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ORIGINAL ARTICLE

Preparation and characterization of alkyl methacrylate capillary monolithic columns

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Abstract Capillary columns were designed and prepared by *in situ* polymerization using either butyl or hexyl methacrylate monomers in a surface treated fused-silica tubing. The pressure drop across the columns was measured as a function of linear velocity using water and acetonitrile, the results showed a good permeability and a high mechanical stability for both columns. The monolithic stationary phases were also characterized by optical microscopy, scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR). The column efficiency was evaluated by calculating the number of theoretical plates, while the influence of several parameters was investigated, such as, flow rate, mobile phase composition and column temperature. To show the applications, mixtures of aromatic hydrocarbons were separated by using both columns.

1. Introduction

In the few last years, many research groups have paid great attention to the study and development of new separation methods (miniaturized) offering the possibility to achieve good resolutions and high efficiency in a short analysis time making use of very low volumes of both samples and reagents. Among these separation methods, capillary liquid chromatography (also called micro-LC) seems to be very promising even for practical applications in different fields, especially in the analysis of chiral compounds of pharmaceutical, clinical, environmental and/or agro-chemical interest. However, the successful development of these techniques is closely related to the technical challenges associated with the column manufacturing. The obtained columns should offer several advantages over classical packed columns, e.g., rapid separations achieved in a short analysis time with high efficiency, use of minute volumes of mobile phases as well as small amounts of packing materials (Nischang et al., 2009).

Monolithic stationary phases are relatively new structures and have attracted increasing interest in all areas of chromatographic methods as an alternative to particulate columns (Dear et al., 2001; Desmet et al., 2006; Unger et al., 2008; Smith and Jiang, 2008; Wu et al., 2008; Zou et al., 2002). They consist of a single rigid piece of porous material that possesses a unique bimodal pore structure distribution with μm-sized through

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pores (macropores) and nm-sized mesopores. Macropores dramatically increase the column porosity, thereby considerably reducing the analysis time, while mesopores form the fine porous structure and provide a very large active surface area for high efficiency separations (Minakuchi et al., 1997; Gritti and Guiochon, 2004; Lubbad and Buchmeiser, 2010). Due to this unique and unusual structure as well as their ease of preparation, monolithic columns offer improving chromatographic performance and favorable properties for high efficiency, fast separations, high reproducibility, low back pressure drop across the column, fast mass transfer kinetics between the mobile and stationary phases and a high binding capacity.

The first attempt to use a monolith material for separation was reported by Kubin (Kubin, 1967), several different monolithic supports were described in the literature since the late 1980s or early 1990s. Two types of monolithic columns have been developed for chromatography: macroporous organic polymer based monolithic columns produced by a simple molding process (Svec and Frechet, 1992) and silica based monolithic columns made by using the sol–gel approach (Minakuchi et al., 1996, 1997, 1998a, 1998b. These stationary phases are basically synthesized from silica or organic monomers, such as acrylamide, styrene and methacrylate monoliths (Hjertén et al., 1989; Nakanishi and Soga, 1991; Wang et al., 1993; Tennikova et al., 1990). Since the 1990s, monolithic supports became widely used in various applications such as environmental, food, pollutants, ions, and chiral analysis (McCalley, 2002, 2003; Spoof and Meriluoto, 2002; Xu et al., 2003, 2004; Lubda et al., 2003; Lubda and Lindner, 2004; Jandera et al., 2010).

Furthermore, the preparation of this type of columns is comparatively inexpensive, because they require smaller amount of stationary phase materials. The present work was aimed to the preparation and characterization of capillary columns suitable for micro-scale high performance liquid chromatography. Since the columns are going to be used at HPLC mode, the monolith must be firmly bounded to the capillary wall to prevent displacement of the monolithic stationary phase by the mobile phase at high pressure.

2. Experimental

2.1. Chemicals and column

Polyimide coated fused silica tubing (0.53 mm i.d., 0.69 mm o.d.) was purchased from Restek (USA). The chemicals used for monolithic column preparation in this work were purchased from Aldrich (Steinheim, Germany) as follows: 3-(trimethoxysilyl)propyl methacrylate (TMSM) 98%, ethylene dimethacrylate 98% used as crosslinker, azo-bis-isobutyronitrile AIBN as initiator and hexyl methacrylate 98% as monomer. Toluene, ethanol 99.7–100%, hydrochloric acid, sodium hydroxide, orthophosphoric acid 85% and disodium phosphate were acquired from BDH (England). All chemicals were used without further purification.

