Monolithic Silica Stationary Phases in Liquid Chromatography

Imran Ali[1], **Vinay D. Gaitonde**[2], and **Hassan Y. Aboul-Enein**[3,*]

[1]Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi – 110025, India; [2]Prochrome India, A/2,Varsha Milan, Sahar Road, Andheri East, Mumbai – 400999, India; and [3]Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12311, Egypt

Abstract

During the last few decades, monolithic stationary phases (based on silica and polymers) have been used for fast separations in high-performance liquid chromatography (HPLC) and capillary electro-chromatography (CEC). The present article describes the preparation, properties, and applications of these stationary phases. Attempts have been made to discuss the preparation of reversed-phase monolithic HPLC columns and CEC capillaries. The chromatographic properties of these phases have been described. The applications included their use in HPLC and CEC modalities of liquid chromatography. The optimization of separations of various molecules on these phases has been discussed. Efforts were also made to predict the future perspectives of monolithic stationary phases.

Introduction

High-performance liquid chromatography (HPLC) is the backbone of separation science, as it is being used in almost all industries, including pharmaceuticals, chemicals, agro- and food-processing, etc. Many pharmaceuticals are bases and their analyses remains problematic due to poor peak shapes, which are often experienced in reversed-phase chromatography (1). Besides, the separation of large molecules such as proteins, nucleic acids, etc. are also not good on commercially available columns. In view of these facts, some workers have attempted to decrease analysis times by using short columns with smaller particles (smaller than the standard 5 µm). In spite of good efficiency with high flow-rates, these columns, sometimes, resulted in high plug and back-pressure (2). Besides, the separation of big molecules is not easy in HPLC. Therefore, researchers have tried to overcome the problems of high-pressure drop by employing ultra high-pressure liquid chromatography (3), capillary electro-chromatography (CEC) (4), and open tube liquid chromatography (5). During the last decade, columns made of a single piece of monolithic silica were introduced as the alternatives to particle-based columns. These columns have a biporous structure of larger macropores (2.0 µm), which permits high flow-rates with low back-pressure and smaller mesopores (13.0 nm); they also provide a high surface area for enough efficiency (6). The unique features and good advantages of monolithic silica HPLC columns over packed microparticulate columns are the ability to independently control the macro- and mesopore diameters as well as the silica skeleton diameter. Nowadays, it is possible to perform analyses with high linear flow velocity without significantly reduced separation efficiency. Besides, monolithic columns made of organic materials (polymer) enable high-speed separation of polypeptides and proteins in reversed phase and ion exchange modes but showed a relatively low efficiency for small molecules. Some reviews (7–21) have been published during the last few decades claiming fast and economic analyses for a variety of compounds on these columns. Due to these facts, attempts have been made to review monolithic phases in HPLC and CEC. The applications of these columns and capillaries have also been included. Efforts were also made to discuss the future perspectives of these stationary phases. The state-of-art of these phases is discussed in the following sections.

* Author to whom correspondence should be addressed: Professor Hassan Y. Aboul-Enein, Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research, Division, National Research Centre, Dokki, Cairo 12311, Egypt; email: enein@gawab.com.

Preparation of Monolithic Silica Gel

Basically, monolithic silica gel is prepared by mixing appropriate amounts of tetramethoxysilane (TMOS) as a silica source and polyethylene oxide (PEO) as a polymer in an aqueous acidic solution. The processes of phase separation and sol-gel transition occur simultaneously and control the structure of the monolithic products. Some publications have described the syntheses using different quantities of reactants (22–29).

TMOS and PEO Columns

The macropore and skeleton diameter or domain size can be controlled by PEO in the reactant mixture while a simulta-

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

neous increase in TMOS is responsible for skeleton diameter and lower macropore volume or thinner skeletons and higher macropore volume. Tanaka et al. (22) described the preparation of monolithic silica with skeleton diameters of 1.0–1.7 µm and macropore diameters of 1.5–1.8 µm. The resulting columns were derivatized to C_{18} material, giving to reversed-phase of 10–15 µm theoretical plate height. Furthermore, these authors (26) developed and investigated a number of reversed-phase monolithic columns with decreased sizes (5.7–2.32 µm). It has been reported by the authors that a subsequent surface modification of C_{18} monoliths silica lead to a reduction of mesopore size and volume, which affected column performance due to different mass transfer kinetics. Ishizuka et al. (30) also described the preparation of monolithic silica columns. The authors converted normal silica into reversed phase mode by continuously feeding octadecyldimethyl-*N,N*-diethylamnosilane (2.0 mL) in 8.0 mL of toluene under the pressure of 0.05 kg/cm^2 at 60°C for 3 h.

Recently, Altmaier and Cabrera (31) prepared monolithic columns for HPLC. The authors varied the concentrations of TMOS and PEO. As per the authors, the resulting monoliths showed differences in the macropore and silica skeleton diameter as well as in the corresponding domain sizes. The authors also synthesized all monoliths with a diameter of 4.6 mm and cladded with polyaryletheretherketone (PEEK). Two types of silica gels were synthesized and these were (*i*) ones where the amount of PEO was decreased step-wise to yield monoliths with identical macropore volumes and variations in domain sizes, and (*ii*) ones that were synthesized by adjusting both TMOS and PEO quantities to yield monolithic columns with identical macropore diameters of ~ 1.80 µm but different skeleton diameters and macropore volumes. The scanning electron microscope (SEM) images of silica gels are given in Figure 1, indicating the effect of homogeneity due to the amount of PEO. A regularly ordered silica monolith with a coral-like structure and large macropores with high quantities of PEO caused the production of columns with a much more inhomogeneous, spongy scaffold with small macropores. The efficiency and permeability were tested by using a mobile phase of *n*-heptane–dioxane (95:5, v/v). As per the authors, the chromatographic results suggested that an increase in the column performance cannot be achieved by decreasing the domain size of a given column. Xu et al. (32) described a monolithic octadecyl silane (ODS) silica gel column by saturating it with lithium dodecylsulfate (Li-DS). Mukai et al. (33) described the formation of monolithic silica gel microhoneycombs using the pseudo-steady state growth of microstructural ice crystals.

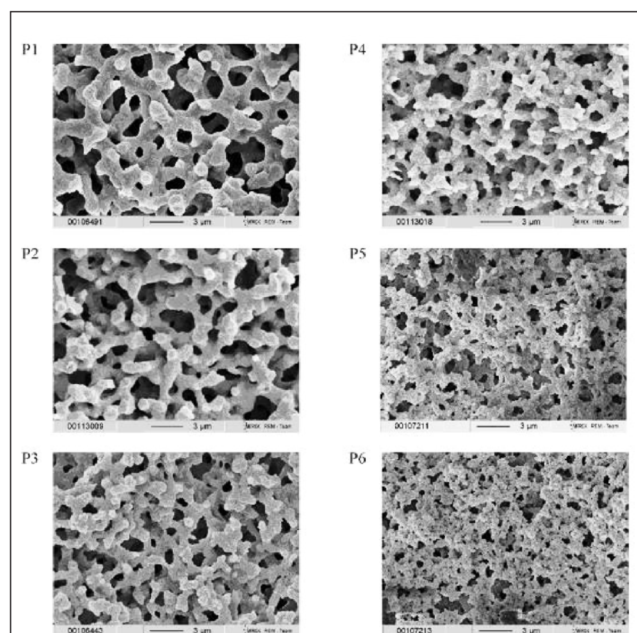

Figure 1. SEM images of P1–P6 monolithic silica gels (31).

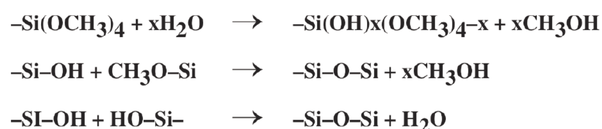

Figure 2. The hydrolysis and polycondensation reactions of TMOS (34).

Preparation of Chromolith Columns

The monolithic silica columns can be prepared either in a mold (polymeric cladding material) similar to the size of conventional HPLC column or in a fused silica capillary. The preparation in a mold is carried out by volume reduction of the whole structure. Normally, straight monolithic columns cannot be more than 15 cm long. The resulting silica monoliths are covered with PTFE tubing or with PEEK resin to fabricate a column for HPLC. PTFE-covered monoliths (MS-PTFE) are used in an external pressurizing device. The PEEK-covered columns (MS-PEEK) can withstand for a long time. This type of monolithic silica column (i.e., Chromolith) is commercially available in 5- and 10-cm length. Gao et al. (34) prepared a biporous monolithic silica gel column, showing both µm-sized through pores and nanometer-sized mesopores located in a silica skeleton. A high

Table I. The Effect of the Different TMOS/PEG Ratio on the Monolithic Silica Samples (34)*

TMOS/PEG ratio	7.1	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.7
Skeleton size (µm)	AGP†	2.5	2.0	1.5	1.2	0.9	0.6	0.3	GM‡
Through-pore size (µm)	AGP†	4.3	3.0	2.5	2.0	1.2	0.8	0.4	GM‡
Linear shrinkage ratio (%)	25.8	25.9	26.6	27.0	27.9	27.2	26.2	24.6	crack

* The contents of TMOS and 0.01 M acetic acid are 28.4 and 52.0 mL, respectively. Mesopores were not fabricated in the batch experiment.
† AGP = Aggregate of gel particles.
‡ GM = Gel matrix.

concentration of progen (2.0 M ammonium hydroxide solution) was used to increase the mesopore size. The authors studied the effect of PEO and reported that a lower concentration was suitable for forming the interconnected porous structure for the silica gel. The hydrolysis and polycondensation reactions of TMOS are given in Figure 2. The effects of different TMOS/PGE ratios and porogen concentrations on the monolithic silica gel are given in Tables I and II, respectively, indicating different textures of monolithic silica gel.

