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Review Article

An Overview of Monolithic Column: Types, Parameters and Applications

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Abstract

The column is the main component for chromatographic separation. Nowadays, monolithic columns are graining more popularity in the field of separation media for liquid chromatography. The monolith columns possess great potential as compared to the conventional packed column in terms of preparing complex mixtures. These columns provide various properties like higher permeability, high-efficiency fast separations, high flow rate with lower backpressure, fast mass transfer kinetics with a high binding capacity. It is categories into three columns and they are organic monolithic column, inorganic monolithic column and hybrid monolithic column and all three types of monolithic column differ through their porous properties. In this review, the various advantage of the high-efficiency monolithic column with recent advances, the origin of the concept, the various parameter of the monolithic stationary phase and the application of monolithic columns are illustrated. It is better column in comparison of selectivity, reproducibility and performance.

Keywords: Monolithic column, Packed columns, Inorganic and organic monolithic column, Column parameters, Pharmaceutical Applications

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INTRODUCTION:

Chromatography is a crucial biophysical technique that enables the separation, identification and purification of the components of a combination for qualitative and quantitative analysis. The fundamental importance chromatography method is the column, which enables the resolution of compounds based on selectivity and column performance. Traditional columns are mostly packed columns that are packed by particles. [1] There are different types of chromatographic separation media and columns split into numerous categories depending on their packaging and particle technologies. Every column category like perfusion column, superficially porous column (fully porous and Core-shell columns), monolithic columns have multiple advantages and drawbacks.

The core-shell columns are also known as fused core, solid core and superficially porous particles. It was developed to achieves separation of the compound in a faster rate with better flow throughput and maintaining column accuracy.[2] It was commercially available in the various brand name as Poroshell (Agilent Little River, USA), Halo (Advanced Material Technology, USA), Kinetex (Phenomenex Torrance, USA), Accucore (Thermofisher scientific), Cortecs (Waters), etc. Poroshell has a 5 µm particle size with 0.25 µm thickness and also has 50% more peak capacity as compare to Zorbax column (fully porous silica particle).[3] Halo HPLC column contains 2.7 µm particle size by fusing small silica particle of 0.5 µm thickness and the solid silica particle is of 1.7 µm size.[4] In 2009, kinetex was available commercially and the particle size of the column is 2.5 µm. The efficiency of Kinetex is better as compared to the Halo HPLC column.[5] Superficially porous particle column reduces band broadening and provides excellent column efficiency because it contains inert fused core-sphere circulated with sub-2 µm active adsorbent porous shell. There are several advantages of fused core-shell columns and they are fast elution, great mass transfer, kinetic with greater performance at high mobile phase velocity. Core-Shell (Superficially porous particle) showed similar column efficiency compared with Sub 2 µm (fully porous particle) with significantly higher permeability.[6]

Sub-2 µm column is known as a totally porous particle or fully porous particle and it exhibits high retention time with great efficiency but requires higher backpressure (nearly 100MPa). It is designed basically for Ultra-High-Performance Liquid Chromatography (UHPLC) system and 1.7 µm hybrid particle was introduced in 2004. In the market, more than 100 different columns are available with 1.5-2 µm practice and also more than 20 different UHPLC equipment available with the limited pressure as 60-130 MPa.[7] Absorption capacity is higher in monolithic columns compared to other columns due to accessibility of surface area greater in monolithic column and it is related to macropores and mesopores of the column.

The historical background of the monolithic column of the first-generation was in the 1960s-1970s: the first production of monolithic porous stationary phase (strongly swelled polymer gel and also porous polyurethane) was unsuccessful.[8] In 1980s, research has been carried out due to the development of a new approach to prepare monolithic porous stationary phase by using polymeric ultrafiltration membrane. The development of this column in 1989 by a joint team (Belenkii, Svec, and Tennikova) for the high-performance chromatography of biopolymers, protein, and nucleic acids.[9] In

the mid-1900s, Belenkii et al. reported that the chromatography of protein by using monolithic column and suggested to use gradient elution for this purpose due to better resolution.[10] Then the joint team had developed new porous column to overcome all the disadvantages before as high permeability from microparticles owing to radial heterogeneity in their packaging and formation of a channel. Further development of monolithic stationary column of the second-generation: Tanaka et al. modified monolithic column based on silica manufactured by polycondensation of tetramethoxysilane.[11] These columns were acquired by Merck KGaA (Darmstadt, Germany) and Phenomenex (Torrance, CA, USA) and the product are available under trade names Chromolith® and Onyx™, respectively. Merck has recently enlarged silica-based monolithic column with the release of 100-µm as internal diameter of capillary columns with the trade name of Chromolith CapRod.

