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Novel monolithic columns with templated porosity

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Abstract

A new type of monolithic stationary phase was prepared within the confines of fused-silica tubing by in situ polymerization of divinylbenzene or ethylene dimethacrylate either with styrene or butyl methacrylate. The porosity of the monolith was dictated by silica beads packed in the capillary prior to flushing the column with the monomeric solution. Subsequent washing of the polymeric rod with sodium hydroxide rendered a porous monolith that was used for both micro-LC and capillary electrochromatography. The novelty of the approach presented herein lies in preparing the polymer within the confines of a fused-silica capillary. The challenges posed in this new context and their resolution are presented in detail. In addition, this study proposes that in addition to tailoring the pore size, the silica beads, through their surface chemistry, can influence the surface characteristics of the finished polymer monolith. For example, the data suggests that octadecyl modified silica particles interacted with hydrophobic moieties of monomers before initiation of polymerization, thus dictating their orientation in the resulting polymer.

Keywords: Monolithic columns; Porosity; Stationary phases, LC; Stationary phases, electochromatography; Electrochromatography; Peptides; Proteins

1. Introduction

The challenges prompted by miniaturization of chromatographic systems have led scientists to explore new avenues in the synthesis of stationary phases [1,2]. Packed column capillary electrochromatography (CEC) and micro-LC have found limited application in routine analysis due to difficulties such as non-specific interaction, increased backpressure in micro-LC, gas-bubble formation in CEC, column-to-column irreproducibility, and increased fragility of capillaries, all of which are often attributable to the frits. New column designs insure the use of stationary phases with high surface area in columns that do not require frits.

One approach considers immobilizing the packed bed within the confines of the silica walls by using a fine matrix evenly distributed over the entire length of the column [3–8]. The extent to which the chromatographic performance of these entrapped columns deviates from that of conventional columns depends on the synthetic procedure employed; in some instances this difference is minimal [6–8].

In the search for fritless columns the concept of replacing the particulate packing with a continuous rod has been investigated. The fact that synthetic polymers can be produced in virtually any geometry allows for in situ preparation, which simplifies column fabrication. Both silica [9–11] and organic [12–17] based monolithic columns have been synthesized in situ by pumping a monomeric solution into a capillary column blank. Thermal or UV polymerization will produce a solid support that can be further derivatized or used as-is for chromatographic separations.

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Organic based polymeric rods evolved from solvent-swollen hydrophilic acrylates [12,13] to efficient methacrylate based polymers [14–16], and then to more rigid polystyrene–divinylbenzene columns [17], which have found applications in peptide and protein separations.

To control the porosity, i.e., pore size and pore size distribution, of the monolithic polymers, solvents are employed as porogenic agents. It is common knowledge that finding the appropriate porogen is the key to the synthesis of polymers showing good performance in chromatographic applications. The ability to independently vary the porosity and composition of the monolith is, however, quite limited. More often a minor alteration in composition of the monomer solution (while all other parameters are left unchanged) has a significant effect on the porosity of the resulting structure. An extensive study performed by Peters et al. on methacrylate-based matrices [15] demonstrates the complex interplay between the nature and the amount of monomers and the concomitant porosity. While ingenious solutions were employed to solve the problem, it is clear that each variation in the composition of monomers requires a new search for optimal conditions. This issue became critical in the synthesis of monolithic polymers when molecular recognition is to be a design feature, where the solvent must dictate porosity and yet not interfere with the imprinting process [18,19]. Since solvents adequate to this task were hard to find, in order to obtain permeable monoliths the polymerization reaction was timed to achieve incomplete polymerization. This method drastically limits the reproducibility of monolith synthesis.

This preliminary study reports the preparation of novel continuous bed columns with porosity dictated by spherical particles. The use of silica beads as sacrificial material for the templating of porosity is a concept that has been applied in the past [20,21]. The novelty of the present study lies in preparation of the polymer within the confines of a fused-silica capillary. Although a more involved manufacturing procedure, relative to presently employed in situ monolithic synthesis, this approach eliminates one of the variables involved in the design of polymer composition: the need for a porogenic solvent. In addition, altering the surface characteristics of the templating beads can influence the composition of the finished monolith surface.