Preparation of Methacrylate Columns

In addition, monolithic silica gels and methacrylate monolithic columns have been developed and fabricated. The methacrylate monolithic columns are the alternatives tools for developing fast, efficient, and highly productive purification processes for large biomolecules (35,36). Some reviews (7,37–39) have appeared in the literature on methacrylate monolithics, describing the preparation and characterization of methacrylate-based monolithic columns. Like monolithic silica gel, methacrylate monolithic columns also do not need column packing or validation at the production site. Besides, the enhanced mass transfer properties, pressure/flow characteristic, specific permeability, and morphological and structural characteristic features make these monolithic columns ideal for separation and purification purposes. Recently, Barut et al. (40) and Urban and Jandera (18) reviewed the preparation, properties, and application of porous monolithic methacrylate-based polymer. The authors described the design and some other features for the purification of large biomolecules (immunoglobulins G and M, plasmid, DNA, and viral particles). They cited some examples from the bioprocess development schemes for methacrylate-based monolithic columns, which represented a novel technology that emerged ~ 15 years ago (39,41). Methacrylate monoliths are formed via a free radical polymerization in the presence of a precipitant (thermodynamically poor solvent) (42). Normally, monovinyl and divinyl methacrylate monomers are used, and the polymers are formed by solution polymerization precipitate that become insoluble in the reaction medium as a result of both cross-linking and the presence of porogen. The pore size distribution is optimized so that the flow through the medium should be achieved at a reasonable backpressure for chromatographic applications. Therefore, polymerization should be carried out for a sufficient volume of large pores, nanometer range sized pores, and for a high specific surface area. Tennikov et al. (43) described monoliths of different pore sizes and reported an optimal separation of medium size proteins.

Table II. The Effect of the Porogen Concentration on the Structure of Monolithic Silica Samples (34)

Concentration of porogen	2*	1†	0.1†	0.01†	0†
pH of porogen	12.62	12.11	11.12	10.17	6.90
Skeleton size (µm)	0.9	2.0	1.8	1.5	1.5
Through-pore size (µm)	1.0	2.2	2.0	2.0	2.0
Mesopore size (nm)	28	25	18	14	micropores

* Experimental conditions of TMOS–PEG ratios of 8.2 at 120ºC.
† Experimental conditions of TMOS–PEG ratios of 7.8 at 120ºC.

Properties of Monolithic Silica Gel

Basically, the properties of monolithic silica depend on the starting materials and the method of preparation. The size of the silica gel skeleton can be controlled by using different concentrations of TMOS and polyethylene glycol (PEG). Furthermore, it is possible to produce monolithic silica columns of different pores and skeleton sizes ratio. Tanaka et al. (44) described the texture of monolithic silica by means of SEM photographs (Figure 3). The authors reported that both the silica skeletons and the pores are co-continuous and that the domain size decreases with the increase in PEG contents in the starting mixture. Minakuchi et al. (26) described the composition of monolithic silica gel and the types of silica rod, amount of PEG, ammonium hydroxide, mesopores, domain

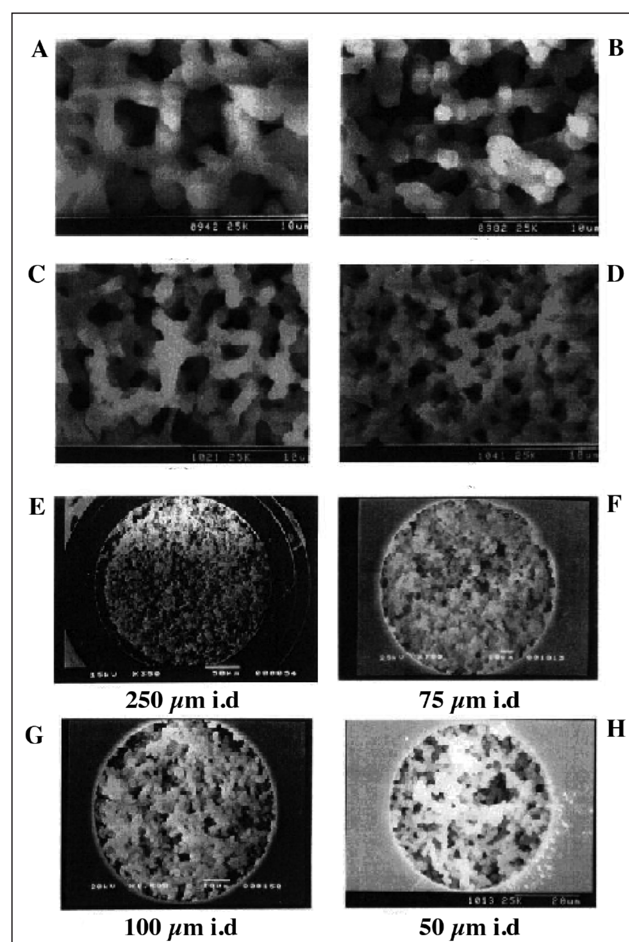

Figure 3. SEM photographs of superficially porous and gigaporous monolithic silica particles showing the silica skeletons (A–D) and with various internal diameters (E–H).

size, and through pore size, which clearly indicates different values of these parameters for different silica rods. Similarly, the porosity of monolithic silica gel columns was studied by Tanaka et al. (45) and Ishizuka et al. (46). The values of porosity of different columns are given in Table III, which clearly indicates a scattered pattern which is responsible for different chromatographic behavior. The porosity of the monolithic silica columns is much greater than that of a particle packed column (45,46).

Table III. The Porosity of Different Monolithic Columns (45,46)

Column type	Particle packed*	Porosity† MS-PTEF(C)-S	MS-PEEK	MS-FS
Column diameter	4.6 mm	7 mm	4.6 mm	100 µm
Column length	15 cm	8.3 cm	10 cm	25 cm‡
Total porosity	0.78	0.86	(0.87)	0.96§
Through-pored	0.39	0.62	(0.69)	0.86
Mesopore	0.40	0.24	(0.18)	0.10

* Develosil C18 particles packed in a column, 4.6 mm diameter, 10 cm in length.
† Porosity of monolithic silica column measured by size exclusion chromatography in THF using polystyrene standards and alkylbenzenes. The porosities in parentheses were obtained with C18 bonded phase.
‡ Effective length between the inlet and the detection window. Total length, 33.5 cm.
§ External porosity.

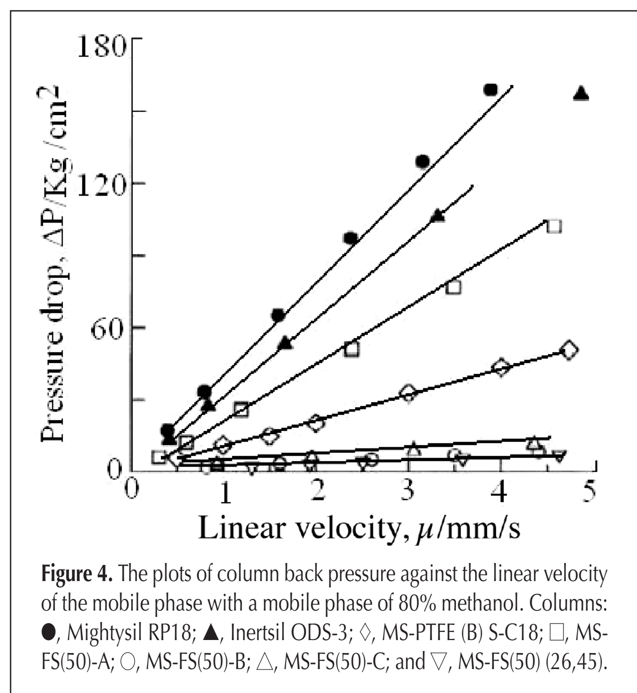

Figure 4. The plots of column back pressure against the linear velocity of the mobile phase with a mobile phase of 80% methanol. Columns: ●, Mightysil RP18; ▲, Inertsil ODS-3; ◊, MS-PTFE (B) S-C18; □, MS-FS(50)-A; ○, MS-FS(50)-B; △, MS-FS(50)-C; and ▽, MS-FS(50) (26,45).

The chromatographic properties of monolithic silica gel depend on large through pore size/skeleton size ratio and high porosities, resulting in high permeability and a large number of theoretical plates per unit pressure drop. Tanaka et al. (26,45) compared the pressure drop of monolithic columns with the packed one (Figure 4), and it was reported that the pressure drop of the MS-PTFE (through pore size 2.2 µm, skeleton size 1.6 µm) was one-fourth of the Mightysil packed column (5 µm ODS particle size). Furthermore, the pressure drop of the MS-FS (50) (through pore size 8.0 µm, skeleton size 2.0 µm) was even lower than the MS-PTFE column. The authors also calculated typical K values (reflecting column permeability) and reported as 4×10^{-13}, 1×10^{-2}, 7×10^{-14}, and 4×10^{-14} m^{-2} for MS-PTFE, MS-FS, and MS-PEEK packed with 5 µm particle size, respectively. As per the authors, these values suggested large-sized through pores and high porosity of monolithic columns. The authors also compared the functionality of the MS-PEEK (10 cm) column with ODS-3 (15 cm) and reported better efficiency of the former column. Similarly, Mihelic et al. (47) studied the pressure drop in convective interaction media (CIM) disk monolithic columns. The authors observed 50% decreases in pressure as compared with columns having the same dimensions. This might be due to the different porous structure, which was quite different in terms of the pore size distribution and parallel pore non-uniformity, compared with the one in conventional packed beds.