2.2. Preparation of butyl (or hexyl) methacrylate capillary monolithic column

In order to clean and activate the inner surface of capillary, the fused-silica capillary (150 × 0.53 mm i.d.) was rinsed first with 1.0 M NaOH solution for 5 min and left for 10 min with the same solution, then rinsed with water and dried in air for 2 min two times for each. The column was then flushed with 1.0 M HCl for 2 min and dried with air for 5 min, after that the capillary was rinsed with toluene for 10 min then flushed with 10% 3-(trimethoxysilyl)propyl methacrylate solution in toluene for 8 min and let with the same solution for 2 h, then rinsed with toluene for 5 min and dried with air for 5 min. This reaction scheme is shown in Fig. 1.

The monomer mixture was prepared as follows (weight%): 20% butyl (or hexyl) methacrylate, 15% ethylene dimethacrylate as crosslinker and 1% AIBN as initiator. The porogen mixture was 64% of the total solution and prepared as follows (weight%): 60% acetonitrile, 20% ethanol, and 20% of 5 mM phosphate buffer at pH 7.1. The monomer mixture and the porogen solvents were mixed into a homogenous solution then sonicated and purged with helium gas for 3 min. The capillary column was then filled with the reactant solution and both ends were plugged with a piece of rubber. The polymerization was performed in an oven at 60 °C for 24 h. After the polymerization, the seals were removed; the resulted column was connected to an HPLC pump and washed with ACN to remove any unreacted materials and the remaining porogenic solvents.

2.3. Characterization of the monolithic columns

After all chromatographic experiments had been completed; each column was washed with the mobile phase then dried with air and cut into pieces with a razor blade. The columns and the monolith materials were then characterized by optical microscopy, scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectroscopy.

The optical microscope images were obtained by a Micromaster Fisher Scientific optical microscope (G2009-A 702-042, China) with typically 100-fold magnification. The pore properties and microscopic morphology of the monolith were examined by a Jeol (JSM-6380LA) scanning electron microscope (Japan) at 5 kV after the column and the monolith material were sputtered with gold.

The Fourier transform infrared (FT-IR) spectrum was determined by using a Thermo Nicolet 6700 FT-IR spectrophotometer (USA). The resulted monolith was removed from the vial then crushed. The powder was immersed in 1 mL (50:50, v/v) acetonitrile/water and shaken for 10 min to remove any soluble compounds, these processes were repeated two times. After vacuum drying, the monolith was thoroughly mixed with KBr in an approximate ratio of 1:20 and pressed into a pellet and the FT-IR spectrum was then recorded at a resolution of 4 cm^{-1} over the full mid-IR range (400–4000 cm^{-1}).

2.4. HPLC analysis

All analyses were performed with a Waters Acquity UPLC instrument including: a binary solvent manager, a thermally conditioned sample manager, a column heater and a dual-wavelength ultraviolet/visible TUV detector. The mobile phase consisted of an acetonitrile/water mixture in isocratic mode.

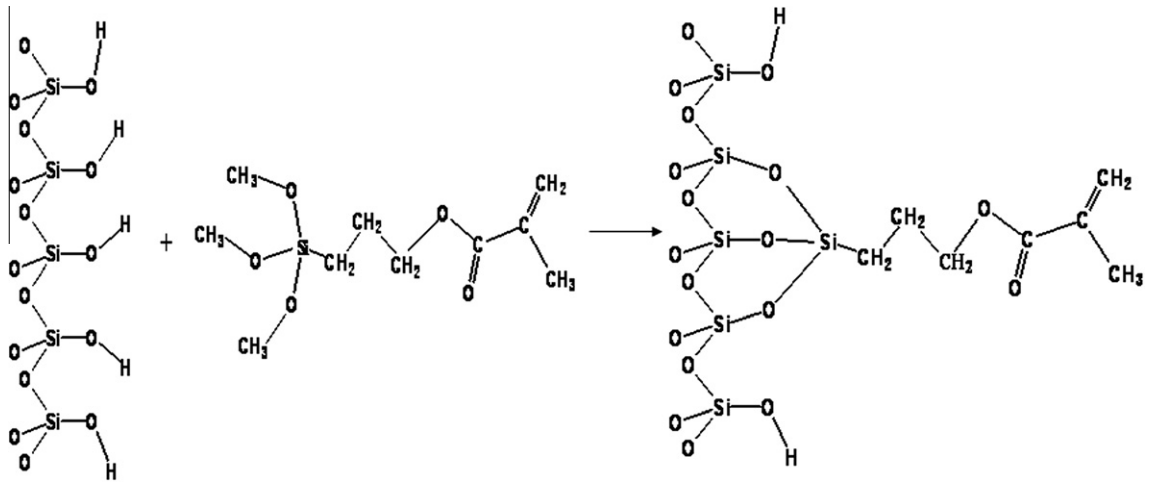

Figure 1 Modification of the superficial silica silanol groups using the difunctional 3-(trimethoxysilyl)propyl methacrylate reagent.