A monolithic column is a novel chromatographic column and plays an important role in analytical investigations within the field of pharmaceutical analysis, food, and chemistry. In terms of flow resistance, monolithic columns have great potential in offering a stable and lower resistance than conventional liquid chromatography columns, good permeability, and high mass transfer speed for both analytical and preparative separations.[12] It is also known as a single piece of a continuous rigid porous polymer that contains pores and also possesses an interconnected skeletal structure. It has a unique chromatographic feature: high column efficacy as well as permeability at the same time. The primary feature of the monolithic column is that they need high as well as variable external porosity. The monolithic column exhibits sterling selectivity with high permeability and fast analysis time and it is prepared by organic or inorganic polymerization method in the column possess continuous stationary phase and superior porosity.[13] There are three types of monolithic columns depend upon the types of substrates: organic polymer monolithic column, Inorganic monolithic columns, and hybrid monolithic columns(organic-Inorganic).[14-15] A monolithic column consists of a single piece of solid materials that consists of interconnected skeletons and flow paths. To determine accurate result of separation behavior of monolithic column is through analyze the elution peak profile of external and internal porosity. In LC condition, the porosities and pore-size distribution of monolithic column is determined through Inverse Size-exclusion chromatography. The mass transfer resistance decreases by increasing external porosity and flows through the size of the pores of the packaging. If porosity increases, then high permeability and also backpressure is low with higher flow rate and faster separation. The small size skeleton offers high efficiency. It is known as molecularly imprinted monoliths because of imprinting template-specific sites. The pore size distribution volume fraction of macropore is having higher than 0.3 µm (75 to 80% pore volume) while mesopore and micropore was 10-12% or lesser than few percentages.

COMPARISON BETWEEN MONOLITHIC AND CONVENTIONAL PACKED COLUMNS:

The column quality is determined by the quality of packing of the particles within the column, the distribution, and also particle size. In HPLC, packed columns and monolithic columns are used as stationary phases. The packed column used silica microspheres and consist of tubes packed with 3-5 µm porous silica microparticles.[16] The plate number is inversely proportional to the particle diameter in the packed column and by using small particles the efficiency increased and the high back pressure was generated, and therefore short columns are used or low flow rates are applied.

In comparison with HPLC-packed columns, the monolithic columns are knowns as single rods and are made of a single piece of porous material. The major advantage of this column is that without generating backpressure it can work at high flow rates (up to 10 mL min^{-1}) in conventional column length (4.6 mm internal diameter) without loss of efficiency because of the nature of monolithic media and enhanced mass transfer from mobile phase to stationary phase was due to interconnected pores provide convective flow.[17] In a packed column, due to slow diffusion mass transfer from stagnant mobile phase into the pore of the column may cause peak broadening and reduction in column efficiency. The major benefit of monolithic columns is that it requires lesser time for analysis and due to more productivity, it may lead to a reduction in cost per analysis.[18] For monolithic columns, the band broadening due to the stagnant mass transfer of mobile phase is reduced because of its high permeability, and therefore, efficiency is maintained at high linear velocities. The total porosity of the monolithic silica column is near about 81%.[19] In comparison, the packed bed columns had a porosity of 66%, and similar porosity results have been reported elsewhere.[20-21] Furthermore, the monolithic stationary phase does not need frits in comparison with the packed particles which have mechanical problems related to column fragility and clogging of the column during use.[22]

The monolithic column pores are categorized into two main types based on its size and function and they are flow pores (macropores) and mesopores which is filled with the stagnant mobile phase and solute molecule migrates at active adsorption sites. They allow LC separations at low pressures are due to large flow pores and they are responsible for the permeability of the monolith. The particles present in the inner pores of packed column are similar to mesopores in the monolithic columns. The mesopores increase the total surface area of pores and sample capacity of monolithic beds. This can increase the efficiency of extraction or separation and decrease the backpressure.[23] According to the IUPAC classification, a micropore is a pore with a diameter smaller than 2 nm, having a diameter of 2-50 nm mesopore, and macropore greater than 50 nm. Several methods are commonly used for physical characterization and also for measuring the porous properties of monolithic materials. For surface characterization methods, they can be studied using optical methods such as scanning electron microscopy (SEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM). They are used to estimate the size of the pores, which will in turn determine the hydrodynamic properties and mechanical strength of the column.[24]