Recently, Altmaier and Cabrera (31) prepared ~ 25 pieces of the same material columns in order to get representative data and cladded them with a suitable PEEK polymer in a standardized and optimized manner for the subsequent chromatographic evaluation. The columns were tested under normal-phase conditions using *n*-heptane–dioxane (95:5, v/v) as a mobile phase and 2-nitroanisole as a test compound for the determination of separation efficiency and permeability. The authors prepared six different monoliths (P1–P6) by keeping the amounts of TMOS and acetic acid constant and changing the fraction of PEO introduced into the initial synthesis mixture (Table IV). Sugrue et al. (48) described ion exchange properties of monolithic and particle type iminodiacetic acid modified silica. The peak efficiencies for most metal ions were of a similar order for both column types, except for Zn(II), which showed significant peak broadening on the IDA monolithic column. Lubda et al. (49) studied comprehensive pore structure characterization of silica monoliths with controlled mesopore size and macropore size by nitrogen sorption, mercury porosimetry, transmission electron microscopy, and inverse size exclusion chromatography.

Table IV. The Structural and Chromatographic Data of Six Different Silica Monoliths Synthesized by PEO (Increasing from P1 to P5) (31)

Sample name	Skeleton dia. (µm)	Macropore dia. (µm)	Domain size (µm)	Back-pressure (bar)	Plate number N (1/m)	Plate height Hmin (µm)
P1	1.25	2.03	3.28	12.0	93 000	10.7
P2	1.05	1.77	2.83	14.0	100 000	10.0
P3	0.82	1.20	2.02	32.0	106 000	9.4
P4	0.78	1.15	1.93	42.0	107 000	9.3
P5	0.63	0.92	1.55	67.0	135 000	7.4
P6	0.50	0.56	1.06	170.0	58 000	17.2

The hydrodynamic characteristics of methacrylate monoliths depend on their structure (i.e., porosity, pore size, and pore

size distribution), which are controlled by the preparation/polymerization of the monolith. Normally, methacrylate monolithic columns are characterized by short column length and an extremely high surface-to-volume ratio. Even small scale synthesized monolithic silica capillaries (SMCs) rigid disks are few millimeters long and can be used for a variety of complex separations. The pressure drop is the linear velocity, which is directly proportional to pressure drop. Bencina et al. (50) reported the effect of large molecule size (i.e., DNA) and reported that pore size of ~ 1.5 µm is quite good for this purpose. Minakuchi et al. (45) and Tanaka et al. (44) reported SEM image of methacrylate monoliths, indicating small particle agglomerates transected by large pore channels. The specific surface area (m^2/g), pore volume (mL/g), median pore radius (nm), porosity (%), and equivalent particle diameter (µm) are 7.19, 1.35, 750, 64, and 0.75, respectively.

Applications of Monolithic Phases

Due to their unique features as discussed earlier, monolithic silica- and polymer-based columns have been used in HPLC. A few reports are also available in CEC. The applications of these monolithics in HPLC and CEC are discussed next.

Applications in HPLC

During last few decades, monolithic columns have been used for fast, sensitive, and reproducible analyses of many compounds, especially for large molecules. Several computational studies were performed to determine the optimum (relative) geometry of monolithic column materials for their application in HPLC (51,52). The parameters that are crucial for a high chromatographic performance of these systems are mesopore, macropore, and skeleton diameter, as well as the homogeneity of these variables, especially a small pore size distribution (53–57). Sutton and Nesterenko (58) described the separation of aromatic hydrocarbons in petroleum fraction by normal-phase mode using bare silica and aminopropyl-modified silica Chromolith-type monolithic columns. Miyabe et al. (59) studied the kinetic parameter concerning mass transfer in silica monolithic and particulate stationary phases measured by the peak parking and slow elution methods.

The methacrylate monolithics have been optimized for the purification of large molecules as exhibited by the highest capacity among other resins. This has been exemplified on the purification of large proteins, DNA, and viruses. Zmak et al. (60) separated various macro-molecules on methacrylate monolithic columns (Figure 5). A perusal of this figure indicates that all the molecules are baseline separated using different mobile phases. The flow did not affect the properties of small methacrylate monolithic columns, which enabled the performance of extremely fast analyses (in seconds). Hence, monolithic columns can be considered as versatile chromatographic sensors. Kramberger et al. (61) and Oulette (62) concentrated and separated plant and adenovirus, respectively. Smrekar et al. (63) presented a strategy for the purification and concentration of the bacteriophage T4 with the SMCs (Figure 6). As per the authors, the methods were very robust and reproducible, giving phage recoveries between 60% and 70% with a relative standard deviation of 9%. Branovic et al. (64) reported quality control for Immunoglobulin G (IgG) by using SMCs. Barut et al. (17) described the analytical applications of a monolithic column of 3–5 mm length with a small diameter. The authors discussed the capacities of these columns in protein separations (Figure 7). The flow-rates can range from 0.5 to 10.0 mL/min. Ali et al. used a Chromolith RP-18e mono-

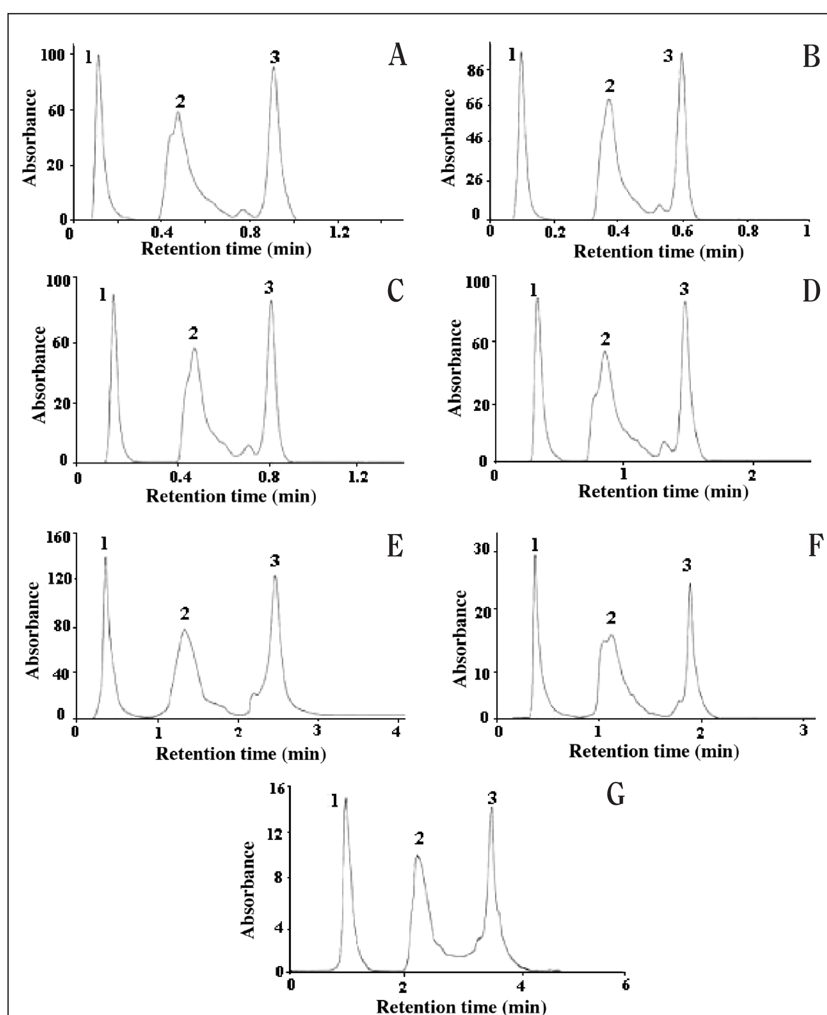

Figure 5. The chromatograms of standard protein mixture on axial and radial monolithic columns. Mobile phases: buffer A, 20 mM Tris–HCl buffer, pH 7.4; buffer B, 20 mM Tris–HCl buffer + 0.7 mol/L NaCl, pH 7.4. Columns: CIM diethylaminoethyl (DEAE; weak anion exchange) disk monolithic columns; 0.34 mL (A), 0.68 mL (B), 1.02 mL (C), 1.36 mL (D); and CIM tube monolithic columns with the monolith volumes: 8 mL (E), 80 mL (F), and 800 mL (G). Detection at 280 nm. Sample: myoglobin (first peak), conalbumin (second peak), trypsin inhibitor (third peak) (60).

lithic column and separated various compounds such as haloperidol (65,66), tadalafil (67), phenols (68), and chloramphenicol (69) in various matrices. The authors optimized the separations of these compounds by varying mobile phase compositions, pH, and flow-rates. Figure 8 (68) indicates the effect of the flow-rates (1 to 10 mL/min) on the separation pattern of phenols. It was observed that the studied phenols were resolved successfully at all flow-rates (1.0 to 10.0 mL/min) and the peaks were slightly broad at 1.0 and 2.0 mL/min flow-rates. It is interesting to note that the detection was poor at flow-rates ranging from 4.0 to 10.0 mL/min and, therefore, 3.0 mL/min was selected as the optimum and most suitable flow-rate. It is also interesting to observe from Figure 8 that the capacity factors changed rapidly from 1.0 to 4.0 mL/min while these become almost constant from 5.0 to 10.0 mL/min flow-rate. These observations lead to a high Eddy diffusion and high mass transfer of phenols in the stationary phase; at high flow-rates, the reverse is true. Kalashnikova and co-workers (70) studied influenza vaccine and virus-like synthetic particles as model objects on monolithic columns (CIM disks). The maximum value of adsorption capacity was registered for a monolithic disk, modified subsequently by chitosan and 2,6-sialyllactose and found to be equal to 6.9×10^{12} virions/mL support.

