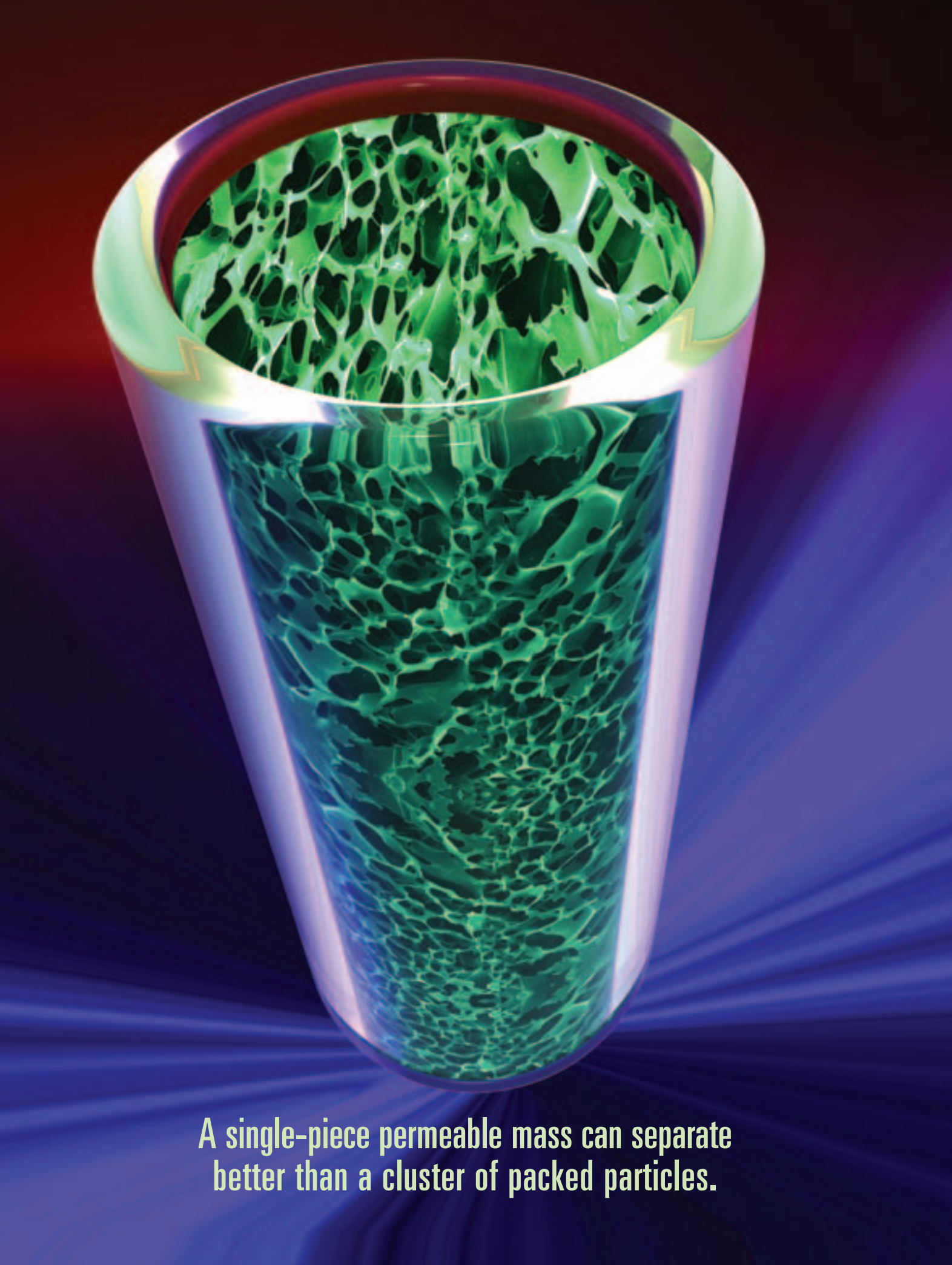


A single-piece permeable mass can separate better than a cluster of packed particles.