3. Results and discussion

3.1. Preparation of the monolithic columns

The general scheme for preparation of the capillary butyl (or hexyl) monolithic columns is illustrated in Figs. 1 and 2. This preparation includes the following steps:

(a) activation of the inner surface of the fused-silica capillary by an alkaline treatment
(b) derivatization of the silanol groups using 3-(trimethoxysilyl)propyl methacrylate
(c) thermal *in situ* polymerization of butyl (or hexyl) methacrylate cross-linked with ethylene dimethacrylate
(d) connection of the prepared column to a HPLC pump and thorough solvent washing.

The preliminary alkaline treatment of the fused-silica capillary, aims to increase the density of silanol groups at its surface and favor chemical bonding of the difunctional TMSM spacer. Therefore, these steps are essential to promote the *in situ* formation of a cross-linked polymer firmly linked to the capillary inner walls and avoid the need of inlet and outlet frits. The monolith pore size is adjusted during the preparation process through the use of a suitable porogen which controls the porous properties of the synthesized polymer.

Before preparing the reaction mixture, the solubility of both monomer and crosslinker in the selected porogenic mixture

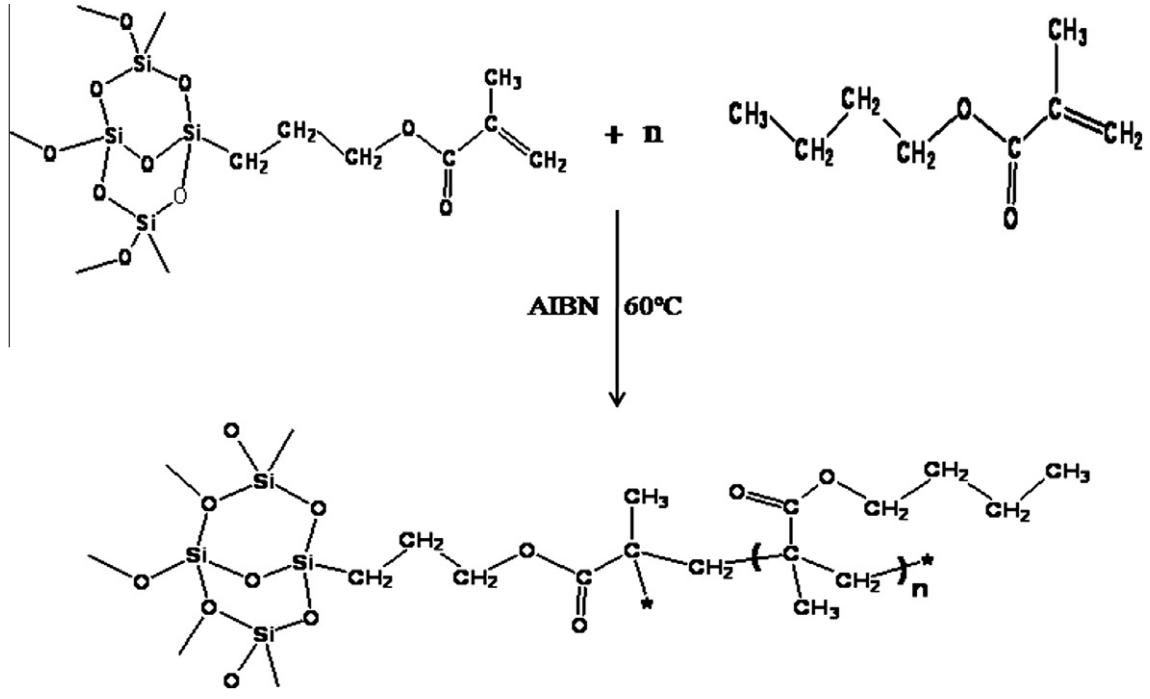

Figure 2 Polymerization of butyl methacrylate on the modified silica.

was carefully checked, in order to obtain a homogeneous and stable solution. This reaction mixture was then deaerated by sonication and helium purging before filling the fused-silica capillary. The two columns selected for the present work were labeled B1 (for butyl methacrylate) and H1 (for hexyl methacrylate).

3.2. FTIR characterization of the monolithic polymers

The Fourier Transform Infra Red spectra of the butyl and hexyl crosslinked polymethacrylate materials are shown on Figs. 3 and 4, respectively. The same profile appears in both spectra, the main observed frequencies on the spectrum can be attributed as follows (Patterson et al., 2003; Peiqiang Yu):

- 2958 cm^{-1}: CH_3 asymmetric stretching band.
- 2866 cm^{-1}: CH_3 symmetric stretching band.
- 1733 cm^{-1}: C═O stretching band of ester.
- 1165 cm^{-1}: C–O stretching band of ester.
- 1457 cm^{-1}: CH_2 bending band.
- 1381 cm^{-1}: CH_3 bending band.