2. Experimental

2.1. Chemicals and materials

Butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), trimethylolpropane trimethacrylate (TRIM), 3-(trimethoxysilyl)propyl methacrylate (TMSPM), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), 2,2′-azobisisobutyronitrile (AIBN) and poly(ethylene glycol) (PEG) average molecular weight ca. 10,000 were purchased from Aldrich (Milwaukee, WI, USA) and used as received. Styrene (S) and divinylbenzene (DVB) obtained from Sigma (St. Louis, MO, USA) were washed with 10% (w/v) aqueous sodium hydroxide to remove the inhibitors. The proteins aprotinin (bovine lung), insulin (porcine pancreas), ribonuclease A (bovine pancreas), lysozyme (chicken egg white), cytochrome *c* (horse heart), myoglobin (horse skeletal muscle), and the peptides FGFG, FLEEI, angiotensin I (DRVYIHPF) and angiotensin II (DRVYIHPFHL) were purchased from Sigma.

The solvents employed in the CEC and micro-LC runs were HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA) or Mallinckrodt (St. Louis, MO, USA).

Fused silica tubing of 100, 180 and 250 μm I.D.×375 μm O.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA). Capillaries cut to a total length of 25 cm were filled with packing material as described herein. The sorbent materials used were as follows: unmodified silica of 10 μm diameter, 1000 and 4000 Å pore size from MetaChem Technologies (Torrance, CA, USA); Nucleosil silica of 3 μm diameter, 100 Å pore size and 7 μm diameter, 300 Å pore size from Macherey–Nagel (Düren, Germany); unmodified silica 5 μm diameter, 250 Å pore size from Bio-Rad (Hercules, CA, USA); wide pore silica 55× (unspecified particle diameter and mean pore size) from Phase Separations (Deeside, UK).

Temporary frits were prepared by tapping one end

of the capillary in a paste prepared from the Nucleosil silica particles of 10 μm diameter, 1000 Å pore size and potassium silicate solution (Kasil No.1) purchased from PQ (Valley Forge, PA, USA).

2.2. Instrumentation

An Isco 100 DX (Isco, Lincoln, NE, USA) syringe pump coupled to an inline filter and a cartridge guard column holder (Upchurch Scientific, Oak Harbor, WA, USA) were employed in column manufacturing. The frits were sintered in place using a Fujikura FSM 05S arc fusion splicer (Alcoa Fujikura, Duncan, SC, USA). The columns were filled with the mixture of monomers using a typical solvent rinsing kit. Using a small piece of PTFE tubing the monolithic column was joined to a fused-silica open tube onto which a detection window was burned. For better coupling, an Agilent (Waldbronn, Germany) capillary column cutter with rotating diamond blade was used to obtain clean, straight cuts of fused-silica tubing.

Examination of capillary columns during packing and monolith preparation was achieved with a simple Stereomaster optical microscope (Fisher Scientific, Houston, TX, USA) with ×40 magnification. Scanning electron microscopy (SEM) images of the gold sputter-coated porous monoliths were taken with an AmRay instrument (Bedford, MA, USA) operated at 10 kV.

All CEC runs were performed on an Agilent/HP^{3D} CE (Waldbronn, Germany) instrument, modified such that pressures of up to 12 bar can be applied on the inlet and/or outlet vials. This feature was used only for column conditioning, since gas-bubbles were not generated during operation of these monolithic columns. The cassette temperature was held at 20°C. Electrokinetic injection (5 kV for 3 s) was used for sampling the analytes. Data acquisition and processing were done using ChemStation software (Agilent).

A laboratory-built instrument was used to conduct the micro-LC runs. This instrument comprised an Agilent 1100 series binary high-pressure gradient pump used with a 1:100 splitter, a Valco micro injection valve of 60-nl loop volume (Valco, Houston, TX, USA), and a Unicam 4225 UV detector (Thermo Separation Products, San Jose, CA, USA). The detector signal was recorded on Apple Power Macintosh 6100/66 (Apple Computer, Cupertino, CA, USA) using a PowerChrom System 2.0 (ADInstruments, Milford, MA, USA) chromatography data system.