MONOLITHIC MATERIALS

PROMISES, CHALLENGES, ACHIEVEMENTS

Nowadays, there is a lot of buzz in separations science about the porous materials known as monoliths. Monoliths are used mostly as separation media and supports, and each one can be roughly compared to a single porous "particle". Monoliths are an alternative to columns packed with particulate stationary phases, which chromatographers have used for >100 years. Although that technology is well developed, it is not flawless. Packed columns have rather large void volumes; this is an inevitable result of the particulate character of the packing. Even in a perfectly organized array of monodisperse spheres, ~30% of the volume is interstitial voids. In reality, the percentage is even larger. As a consequence, a significant part of the column volume is not used for separation.

However, this is not the only problem. Consider what happens when a mobile phase is pumped through a column packed with a standard porous packing. The liquid flows readily through the interstitial voids between the particles, where resistance to its flow is the smallest (Figure 1a). In contrast, the liquid in the pores remains stagnant. If a sample is injected into the stream of the mobile phase, the compounds in the sample will also be carried through the voids. However, diffusion occurs because of the concentration gradient between the compounds in solution flowing through the interstices and the stagnant liquid within the pores of the packing, and transport of these compounds into the pores results. Once the concentration "pulse" has passed by the bead, the compounds diffuse back from the pores into the surrounding liquid; eventually, only the original stagnant phase remains within the pores.

Frantisek Svec
The Molecular Foundry, Lawrence Berkeley National Laboratory

Christian G. Huber
Saarland University (Germany)

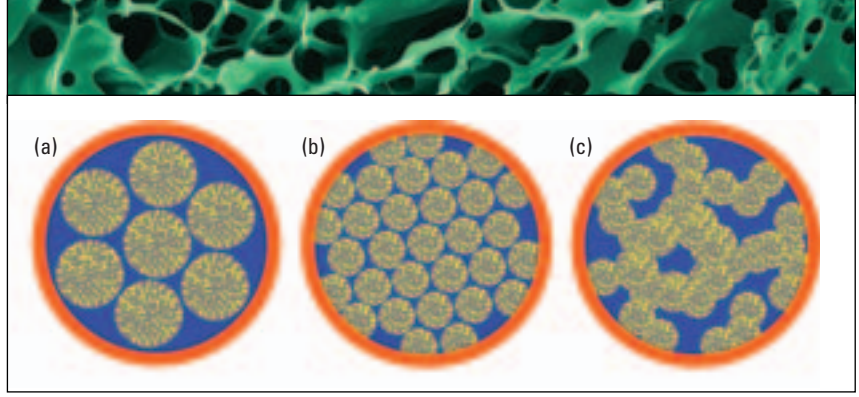


FIGURE 1. Evolution of columns from (a) highly permeable and less-efficient packed columns to (b) less-permeable and highly efficient packed columns to (c) highly permeable and highly efficient monolithic columns.

Typically, entities such as gases, small organic molecules, and ions diffuse relatively quickly. However, the transfer of large molecules such as proteins, nucleic acids, and synthetic polymers is considerably slower, because their diffusion coefficients are several orders of magnitude smaller than those of low-molecular-weight compounds. This effect is detrimental to processes when the speed of the mass transfer limits the overall rate, as in high-speed chromatography.

The logical way to improve mass transfer in porous particles is to reduce their size and the diffusional path lengths within the pores (Figure 1b). However, along with this improvement, the size of the interstitial spaces between the particles diminishes. This leads to a decrease in permeability and an increase in the back pressure of the chromatographic column. In contrast, a monolithic column can be thought of as fused together to form a continuous phase of a porous material (Figure 1c). In this configuration, the size of the flow-through channels and the depth of the pores that are accessible only by diffusional mass transfer may be independently optimized. Thus, this configuration enables column permeability and mass transfer to be maximized simultaneously. Such a configuration provides highly efficient separations with low resistance to hydraulic flow. In this article, we focus on the development of various monoliths and their applications in separation devices, flow-through reactors, and miniaturized systems.

Brief historical background

In the 1950s, Nobel Prize winner Robert Synge first postulated structures similar to what we now call monoliths (*1*, *2*). However, he realized that the soft materials available at the time for such devices would collapse under hydrostatic pressure. Kubín et al. confirmed this 15 years later when they tested a monolith prepared from 2-hydroxyethyl methacrylate hydrogel (*3*); its permeability was very poor. In contrast, columns filled with open-pore polyurethane foams created via in situ polymerization were more successful, and decent separations could be obtained in GC and LC (*4*–*6*). However, none of these early technologies lasted long, and the modern era of monolithic columns began much later.

