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# Advances in monolithic silica columns for high-performance liquid chromatography

Gaurav Sharma[1*], Anjali Tara[2] and Vishnu Dutt Sharma[3]

## Abstract

In a monolithic column stationary phase contains a continuous porous material, sealed against the wall of a tube, instead of beads. Due to the decrease in chemical usage, sample usage, improved sensitivity, reusable frit-less columns and less back pressure monolithic column are very popular in the field of capillary electrochromatography (CEC). This review attempts to give an overview of three different types of monolithic columns, i.e. silica-based, polymer-based, and hybrid monolithic column. Moreover, the review also focuses on its advantages and applications over last 5 years. Relevant electronic journals such as Entrez, Science Direct, and different database, namely, PubMed, Scirus, Embase, NIH.gov, Medknow.com, Medscape.com, Scopus, Google Scholar, MedHelp.org, Cochrane Library, WebMD.com, and World Health Organization Hinari were used.

**Keywords:** Monolithic column, Stationary phase, Frit-less column, Column back pressure

## Review

### Introduction

Since late 1970s, particulate columns (5 μm) have been widely used in the field of chromatography. The decrease in the particle size gives better column efficiency but results in a higher back pressure. With the advent of monolithic columns, a higher column efficiency can be provided with minimum back pressure. Georges Guiochon about monolithic column stated that "The recent invention and development of monolithic columns is a major technological change in column technology, indeed the first original breakthrough to have occurred in this area since Tswett invented chromatography, a century ago" (Al-Bokari, Cherrak, and Guiochon, 2002). The monolithic column is made up of continuous porous material, sealed against the wall of a tube, instead of beads. The decrease in chemicals and samples usage, improved sensitivity, reusable frit-less columns, and lower back pressure make it more efficient and user-friendly columns in the present world (Jiang, Qi, Chu, Yuan, and Feng, 2015). Various papers have already mentioned the use of monolithic columns in the field of chromatography. The current review is focused on the three different types of monolithic columns, i.e., silica-based, polymer-based, and hybrid monolithic columns. Relevant publications until December 2016 are reviewed in this paper. Information on monolithic columns from previous publications was supplemented and systemized (Table 1).

There are two main types of pores in the monolithic column; flow-throughpores and mesopores. Mobile phase passes through flow-throughpores or macropores and determines the column permeability (Fig. 1); however, mesopores are found in lumps (porons) of porous solid located between the channels made by throughpores and determine the column performance (Fig. 3) (Guiochon, 2007). The average size of throughpores as determined by mercury intrusion porosimetry is 1.7 μm, and the average size of mesopores ranges from 2 to 50 nm (Fig. 2) (Guiochon, 2007). Due to the presence of macropore, the backpressure of the monolithic columns is less than the conventional column (3.5 and 5 μm). Therefore, the monolithic column can be operated at a higher flow rate with faster separation. Mesopores form the internal porosity of the column which is approximately 0.20 for the neat silica (Minakuchi, Nakanishi, Soga, Ishizuka, and Tanaka, 1996). The presence of the mesopore in the column increases the efficiency (by increasing the plate number and decreasing the height equivalent to theoretical plate (HETP)) of the column (Fig. 3). It also results in a higher surface area and hence higher absorption capacity.

* Correspondence: gxs183@miami.edu
[1]Department of Chemistry, University of Miami, Miami, Florida, USA
Full list of author information is available at the end of the article

**Table 1** Difference between particulate and monolithic column

| S.No. | Particulate column | Monolithic column | Reference |
|---|---|---|---|
| Characteristics of the column | | | |
| | Inter-particle void volume determines the permeability and column back pressure | Macropore determines the permeability and column back pressure | (Cabrera, 2004) |
| | Inter-particle volume depends on particle size Dia < 3 μm (small permeability) Dia > 11 μm (large permeability) | Macropore size determines the particle performance | (Cabrera, 2004) |
| | Plate number (N) is inversely proportional to particle diameter | Plate number (N) is directly proportional to particle diameter | (Cabrera, 2004) |
| | Performance and permeability cannot be controlled independently by particle packed column | Performance and permeability can be controlled independently by controlling over macropore and mesopore size | (Cabrera, 2004) |
| Column performance | | | |
| Flow rate | Lesser | Higher | (Cabrera, 2004) |
| Back pressure | Higher | Lesser | (Cabrera, 2004) |
| Porosity | Lesser | Higher | (Cabrera, 2004) |
| Run time | Higher | Lesser | (Cabrera, 2004) |
| Efficiency (HETP, plate height) | Lesser | Higher | (Cabrera, 2004) |
| Precision and reproducibility | | | |
| Retention time | Higher | Lesser | (Deeb, Preu, and Wätzig, 2007), 90 |
| Resolution factor | Lesser | Higher | (Deeb et al. 2007), 91 |
| Tailing factor | Higher | Lesser | (Deeb et al. 2007) |
| Structure | | | |
| Frit | Present | Absent | |
| Sample and mobile phase usage | More required | Less required | |
| Absorption and separation capacity | Lesser | 30–40% higher capacity | (K. Nakanishi et al. 1998) |
| Surface area | Lesser | Larger | (K. Nakanishi et al. 1998) |
| Loadability | Less | More | |