3. Preparation of monolithic columns

3.1. Silanization of the fused-silica walls

For columns in which the monolith was anchored to the fused-silica capillary wall, functionalization of the walls was required. A 3-m length of fused-silica tubing was washed and filled with a solution of sodium hydroxide (1 *M*) using a solvent rinsing kit. The tubing was then sealed at both ends with rubber septa and heated in an oven at 100°C for 1 h. Subsequent flushing with deionized water, diluted hydrochloric acid, deionized water and acetone, each for about 10 min, was followed by a drying step in which the column was purged with nitrogen at 100°C for 20 min. The derivatization reaction employs TMSPM, a bifunctional coupling agent, which reacts with the silanol groups on the silica surface through the trimethoxysilyl moiety; the other functionality, the methacrylate group, is the anchor for the monolith to be synthesized in a radical polymerization reaction. The attachment of TMSPM to the silica surface takes place at elevated temperatures. In order to minimize the premature occurrence of the methacrylate polymerization reaction, favored at high-temperatures, the inhibitor DPPH was added following a procedure described by Gusev et al. [16]. A solution of TMSPM in dimethylformamide (DMF) 50% (v/v) with 0.01% (w/v) DPPH was then prepared separately and purged with nitrogen for 10 min. The capillary was flushed and filled with this mixture, sealed at both ends using rubber septa and heated in an oven at 100°C for 4 h. The tubing was subsequently flushed with acetone and purged with nitrogen for about 30 min.

3.2. Packing the columns

Capillaries of 100, 180 and 250 μm I.D. were

packed using the slurry packing method. A detailed description of the packing procedure is given elsewhere [5,22]. The only modification from the earlier method is the replacement of the packing solvent with the pore filling mixtures described in Table 1.

3.3. Synthesis of the organic-based polymer

The packed columns were flushed with nitrogen at about 200 p.s.i. for 15–30 min to remove liquid from the interstitial space (between particles of packing material) (1 p.s.i.=6894.76 Pa). The columns were inspected under the optical microscope and nitrogen flushing was stopped when the bed appeared to be dry. Each monomer solution was then purged with nitrogen for 10 min. Using the solvent rinsing kit in a setup described elsewhere [23] at an applied gas pressure of 50 p.s.i., the columns were filled with the solution of monomers in about 5 min. The columns were sealed with rubber septa and placed in a water bath at 70°C for 24 h. The various compositions of cross-linkers, monomers and solvents tested in this study are summarized in Table 1.

3.4. Removal of silica particles

After polymerization, the monoliths were coupled to a syringe pump and pressures up to 2000 p.s.i. were applied. A combination of diffusive and convective flow allowed the sodium hydroxide solution to advance through the small pores at a flow velocity of about 50 cm/h. Interconnectivity between the silica beads might be facilitated by residual pore filling solution at points of contact in between the silica particles and/or pore spaces in the organic polymer itself (for compositions of organic monolith containing porogenic solvent). Prolonged flushing with the alkaline mixture (24–48 h) slowly dissolved the silica beads.

3.5. Characterization of monolithic columns

After the removal of silica particles, the chromatographic performance of the monolithic capillary columns was tested in the CEC or micro-LC format. Later, the monoliths that were not anchored to the capillary walls were left at room temperature for a week, then extruded from the capillary and cut into small pieces with an exacto knife. The polymer was sputter-coated with gold and examined with a scanning electron microscope. The SEM images presented in Fig. 1 demonstrate that the procedure for synthesis described herein renders a highly permeable monolith with a uniform porosity.

Table 1
Factors affecting the results of column preparation include the choice and amount of monomers and crosslinking agents, diameter and surface characteristics of the templating beads, and the nature of the pore-filling liquid

Column	Anchored to capillary walls	Packing material	Pore filling liquid	AMPS (mg/ml)	Polymer composition	Solvent (%, v/v)	Cross-linker (CL)	Monomer (*M*)	Vol. ratio (CL: *M*)
1	No	Si 10 μm, 1000 Å	–	–	EB1	–	EDMA	BMA	50:50
2	No	Si 10 μm, 1000 Å	–	–	EB2	10	EDMA	BMA	50:50
3	No	Si 10 μm, 4000 Å	10% PEG_{aq}	–	EB3	40	EDMA	BMA	50:50
4	No	Si 5 μm, 250 Å	10% PEG_{aq}	–	EB4	20	EDMA	BMA	50:50
5	No	Wide pore Si, 55×	5% PEG_{aq}	2	EB5	20	EDMA	BMA	40:60
6	No	Wide pore Si, 55×	10% PEG_{aq}	2	EB5	20	EDMA	BMA	40:60
7	No	Wide pore Si, 55×	10% PEG_{aq}	2	TB6	10	TRIM	BMA	50:50
8	No	Wide pore Si, 55×	10% PEG_{aq}	2	VB7	10	DVB	BMA	50:50
9	No	C_{18} 7 μm, 300 Å	10% PEG_{aq}	2	VB7	10	DVB	BMA	50:50
10	Yes	Si 5 μm, 250 Å	10% PEG_{aq}	2	VS8	–	DVB	S	50:50
11	No	Wide pore Si, 55×	10% PEG_{aq}	2	VB9	–	DVB	BMA	50:50
12	Yes	Si 3 μm, 100 Å	20% Glycerol	–	VS10	10	DVB	S	50:50

The corresponding parameters employed in the fabrication of each column are listed below. The nomenclature used for each composition contains two letters designating the crosslinker (E for EDMA, T for TRIM, V for DVB) and the monomer (B for butyl methacrylate, S for styrene), and a numeral indicating the current number of the trial.