On the other hand, the absence of C═C and ═C–H stretching bands at 1650 and 3090 cm^{-1}, respectively confirms completion of the polymerization reaction.

3.3. Morphology of the monolithic columns

The prepared monolithic materials were first checked by optical microscopy in order to inspect distribution of the polymeric bed in the capillary tubing.

The pictures recorded on an optical microscope, at ×100 magnification, are shown in Fig. 5. They revealed a continuous and homogeneous polymeric phase filling the whole capillary and firmly attached to the wall.

In order to characterize the prepared monolithic materials, the polymers were sputter-coated with gold and their morphology was examined by scanning electron microscopy (SEM). As an example, some SEM pictures are shown in Fig. 6; they demonstrate that for both columns the preparation procedure renders a permeable monolith with a uniform porosity. Moreover, the well-developed macroporous structure, typical for monolithic supports, is clearly illustrated and is similar for the two monolithic columns B1 and H1.

Monoliths usually contain two types of pores, classically referred to as mesopores (size between 2 and 50 nm) and macropores (size > 50 nm), the former ones allowing diffusive mass transport and interactions whereas the latter ones allow mobile phase flow and convective mass transport.

The microphotographs of both capillary cross sections illustrate a uniform structure of the monolith bed with the presence of interparticle spaces. The monolithic bed of columns B1 and H1 represent a continuous and relatively dense polymeric phase with low interstitial porosity. The figures show that the synthesized monoliths are composed of spherical microglobules with an average diameter in the range 1–2 μm, linked together and forming a skeleton around macropores. It can be observed that larger macropores and microglobules are obtained for column B1 as compared to the other column H1 which is characterized by a relatively higher polymer density. In addition to the macropores, some larger channels are observed in the bulk material, resulting in higher permeability but lower mass transfer rate. This macroporous structure facilitates the mobile phase flow, promotes effective solute/stationary phase interactions and allows the high permeability of the prepared capillary columns.

3.4. Mechanical stability of the capillary monolithic columns

Acetonitrile and water were used for the measurement of the pressure drop across the column with increasing flow rates, in order to evaluate the mechanical stability and permeability of our monolithic material. Fig. 7 shows the effect of the flow rate through column on backpressure using either water or

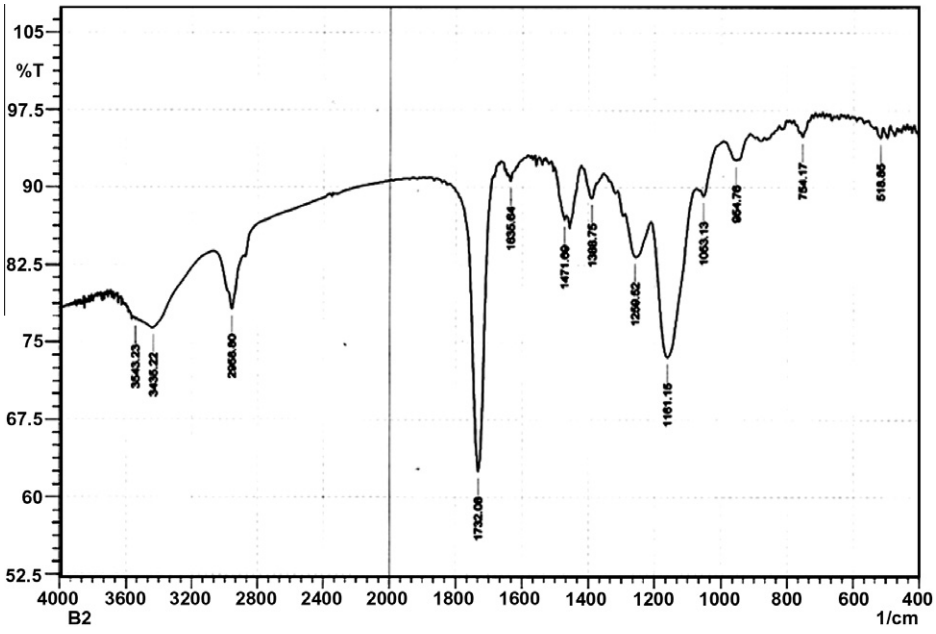

Figure 3 FTIR spectrum of the crosslinked butyl methacrylate polymer.