The van Deemter curve for the monolithic column is better than 3.5 and 5 μm column (Fig. 4). However, at the same flow rate in 3.5 μm column provides a higher resistance system. One of the most important properties of porous beds in HPLC is the degree of radial homogeneity (Yew, Drumm, and Guiochon, 2003a; Yew, Ureta, Shalliker, Drumm, and Guiochon, 2003b). Mobile phase should percolate through the bed or it may cause a deleterious increase in bandwidth (Guiochon et al. 1997). Monolithic columns with larger diameters (4.6 mm) are coated with a suitable material so that the silica rod is attached to column end fittings (Cabrera, 2004; Siouffi, 2003). Many exothermal reactions like polymerization, polycondensation, and cross-linking take place during the preparation of monolithic columns, resulting in heat transfer across the column and the mold wall. Therefore, the center of the bed is hotter than sides closer to the wall (Kazuki Nakanishi and Soga, 1992b). Ishizuka et al. determined that efficiency of the columns increases with a decrease in the internal diameter of the column and made a column with an internal diameter of 75 μm (Ishizuka et al. 2002; Motokawa et al. 2002). Later, columns with a higher internal diameter of 200 (Tanaka et al. 2002) and 530 μm were made (Hara, Kobayashi, Ikegami, Nakanishi, and Tanaka, 2006; Motokawa, Ohira, Minakuchi, Nakanishi, and Tanaka, 2006).

## Column permeability

The pressure required for the percolation of mobile phase through the porous bed plays very important part in chromatography procedure. Particle/globule size and pore-size

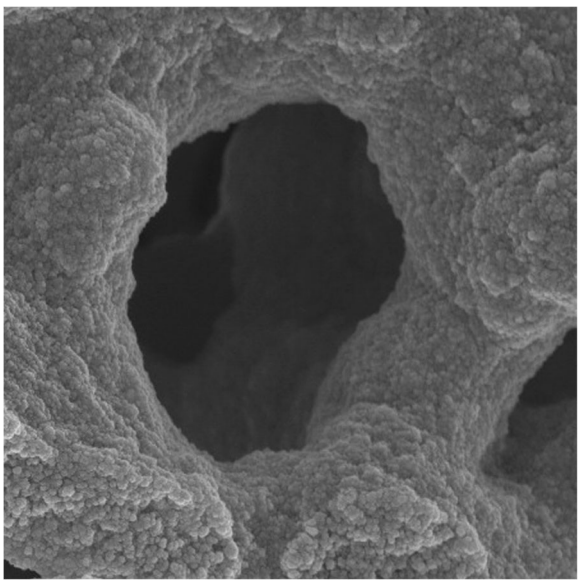

**Fig. 1** Macropore in a monolithic column (Cabrera, 2004)

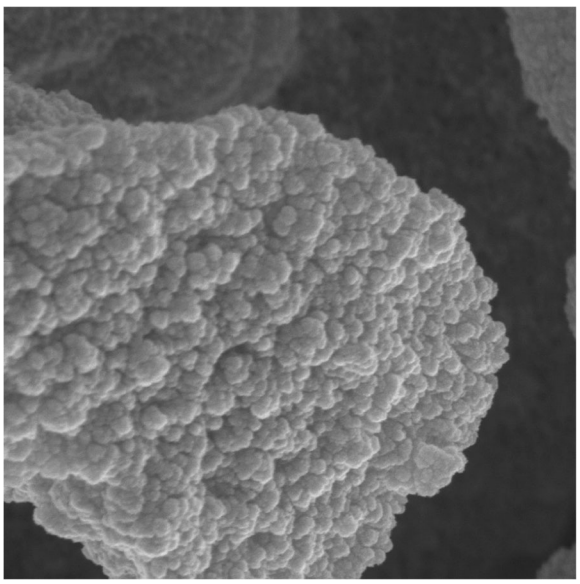

**Fig. 3** Mesopore in a monolithic column (Cabrera, 2004)

distribution mainly determine the performance of the monolithic columns. Methods for measuring the size and distribution include microscopic techniques like scanning electron microscope (Liang, Dai, and Guiochon, 2003), X-ray analysis, and transmission electron microscope (Courtois, Szumski, Georgsson, and Irgum, 2007).