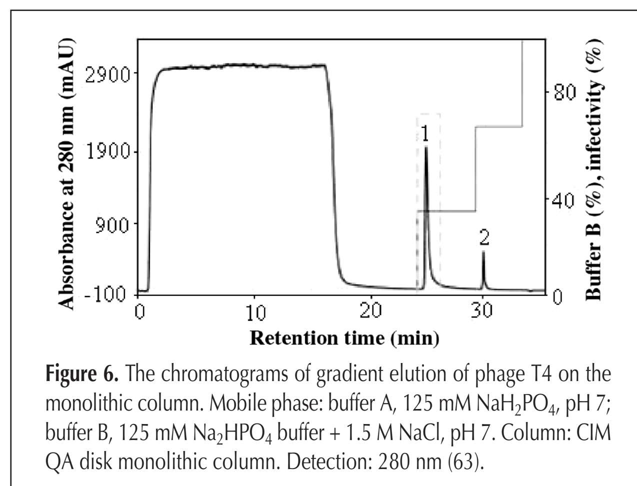

Figure 6. The chromatograms of gradient elution of phage T4 on the monolithic column. Mobile phase: buffer A, 125 mM NaH_2PO_4, pH 7; buffer B, 125 mM Na_2HPO_4 buffer + 1.5 M NaCl, pH 7. Column: CIM QA disk monolithic column. Detection: 280 nm (63).

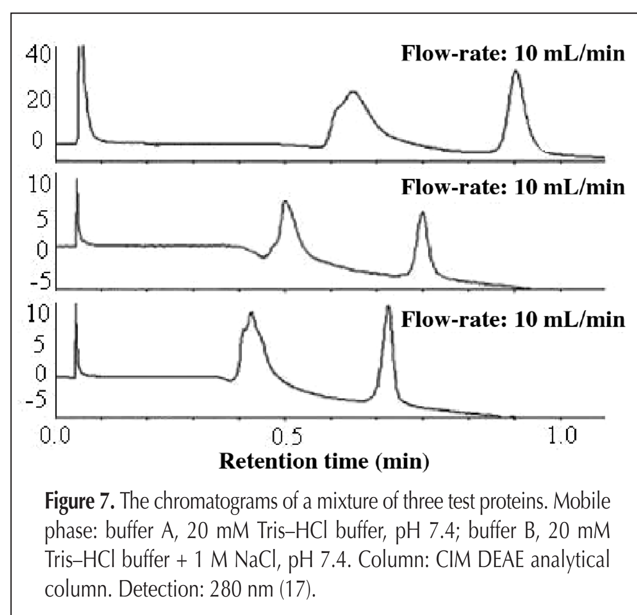

Figure 7. The chromatograms of a mixture of three test proteins. Mobile phase: buffer A, 20 mM Tris–HCl buffer, pH 7.4; buffer B, 20 mM Tris–HCl buffer + 1 M NaCl, pH 7.4. Column: CIM DEAE analytical column. Detection: 280 nm (17).

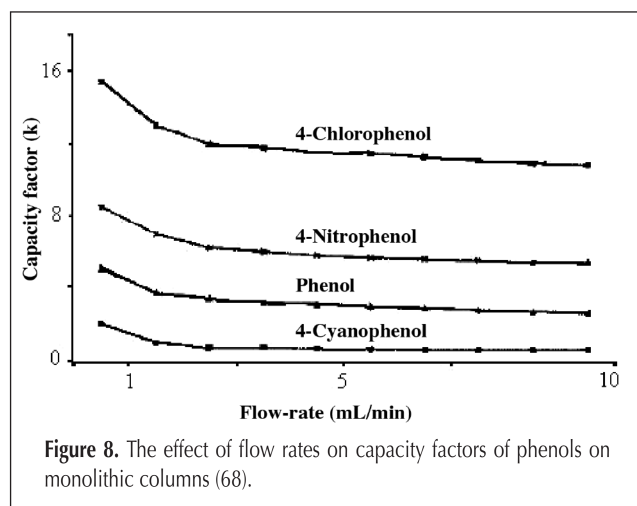

Figure 8. The effect of flow rates on capacity factors of phenols on monolithic columns (68).

Satínský et al. (71) determined ambroxol hydrochloride, methylparaben, and benzoic acid in pharmaceutical preparations on a sequential injection technique coupled with a monolithic column. The porous monolithic columns (Chromolith SpeedROD RP-18e, 50–4.6 mm column with 10 mm) showed high performance at relatively low pressure. The mobile phase used was acetonitrile–tetrahydrofuran–0.05 M acetic acid (10:10:90, v/v), with pH 3.75 (adjusted with triethylamine) and a flow-rate of 0.48 mL/min. As per the authors, the method was found to be useful for the routine analysis of the reported compounds in various pharmaceutical syrups and drops. Recently, Gomez et al. (72) described flow through low pressure chromatographic separations on the sequential injection chromatographic (SIC) concept coupled to second order multivariate regression models based on multivariate curve resolution-alternating least-squares (MCR–ALS) on short monolithic columns along with isocratic elution. To ascertain the improved peak capacity of the SIC–MCR–ALS procedure, five phenolic compounds commonly used in disinfectant products and featuring similar UV spectra and close retention times in short reversed-phase silica-based monolithic phases were selected as model compounds by the authors. Zacharis et al. (73) carried out a sequential injection analysis on monolithic strong anion-exchanger column for on-line drug–protein interaction studies. Ciprofloxacin was selected as a model drug with BSA. The effect of incubation time was studied and on-line binding assays and binding constants were determined as $(3.16 \pm 0.21) \times 10^6$ and $(1.27 \pm 0.48) \times 10^4$ per moles. As per the authors, the results were evaluated for the determination of accuracy of the developed method. The authors compared their results with ultra-filtration experiments and found in good agreement.

Applications in CEC

Besides HPLC, monolithic capillaries are more effective and efficient for the separation of different molecules. An increase in efficiency was observed in CEC compared to pressure-driven elution, indicating A-term contribution responsible for poor efficiency of the column in HPLC. Several other data on the

correlation of column performance and variation of the domain sizes were obtained utilizing monolithic silica capillaries (28). So far, the best results were achieved with a capillary possessing a domain size of 2.2 µm and a corresponding H_{min} of 5 µm (29). It is possible to generate 50,000 to 1,00,000 theoretical plates in capillaries of 20–30 cm length in CEC (74). Moravcová et al. (75) compared the chromatographic properties of the organic polymer monolithic columns with those of commercial silica-based particulate and monolithic capillary. As per the authors, organic polymer monolithic capillary columns showed similar retention behavior to chemically bonded alkyl silica columns for compounds with different polarities characterized by interaction indices but had low methylene selectivities and did not show polar interactions with sulphonic acids. Puy et al. (76) synthesized SMCs by a sol-gel process. These capillaries were evaluated in electro-chromatography and showed high efficiencies (H = 5 µm). The effect of skeleton size on the EOF was investigated with unmodified SMCs used under various experimental conditions. Ishizuka et al. (46) prepared and used a monolithic silica column in a capillary under pressure-driven and electro-driven conditions. The considerable dependence of column efficiency on the linear velocity of the mobile phase was observed. As per the authors, the performance of the continuous silica capillary column in the electro-driven mode was much better than that in pressure-driven mode. Breadmore et al. (77) described microchip-based sol-gel phases for capillary electro-chromatography for monolithic silica gel. The chromatographic performance of the monolithic columns was evaluated by ion-exchange electro-chromatography, with ion-exchange sites introduced via dynamic coating with the cationic polymerpoly(diallyldimethylammonium chloride).

Gatschelhofer et al. (78) described chiral separation of glycyl-dipeptides by CEC using particle-loaded monoliths; prepared by ring opening metathesis polymerization. The chiral selectors used were teicoplanin aglycone, which was chemically bonded to silica gel. Chankvetadze et al. (79) modified monolithic capillary of silica gel with cellulose *tris*-(3,5-dimethylphenylcarbamate) and used for enantio-separations. High efficiency enantioseparations of several chiral drugs were achieved in a short time. The baseline enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol was achieved in less than 30 s.