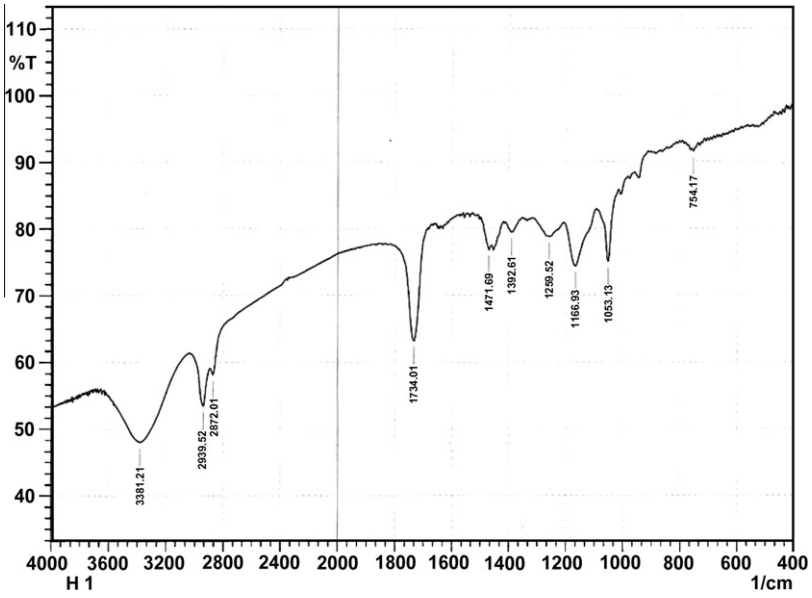

Figure 4 FTIR spectrum of the crosslinked hexyl methacrylate polymer.

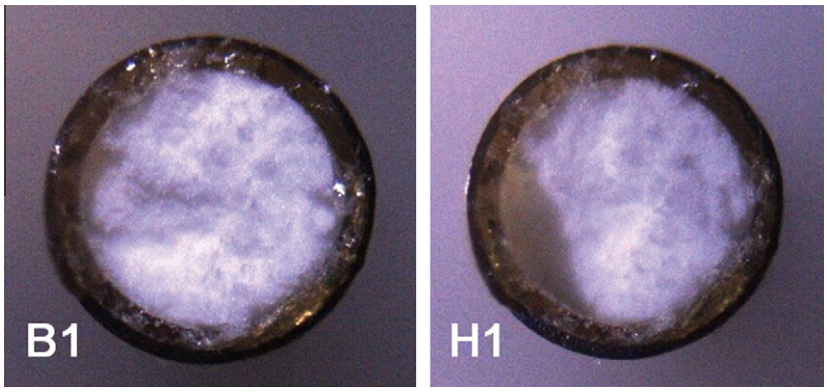

Figure 5 Optical micrographs of the prepared capillary columns B1 and H1.

acetonitrile as mobile phase with columns B1 and H1. An excellent linear dependence of the column inlet pressure on the flow rate is observed for all curves. When the plot of the pressure drop versus the velocity of the fluid shows a linear relationship, this indicates that permeability and mechanical stability of the prepared monolithic stationary phases are excellent. This linear behavior is characteristic of a rigid crosslinked polymer with a suitable porosity. Comparison of the two plots shows that, in the same conditions, the backpressure measured for column H1 is higher than for B1; it confirms that, as observed by SEM, the polymer density of the latter is lower.

3.5. Efficiency of monolithic column

A reversed-phase mechanism was observed for the prepared columns throughout all separation experiments, meaning that solution partitioning between the mobile and stationary phases was the main mechanism responsible for the retention of model solutes. Band broadening in chromatographic columns is conveniently described by the well known Van Deemter equation as the dependence of the height equivalent to a theoretical plate, H, on the flow rate of the mobile phase, u.

$$H = A + B/u + C \cdot u$$

The reference compounds used to evaluate the prepared capillary columns efficiency were alkyl benzenes and polycyclic aromatic hydrocarbons. The Van Deemter curves were plotted for benzene and phenanthrene by varying the mobile phase flow rate in the range 10–50 μL min^{-1}.

Fig. 8 shows the Van Deemter curves obtained for benzene and phenanthrene on columns B1 and H1 at 25 °C using water/acetonitrile (50:50, v/v) as mobile phase. Measured values of the height equivalent to a theoretical plate (HETP) were in the range 0.3–3 mm and are increasing while raising the flow rate, indicating a lower performance of the column. Thus, the use of a low flow rate means a higher number of theoretical plates but induces a longer retention. Although the two columns showed different polymer density and permeability, their efficiency is quite similar on the whole flow range. Meanwhile, column efficiency measured for phenanthrene was only slightly affected by increasing the flow rate, this means that faster anal-