## Silica-based monolithic columns

Svec and Frechet in 1992, first prepared a continuous porous polymer rod by using a porogen solvent (by in situ polymerization of glycidyl methacrylate and ethylene dimethacrylate) (Svec and Frechet, 1992) with a better external porosity and permeability than a packed bed columns. Monolithic silica rods were first prepared by a sol-gel process of hydrolysis and polycondensation of organo-silicium compounds used for the preparation of fine particles of porous silica. Reagents used for the synthesis of columns are tetraalkoxysilanes, tetraethoxysilane (TEOS), methyltrimethoxysilane (MTMS), aminopropyltriethoxysilane, and N-octyltriethoxysilane (Guiochon, 2006, 2007). The conventional silica-based monolithic columns (rod columns) have a range of internal diameters (i.d.) from 3 to 25 mm, but mainly, they are of 4.6-mm i.d. These are characterized by round pores, large through-pore/skeleton size

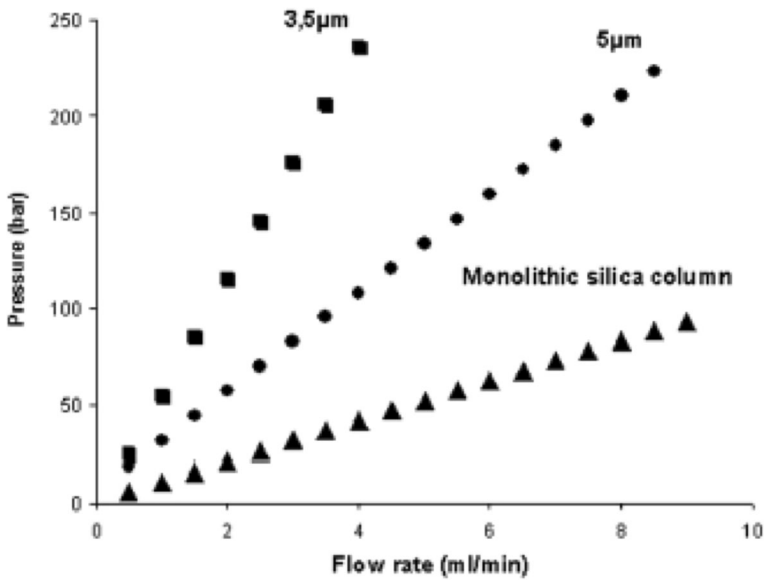


**Fig. 2** Back pressure of conventional and monolithic silica column. (Cabrera, 2004)

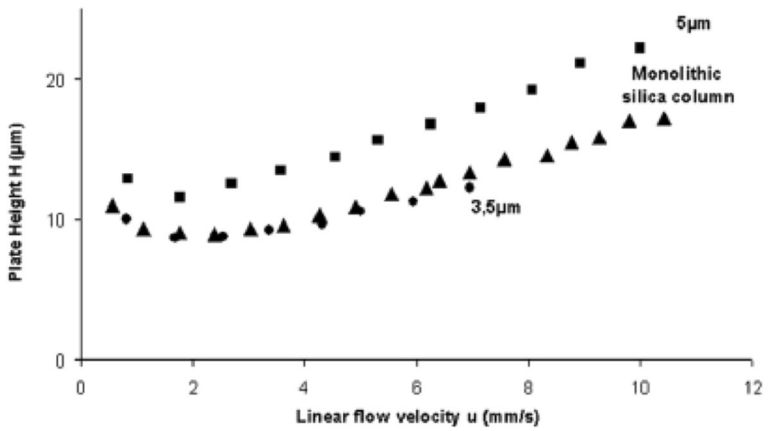


**Fig. 4** van Deemter curves for the conventional and monolithic columns (Cabrera, 2004)

ratio (high external porosity) (Knox and Scott, 1984), and bimodal pore-size distribution with a significant fraction of mesopores and a network skeleton structure.

Silica bases monolithic columns are of two types, i.e., first generation and second generation. The first generation columns have a narrow bore, made up of a long silica capillary tube having 50-μm i.d. and containing a silica monolith derived from tetramethoxysilane (TMOS). The second generation monolithic columns also have a narrow-bore, derived from mixtures of TMOS and MTMS and have a wider diameter (up to 500 μm). The macroporous channels of the flow-throughpores in monoliths are less constricted and less tortuous than the inter-particle volume of packed bed, which decreases the eddy diffusion effect in monoliths due to the absence of reticulation zones (Mason and Bernal, 1960). Gritti et al. measured the mass transfer mechanism in silica monolithic columns of the second generation. They measured HETP and coefficients of van Deemter equation and showed that HETP of first generation monolithic columns are 6–7 μm which is three times lower than those observed for monolithic columns of the first generation (Gritti and Guiochon, 2012a). Later, they demonstrated that HETP of these columns ranges between 4 and 5 μm which were three to four times lower than the monolithic columns observed in the first generation (Gritti and Guiochon, 2012b). The main reason for a decrease in the HETP was an increase in the radial homogeneity of monolithic rods (Gritti and Guiochon, 2012b). The column length was 25-cm, 100-μm i.d., with an external porosity of 86% and total porosity of 90% making it more permeable (Ishizuka et al. 2002; Motokawa et al. 2002; Tanaka et al. 2002). Martinez et al. showed that monolithic columns are better than the particulate columns for the separation of organic pollutants like simazine, atrazine and terbutylazine (triazines), chlorfenvinphos and chlorpyrifos (organophosphorous), diuron and isoproturon (phenylureas), trifluralin (dinitroaniline), and di(2-ethylhexyl)phthalate. Monolith gives better selectivity, sensitivity, and faster separation of water pollutants (Moliner-Martínez, Molins-Legua, Verdú-Andrés, Herráez-Hernández, and Campíns-Falcó, 2011).