Table V. Applications of Monolithic Phases in HPLC and CEC

Compounds	Stationary phases	Mobile phases	Refs.
HPLC applications			
Alkylbenzenes	Monolithic silica	90% acetonitrile–10% Tris–HCl buffer	44
Bacteriophage T4	Monolithic silica	—	63
IgG	Monolithic silica	—	64
Toulene, nitrobenzene, & 2-nitroanisole	Monolithic silica	*n*-Heptane–dioxane (95:5)	31
Glycyltyrosine, leucine-enkephalin, insulin, cytochrome C, lysozyme, transferrin, BSA, β-blactoglobulin, & ovalbumin	Monolithic silica	MeCN–H_2O–TFA (60:40:0.1)	82
Hydroxide ion	Monolithic silica gel	10 mM sodium sulfate eluent at pH 8.2	83
Pectin methylesterases & polygalacturonases	Monolithic columns	0 to 500 mM NaCl with 20% acetonitrile	84
Low abundance membrane proteins	Monolithic columns	—	85
DNA	Monolithic columns	—	86
DNA	Monolithic columns	—	87
Pectin methylesterase isoenzymes	Monolithic columns	0 to 700 mM NaCl	88
H^+, Na(I), NH^4(I), K(I), Mg(II), & Ca(II)	Monolithic ODS silica gel	5 mM EDTA-2K with 0.10 mM Li-DS (pH 4.80)	32
Zonisamide (1,2-benzisoxazole-3-methanesulfonamide, ZNS), & its raw material (1,2-benzisoxazole-3-methanecarbonic acid) & intermediate (sodium 1,2-benzisoxazole-3-methanesulfonate)	Monolithic ODS-silica gel column dynamically coated with cetylpyridinium chloride	40 mM sodium perchlorate (pH 7.0)–acetonitrile (90:10)	89
Macromolecules	Methacrylate monolithic	Different mobile phases	60
Tumor necrosis factor-α-(TNF-α) analog LK-801 & green fluorescence protein with 6 histidine tag (GFP-6His)	Metal-chelate methacrylate monoliths-CIM	—	90
Microbial enzymes	CIM monolithic column	—	91
DNA isolation	CIM monolithic columns	—	92
Plant viruses	CIM disk monolithic columns	—	93
Tomato pectin methylesterase & polygalacturonase	Monolithic methacrylate disk columns	20% of acetonitrile	94
IgM* from IgG	Ion-exchange methacrylate monoliths	—	95
PAHs†	Chromolith RP-18e	—	58
Uracil, pyridine aniline, ethylaniline, quinine, nortriptyline, & benzene	Chromolith RP-18e	MeCN–H_2O (40:60)	96

* Immunoglobulin M.
† Polynuclear aromatic hydrocarbons.

Table V. (continued) Applications of Monolithic Phases in HPLC and CEC

Compounds	Stationary phases	Mobile phases	Refs.
Cyclooxygenase II inhibitors rofecoxib & 3-isopropoxy-4-(4-methanesulfonylphenyl)-5,5'dimethyl-5H-furan-2-one	Chromolith RP-18e	Different ratio of phosphate buffer & acetonitrile	97
Chloramphenicol	Chromolith RP-18e	Phosphate buffer (100 mM, pH 3.0)–acetonitrile (75:25)	69
Haloperidol		Chromolith RP-18e Phosphate buffer (100 mM, pH 3.0)–acetonitrile (70:30)	65,66
Tadalafil		Chromolith RP-18e Phosphate buffer (100 mM, pH 3.0)–acetonitrile (80:20)	67
Phenols	Chromolith RP-18e	water–acetonitrile (80:20)	68
Alkylbenzene	MS-PEEK	MeOH–water (80:20)	30
Alkylbenzene	MS-PEEK	MeOH–water (80:20)	44
Nimesulide, tetracycline, ethylbenzene, butylbenze, o-terphenyl, amylbenzene, & triphenylene)	Speed ROD	MeCN–$NH_4H_2PO_4$, 1.15 g/L, alkylbenzene (toluene, pH 7.0 (30:70)	98
IgG, HAS, & IgM	CIM QA, CIM DEAE, & CIM EDA	20 mM phosphate buffer pH 7.2 + 1 M NaCl	50
Digested proteins	A trypsin-immobilized monolithic silica	–	99
Aluminium	Diethylamine monolithic disk	100% buffer A (0.05 M Tris–HCl + 0.03 M $NaHCO_3$) to 100% buffer B (A + 1M NH_4Cl)	100
CEC Applications			
Alkylbenzenes	MS-FS(50)D	MeCN–Tris–HCl buffer (50 mM, pH 8.0), (80:20)	44
Hexylbenzene	Fused-silica capillary containing	MeCN–H_2O (90:10) continuous macroporous silica gel	46
PAHs	Monolithic capillaries	MeCN–4 M sodium acetate, pH 7.4 with 1 mM SDS	101
Chiral Resolutions			
Glycyl-dipeptides	Teicoplanin aglycone monolithic silica	–	78
2,2,2-Trifluoro-1-(9-anthryl)ethanol	Tris-(3,5-dimethylphenyl carbamate) monolithic silica	–	79
Chiral drugs	3,5-Disubstituted phenylcarbamates of cellulose and amylose monolithic silica	–	80

* Immunoglobulin M.
† Polynuclear aromatic hydrocarbons.

Furthermore, the same group (80) developed monolithic capillary columns having silica gel covalently bonded with 3,5-disubstituted phenylcarbamate derivatives of cellulose and amylose. The authors used these capillaries for chiral separations. The effects of the type of polysaccharides and the substituents, as well as of multiple covalent immobilization of polysaccharide derivatives, were studied. As per the authors, the capillary columns obtained using this technique were stable in all solvents and exhibited promising enantiomer-resolving ability. Other applications of monolithic phases are given in Table V.

Monolithic silica gel in HPLC vs CEC

Of course, monolithic phases are effective and efficient phases for macromolecules. There are some papers on analytical scale using monolithic columns with good results in both HPLC and CEC. However, some band broadening in HPLC of monolithic silica columns was observed due to slow mass transfer, which does not occur in CEC. The theoretical plate numbers in CEC were found to be 3 to 4 times greater in CEC than in HPLC (Figure 9) (46). Besides, CEC utilized high permeability of monolithic silica columns. The authors compared the efficiency of monolithic silica gel in HPLC and CEC modes. The monolithic silica capillary columns provided high permeability; the pressure-driven operation at a very low pressure can afford a separation speed similar to CEC at a high electric field. Under most favorable conditions, the MS-FS (50) column (25 cm) showed ~ 80,000 theoretical plates in 80% acetonitrile–20% aqueous buffer (pH 8) (81).

A comparison of monolithic silica columns with other particle-based columns is shown in Figure 10 (82) at a mobile phase velocity of 4.0 mm/s and gradient time of 5 min for the separation of polypeptides. It was observed that the efficiency of monolithic column was almost similar with small non-porous particles but much higher than that of conventional 5 µm particle size. Besides, monolithic silica columns showed similar selectivities with higher performance than particle-packed columns. These authors compared the performance of monolithic columns in HPLC and CEC, and the chromatograms are given in Figure 9. The studied compounds were alkylbenzenes in 90% acetonitrile–10% Tris-HCl buffer. The theoretical plates were 50,000 and 16,000 in HPLC and CEC, respectively. The monolithic silica in a capillary produced a much higher efficiency than in the pressure-

driven mode. Besides, Figure 9 indicates that the peaks are sharp in CEC with good separations. Of course, the use of an HPLC column requires a longer separation time than CEC to achieve similar efficiency as the former has less practical difficulty and is easy to hyphenate with an MS detector. Briefly, the separation power of CEC is higher than HPLC, but the former cannot be used at preparative scale.

Future Perspectives and Conclusion

To the best of our experience, the future of monolithic columns is quite bright for several reasons. These columns are economical, as they require less costly chemicals and labor. In addition, polymer-based columns can withstand and work under different mobile phases, including drastic conditions. The working capacity and efficiency of these phases for large molecules make them ideal phases. Moreover, these columns do not require packing into PEEK or steel columns, which is a tedious job and requires training/expertise, which might not be available in all labs. All of these points indicate the advantages of monolithic columns.

The applications and other issues related to monolithic phases discussed in this article clearly show that monolithic phases have been used for successful separations of a variety of compounds. They are ideal phases for large molecules but still need more modifications and developments, especially in reversed-phase modes. Normally, these columns have low back-pressure, which requires a mobile flow pump of low pressure capacities. Therefore, these columns may have great applications in sequential injection chromatography. It is desirable to prepare monolithic phases that can produce high electroosmotic flow without affecting the chemical and chromatographic properties of monolithic silica columns. Moreover, monolithic chiral phases are also becoming commercially available because the need of enantiomeric separations is increasing continuously. Recently, Advanced Material Technology, Inc. (Wilmington, DE) introduced Halo columns (silica particle of 2.7 µm size and manufactured by fused core technology). These columns have good load abilities with fast speed and high back-pressures, so they have a good future. Therefore, there will probably be a tight competition of monolithics with Halo columns in near future. Briefly, the future will decide the applications, importance, and market of monolithic columns.