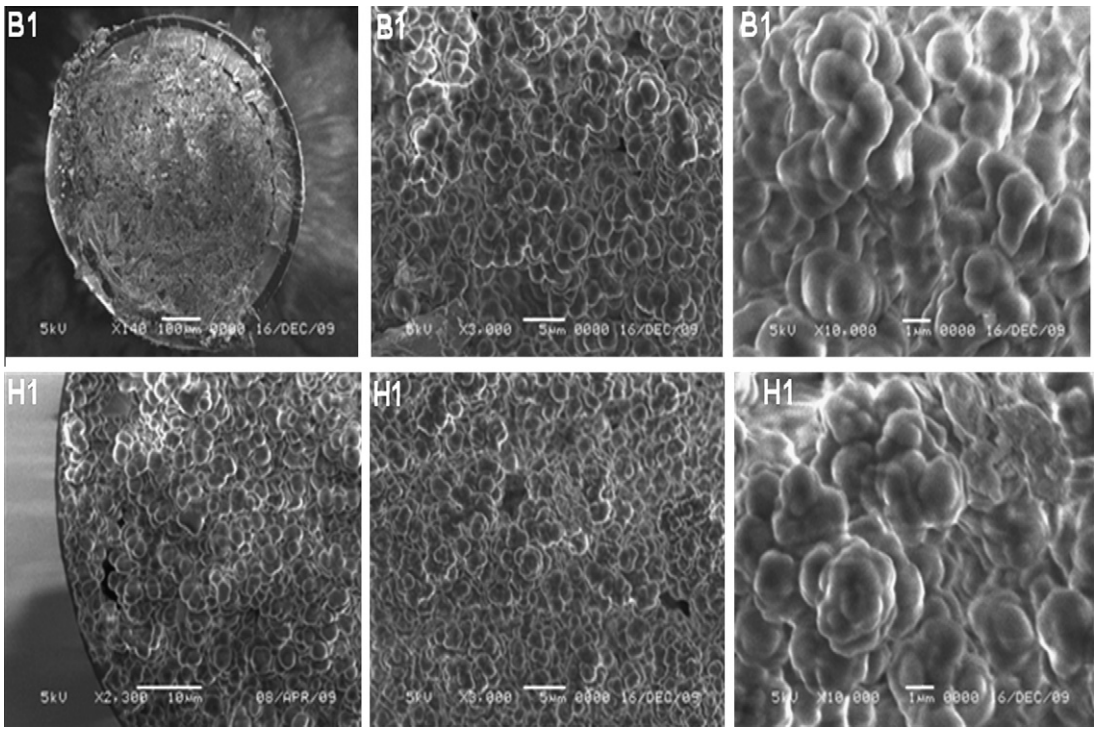

Figure 6 Scanning electron micrographs of the prepared capillary columns B1 and H1.

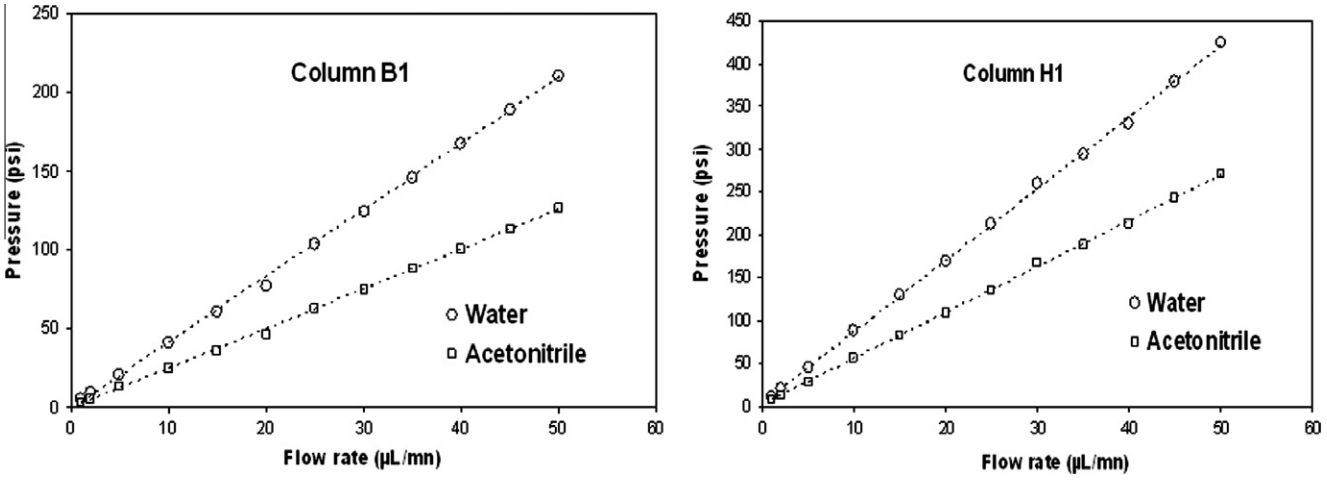

Figure 7 Plots of pressure drop vs. flow velocity using water and acetonitrile as mobile phase, columns B1 and H1 (150×0.53 mm), temperature: 24 °C.

yses are possible without greatly affecting the separation quality. This is one of the main advantages of capillary columns and was previously established by many research groups (Siouffi, 2006; Vlakh and Tennikova, 2009; Guiochon, 2007; Matusova et al., 2008).