Rogeberg et al. demonstrated the effect of temperature on the separation of silica monolithic columns. They separated tryptic peptides with MS detection and found that flow rate of 2000 nL/min at 80 °C resulted in higher peak capacity per time unit. The separation gave 70% more protein identification and 40% reduction in run time compared to conventional packed column (Rogeberg et al. 2011). Eghbali et al. used the peptide mixture to show that flow resistance is consistent with the different length monolithic columns (0.25, 1, 2, and 4 m) indicating that it has a structural homogeneity over its entire length (Eghbali et al. 2011). With an increase in polymerization, the rate of reaction decreases (heat generation decreases) so it can easily get cool and be poured into a mold before the sol-gel transition takes place (Kazuki Nakanishi, 1997).

## Preparation

During the preparation of monolithic columns, an exothermal reaction takes place in which water reacts with tetraalkoxysilane and alkoxy groups are replaced by silanol groups. The reaction is forwarded into a dimer and finally into a polymer formation (Kazuki Nakanishi and Soga, 1992a). During the polycondensation process, the viscosity of the solution increases rapidly. For aqueous solutions, the sol-gel sol transition occurs in a single phase system. However, for water-soluble polymer solubility of the gel is limited and hence phase separation occurs as the reaction proceeds (Kazuki Nakanishi and Soga, 1992a). The gel morphology depends on the cooling of miscible oxides solutions into an immiscible glass. Hence, the sol-gel transition depends on glass transition, segregation strength at bimodal temperature, and polymerization rate on cooling rate (Kazuki Nakanishi and Soga, 1992a). For

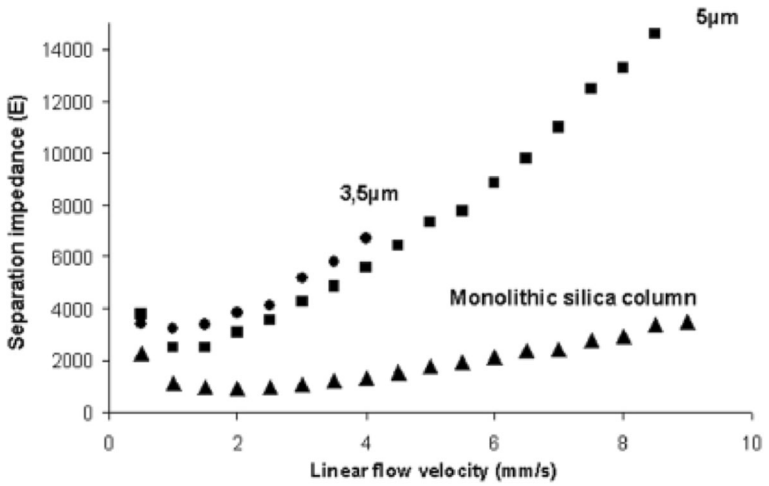


**Fig. 5** Separation impedance for the conventional and monolithic column. Lesser the separation impedance better is the separation (Cabrera, 2004)

better and more efficient separation long monolithic columns are required; however, after drying, they shrink and cannot stick to the wall of the mold (Kazuki Nakanishi and Soga, 1992a). Ishizuka et al. are the first to make the monolithic silica beds in a narrow-bore fused-silica columns (Ishizuka et al. 1998). During the preparation of the column, the mesopore fraction can be regulated by aging and drying (solvent exchange) method. Temperature variation controls the pore network and pore-size distribution of the columns, while the pH of the liquid influences only the pore-size distribution (K. Nakanishi, Minakuchi, Soga, and Tanaka, 1998). Chen et al. prepared C18 monolithic silica column by using ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate ([bmin]BF4)). In this process, the throughpores and mesopores were formed simultaneously during a sol-gel reaction, hence simplifying the preparation process of the silica-based monolithic columns (J. Chen, Zhang, and Jia, 2011). Zhang et al. incorporated N-methylimidazolium during monolithic column preparation and used in the separation of multiple compounds (like inorganic anions, aromatic acids, nucleotides, polycyclic aromatic hydrocarbons, alkylbenzenes, and phenols). The interactions which control separation mechanism of these compounds were mixed interaction including anion-exchange, hydrophilic, π–π, dipole–dipole, and hydrophobic interactions (P. Zhang, Chen, and Jia, 2011) (Fig. 5).