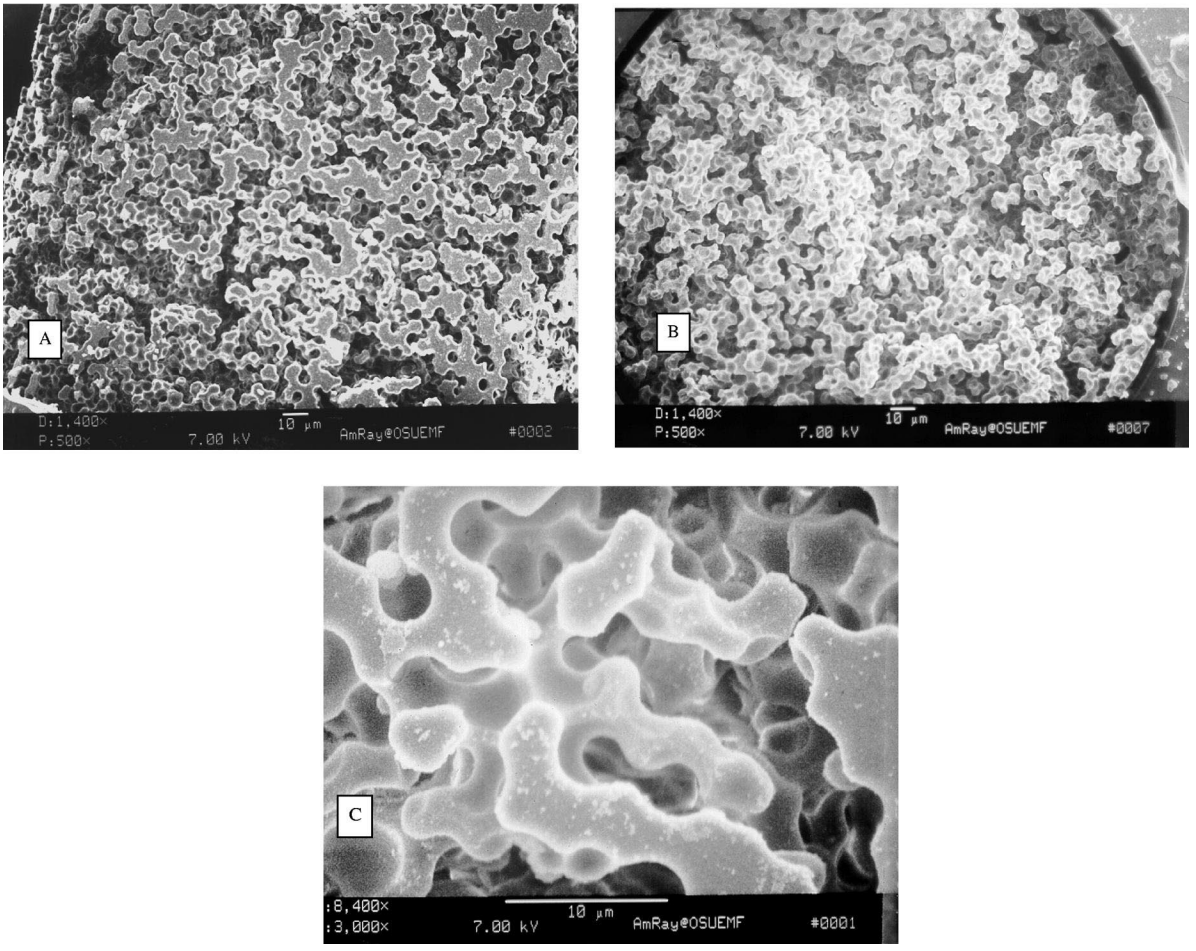

Fig. 1. SEM images of the porous monoliths obtained from columns 10 and 11 (see Table 1 for detailed description of polymer composition). (A) Radial image of an extruded rod of monolith 11 depicts the structure of the polymer in contact with fused-silica wall. (B) Axial image of the monolith 11 indicates that the core of the polymer has a permeable structure. (C) A closer look at monolith 10 indicates that the polymer is a fairly accurate negative image of a packed bed.