Fig. 9 shows also the effect of column temperature on benzene and phenanthrene HETP using column H1 in the range 25–55 °C. While it is generally admitted that the temperature has no great influence on HPLC experiments, the results obtained show that the plate number can be drastically improved when increasing temperature from the ambient to 65 °C. Increasing temperature induces a lower plate height for benzene while for phenanthrene the plate number rapidly increases. This is due to the fact that the phenanthrene retention is higher and is more affected by any change in temperature. In spite of this well established effect, the temperature influence on the separation of homologous series is considered to be minor, in comparison with the importance of the mobile phase composition (Lubbad and Buchmeiser, 2010). These results confirm the importance of temperature control in capillary liquid chromatography. Another important result should be highlighted: although the column temperature was changed over a quite long period, its performance remained constant, indicating a high stability of the monolithic polymeric bed.

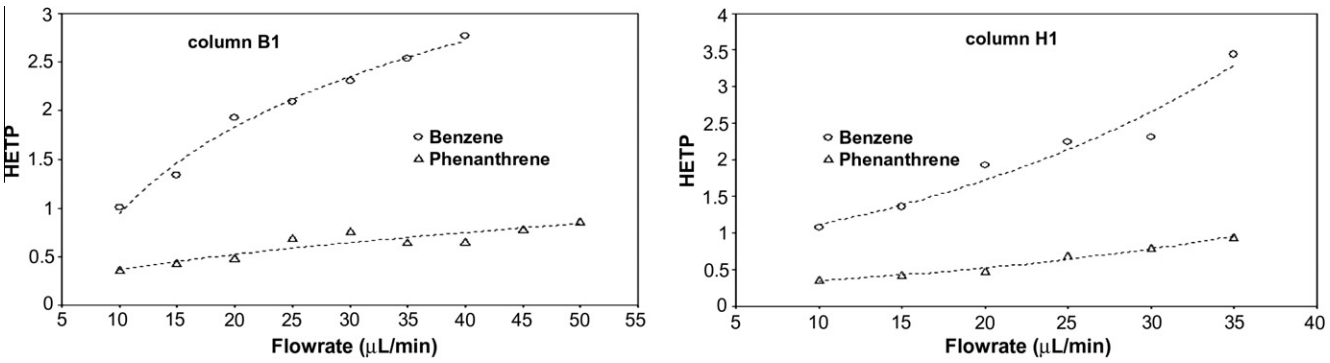

Figure 8 Van Deemter plot of the height equivalent to a theoretical plate as a function of flow rate for benzene and phenanthrene. Experimental conditions: monolithic capillary columns B1 and H1, 15 cm × 530 μm i.d., mobile phase: acetonitrile/water (50:50, v/v), 1.0 ppm solution, injection volume: 1 μL, UV detection at 215 nm.

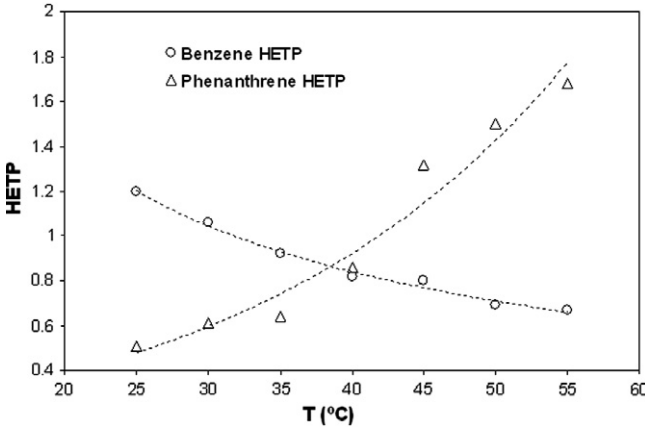

Figure 9 Height equivalent to a theoretical plate of benzene and phenanthrene as a function of column temperature. Experimental conditions: monolithic capillary columns H1, 15 cm × 530 μm i.d., mobile phase: acetonitrile/water (50:50, v/v), flow rate: 10 μL min^{-1}, 1.0 ppm solution, injection volume: 1 μL, UV detection at 215 nm.

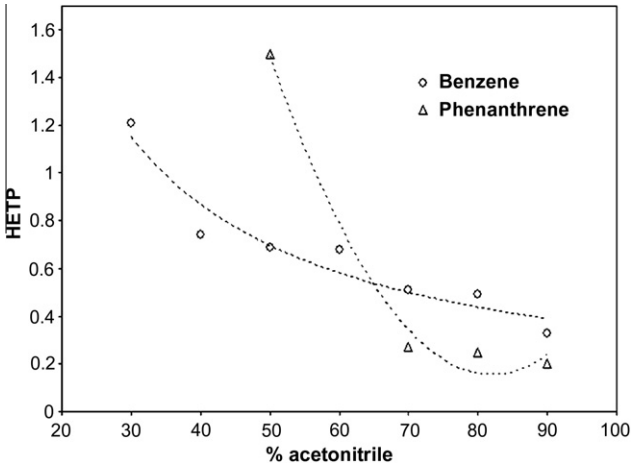

Figure 10 Height equivalent to a theoretical plate of benzene and phenanthrene as a function of mobile phase composition. Experimental conditions: monolithic capillary column H1, 15 cm × 530 μm i.d., flow rate: 10 μL/mn, temperature: 50 °C, 1.0 ppm benzene solution, injection volume: 1 μL, UV detection at 215 nm.