## Applications

Yang et al. prepared a macroporousboronate affinity monolithic column using a metal-organic gel as a porogenic template. The prepared column had a better binding capacity towards glycoproteins compared with non-glycoproteins (Yang, Lin, He, Chen, and Zhang, 2011). Iwasaki et al. developed one-dimensional liquid chromatography-tandem mass spectrometry system with a reversed-phase monolithic silica-C18 column for human proteome analysis. It gave 5-fold improvements in MS response (Iwasaki, Sugiyama, Tanaka, and Ishihama, 2012). Hu et al. prepared a porous monolithic capillary column and extracted estrogen from urine samples (Hu, Fan, and Li, 2012). Miyazaki et al. prepared a 25-cm monolithic column by using tetramethoxysilane and octadecylsilyl moieties, closed in a stainless-steel protective column with two polymer layers between the silica and stainless-steel tubing. It has 45,000 theoretical plates (5.5 μm of HETP) for aromatic hydrocarbons, a flow rate of 2.3 mm/s and backpressure of 7.5 MPa in an acetonitrile-water mobile phase (Miyazaki et al. 2011). Kato et al. demonstrated the separation of nanometer size particle by a silica monolithic column, which can be used for a quality control of nanomaterials in nanotechnology experiments. They separated colloidally dispersed nanoparticles with a back pressure of 5.8 MPa at a flow rate of 1 μL/min (Sakai-Kato, Ota, Takeuchi, and Kawanishi, 2011). Kumar et al. performed a separation of eight chiral β-blockers on a cellulose tris(3,5-dimethylphenylcarbamate)-coated zirconia monolithic column by capillary electrochromatography (CEC), in less than a minute (Kumar and Park, 2011). Garcia et al. demonstrated the separation of triacylglycerols in vegetable oils by CEC with UV-Vis detection within 12 min (Lerma-García, Vergara-Barberán, Herrero-Martínez, and Simó-Alfonso, 2011). Urbas et al. used monolithic butyl and styrene-divinyl benzene columns for the separation of degraded influenza viruses. They showed that the method can separate HA1 subunit of H3N2 influenza virus and the influenza B virus (Urbas et al. 2011).

## Polymer-based monolithic columns

### Preparation

Preparation of polymeric-based monolithic column is divided into three steps:

1. Wall treatment. So the polymer synthesized sticks properly to the wall and prevents the flow of mobile phase between the wall and the polymer.
2. Polymerization or polycondensation. In the case of hydrophilic gels, the reagents include a monomer (N,N′-methylenebisacrylamide or piperazine diacrylamide), a catalyst (TEMED or N,N,N′,N′-tetramethylethylenediamine), and precipitation inducer (ammonium sulfate, dextran, polyethyleneglycol). In the case of hydrophobic gels, the reagents include a monomer (glycidylmethacrylate and ethylene dimethacrylate, sterrene, divinylbenzene), porogen (propanol, butanediol, cyclohexanol, dodecanol), and a catalyst (AIBN or azobisisobutyronitrile, 2, 2′-azobis (iobutyronitrile)).
3. Modification of surface chemistry of polymer bed (Guiochon, 2007).

Tong et al. prepared a monolithic column with embedded graphene (to increase the loading capacity of a column) coupled with LC-MS and compared it with a poly(BMA–EDMA) column and an increase in the loading capacity of the column were observed (Tong et al. 2012). Chen et al. prepared sulfo/vinyl biphasic silica hybrid monolithic capillary column by polymerization of 3-sulfopropyl methacrylate potassium salt (VTMS) with tetramethoxysilane (TOMS) and separated closely related amines including p-phenylenediamine, aniline, p-toluidine, N-methyl aniline, N,N′-dimethyaniline, and diphenylamine (Y. Chen et al. 2012). Han et al. prepared polymeric ionic-modified organic-silica hybrid monolithic columns by in situ co-condensation of tetramethoxysilane and 3-mercaptoprpyltrimethoxysilane by the sol-gel process and showed a good reproducibility while separating compounds like aromatic hydrocarbons, four alkylbenzenes, and five phenols (Han, Wang, Liu, and Jiang, 2012). Wang et al. developed a microfluidic chip-based liquid chromatography system with valveless gates sample injection method which have a simple system structure, ease of operation, convenience for varying injection volume, and high sample loading capacity (Wang, Zhu, and Fang, 2012b).