4. Results and discussion

The goal of this study was to synthesize monoliths with porosity dictated by the size of spherical silica particles. This involved building a bed of beads followed by filling the space in between the particles with a solution of monomers. Radical polymerization was thermally initiated, and subsequent dissolution of the silica particles produced a rod of polymer with a uniform porosity dictated by the diameter of the silica beads. As mentioned before, this general type of polymer was prepared previously [20,21]. The method described by Johnson et al. [21] involved sintering the silica beads at high-temperature and the use of gaseous HF to dissolve the beads. However, developing a similar monolith within the confines of silica walls does not allow bead sintering and the use of gaseous HF. Additional complexity is introduced by the need for a fairly long monolith that would afford chromatographic use. Our approach employs the use of a bed packed within a capillary column to obtain a fairly uniform bead-based structure and a relatively long monolithic rod.

In the first attempt to synthesize a monolith,

column 1 was packed with silica beads of 10 μm diameter and 1000 Å pore size, then filled with polymer composition EB1 (see Table 1 for detailed description). Upon polymerization an impervious block of polymer was obtained. Pieces of monolith were extruded from the capillary for observation and treated with HF (48%) solution for more than 24 h. Even after this rigorous treatment, the monolith remained nonporous. A second column was prepared in a similar fashion and this time a solvent (10%, v/v, solvent) was incorporated in the polymerization mixture to aid the access of HF solution to the silica beads. Again, the treatment did not dissolve the silica beads.

It became obvious that the silica would be dissolved only by a solution that could flow through the polymer. Adding more solvent to the mixture would decrease to an even greater extent the amount of stationary phase in the monolith; with no solvent in the monolithic mixture complete dissolution of silica would provide 60% porosity. A stationary phase having even greater porosity, hence much lower surface area, would be non-ideal for chromatographic use. The only option was to fill the pores of the silica beads with a liquid having a high surface tension, such as water, in order to keep the pores accessible for later flushing. To minimize the number of steps required in the preparation of the monolith, the pores were filled during column packing by employing the ''pore-filling liquid'' as a slurry solvent. Subsequent purging of the packed bed with nitrogen at low pressure for a short period of time removed the pore-filling liquid from the interstitial space. This step afforded control over the purging process through visual examination of the column using the optical microscope; the advance of nitrogen (or the monomeric mixture in the next step) was easily observed. After filling the column with the monomer solution and thermal polymerization, the silica beads were removed by passing a solution of sodium hydroxide through the column for about 30 h. A schematic representation of this procedure is provided in Fig. 2. As the study progressed, the main parameters that affected the permeability of the monolith and the characteristics of the stationary phase were identified. These are listed in Table 1, and their successive observation and optimization is described in the following paragraphs.

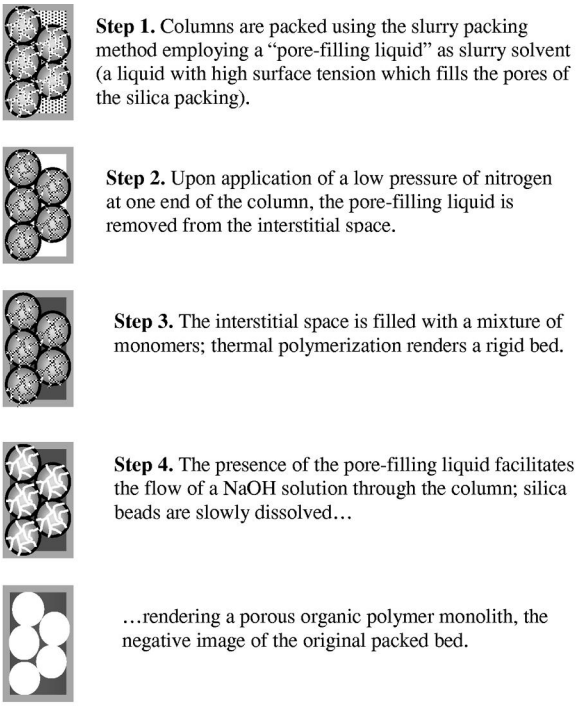

Fig. 2. Schematic representation of the synthesis of monoliths with porosity templated by silica beads.

One parameter essential to this procedure was the composition of the pore-filling liquid. Such a liquid should possess a high surface tension to ensure that it remains in the pores during steps 2 (nitrogen flush) and 3 (filling with monomer solution). Water was also tested as a pore-filling liquid; some of the columns prepared using water were not permeable for the NaOH flushing step. The pore-filling liquid employed in the preparation of the columns described herein consisted of a solution of PEG or glycerol in water in the percentages indicated in Table 1. The increase in viscosity of the pore-filling liquid on addition of PEG or glycerol might be responsible for the improvement in permeability realized during the silica bead dissolution step.