Monolithic column materials possess higher column efficiency at a high linear velocity while conventional packed column separation efficacy is low. Monolithic columns also offer greater mechanical stability versus polymer particle-packed columns, where the combination of high backpressure with large-pore beads can lead to the collapse of particles. The stable monolithic chromatographic beds also do not advance the voids. The adsorption capacity of the monolithic column is 30 to 40% higher than that of particle packed column. In monolithic silica columns, performance and permeability are independent of each other while in conventional packed particle column permeability is directly proportional to particle diameter and inversely proportional to plate number. Therefore, permeability and performance are dependent on each other. Figure 1 shows the structural differences between the packed column and the monolithic column. In figure 1(A) the particles are tightly packed and in figure 2(B) a monolithic column fabricated of a single piece of a porous solid with relatively large channels for convective flow. The scanning electron microscope of the packed and monolithic chromatographic beds demonstrated that the monolithic bed

contains a much greater number of channels penetrating the chromatographic bed compared with the column packed with particles.[25]

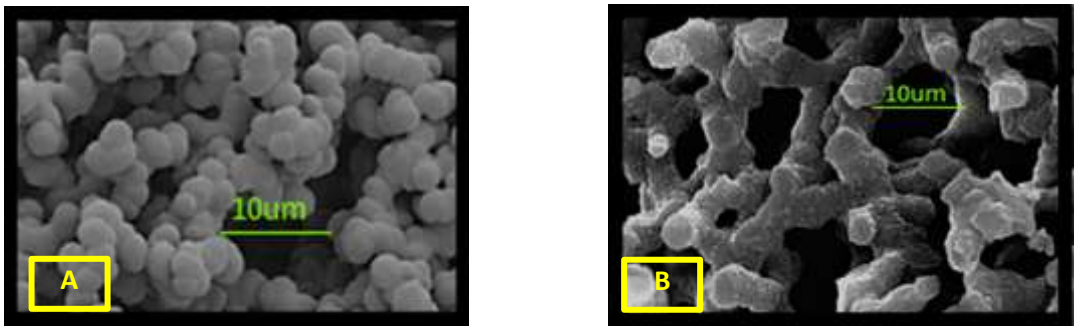

Figure (1): Structural characteristic of (A) Packed chromatographic bed and (B) Monolithic chromatographic bed

TYPES OF MONOLITHIC COLUMNS:

The monolithic column can be roughly divided into three categories according to different types of materials as shown in figure (2)

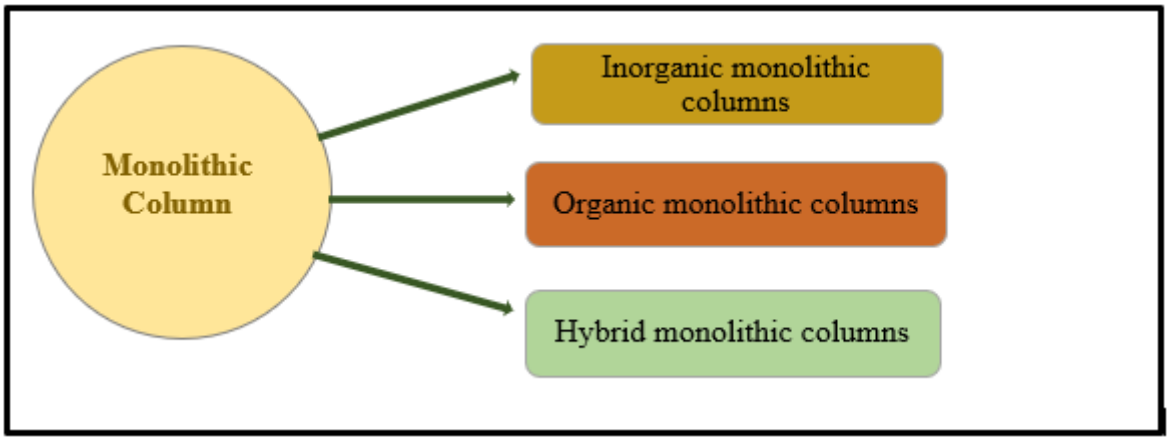

Figure (2): Types of Monolithic Column

INORGANIC MONOLITHIC COLUMNS:

The inorganic silica monolithic columns were first prepared in the year 1990s. The advantage of this column is that it can be easily synthesized and has high mechanical strength.[26] The internal diameter of conventional silica-based monolithic columns (rod columns) in the range of 3 to 25 mm but they usually in the range of 4.6 mm internal diameter.

The main material used by the inorganic silica monolithic column is alkoxysilane, with columns prepared by a direct sintering process or sol-gel process. The first monolithic silica rods were prepared by a multi-step process of sol-gel process of hydrolysis and for preparing fine particles of porous silica polycondensation of organo-silicium compounds used. Especially for the separation of small particles, it provides the separation process with high efficiency. The silica based monolithic column possess both macropores and mesopores which can provide desired surface area and allow a high flow rate at moderate pressure. Hence, silica-based monolithic column has large surface area as compared to polymer based monolithic columns and silica based monolithic column are utilizing for separation of small molecule. The sol-gel technology allows the formation of highly porous material that contain macropores of 2 nm and responsible for a low flow resistance and hence enable high eluent flow rates and mesopores of 12 nm provide sufficient surface area for separation efficiency in its structure. The separation characteristics make these columns for faster pharmaceutical analysis that needs to assay many samples in a short time with high accuracy and robustness. By using a monolithic column at a high flow rate may leads to increasing the volume of the mobile phase and also the total amounts of organic solvent increase. These may be better columns because of toxicity and environmental consideration.[27]

Compared to organic polymer monolithic, the inorganic silica has better mechanical stability and proportionately experienced swelling in the solvent. Although, pH stability is low in silica-based monolithic columns nearly it may be used at pH 2-8. There are various disadvantages if the pH level varies as if pH higher than 8, it will cause high backpressure, the silica will get degraded and also causing poor reproducibility as well as efficiency and if pH lower than 2, the loss of ligand bond due to silica silylether bond will be hydrolyzed. Silica monolith rapidly degraded in phosphate buffer. There are two types of generation of inorganic silica-based monolithic columns: first-generation and second-generation. The first generation of silica based monolithic HPLC column is Chromolith® (Merck Millipore) and it was launched in 2000 and commercially available for more than 10 years. The Merck Chromolith® series is Chromolith® Performance RP (18e, 100 × 4.6 mm) is mostly used.[28] However, Gritti et al.[29] designated that the drawback of the

first-generation monolithic column is the radial heterogeneity. To enhance the overall performance of first-generation monoliths, changes to the production process had made, so it derived second-generation monoliths column by using an increasing amount of porogen.[30] The second generation of the silica-based monolithic column is under the trade name of Chromolith® High Resolution and launched by Merck in 2011. The second-generation monolithic column has more advantages as compared to first-generation as it has finer control to production process also decrease the size of macropores. The major purpose to produce a second-generation silica-based monolithic column was to improve the separation efficiency and to reduce the peak asymmetry.[31] The chromolith column can be operated at 25 bar (~ 370 psi) and separation efficacy around N/M>80,000. The second generation of silica monolithic column is Chromolith High Resolution [HR]. The macropore size of Chromolith HR column is around 1.1 to 1.2 µm. The Chromolith HR shows column backpressure of around 65 bar (~ 950 psi) and possesses higher separation efficacy than first- generation column N/M>1,40,000. The porous silica structure of Chromolith HR is much more homogeneous than that of the first-generation.[32]

The first-generation columns are made of long silica capillary tube with 50 µm internal diameter and it of derived from tetramethoxysilane (TMOS) and the second-generation columns are derived from a mixture of TMOS and MTMS (methyltrimethoxysilane) and it has a wider diameter nearly 500 µm.[33] The primary goals of this investigation are: (a) to gain a better understanding of the chromatographic theory of these monolithic columns; (b) to study analytical parameters (selectivity); (c) to study the run-to-run and column-to-column performance (retention times and peak areas) and (d) to develop novel fast HPLC applications for these columns.[34]