Takahashi et al. prepared a methacrylate-based anion-exchange monolithic column by UV photocopolymerization of [2-(methacryloyloxy) ethyl]-trimethyl ammonium chloride, butylmethacrylate, and ethylene dimethacrylate at a temperature of −15 °C and got an HETP of about 12.2–15.6 μm (Takahashi, Hirano, Kitagawa, and Ohtani, 2012). Chambers et al. made a monolithic poly(glycidyl methacrylate-co-ethylene dimethacrylate) capillary columns by incorporating multiwalled carbon nanotubules for the better performance of the separation. They observed an 1800 plates/m (without nanotubes) and 15,000 and a 35,000 plates/m at a flow rate of 1 and 0.15 μL/min, respectively (Chambers, Svec, and Fréchet, 2011). Urban et al. prepared hypercrosslinked porous polymer monolithic capillary columns by using poly (styrene-co-vinylbenzyl chloride-co-divinylbenzene) precursor monolith that was swollen in 1,2-dichloroethane and hypercrosslinked via Friedel-Crafts reaction catalyzed by ferric chloride. They obtained high efficiency in the separation of molecules such as uracil and alkylbenzene with a column efficiency of an 80,000 plates/m (Urban, Svec, and Fréchet, 2010). Li et al. prepared a biocompatible poly (ethylene glycol methyl ether acrylate-co-polyethylene glycol diacrylate) monoliths for the separation of four proteins in 20 mM of sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl. They observed an increase in mesoporosity which intern improves separation of protein with high molecular weight and decrease column backpressure (Li, Tolley, and Lee, 2010). Huang et al. prepared poly(4-vinylphenylboronic acid-co-pentaerythritol triacrylate) monolithic column and found that the selectivity of the monoliths increases by increasing the content of 4-vinylphenylboronic acid. They separated alkylbenzenes, amides, and anilines and found that the monolith exhibited high column efficiencies of 43,000–100,000 plates/m (Huang et al. 2012).

#### *Factors affecting*

Thermal initiation: Svek and Frechet (Svec and Frechet, 1992) first used AIBN as a thermal initiator. They also showed the effect of polymerization time and temperature, type, and concentration of thermal initiator on the morphology of the monoliths (Svec and Frechet, 1995b). Svec et al. showed that as polymerization time decreases, the mesopore fraction increases (Svec and Frechet, 1995a). Later, Trojer et al. (Trojer, Bisjak, Wieder, and Bonn, 2009) and Nischang et al. (Nischang and Brüggemann, 2010) explained that it is due to less crosslinking with a shorter polymerization time. Photo initiation: as the photo polymerization step reduces the polymerization time and increases the range of solvents (porogen), the morphology and porosity of the polymeric column are greater than silica monolithic columns. The factors that govern photo polymerization are light intensity, wavelength (remain constant with lamp) nature, and concentration of the initiator (does not remain constant). Khimich et al. showed that an increase in an initiator concentration gives a uniform pore structure (Khimich and Tennikova, 2005) but results in the cracking of the monolithic column (Viklund et al. 1997).

### *Effect of porogens*

Porogen influences the solubility of the growing polymer chains and hence controls the pore properties of the monoliths (Peters, Svec, Fréchet, Viklund, and Irgum, 1999). Viklund et al. showed that the effect of the addition of poor solvent (dodecanol) results into increase in the pore size of the column (Viklund, Svec, Fréchet, and Irgum, 1996). Santora et al. demonstrated the effect of porogen nature on the surface area of the monolith. They showed that in a divinylbenzene-styrene monolith, non-polar (n-hexane) porogen gives a higher surface area where for polar (methanol) porogen gives a smaller surface area. But with the use of more polar ethylene dimethacrylate-methylmethacrylate monolith, the solvent role was reversed, i.e., n-hexane gives smaller and methanol gives larger surface area (Santora, Gagné, Moloy, and Radu, 2001). Premstaller et al. demonstrated that porogen mixture (decanol and tetrahydrofuran) forms large throughpores in poly(styrene) divinylbenzene. They used it to separate oligo-nucleotides with high resolution (Premstaller, Oberacher, and Huber, 2000). Courtois et al. used poly(ethyleneglycol) and 2-methoxyethanol as a porogen solvent for a glycidyl methacrylate-co-trimethylolpropane trimethacrylate-co-triethylene glycol dimethacrylate monolith. They showed that the larger the molecular weight of polyethyleneglycol, the larger is the pore size of the monolith (Courtois, Byström, and Irgum, 2006). In a different study, Aoki et al. used polystyrene and chlorobenzene mixture as a porogen for the poly(glycerol demethacrylate) monolith. They observed that PS gave a continuous skeletal structure whereas toluene (poor porogen) resulted in an agglomerated globular structure (Aoki et al. 2006).

### *Effect of monomer to porogen ratio*

Trojer et al. increased the monomer to porogen ratio from 35 to 45% *v*/*v* in a poly[p-methylstyrene-co-1,2-(p-vinylphenyl)ethane] monolith and resulted in a decrease in the macropore distribution from 8.75 to 0.09 μm. It is because of a large number of nuclei formed due to a high concentration of monomer resulting in an increase in the resistance of the column (Trojer et al. 2009; Trojer, Lubbad, Bisjak, and Bonn, 2006). Hebb et al. showed that a monomer concentration of <0.5 g/ml for trimethylolpropane trimethacrylate monolith which resulted into powder (Hebb, Senoo, and Cooper, 2003). Eeltink et al. showed that using a monomer up to 20% resulted in a low density of methacrylate monoliths (Eeltink, Herrero-Martinez, Rozing, Schoenmakers, and Kok, 2005). It was concluded that the density and rigidity of the monolith decrease with the decrease in the concentration of monomers.