Column 3 was prepared using the procedure described in Fig. 2, and a permeable bed was readily obtained. To ease the access of NaOH to the silica beads, the monomer solution contained a solvent in addition to monomers, crosslinker, and initiator. At this stage the nature of the solvent was not critical to the structure of the monolith; in fact, as the study

progressed the solvent was gradually eliminated from the monomer solution, and found to be an unnecessary component of the mixture. However, the high porosity dictated partly by the large diameter silica beads and partly by the solvent present in the monomer solution resulted in a low surface area. As the monolith was tested in the micro-LC setup, hydrophobic analytes were not retained even in a relatively weak mobile phase consisting of acetonitrile–sodium acetate (5 m*M*, pH 3.0) (20:80).

To increase the surface area, the amount of solvent added to the monomeric mixture was minimized and the particle size of the silica beads was decreased. A further increase in the amount of hydrophobic monomer versus crosslinker, in the case of columns 5 and 6, did not provide the expected improvement in retention.

One of the advantages inherent to polymeric sorbents is the facile incorporation of various functionalities by simple addition of monomers that contain them. For example, the synthesis of stationary phases for CEC might include a monomer with an ionizable moiety to support electroosmotic flow. A reagent commonly employed in the preparation of these sorbents is AMPS. Due to its polar sulfonic group this compound has limited solubility in the hydrophobic mixture of monomers; this limits the range of porogenic solvents currently employed in the synthesis of monoliths and impacts the ability of the researcher to control the porosity of the monolith. One advantage introduced by our approach is the ability to include AMPS in the matrix even if no solvent is added to the monomeric mixture. This is achieved by dissolving a certain amount of AMPS in the pore-filling liquid; the ionizable monomer then becomes available for copolymerization and can be incorporated at the surface of the polymer. Electroosmotic flows of 2.5 cm/min were achieved at 1000 V/cm field strength using this approach.

All of the columns prepared as of this point in the study were built of a methacrylate matrix with EDMA as crosslinker. In some instances, upon application of pressure it was observed that the bed was compressed. This was especially true for columns in which more monomer (BMA) was added to increase the active surface area. This prompted us to experiment with other crosslinkers, such as TRIM and DVB, known to provide a more rigid bed. A significant improvement was noticed with the use of DVB as crosslinker of columns 8 through 12. The columns were prepared more rapidly, and lower pressures were employed in the silica dissolution step. This allowed for the synthesis of monoliths with a uniform structure both radially and axially (Fig. 2).

Extensive studies of the monoliths suggest that altering the nature and amount of crosslinkers, monomers and porogenic solvent are the primary tools affording control over the structure and properties of the finished monoliths. The synthetic approach described here also offers the possibility of tailoring the nature of the surface area of the resulting monolith by selecting silica beads with specific surface chemistry. For example, column 9 employed C_{18} modified particles for pore templating in a matrix of BMA and DVB. Fig. 3 illustrates the separation of thiourea, fluorene, naphthalene, phenanthrene and chrysene achieved on this column. On the other hand, column 8 (prepared following a similar procedure, with the exception of the nature of the templating particles which were not C_{18} modified), demonstrated virtually no selectivity towards these PAHs. Column 9 was washed with NaOH solution for more than 48 h; inspection of pieces of monolith by SEM indicated virtually complete disso-

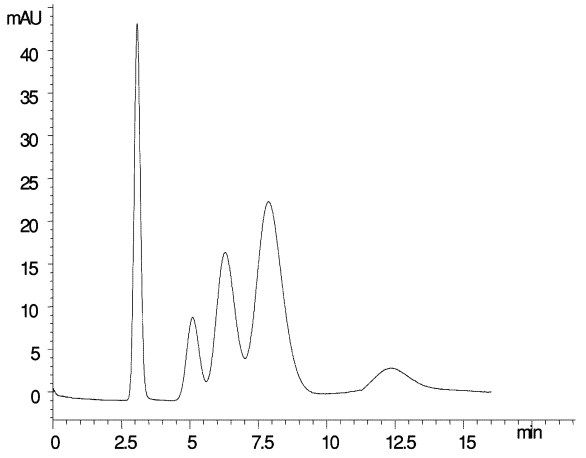

Fig. 3. Capillary electrochromatographic separation of thiourea, fluorene, naphthalene, phenanthrene and chrysene (in order of elution) on column 9 (see Table 1 for detailed description of monolith composition). Column: effective length 15 cm×250 μm I.D., UV detection at 254 nm. Mobile phase acetonitrile–sodium acetate buffer (25 m*M*, pH 3) (60:40).

lution of the templating particles. This suggests a significant increase in the hydrophobicity of the surface of the monolith itself, and further suggests that prior to the onset of polymerization the monomers oriented their hydrophobic moieties towards the surface of the C_{18} modified particles. This arrangement is made permanent by the thermal polymerization of the mixture.