The influence of the mobile phase composition on the column performance was also investigated for benzene and phenanthrene on column H1, the results are described in Fig. 10. Using higher acetonitrile content induces for both solutes an apparent rapid decrease in the height equivalent to a theoretical plate. Indeed, increasing the eluent strength lowers their retention while the corresponding peaks become narrower, the result being an increase in the plate number. Similar results were observed for the separation of alkylbenzenes on a polystyrene/divinylbenzene based monolithic column prepared by microwave irradiation (Zhang et al., 2009).

A typical chromatogram showing the separation of some aromatic compounds are depicted in Fig. 11. It should be mentioned first that the elution order is characteristic of a typical reversed phase behavior: retention increases with the carbon chain length. This could be explained by the hydrophobic character of interactions between aromatic hydrocarbons and the non-polar polymeric surface. In order to obtain a complete baseline separation of the mixture compounds, it is possible to modulate the different parameters which were investigated, as they can affect the column efficiency: flow rate, mobile phase composition and column temperature. The calculated R_s values for both columns are given in Table 1.

4. Conclusion

The described procedure allowed the preparation of two capillary columns packed with a butyl or hexyl methacrylate monolithic stationary phase chemically bonded to the inner silica surface. The polymer morphology was characterized by both optical and scanning electron microscopy which showed a uniform porous structure with microglobules having an average diameter in the range 1–2 μm. Fourier transform infrared spectra of the synthesized polymers illustrated the presence of the characteristic frequencies corresponding to the main functional groups. A chromatographic evaluation was carried out by evaluating the performance of each column and its dependence on several parameters: flow rate, mobile phase composition and column temperature. Both columns were used to separate different mixtures of alkylbenzenes and polycyclic aromatic

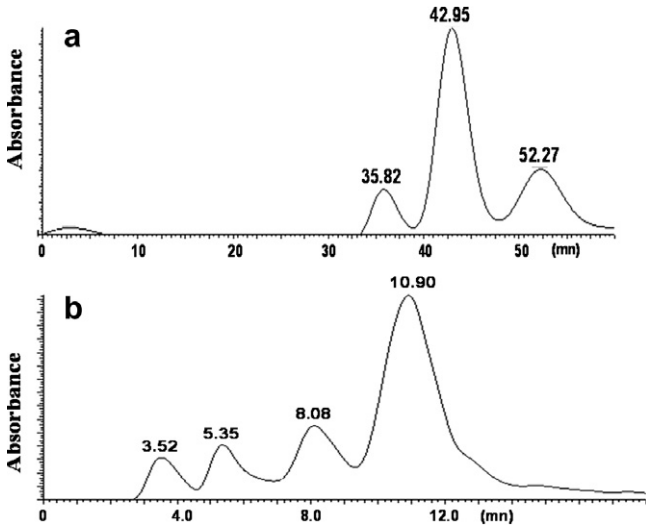

Figure 11 Chromatograms: (a) aromatic hydrocarbons (benzene, toluene and ethylbenzene, 100 ppm each in pure form) column B1, mobile phase: ACN/H_2O 50:50, flowrate: 5 μL min^{-1}, temperature: 25 °C (b) aromatic hydrocarbons (benzene, naphthalene, fluorene and phenanthrene, 100 ppm each in pure form), column H1, mobile phase: ACN/H_2O 50:50, flowrate: 35 μL min^{-1}, temperature: 25 °C, UV 244 nm. The numbers on the peak indicates the retention time.

Table 1 The calculated R_s value of Columns B1 and H1.

Peak position	$W_{1/2}$	t_R	R_s
Column B1			
Peak 1	6	82.5	–
Peak 2	8	99	1.39
Peak 3	12	121	1.30
Column H1			
Peak 1	7	27	–
Peak 2	7.5	41	1.14
Peak 3	11.5	62	1.30
Peak 4	13	84	1.06

hydrocarbons. In spite of using the prepared columns under high pressure, during long runs with various eluents and flow rates, good reproducibilities of the retention time and efficiency were observed. This fact proves a high stability and robustness of capillary monolithic columns which are considered as an interesting and economical alternative to conventional particle packed columns.

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