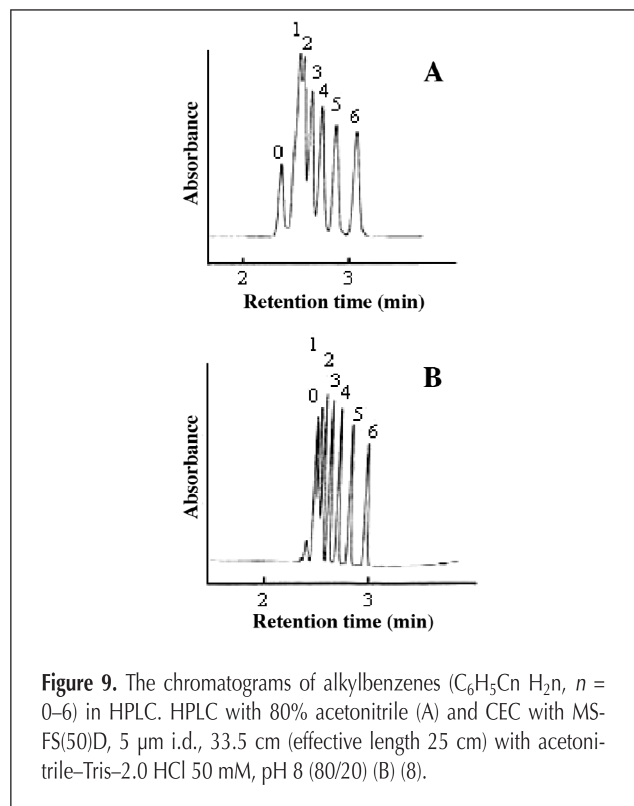

Figure 9. The chromatograms of alkylbenzenes ($C_6H_5Cn\ H_2n$, n = 0–6) in HPLC. HPLC with 80% acetonitrile (A) and CEC with MS-FS(50)D, 5 µm i.d., 33.5 cm (effective length 25 cm) with acetonitrile–Tris–2.0 HCl 50 mM, pH 8 (80/20) (B) (8).

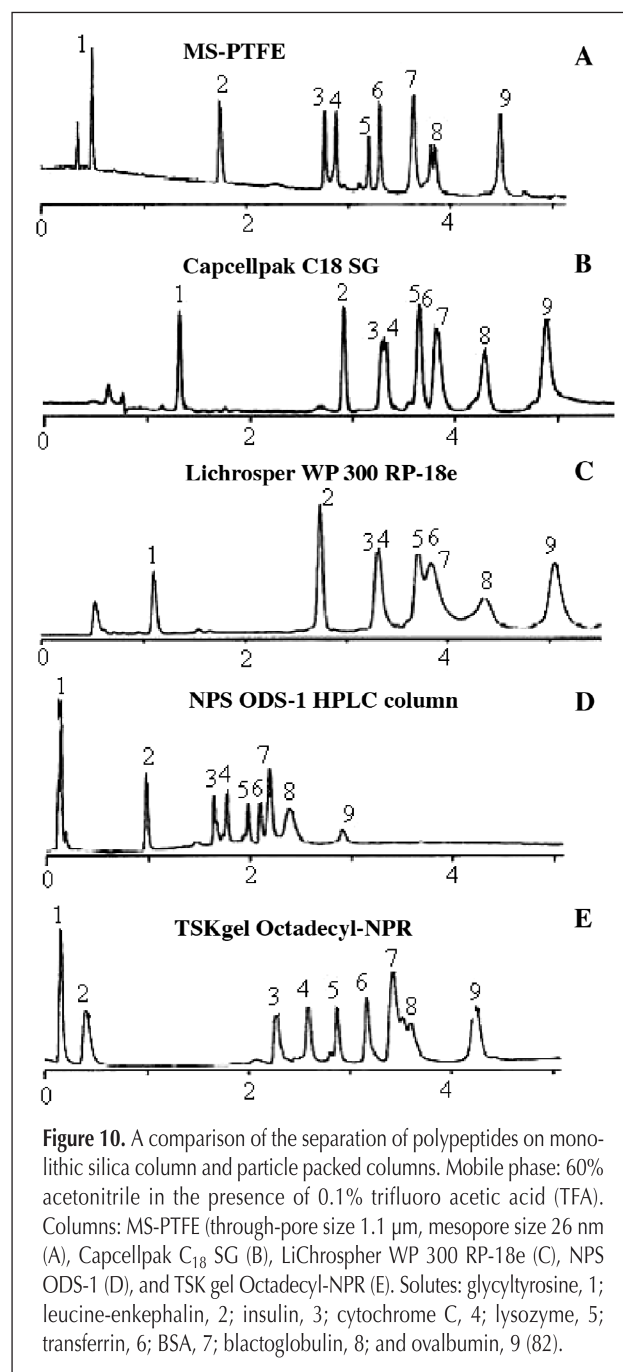

Figure 10. A comparison of the separation of polypeptides on monolithic silica column and particle packed columns. Mobile phase: 60% acetonitrile in the presence of 0.1% trifluoro acetic acid (TFA). Columns: MS-PTFE (through-pore size 1.1 µm, mesopore size 26 nm (A), Capcellpak C_{18} SG (B), LiChrospher WP 300 RP-18e (C), NPS ODS-1 (D), and TSK gel Octadecyl-NPR (E). Solutes: glycyltyrosine, 1; leucine-enkephalin, 2; insulin, 3; cytochrome C, 4; lysozyme, 5; transferrin, 6; BSA, 7; blactoglobulin, 8; and ovalbumin, 9 (82).

