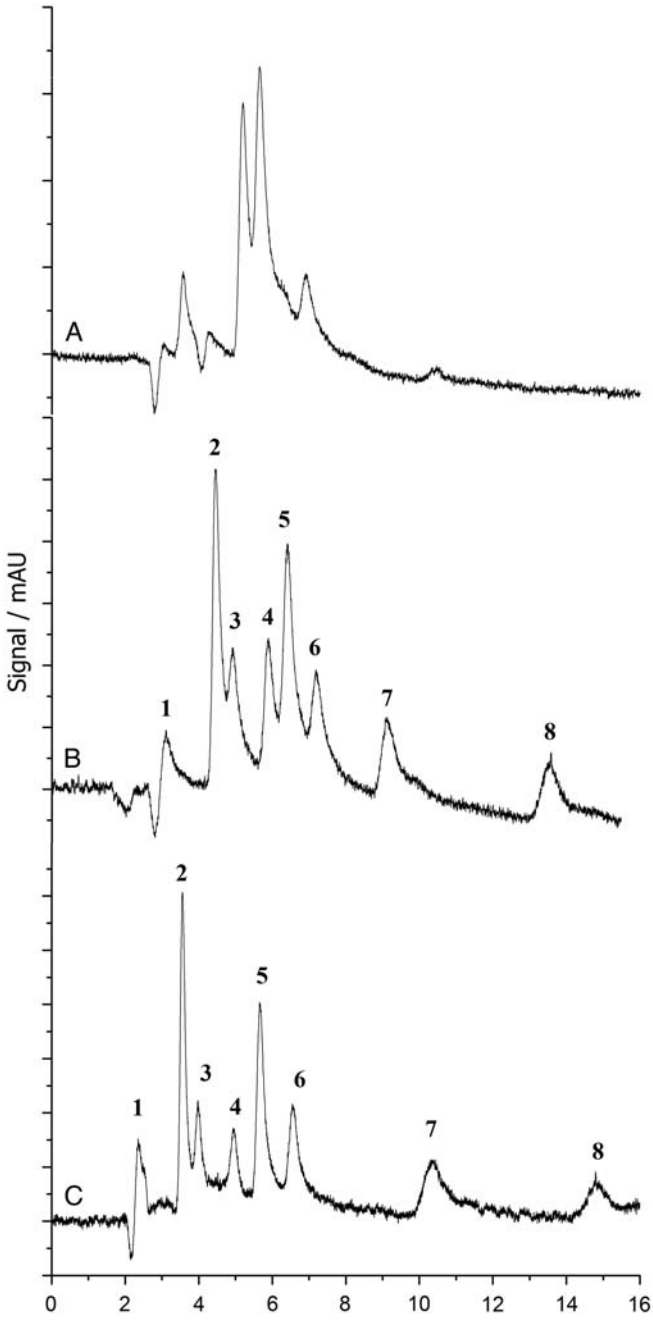

Figure 7. Separation of a mixture containing prometryn (1), ametryn (2), simetryn (3), atrazine (4), carbofuran (5), simazine (6), tebutiuron (7) and imidacloprid (8) using monolithic ormosil column B. Injection volume: 0.05 μL, detection: UV at 220 nm; mobile phase: acetonitrile–water, 30:70 (v/v) (A); acetonitrile–water, 70:30 (v/v) (B); acetonitrile–water, 90:10 (v/v) (C); flow rate: 0.5 μL/min.

Conclusions

The results presented herein demonstrate a simple approach to the preparation of two monolithic stationary phases, using a synthetic approach based on the sol-gel process. The potential for operation of monolithic silica capillary columns was explored using the HILIC mode in nano-LC. The columns were successfully tested for the separation of different test mixtures of polar compounds, using mobile phases with high concentrations of acetonitrile. The repeatability of the column preparation was evaluated and this study confirmed the good performance of

Table II
Chromatographic Parameters for Herbicides with Monolithic Ormosil Column B using ACN–H_2O, 90:10 (v/v)

Analyte	Efficiency (N/m)	$As_{10\%}$	Rs*
Prometryn	7,300	2.9	—
Ametryn	21,500	1.2	5.9
Simetryn	13,500	0.9	1.9
Atrazine	25,400	1.2	3.5
Carbofuran	21,300	1.0	1.9
Simazine	28,800	1.1	2.6
Tebuthiuron	43,400	1.1	6.6
Imidacloprid	79,400	1.1	14.2

*Rs: calculated between adjacent peaks.

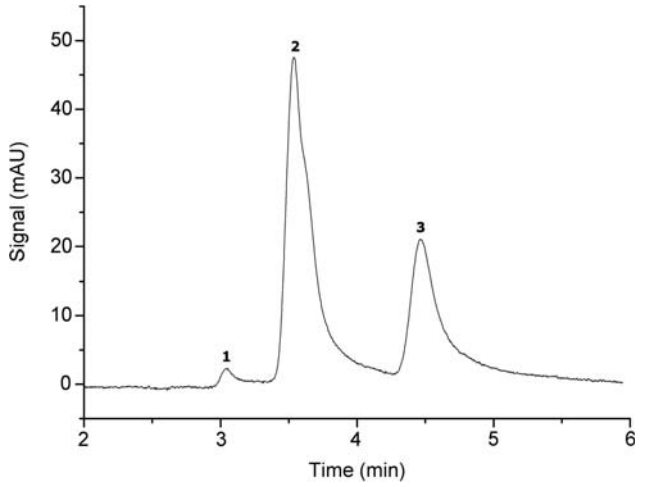

Figure 8. Separation of a mixture of antioxidants containing kaempferol (1), caffeic acid (2) and gallic acid (3) using monolithic ormosil column B. Injection volume: 0.05 μL, detection: UV at 270 nm; mobile phase ACN–H_2O, (85:15 v/v); flow rate: 0.5 μL/min.

Column B. The monolithic ormosil column was successfully used for the separation of mixtures of herbicides and of antioxidants.

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