### *Performance of the organic monolith*

Nischang et al. (Trojer et al. 2009) and Trojer et al. (Nischang and Brüggemann, 2010) have shown that the separation of small molecules is more appropriate when the polymerization time decreases as the mesopore concentration increases.

### *Type of porogen used to determine the porosity of monolith, pore size, and pore distribution*

Premstaller et al. demonstrated that the use of the micropellicular morphology monolithic column will separate molecules like oligodexoynucleotide (large molecules) (Premstaller et al. 2000). On the other hand, bisphenol A dimethacrylate monolithic columns are suitable for the separation of alkylbenzenes and alkylparabens (small molecules) (Li, Tolley, and Lee, 2011). Smirnov et al. demonstrated that with the increase in poly(2-hydroxyethyl methacrylate-co-1,5-naphthalene bismaleimide) content (from 4 to 8%), plate height decreases from 188 to 51 μm due to the decrease in the globule size (Smirnov, Dyatchkov, Telnov, Pirogov, and Shpigun, 2011). Xu et al. showed that crosslinker ethylenedimethacrylate gives 11,000 plates/m and crosslinker 2-methyl-1,8-octanediol dimethacrylate gives 83,000 plates/m. This is due to an increase in the number of mesopores (Xu, Yang, and Wang, 2009).

## Applications

For the separation of large molecules, polymeric monoliths (due to their biocompatibility and large domain size morphology) showed better performance than silica monoliths (Smith and Jiang, 2008; Vlakh and Tennikova, 2009). But for isocratic separation of the low-molecular-weight, organic compound is relatively poor in polymeric monoliths; it is because of an absence of mesopore, a presence of micropore, and a structural inhomogeneity (causing flow dispersion) (Guiochon, 2007; Svec, 2010). Eeltink et al. studied the separation of complex proteolytic digest by using 50-mm, 250-mm, and 1-m-long poly(styrene-co-divinylbenzene) monolithic capillary column by coupling with LC-MS/MS separation. They observed that 50-mm column has the maximum peak capacity of 400. Moreover, 20% of peak capacity increases with 5-fold increase in the column length, which could be explained by the larger macropore size of the 250-mm-long monolith. Taking gradient time, dwell time, and equilibrium time into consideration, 50-mm monolithic column has better peptide separation than 250-mm monolithic column. On the other hand, 250-mm monolithic column has the highest peak production rate (Eeltink et al. 2010). Zhang et al. showed that sulfonate strong cation exchange (SCX) hybrid monolithic columns had seven times more permeability and three times more sample loading capacity compared to the commercially available particulate SCX column. They also compared

sulfonate SCX hybrid monolithic columns with phosphate SCX polymer monolithic column and found a 19% increase in the phosphopeptides identification (sulfonate group bind peptide cation stronger than phosphate group) (Z. Zhang, Wang, et al. 2012a). Cambra et al. demonstrated that the separation of tryptic digest of industrial enzymes is best done by particulate shell-core C18 columns (Kinetes, 2.6 μm) followed by silica monolithic column and (Chromolith RP-18e) conventional C18 columns (Gemini, 5 or 3 μm) and then by polymeric monolithic column (ProSwift) (Beneito-Cambra, Herrero-Martínez, Ramis-Ramos, Lindner, and Lämmerhofer, 2011). Eeltink et al. used a poly(styrene-co-divinylbenzene) monolithic capillary column for the separation of protein isoforms coupled with MS. Formic acid were added as an ion pairing agent (Eeltink et al. 2011). Liu et al. used a polymeric weak anion exchange monolithic capillary for high-resolution separation of glycoprotein isoforms. They separated glycoproteins and found them to be distinct glycoforms (Liu, Ren, Liu, Li, and Liu, 2012). Ivanov et al. separated tryptic digest peptide mixtures by using polymeric polystyrene-divinylbenzene monolithic nanocapillary columns of an internal diameter of 20 μm (Ivanov, Zang, and Karger, 2003). He et al. prepared an amino acid-based polymeric monolithic column by using chiral amino acid surfactant containing acryloyl amide tail, a carbamate linker, and leucine headgroup. They showed the separation of ephedrine and pseudoephedrine-containing multiple chiral centers by coupling CEC to MS (He, Wang, Morill, and Shamsi, 2012). Hutchinson et al. prepared latex coated polymeric monolithic ion-exchange stationary phase by using sulfonated methacrylate monolithic polymer and coated it with quaternary latex particles. They separated seven inorganic anions like bromide, nitrate, iodide, iodate, bromate, thiocyanate, and chromate within 90 s (Hutchinson et al. 2005).