In the preparation of columns 10 and 12 the solution of monomers was based on divinylbenzene and styrene. This type of polymer is more rigid and less prone to swelling in organic solvents than the methacrylate based polymer. Since swelling does not occur, when sufficient pressure is applied to a monolithic column this type of polymer slowly advances in the capillary. To prevent this movement, the capillary surface was modified (following a procedure described previously) to afford attachment of the monolith to the walls. Additionally, the silanization reaction renders a hydrophobic surface that eliminates the possibility of secondary interactions between the analytes and the silanol groups on the fused-silica.

In the case of column 10, no solvent was added to the mixture and the resulting monolith was a polymeric rod having a mean pore diameter equal to or larger than 5 μm. Fig. 1 presents a close-up SEM image of this monolith. A separation of small proteins was achieved by operating the column in gradient CEC mode (Fig. 4). The Agilent/HP^{3D}CE instrument was programmed to switch after predetermined periods of time from one set of vials with a weak mobile phase to different vials containing stronger mobile phases.

Horvath's group has previously demonstrated the feasibility of the styrene–DVB type of monolith for the separation of biomolecules [16]. Essentially the same composition of polymer (50% styrene and 50% DVB) was employed in the preparation of column 12. A mixture of five peptides was separated with the home-built micro-LC instrument by employing a shallow gradient (Fig. 5). The corresponding amino acid sequences of the peptides contained in this mixture are presented in Table 2. The pores of the monolith that is column 12 were templated by silica particles with a mean diameter of 3 μm. The resulting surface area of the bead templated monolith was much smaller than that of a similar monolith

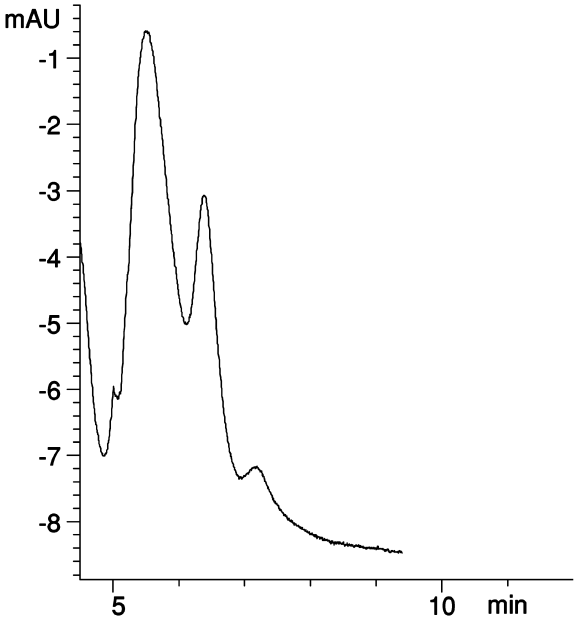

Fig. 4. Lysozyme and myoglobin were separated on column 10 (see Table 1 for monolith composition) by applying 8 kV by CEC employing a step gradient. Agilent/HP^{3D}CE instrument was programmed to use consecutively, for 2 min each, vials containing 80% B, 70% B and 60% B, respectively. Solvent A was potassium phosphate buffer (50 m*M*, pH 2.5) and solvent B was acetonitrile. Column: effective length 15 cm×250 μm I.D., UV detection at 220 nm.

prepared employing a porogenic solvent. This decrease in surface area might explain the lack of selectivity necessary for the separation of the two angiotensins, which differ by only two amino acids. By employing porous particles with a smaller diameter, significant improvement in the chromatographic properties of the bead templated monoliths is expected. Regrettably, such particles were not (commercially) available during the time of this study. Using the laboratory-built micro-LC setup the monolith demonstrated an ability to separate a mixture of six proteins when a steeper gradient was employed (Fig. 6).

Further work will be directed towards synthesis of monoliths employing templating particles having smaller diameters. The feasibility of other types of stationary phases, such as ionic exchange materials, will be tested. Further efforts will focus on achieving improved column-to-column reproducibility.