References

1. S.D. Rogers and J.G. Dorsey. Chromatographic silanol activity test procedures: the quest for a universal test. *J. Chromatogr. A* **892:** 57–65 (2000).
2. L.R Synder, J.J. Kirkland, and J.L. Glajch. The Column in Practical HPLC Method Development. John Wiley & Sons Inc., New York, NY, 1997, pp. 174–232.
3. J.E. McNair, K.C. Lewis, and J.W. Jorgenson. Ultra-high pressure reversed-phase liquid chromatography in packed capillary columns. *Anal. Chem.* **69:** 983–89 (1997).
4. M.M. Dittmann and G.P. Rozing. Capillary electrochromatography—A high efficiency microseparation technique. *J. Chromatogr. A* **744:** 63–74 (1996).
5. P.P.H. Tock, P.P.E Duijsters, J.C. Kraak, and H. Poppe. Theoretical optimization of open tubular columns for liquid chromatography with respect to mass loadability. *J. Chromatogr. A* **479:** 200–208 (1990).
6. K. Cabrera, D. Lubda, H.M. Eggenweiler, H. Minakuchi, and K. Nakanishi. A new monolithic-type HPLC column for fast separations. *J. High Resolut. Chromatogr.* **23:** 93–99 (2000).
7. D. Josic, A. Buchacher, and A. Jungbauer. Monoliths as stationary phases for separation of proteins and polynucleotides and enzymatic conversion. *J. Chromatogr. B* **752:** 191–205 (2001).
8. N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, and T. Ikegami. Monolithic silica columns for high-efficiency chromatographic separation. *J. Chromatogr. A* **965:** 35–49 (2002).
9. A.M. Siouffi. Silica gel-based monoliths prepared by the sol-gel method: facts and figures. *J. Chromatogr. A* **1000:** 801–818 (2003).
10. F. Svec, J.M.J. Frechet, F. Svec, T. B. Tennikova, and Z. Deyl, eds. Monolithic Materials: Preparation, Properties and Applications. Elsevier, Amsterdam, The Netherlands, 2003.
11. K. Cabrera. Applications of silica-based monolithic HPLC columns. *J. Sep. Sci.* **27:** 843–852 (2004).
12. A. Podgornik and A. Strancar. Convective interaction media (CIM)—short layer monolithic chromatographic stationary phases. *Biotechnol. Ann. Rev.* **11:** 281–333 (2005).
13. F. Svec. Recent developments in the field of monolithic stationary phases for capillary electrochromatography. *J. Sep. Sci.* **28:** 729–745 (2005).
14. F. Svec and C.G. Huber. Monolithic materials: promises, challenges, achievements. *Anal. Chem.* **78:** 2100–2107 (2006).
15. H. Kobayashi, T. Ikegami, H. Kimura, T. Hara, D. Tokuda, and N. Tanaka. Properties of monolithic silica columns for HPLC. *Anal. Sci.* **22:** 491–501 (2006).
16. G. Guiochon. Monolithic columns in high-performance liquid chromatography. *J. Chromatogr. A* **1168:** 101–168 (2007).
17. M. Barut, A. Podgornik, L. Urbas, B. Gabor, P. Brne, J. Vidic, S. Plevcak, and A. Strancar. Methacrylate-based short monolithic columns: enabling tools for rapid and efficient analyses of biomolecules and nanoparticles. *J. Sep. Sci.* **31:** 1867–80 (2008).
18. J. Urban and P. Jandera. Polymethacrylate monolithic columns for capillary liquid chromatography. *J. Sep. Sci.* **31:** 2521–2540 (2008).
19. T. Ikegami, K. Tomomatsu, H. Takubo, K. Horie, and N. Tanaka. Separation efficiencies in hydrophilic interaction chromatography. *J. Chromatogr. A* **1184:** 474–503 (2008).
20. O. Nunez, K. Nakanishi, and N. Tanaka. Preparation of monolithic silica columns for high-performance liquid chromatography. *J. Chromatogr. A* **1191:** 231–52 (2008).
21. R. Wu, L. Hu, F. Wang, M. Ye, and H. Zou. Recent development of monolithic stationary phases with emphasis on microscale chromatographic separation. *J. Chromatogr. A* **1184:** 369–92 (2008).
22. H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, and N. Tanaka. Effect of skeleton size on the performance of octadecylsilylated continuous porous silica columns in reversed-phase liquid chromatography. *J. Chromatogr. A* **762:** 135–146 (1997).
23. K. Nakanishi. Pore structure control of silica gels based on phase separation. *J. Porous Mater.* **4:** 67–112 (1997).
24. K. Nakanishi, H. Minakuchi, N. Soga, and N. Tanaka. Double pore silica gel monolith applied to liquid chromatography. *J. Sol-Gel Sci. Technol.* **5:** 547–552 (1997).
25. K. Nakanishi, H. Minakuchi, N. Soga, and N. Tanaka. Structure design of double-pore silica and its application to HPLC. *J. Sol-Gel Sci. Technol.* **13:** 163–169 (1998).
26. H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, and N. Tanaka. Effect of domain size on the performance of octadecylsilylated continuous porous silica columns in reversed-phase liquid chromatography. *J. Chromatogr. A* **797:** 121–31 (1998).
27. K. Nakanishi, H. Shikata, N. Ishizuka, N. Koheiya, and N. Soga. Tailoring mesopores in monolithic macroporous silica for HPLC. *J. High Resolut. Chromatogr.* **23:** 106–110 (2000).
28. M. Motokawa, H. Kobayashi N. Ishizuka, H. Minakuchi, K. Nakanishi, H. Jinnai, K. Hosoya, T. Ikegami, and N. Tanaka. Monolithic silica columns with various skeleton sizes and through-pore sizes for capillary liquid chromatography. *J. Chromatogr. A* **961:** 53–63 (2002).
29. T. Hara, H. Kobayashi, T. Ikegami, K. Nakanishi, and N. Tanaka. Performance of monolithic silica capillary columns with increased phase ratios and small-sized domains. *Anal. Chem.* **78:** 7632–7642 (2006).
30. N. Ishizuka, H. Kobayashi, H. Minakuchi, K. Nakanishi, K. Hirao, K. Hosoya, T. Ikegami, and N. Nakanishi. Monolithic silica columns for high efficiency separations by high performance liquid chromatography. *J. Chromatogr. A* **960:** 85–96 (2002).
31. S. Altmaier and K. Cabrera. Structure and performance of silica-based monolithic HPLC columns. *J. Sep. Sci.* **31:** 2552–2559 (2008).
32. Q. Xu, K. Tanaka, M. Mori, M.I. Helaleh, W. Hu, K. Hasebe, and H. Toada. Monolithic octadecylsilyl-silica gel column for the high-speed ion chromatographic determination of acidity. *J. Chromatogr. A* **997:** 183–90 (2003).
33. S.R. Mukai, H. Nishihara, and H. Tamon. Formation of monolithic silica gel microhoneycombs (SMHs) using pseudosteady state growth of microstructural ice crystals. *Chem. Commun. (Camb.)* **7:** 874–75 (2004).
34. W. Gao, G. Yang, J. Yang, and H. Liu. Formation of the monolithic silica gel column with bimodal pore structure. *Turk. J. Chem.* **28:** 379 (2004).
35. P. Gagnon, F. Hensel, and R. Richieri. Purification of IgM monoclonal antibodies: manufacturing, challenging, surround the use of IgM monoclonal antibodies, but these can be overcome with current technology. *Bio. Pharm. Int.* 26–35 (2008).
36. J. Urthaler, R. Schlegl, A. Podgornik, A. Strancar, A. Jungbauer, and R. Necina. Application of monoliths for plasmid DNA purification: development and transfer to production. *J. Chromatogr. A* **1065:** 93–106 (2005).
37. D. Josic and A. Strancar. Application of membranes and compact, porous units for the separation of biopolymers. *Ind. Eng. Chem. Res.* **38:** 333–342 (1999).
38. F. Svec and J.M.J. Frechet. New designs of macroporous polymers and supports: from separation to biocatalysis. *Science* **273:** 205–11 (1996).
39. J.M.J. Frechet, F. Svec, T.B. Tennikova, and Z. Deyl. Monolithic Materials: Preparation, Properties, and Applications. Elsevier, Amsterdam, The Netherlands, 2003, pp. 19–50.
40. M. Barut, A. Podgornik, L. Urbas, B. Gabor, P. Brne, J. Vidic, S. Plevcak, and A. Strancar. Methacrylate-based short monolithic columns: enabling tools for rapid and efficient analyses of biomolecules and nanoparticles. *J. Sep. Sci.* **31:** 1867–80 (2008).
41. A. Strancar, M.A. Barut, A. Podgornik, P. Koselj, Dj. Josic, and A. Buchacher. Convective interaction media: polymer-based supports for fast separation of biomolecules. *LC-GC* **11:** 660–69 (1998).
42. F. Svec and J.M.J. Frechet. Kinetic control of pore formation in macroporous polymers. Formation of "molded" porous materials with high flow characteristics for separations or catalysis. *Chem. Mater.* **7:** 707–715 (1995).
43. M.B. Tennikov, N.V. Gazdina, T.B. Tennikova, and F. Svec. Effect of porous structure of macroporous polymer supports on resolution in high-performance membrane chromatography of proteins. *J. Chromatogr. A* **798:** 55–64 (1998).
44. N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, and T. Ikegami. Monolithic silica columns for high-efficiency chromatographic separations. *J. Chromatogr. A* **965:** 35–49 (2002).
45. N. Tanaka, H. Nagayama, H. Kobayashi, T. Ikegumi, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Cabrera, and D. Lubda. *J. High Resolut. Chromatogr.* **23:** 111 (2000).
46. N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, and N. Tanaka. Performance of a monolithic silica column in a capillary under pressure-driven and electrodriven conditions. *Anal. Chem.* **72:** 1275–80 (2000).
47. I. Mihelic, D. Nemec, A. Podgornik, and T. Koloini. Pressure drop in CIM disk monolithic columns. *J. Chromatogr. A* **1065:** 59–67 (2005).
48. E. Sugrue, P. Nesterenko, and B. Paull. Ion exchange properties of monolithic and particle type iminodiacetic acid modified silica. *J. Sep. Sci.* **27:** 921–30 (2004).
49. D. Lubda, W. Lindner, M. Quaglia, C. du Fresne von Hohenesche, and K.K. Unger. Comprehensive pore structure characterization of silica monoliths with controlled mesopore size and macropore size by nitrogen sorption, mercury porosimetry, transmission electron microscopy and inverse size exclusion chromatography. *J. Chromatogr. A* **1083:** 14–22 (2005).
50. K. Bencina, M. Bencîna, A. Podgornik, and A. Tramcar. Influence of the methacrylate monolith structure on genomic DNA mechanical degradation, enzymes activity and clogging. *J. Chromatogr. A* **1160:** 176–83 (2007).
51. P. Gzil, N. Vervoort, G.V. Baron, and G. Desmet. A computational study of the porosity effects in silica monolithic columns. *J. Sep. Sci.* **27:** 887–96 (2004).
52. G. Desmet, D. Cabooter, P. Gzil, H. Verelst, D. Mangelings, Y. Vander Heyden, and D. Clicq. Future of high pressure liquid chromatography: do we need porosity or do we need pressure? *J. Chromatogr. A* **1130:** 158–66 (2006).
53. N. Vervoort, P. Gzil, G.V. Baron, and G. Desmet. A correlation for the pressure drop in monolithic silica columns. *Anal. Chem.* **75:** 843–50 (2003).
54. P. Persson, O. Baybak, F. Plieva, I.Y. Galaev, B. Mattiasson, B. Nilsson, and A. Axelsson. Characterization of a continuous supermacroporous monolithic matrix for chromatographic separation of large bioparticles. *Biotechnol. Bioeng.* **88:** 224–36 (2004).
55. N. Vervoort, P. Gzil, G.V. Baron, and G. Desmet. Model column structure for the analysis of the flow and band-broadening characteristics of silica monoliths. *J. Chromatogr. A* **1030:** 177–186 (2004).
56. J. Billen, P. Gzil, and G. Desmet. Domain size-induced heterogeneity as performance limitation of small-domain monolithic columns and other LC support types. *Anal. Chem.* **78:** 6191–6201 (2006).
57. P. Gzil, J. De Smet, and G. Desmet. A discussion of the possible ways to improve the performance of silica monoliths using a kinetic plot analysis of experimental and computational plate height data. *J. Sep. Sci.* **29:** 1675–85 (2006).
58. PA. Sutton and P.N. Nesterenko. Retention characteristics of aromatic hydro-