## Hybrid monolithic columns

The hybrid monolithic column is a narrow-bore column prepared from TMOS and MTMS. They give much better results as compared to the particulate column and have more homogenous radial distribution of the throughpores and efficient 200-μm i.d. columns (Tanaka et al. 2002). Moravcov et al. prepared silica-based monolithic column modified with sulfoalkylbenzene zwitterion for LC and showed a long-term stability, high permeability, and efficiency. They showed that zwitterion silica-based monolithic capillary column can be used for isocratic and gradient hydrophilic interaction liquid chromatography (Moravcová, Planeta, Kahle, and Roth, 2012).

### Preparation

Li et al. formed a new boronate-silica hybrid monolithic column by using acrylamidophenylboronic acid as the boronate affinity ligand. It was highly hydrophobic with large surface area, can bind with cis-diol containing compounds at pH 6.5 and also a two-dimensional separation of cis-diol compounds in a single column can be done (Q. Li et al. 2012). Ou et al. prepared hybrid monolithic capillary column synthesized by using (3-chloropropyl)-trimethoxysilane and TMOS by using sol-gel chemistry for enantioseparations in capillary electrochromatography and capillary liquid chromatography (CLC) (Ou et al. 2012). Wang et al. prepared a hybrid-silica monolithic column for the analysis of nucleotides by capillary electrochromatography. Good resolutions and separation of polar and basic nucleic acid bases and nucleosides were also achieved without peak tailing (X. Wang et al. 2012a). Zhang et al. prepared phenyl silica hybrid monolithic column by using benzyl methacrylate and alkoxysilanes. They did the analysis of bovine serum albumin, ovalbumin, α-casein, cytochrome C, and myoglobin by coupling CLC with MS, and hence showed the use of a monolithic column in proteome analysis (Z. Zhang, Lin, et al. 2012b). Lei et al. prepared a monolithic column by incorporating the nanoparticles Fe, wormlike and hexagonal SBA-15 silica to develop a stationary phase. From this, they separated aqueous extract of rhizome gastrodiae with a column efficiency of 290,000 plates/m (Lei et al. 2012).

### Applications

Hera et al. showed that with a decrease in the ratio of MTMS to TMOS, the performance of the column increases. They prepared a monolithic columns with a plate height of 4.6–6.0 μm with a linear velocity of 2 mm/s (Hara et al. 2010). In a further study, they have demonstrated that hybrid columns should be treated at higher temperature for a longer time, than the TMOS column, if the molecular size of the solute increases (Hara, Mascotto, Weidmann, and Smarsly, 2011). Yan et al. prepared a hybrid organic-inorganic phenyl monolithic column from TEOS and PTES by a sol-gel procedure to introduce phenyl group in a silica matrix (Yan et al. 2005). Yan et al. modified the hybrid organic-inorganic phenyl monolithic column by combining with supramolecular template-based approach. They separated eight organic acids with column efficiency up to 267,000 theoretical plates/m (Yan et al. 2004). Wu et al. prepared 'one-pot' process for the preparation of organic-silica hybrid monolithic capillary columns by using TMOS and VTMS as a precursor with allyldimethyldodecylammonium bromide (ADDAB) or acrylamide as an organic monomer and azobisisobutyronitrile as an initiator. They used the prepared ADDAB-silica hybrid capillary monolithic column for the analysis of tryptic digest of bovine serum albumin and mouse liver extract by coupling micro liquid chromatography-tandem mass spectroscopy (Wu et al. 2009).

## Conclusions

In recent scenario, the condition of monolithic silica column is very auspicious. The major reason of monolithic columns popularity in an analytical field is because of their principle. It provides a systematic approach to modify and optimize like in the sizes of the different geometrical elements separately, which is necessary to do chromatographic separations, the throughpores, the mesopores, the domains, and the porons. One of the most important features of a monolithic column is their high permeability; therefore, they can be operated at a high flow rate of up to 10 mL/min, thus allowing fast separations of various mixtures. Many authors have compared monolithic columns and conventional packed silica columns and came to this conclusion that monolithic columns are comparable with respect to selectivity, reproducibility, and performance. Some authors have claimed that monolithic silica columns are more stable than packed ones due to their rigid silica structure. Therefore, monolithic silica columns seem to exhibit a great potential for the near future as further advances may lead to enhanced efficiency which will be needed in the challenging field of high throughput and bioanalytical analysis.

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#### Authors' contributions
GS participated in the design of the study and helped to draft the manuscript. AT participated in the collection and analysis of the data and helped to draft the manuscript. VDS participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

#### Competing interests
The authors declare that they have no competing interests.

## Publisher's Note



#### Author details
[1]Department of Chemistry, University of Miami, Miami, Florida, USA. [2]Department of Biotechnology, NCC College of Engineering, Kurukshetra University, Kurukshetra, Haryana, India. [3]Department of Microbiology and Molecular Biology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, Uttar Pradesh, India.

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