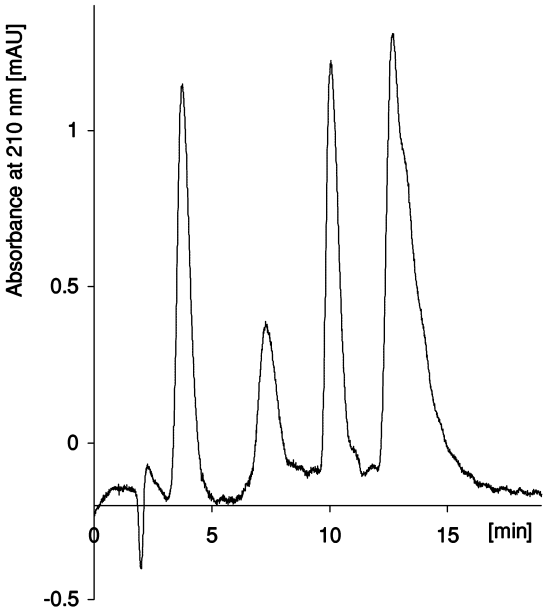

Fig. 5. Peptide separation by micro-LC using column 12 (see Table 1 for detailed monolith composition). In order of elution, the mixture contained the peptides listed in Table 2. A shallow gradient of 10–30% solvent B over 15 min was employed. Solvent A was water with 0.1% TFA and solvent B was acetonitrile with 0.1% trifluoroacetic acid (TFA). Column: effective length 15 cm×250 μm I.D. UV detection at 210 nm.

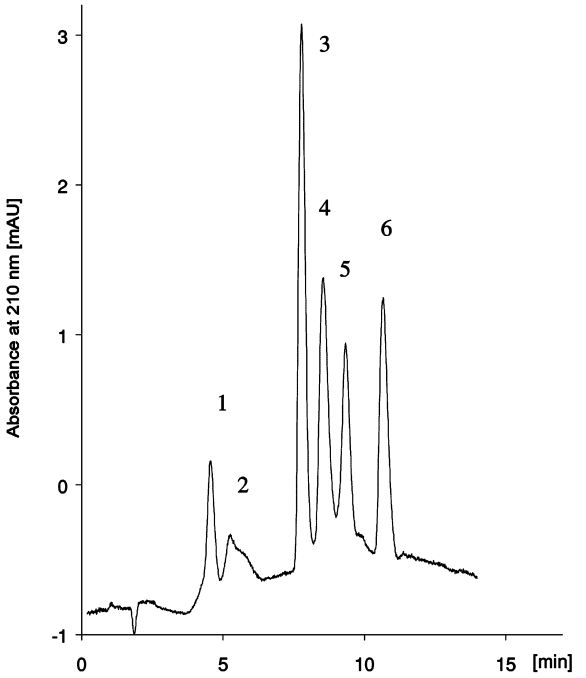

Fig. 6. Micro-LC separation of a mixture of six proteins achieved on column 12 (see Table 1 for detailed description of monolith composition). Insulin (1), aprotinin (2), ribonuclease A (3), lysozyme (4), cytochrome *c* (5) and myoglobin (6) were separated employing a gradient of 20–80% B over 20 min. Solvent A was water with 0.1% TFA and solvent B was acetonitrile with 0.1% TFA. Column: effective length 15 cm×250 μm I.D. UV detection at 210 nm.

5. Conclusion

We report a new procedure for the synthesis of stationary phase monoliths within the confines of fused-silica capillary tubes. To this point in time, adjustments in the amount and nature of porogenic solvents and incomplete polymerization were the only available tools for modifying the porosity of polymeric monolithic sorbents. Aside from affecting porosity, such adjustments alter other properties such as the surface area, nature and swelling properties of the resulting monolith. Our approach minimizes such side effects by employing a solid, fixed template, i.e., silica beads, to template the porosity and, to a certain extent, control the surface characteristics of the resulting material. The feasibility of employing these monoliths in chromatographic applications has been demonstrated.

Table 2
Amino acid sequence of the peptides separated in Fig. 5 (in order of elution)

Peptide	Amino acid sequence (one-letter code)
Phe–Gly–Phe–Gly	FGFG
Phe–Leu–Glu–Glu–Ile	FLEEI
CTIP2	EATILEEDEGLEIEEPSSLG
Angiotensin I	DRVYIHPF
Angiotensin II	DRVYIHPFHL

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