carbons on silica and aminopropyl-modified monolithic columns in normal-phase HPLC. *J. Sep. Sci.* **30:** 2900–2909 (2007).
59. K. Miyabe, H. Kobayashi, D. Tokuda, and N. Tanaka. A kinetic parameter concerning mass transfer in silica monolithic and particulate stationary phases measured by the peak-parking and slow-elution methods. *J. Sep. Sci.* **29:** 2452–62 (2006).
60. P. Milavec Zmak, H. Podgornik, J. Jancar, A. Podgornik, and A. Strancar. Transfer of gradient chromatographic methods for protein separation to convective interaction media monolithic columns. *J. Chromatogr. A* **1006:** 195–205 (2003).
61. P. Kramberger, M. Peterka, J. Boben, M. Ravnikar, and A. Tramcar. Short monolithic columns—A breakthrough in purification and fast quantification of tomato mosaic virus. *J. Chromatogr. A* **1144:** 143–149 (2007).
62. T. Oulette. Chromatographic applications for viruses and recombinant proteins using convective interaction media. Lecture presented at the Second Monolith Summer School, Portoroz, Slovenia, May 28–31 (2006).
63. F. Smrekar, M. Ciringer, M. Peterka, A. Podgornik, and A. Tramcar. Purification and concentration of bacteriophage T4 using monolithic chromatographic supports. *J. Chromatogr. B* **861:** 177–80 (2008).
64. K. Branovic, G. Lattner, M. Barut, A. Trancar, Dj. Josic, and A. Buchacher. Very fast analysis of impurities in immunoglobulin concentrates using conjoint liquid chromatography on short monolithic disks. *J. Immunol. Methods* **271:** 47–58 (2002).
65. H.Y. Aboul-Enein, I. Ali, and H. Hoenen. Rapid determination of haloperidol and its metabolites in human plasma by HPLC using monolithic silica column and solid phase extraction. *Biomed. Chromatogr.* **20:** 760–64 (2006).
66. I. Ali and H.Y. Aboul-Enein. Fast determination of haloperidol in pharmaceutical preparations using HPLC on monolithic silica column. *J. Liq. Chromatogr. Relat. Technol.* **28:** 3169–79 (2005).
67. H.Y. Aboul-Enein and I. Ali. Determination of tadalafil in pharmaceutical preparation by HPLC using monolithic silica column. *Talanta* **65:** 276–80 (2004).
68. I. Ali and H.Y. Aboul-Enein. Fast screening of phenol and its derivatives in wastewater by HPLC using monolithic silica column and solid phase extraction. *Anal. Lett.* **37:** 2351–61 (2004).
69. I. Ali, V.K. Gupta, P. Singh, H.V. Pant, and H.Y. Aboul-Enein. Fast screening of chloramphenicol in wastewater by high performance liquid chromatography and solid phase extraction methods. *J. Liq. Chromatogr. Relat. Technol.* **31:** 2862–78 (2008).
70. I. Kalashnikova, N. Ivanova, and T. Tennikova. Development of a strategy of influenza virus separation based on pseudoaffinity chromatography on short monolithic columns. *Anal. Chem.* **80:** 2188–98 (2008).
71. D. Satínský, J. Huclová, R.L. Ferreira, M.C. Montenegro, and P. Solich. Determination of ambroxol hydrochloride, methylparaben and benzoic acid in pharmaceutical preparations based on sequential injection technique coupled with monolithic column. *J. Pharm. Biomed. Anal.* **40:** 287–93 (2005).
72. V. Gómez, M. Miró, M.P. Callao, and V. Cerdà. Coupling of sequential injection chromatography with multivariate curve resolution-alternating least-squares for enhancement of peak capacity. *Anal. Chem.* **79:** 7767–74 (2007).
73. C.K. Zacharis, G.A. Theodoridis, A. Podgornik, and A.N. Voulgaropoulos. Incorporation of a monolithic column into sequential injection system for drug-protein binding studies. *J. Chromatogr. A* **1121:** 46–54 (2006).
74. K.D. Bartle and P. Myers. Theory of capillary electrochromatography. *J. Chromatogr. A* **916:** 3–23 (2001).
75. D. Moravcová, P. Jandera, J. Urban, and J. Planeta. Comparison of monolithic silica and polymethacrylate capillary columns for LC. *J. Sep. Sci.* **27:** 789–800 (2004).
76. G. Puy, C. Demesmay, J.L. Rocca, J. Iapichella, A. Galarneau, and D. Brunel. Electrochromatographic behavior of silica monolithic capillaries of different skeleton sizes synthesized with a simplified and shortened sol-gel procedure. *Electrophoresis* **27:** 3971–80 (2006).
77. M.C. Breadmore, S. Shrinivasan, K.A. Wolfe, M.E. Power, J.P. Ferrance, B. Hosticka, P.M. Norris, and J.P. Landers. Towards a microchip-based chromatographic platform. Part 1: evaluation of sol-gel phases for capillary electrochromatography. *Electrophoresis* **23:** 3487–95 (2002).
78. C. Gatschelhofer, M.G. Schmid, K. Schreiner, T.R. Pieber, F.M. Sinner, and G. Gübitz. Enantioseparation of glycyl-dipeptides by CEC using particle-loaded monoliths prepared by ring-opening metathesis polymerization (ROMP). *J. Biochem. Biophys. Methods* **69:** 67–77 (2006).
79. B. Chankvetadze, C. Yamamoto, N. Tanaka, K. Nakanishi, and Y. Okamoto. High-performance liquid chromatographic enantioseparations on capillary columns containing monolithic silica modified with cellulose tris(3,5-dimethylphenylcarbamate. *J. Sep. Sci.* **27:** 905–11 (2004).
80. B. Chankvetadze, T. Kubota, T. Ikai, C. Yamamoto, M. Kamigaito, N. Tanaka, K. Nakanishi, and Y. Okamoto. High-performance liquid chromatographic enantioseparations on capillary columns containing cross linked polysaccharide phenylcarbamate derivatives attached to monolithic silica. *J. Sep. Sci.* **29:** 1988–95 (2006).
81. H. Kobayashi, C. Smith, K. Hosoya, T. Ikegami, and N. Tanaka. Capillary electrochromatography on monolithic silica columns. *Anal. Sci.* **18:** 89 (2002).
82. H. Minakuchi, N. Ishizuka, K. Nakanishi, N. Soga, and N. Tanaka. Performance of an octadecylsilylated continuous porous silica column in polypeptide separations. *J. Chromatogr. A* **828:** 83–90 (1998).
83. Q. Xu, M. Mori, K. Tanaka, M. Ikedo, W. Hu, and P.R. Haddad. Ion chromatographic determination of hydroxide ion on monolithic reversed-phase silica gel columns coated with nonionic and cationic surfactants. *J. Chromatogr. A* **1041:** 95–99 (2004).
84. I. Vovk and B. Simonovska. Separation of pectin methylesterases and polygalacturonases on monolithic columns. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* **849:** 337–43 (2007).
85. M. Rucevic, J.G. Clifton, F. Huang, X. Li, H. Callanan, D.C. Hixson, and D. Josic. Use of short monolithic columns for isolation of low abundance membrane proteins. *J. Chromatogr. A* **1123:** 199–204 (2006).
86. M. Brgles, B. Halassy, J. Tomasic, M. Santak, D. Forcic, M. Barut, and A. Strancar. Determination of DNA entrapment into liposomes using short monolithic columns. *J. Chromatogr. A* **1144:** 150–54 (2007)
87. D. Forcic, K. Branovic-Cakanic, J. Ivancic, R. Jug, M. Barut, and A. Strancar. Purification of genomic DNA by short monolithic columns. *J. Chromatogr. A* **1065:** 115–20 (2005).
88. I. Vovk, B. Simonovska, and M. Bencina. Separation of pectin methylesterase isoenzymes from tomato fruits using short monolithic columns. *J. Chromatogr. A* **1065:** 121–28 (2005).
89. J. Li, G. Wu, and Y. Zhu. Determination of zonisamide by a coated monolithic column. *J. Chromatogr. A* **1118:** 151–54 (2006).
90. M. Peterka, M. Jarc, M. Banjac, V. Frankovic, K. Bencina, M. Merhar, V. Gaberc-Porekar, V. Menart, A. Strancar, and A. Podgornik. Characterisation of metal-chelate methacrylate monoliths. *J. Chromatogr. A* **1109:** 80–85 (2006).
91. K. Isobe and Y. Kawakami. Application of two types of CIM tube column for purification of microbial enzymes. *J. Chromatogr. A* **1065:** 129–34 (2005).
92. S. Jerman, A. Podgornik, K. Cankar, N. Cadet, M. Skrt, J. Zel, and P. Raspor. Detection of processed genetically modified food using CIM monolithic columns for DNA isolation. *J. Chromatogr. A* **1065:** 107–13 (2005).
93. P. Kramberger, N. Petrovic, A. Strancar, and M. Ravnikar. Concentration of plant viruses using monolithic chromatographic supports. *J. Virol. Methods* **120:** 51–57 (2004).
94. I. Vovk and B. Simonovska. Isolation of tomato pectin methylesterase and polygalacturonase on monolithic columns. *J. Chromatogr. A* **1144:** 90–96 (2007).
95. P. Brne, A. Podgornik, K. Bencina, B. Gabor, A. Strancar, and M. Peterka. Fast and efficient separation of immunoglobulin M from immunoglobulin G using short monolithic columns. *J. Chromatogr. A* **1144:** 120–125 (2007).
96. D.V. McCalley. Comparison of conventional microparticulate and a monolithic reversed phase column for high efficiency fast liquid chromatography of basic compounds. *J. Chromatogr. A* **965:** 51–64 (2002).
97. P.T. Vallano, R.S. Mazenko, E.J. Woolf, and B.K. Matuszewski. Monolithic silica liquid chromatography columns for the determination of cyclooxygenase II inhibitors in human plasma. *J. Chromatogr. B* **779:** 249–57 (2002).
98. A.M. van Nederkassel, A. Aerts, A. Dierick, D.L. Massart, and Y.V. Heyden. Fast separations on monolithic silica columns: method transfer, robustness and column ageing for some case studies. *J. Pharm. Biomed. Anal.* **32:** 233–49 (2003).
99. S. Ota, S. Miyazaki, H. Matsuoka, K. Morisato, Y. Shintani, and K. Nakanishi. High-throughput protein digestion by trypsin-immobilized monolithic silica with pipette-tip formula. *J. Biochem. Biophys. Methods* **70:** 57–62 (2007).
100. S. Murko, R. Milacic, and J. Scancar. Speciation of aluminum in human serum by convective-interaction media fast-monolithic chromatography with inductively coupled plasma mass spectrometric detection. *J. Inorg. Biochem.* **101:** 1234–41 (2007).
101. J.L. Liao, N. Chen, C. Ericson, and S. Hjerten. Preparation of continuous beds derivatized with one-step alkyl and sulfonate groups for capillary electrochromatography. *Anal. Chem.* **68:** 3468 (1996).

Manuscript received August 22, 2008;
Revision received October 8, 2008;