In the mid-1980s, Belenkii and co-workers studied the chromatography of proteins in gradient elution mode by using stationary phases with a variety of chemistries and column geometries (*7*). They found that a certain distance, often only a very short one,was required to achieve a good chromatographic separation. This finding resulted in the concept of short separation beds. Creating such beds from particulate sorbents was very difficult because of irregularities in packing density and excessive channeling. Therefore, a new type of stationary phase—the monolith—had to be born (*8*). Originally, the highly cross-linked monolithic material was prepared from a polymerization mixture consisting of monovinyl and divinyl methacrylate monomers, a free-radical initiator, and a porogenic solvent in a flat or tubular mold that yielded a sheet or cylinder. The porous monolithic polymer was then removed from the mold, punched or sliced to obtain thin disks that were placed in a cartridge, and used for chromatographic separations.

Continuing research led to the invention of monoliths in typical column formats; the monomers used were extended to styrene, divinylbenzene, and their derivatives (*9*). In contrast to the disks mentioned earlier, these monoliths were polymerized in situ within a chromatographic tube or other tube, in which they remained after preparation was complete. Thanks to the slow thermally initiated polymerization in a vertical position, the initially formed cross-linked polymer nuclei could settle; thus, the liquid polymerization mixture was left on the top and around the solid phase. This mechanism readily compensated for the radial shrinkage that would otherwise create voids at the monolith–column-wall interface and be deleterious to the chromatographic function of the monolithic column (*10*).

At the same time, Hjertén and his colleagues developed a process that led to continuous beds (*11*). In their approach, a highly swollen cross-linked gel was prepared by the polymerization of aqueous solutions of *N*,*N*′-methylene bisacrylamide and acrylic acid in the presence of a salt, typically ammonium sulfate; the gel was massively compressed to a fraction of its original volume. Such a compressed gel completely filled the cross section of the column and exhibited very good permeability to flow, despite the high degree of compression.

Tanaka et al. made the next contribution to modern monolithic materials, finding their inspiration in a very popular inorganic support widely used in chromatography—silica (*12*). In contrast to columns made of organic polymers, silica-based monolithic columns in typical analytical sizes cannot be prepared in situ because of the significant shrinkage when the material solidifies. For example, a rod with a diameter of 4.6 mm is obtained from a 6-mm-i.d. mold. Thus, the porous silica rod is prepared first, removed from the mold, encased within a PEEK tube, and functionalized with a bonded C_{18} chemistry to create a reversed-phase chromatographic column.

Early studies with all these columns clearly demonstrated extremely fast chromatographic separations at high flow rates and at reasonably low back pressure. These advantageous features made monolithic columns particularly suitable for high-throughput applications. For example, Figure 2 shows the separation of 5 proteins in <20 s (*13*). Interestingly, the organic-polymer-based monoliths have always done a better job of separating larger molecules, such as proteins (*11*, *14*), nucleic acids (*15*), and synthetic polymers (*16*); silica-based monolithic columns enable fast separations of smaller molecules (*12*). Thus, these two column technologies are complementary.

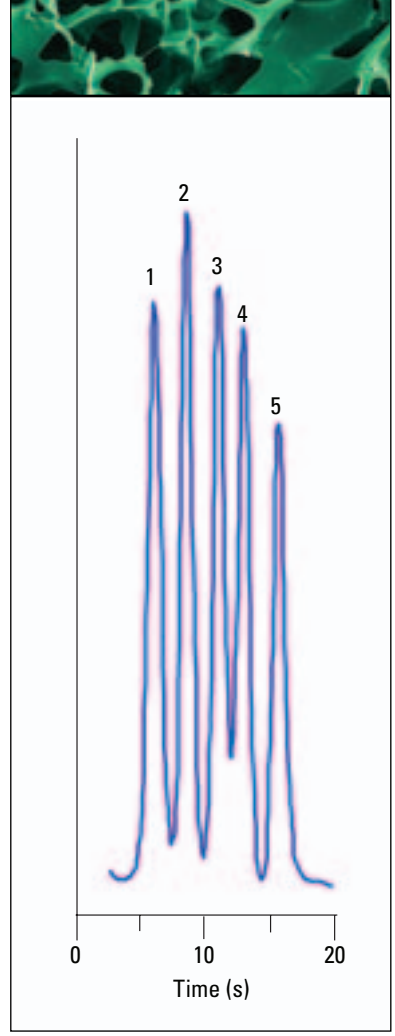


FIGURE 2. Rapid separation of 5 proteins on a 50 × 4.6-mm-i.d. poly(styrene-*co*-divinylbenzene) monolith with a mobile-phase gradient of 42–90% acetonitrile in water. (Adapted with permission from Ref. 13.)