ORGANIC POLYMER BASED MONOLITHIC COLUMNS:

An organic monolithic column material is highly porous in nature and consists of polymer globules that are separated by numerous interconnected pores, and well balanced through extensive crosslinking.[35] The organic polymer-based monolith column can be prepared fast and the method is simple. The organic polymer-based monolithic column has smaller surface area and also used for the separation of macromolecules. As a stationary phase, the organic polymer monolithic column can separate many substances such as proteins, peptides, nucleic acids, and other cells. Based on organic polymer monolithic columns material, it can be classified as polystyrene, polymethylacrylate and polyacrylamide monolithic columns and produced through molding process.[36] The polystyrene-based monolithic column was initial prepared in the early 1990s and has developed for a long time and this column types mainly contain poly (styrene-divinylbenzene) (PS- DVB) and the modified ones. It has good mechanical strength and remain stable over a pH range in between 1 to 14.[37] The polymethylacrylate is one of the most common polymer materials used in the monolithic column. This kind of monolithic column was prepared by using methacrylic acid or its derivatives as the monomer, ethylene glycol dimethacrylate (EDMA), or others as the cross-linking agent, and then the monomer and cross-linking agent are mixed with the porogenic agent and initiator. Compared with silica-based material, the methylacrylated-based polymer is stable at extreme pH condition. The important material of electrophoresis is polyacrylamide is widely used for several years as a biological separation medium and identification of the biological molecules. Additionally, the monomers are acrylamide, N-isopropyl acrylamide, methylacrylamide, N-allyldi- methylamine, and N, N'- methylene bisacrylamide is used as crosslinked agent. The preparation method is a typical aqueous polymerization method and the product gives good biocompatibility. The polyacrylamide-based monolithic column is used for the separation of biological macromolecules, but the mechanical properties of the column are not so good.[38] In recent years, many researchers applied many different polymerizations, and changes in the method had made to improve the separation performance of polyacrylamide monolithic. Organotellurium-mediated living radical polymerization (TERP) is a general living radical reaction applied to many different types of monomers. The polyacrylamide monoliths synthesized by TERP had kept the characteristics of the polyacrylamide monolithic column and also well-defined macropores with small pore size distributions and the surface tension arising in the repetitive swelling and drying.[39]

In terms of mechanical strength, organic polymer-based monolithic columns are sensitive as compared to the inorganic silica-based monolithic columns, and also the mechanical strength is relatively low due to swelling and shrinking of a column when it comes in contact with some organic solvents. The benefits of organic polymer monolith are versatility, high temperature, flexibility, stability nearly pH range in between 2 to 12, also inert to biomolecules and especially in the case of post-modification for more diverse applications.[23] Monolith organic polymers are a single-step process. The hindrance of organic polymer monolithic can be overcome by optimizing the composition of functional monomer, crosslinker, porogen, temperature, and polymerization and time period is used to yield the pore dispersal according to the desired application and better mechanical strength.[40]

HYBRID MONOLITHIC COLUMNS:

The combination of both polymer-based and silica-based monolithic column that is known as organic-inorganic hybrid monolithic column. Nowadays, organic polymer–inorganic silica-hybrid monolithic columns have drawn more attention because of several advantages of using both the columns and the benefits are easily prepared, low density and cost, high surface area, long shelf life with good biocompatibility, and excellent mechanical and pH stability. The hybrid monolithic column provides better result than the particulate column and the radial distribution of pore is more homogeneous. The hybrid monolithic columns can be prepared based on synthetic methodologies. The sol–gel process is well known in the production of hybrid monolithic columns. There are two types of procedures to prepare monolithic columns and they are one-step and acid/base two-step procedures. The "one-pot" and nano-scaled inorganic-organic hybrid reagent is the new method of polyhedral oligomeric silsesquioxane and is also known for the fabrication of hybrid monolithic columns.[41]

PARAMETER OF MONOLITHIC COLUMN

RETENTION TIME:

Retention time is vital parameter for the analysis of any compound as it serves as an index for the identification of any compound. The peak area gives information regarding the quantity of tested compounds. Both are important for accurate and precise methods. However, their repeatability is verified during method validation. The monolithic columns provide the best results for retention time repeatability value (%RSD ranged nearly from 0.00 to 0.09%) as compared to other columns[42]. Column efficiency derived from retention time, peak width and the RSD value of long-term repeatability is in between 1 to 4%.

SEPERATION EFFICIENCY:

The parameter used to interpret the separation performance is the efficiency and that is measured by number of theoretical plates, height equivalent to a theoretical plate (HETP) and van

Deemter curves. The longitudinal diffusion coefficient of the van Deemter equation is given by peak parking experiments. Gritti et al. identified the mass transfer mechanism in second-generation silica-based monolithic columns. They calculated HETP and coefficients of the van Deemter equation. The HETP of first-generation monolithic columns were first identified in the range of 6 -7 µm which is three and then later they observed that HETP was in between 4 and 5 µm because of increase in the radial homogeneity of monolithic rod.[43] The minimum plate height decreases as 66.5% and that observed between first- and second-generation silica-based monolithic column and hence the two major reform can be attributed that are narrow size at increased macropore homogeneity and without presence of radial morphology gradients.[44] Sklenarova H. et al. reported that the new type of column observed good peak symmetry, the highest column separation efficiency as the number of theoretical plates 13,137 and HETP 7.61 µm-twice the number of theoretical plates compared with the CMC and three times more as compared with the NMC.[45]

The Van Deemter equation is,

$$H = A + \frac{B}{u_{av}} + Cu_{av}$$

Where, the plate height minimum (H_{min}) and the Linear velocity ($u_{av,\ min}$) and the coefficients A, B, and C characterize [A-term is known as classical eddy dispersion (multiple path effect), B-term is longitudinal molecular diffusion, and C-term is mass transfer coefficient]. In the monolithic column, the macroporous channels of flow-throughpores are less narrow and twisted than the inter-particle volume of the packed bed, which decreases the eddy diffusion effect in monoliths due to the absence of reticulation zones.[33] The plate number increase and reduction of the height equivalent to a theoretical plate (HETP) is due to the presence of mesopore in the column. It also results in a higher surface area and absorption capacity.

FLOW RATE:

High flow rates may be used in the monolithic columns without loss of efficiency due to the nature of the monolithic media. Interconnected pores allow for convective flow, resulting in enhanced mass transfer between the stationary and mobile phases. Monolithic polymer columns allow the use of high flow rates due to decreased flow resistance resulting in low backpressures. The flow rate determines the retention time of any compound, where a high flow rate causes the compound to elute faster. Moreover, if the flow rate is extremely high then the backpressure will be high and the polymer inside the column may come out in other columns. But in the case of a monolithic column, the backpressure remains in control even if the flow rate is high because the structure and the geometry of the pores in the monolithic columns impart by lowering the flow resistance. The permeability and connectivity to the structure of the monolithic column assure that even when flow rate elevated the interaction between the mobile phase and stationary phase is constant and that leads to an increase in mass transfer and reduction in the peak broadening due to eddy diffusion.[46]

SELECTIVITY:

Selectivity (α) may be defined as the ratio of retention factor for two adjacent eluting compound peaks in a chromatogram. Selectivity for a column is determined by the surface chemistry.[47] The selectivity factor is also known as the separation factor, with a value of $\alpha > 1$. Through the resolution, the selectivity may be seen as if the two or more compounds when injected simultaneously at an equal concentration. The monolithic column has an absorption capacity nearly 1.4 times greater than that of the packed column, whether the surface area and the carbon content were comparable.[48] Resolution value must be greater than 1.5 and it indicates the degree of separation from each analyte,

Selectivity (α) was demonstrated by first calculating the retention factor (k) under gradient conditions by using an equation,

$$K = \frac{tr - t_0}{t_0}$$

where t_r is the retention time and t_o is the dead time (measured by the elution), an unretained compound in a reverse phase system.

The selectivity, (α) was then calculated by using a formula,

$$\alpha = \frac{k_2}{k_1}$$

where k_2 is the later eluting peak and k_1 is the earlier eluting peak

PEAK ASYMMETRY:

Asymmetry factor is the most important parameter for precise integration of compound peaks and also for precise and accurate quantitative information. For peak asymmetry values for all tested analytes, it must be according to pharmacopoeia that recommends value in between 0.8 and 1.5.[49] According to Kele and Guiochon, the tailing factor is known as the ratio of the peak width at 5% of the peak height to twice the forward half-width. The tailing factor was below 1.4 because of a slow mass transfer kinetics or a column bed radial heterogeneity. On the other hand, the extra-column effects and the overloading effects on the peak tailing were ruled out. The tailing factors for basic compounds were greater than the neutral ones. The reproducibility of the tailing factor ranged between 1.2 and 2.6% for neutral compounds and 3.4 and 22.6% for basic ones and these values comparable with the reproducibility values for conventional particulate packing materials.[50]

APPLICATION OF MONOLITHIC COLUMNS:

SILICA BASED MONOLITHIC COLUMN:

- Nakanishi et al. assorted the post-gelation temperature and age time to fix the pore size distribution of the mesopores on the surface of the monolithic skeleton.[51-52] Mesopores of greater than 100 nm were constructed and this is superior for the separation of high molecular weight polymers.[53] The packed columns use for large pore sizes are limited due to loss of mechanical strength.
- Cabooter D et al., described that the second-generation silica monolithic column has 30-40% higher efficiency as compared to first-generation. It was observed that for separation of complex mixture (N > 50,000) the first generation is more preferable than second-generation due to its higher permeability.[54]
- Dolezalova et al. compared the reversed-phase HPLC separation on a C18 silica monolith with micellar electrokinetic chromatography for the analysis of phenoxymethylpenicillin and ampicillin.[55-56] To determine impurities in the drug, a better correlation was obtained between the two methods. Although the MEKC separation was completed in 10 min, whereas the monolith method was 18 min, the precision and limit of quantitation (<0.1% w/w) were better with the HPLC method.
- The application of monolithic C18 silica columns for sequential injection analysis (SIA) provided an alternative to HPLC for the identification of small molecules.[57-59] The

use of a syringe pump in place of the high-pressure LC pump resulted from the reduction in the cost. The method was validated for various pharmaceuticals and parabens, and linear calibration was demonstrated.

- Vallano et al. determined the COX-II inhibitor in human plasma by using monolithic silica HPLC column and result showed by using monolithic columns was up to a five-fold reduction in analysis time and better reproducibility was illustrated for up to 1600 injections using different columns.[60]
- Miyazaki et al. developed a 25 cm monolithic column by using tetramethoxysilane and octadecylsilyl moieties and it was closed in a stainless-steel protective column with a two-polymer layer between the silica and stainless-steel tubing.[61] It has 45,000 theoretical plates (5.5 µm of HETP) for aromatic hydrocarbons with a flow rate of 2.3 mm/s and the mobile phase was acetonitrile-water with a back pressure of 7.5 MPa.
- Kato et al. illustrated that the dissociation of the nanometer-size particle by a silica monolithic column and also it is beneficial for quality control of nanomaterials in nanotechnology experiments and also separated colloidally dispersed nanoparticles at a flow rate of 1 µL/min and the backpressure was 5.8 MPa.[62]
- Yang et al. prepared a macroporous boronate affinity monolithic column by applying a metal-organic gel as a porogenic template and it has a greater binding capacity towards glycoproteins rather than non-glycoproteins.[63]
- Moravcova D. et al. modified silica-based monolithic column with sulfoalkylbenzene zwitterion for LC and exhibit persistent stability and also high permeability and efficiency and the zwitterion silica-based monolithic capillary column may be utilize for isocratic and gradient hydrophilic interaction LC.[64]
- Rogeberg et al. demonstrated the optimal conditions for high-speed separations for silica-based monolithic column with regard to RPLC separation of peptides and resulted that at a maximum flow rate the highest peak capacity was obtained at 80 °C and rat liver sample was analyzed within 30 min.[65]

POLYMER BASED MONOLITHIC COLUMNS:

- The polymer-based monolithic columns mostly used for the separation of complex samples.
- The preparation and evaluation of octadecylated poly(styrene-co-divinylbenzene) monolithic columns.[66] The 60 cm × 200 mm ID fused silica columns were shown to be stable for up to 250 bar column inlet pressure and provide greater than three times the separation efficiency compared to a PSDVB packed pellicular column for oligonucleotides. The total porosity of the monolith was determined to be 70% using inverse size exclusion chromatography and the loading capacity was comparable to a similar packed column.
- Yan et al. adapted by combining the hybrid organic phenyl monolithic column with a supramolecular template-based approach and they separated eight organic acids with column efficiency of up to 267,000 theoretical plates/m.[67]
- Hara et al. reported that the performance of the column rises with a reduction in the ratio of MTMS to TMOS. They developed a monolithic column with a plate height of 4.6–6.0 µm and a linear velocity of 2 mm/s.[68] In a further study, they have reported that hybrid columns must have a higher temperature for a longer time, than the TMOS column, if the molecular size of the solute increases.[69]
- Hydrophobic interaction chromatography was used for the separation of proteins by using an acrylamide-methacrylate based monolithic column and the hydrophobic component of the polymerization mixture was diverse to control the surface hydrophobicity.[70] Cytochrome c, ribonuclease, carbonic anhydrase, lysozyme, and chymotrypsinogen were separated using a 10% butylmethacrylate column, and a mobile phase gradient of ammonium sulfate with sodium phosphate was used. This approach appears to be promising for large-scale preparative purification of proteins.
- Xie et al. produced a typical (4.6 mm i.d.) analytical HPLC organic monolithic column on a poly(styrene-co-divinylbenzene) polymer in 1999 for the separation of proteins and they separated five proteins in less than 20 seconds. However, the separation of five peptides was not carried off, apparently due to a lack of selectivity of the styrene stationary phase.[71]
- Salih et al. prepared hexyl methacrylate-co-ethylene dimethacrylate monolithic capillary column and used for isocratic elution nanoLC-UV produce and validated with better accuracy and precision and compared with standard LC procedure and resulted with higher recovery rate, low cost and green analytical approach with lesser mobile phase consumption.[72]
- The first Poly (Styrene-co-Divinylbenzene) monolithic was reported in 1993 by Svec and Frechet group. The polymer prepared by stainless steel and they demonstrated that the potential of column for faster separation of four protein and other biomolecules.[73] Further, the Poly (sty-co-DVB) monolithic used for the separation of alkylbenzene and peptides in RPLC and it was illustrated that the separation of five proteins within 20 secs due to higher permeability of the column.[74]
- The most commonly used methacrylated based monolithic is poly [butyl methacrylate-co-ethylene dimethacrylate) for RPLC separation prepared using photoinitiation. (Poly [BUMA-Co-EDMA] monolithic used for separation of small molecule of different polarities and then later applied for separation of alkylbenzene.[75-76]
- Dolman et al. investigated the carryover by using four capillary columns with different morphologies and they found that carryover can still be detected best through polymer-based column as compared with other four columns because of abudance of mesopore.[77]
- Vonk et al. developed Titanium-scaffolded organic-monolithic column in narrow bore square conduct to reduce the shrinkage effect and the column applied for UHPLC gradient elution successfully.[78]
- Umemura et al prepared Hexyl methacrylate (HMA)-based monolithic semi-micro columns by in situ polymerization for reversed-phase separation of alkylbenzene and protein and it can operate under wide flow rate, allowing easy coupling to different detectors to provide complementary information.[79]

CONCLUSION:

In recent year, the monolithic stationary phase becomes popular due to its advantageous hydrodynamic characteristics as it has unique chromatographic characteristics as compared to conventional packed column and they are high flow rate up to 10ml/min without significant loss of efficiency and also leading to fast separation of various mixture compound at the same time. The monolithic columns are better compared for selectivity, reproducibility, and performance. Both the types of

the monolithic column have their advantages as the silica-based monolithic column has lower backpressure in high flow rate because of the presence of macropores throughout the column and the polymer-based monolithic column is a relatively simple and easy surface modification and it is used to separate complex sample. Nowadays, major focus on the development of polymer-based monolithic columns as several changes had made in their physical structure for the separation of the low-molecular-weight compound. Comparing with the polymer-based monolithic column, less research has been seen to improve and characterize new silica-based monolithic column with various surface chemistry because more synthetic skills are required to prepare this column and also there are several drawbacks such as radial and longitudinal homogeneity of modified surface, low reaction yield, and blockage of the pores that decrease permeability. So, the surface modification silica-based monolithic column further advance may lead to enhance the reproducibility, stability and also efficiency and also needed in challenging field as it has wide scope in the future.

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