Hydrodynamic properties

The entire mobile phase must flow completely through the monolith that fills the volume of the separation device. Therefore, the first concern is the permeability of the monolith to liquids, which totally depends on the size of the pores. A monolith with the nanometer-sized pores typically found in chromatographic packings would be crushed at the extremely high pressures required for flow. Obviously, an ideal monolith must contain pores large enough to facilitate flow. Indeed, optimization of the conditions under which monoliths are prepared leads to the use of highly porous materials containing a network of interconnected pores with sizes in the low-micrometer range. As a result, the pressure drop remains small even if the liquids are pumped through the monoliths at very high rates. Simple measurements revealed that a silica-based monolithic column (Chromolith) exhibits back pressure comparable to a column of identical dimensions packed with 11-µm beads. However, the chromatographic efficiency of this monolith column is as good as that of an analytical column packed with 3-µm particles (*17*).

Another remarkable feature of monolithic columns is their high porosity, which can reach 80%. In other words, only ~20% of the column may be occupied by porous material. This seems to be much less than the 60% of the column volume typically occupied by particulate packings. However, these values are not comparable. The packed column contains 40% voids through which the mobile phase must flow (in addition to diffusing through the packing). In the case of monolithic materials, however, all of the mobile phase flows through the larger pore volume of the monolithic structure. As a result, the resistance to flow is smaller, and the slow diffusion into and out of the pores of typical particulate packings is replaced with much faster convection. In a slightly simplified view, the analytes are delivered by flow, not by diffusion. Liapis et al. developed a mathematical model that predicts the dynamic behavior, scale-up, and design of monoliths (*18*).

Capillary electrochromatography

Despite their success in fast separations and their commercial availability, monolithic columns with relatively large internal diameters of 4–10 mm initially remained in the shadow of their older relatives, columns packed with particles. Capillary electrochromatography (CEC) re-emerged in the mid-1990s as an exciting miniaturized separation technique promising vastly enhanced efficiencies and peak capacities compared with traditional LC. However, CEC practitioners who had switched from HPLC and CE quickly realized that creating retaining frits in capillaries and packing stable microcolumns with beads were very challenging. This spurred the development of alternative column technologies containing monolithic separation media prepared in situ, a concept adopted from earlier work. As a result of their unique properties and simplicity of preparation, monolithic columns attracted considerable attention, and a plethora of chemistries and approaches soon became available (*19*).

Although the preparation procedure for monolithic CEC columns is somewhat similar to that of the original analytical-size monolithic columns, it is not identical. In contrast to polymerization in stainless-steel tubes, the capillary wall can be functionalized by the reaction of surface silanol groups with [3-(trimethoxysilyl)propyl] methacrylate. These methacrylate units are then inserted into the propagating polymer chains during the formation of the monolith, covalently attaching the monolith to the wall and preventing dislocation. The small-capillary format also enables the in situ preparation of silica-based monoliths, because the surface silanol functionalities are engaged in the polycondensation of an alkoxysilane precursor and bind the monolith to the capillary wall.

Column efficiencies in the range of hundreds of thousands of plates per meter were demonstrated with monolithic capillary columns; the samples were mostly mixtures of very simple organic compounds, such as benzene derivatives. These studies did not have big appeal for the chromatographic community, because they mimicked separations that could be achieved with HPLC on packed columns. As a result, CEC use faded a few years ago.

However, CEC is not dead. It can be extremely useful in applications where high column efficiency can make a big difference. One example is the proteomic separation of complex peptide and/or glycan mixtures, followed by MS detection (*20*, *21*). CEC separations of proteins and peptides are difficult to perform because of the ionizable nature of these compounds. Although the flow of neutral molecules is driven only by electroosmotic flow (EOF), the electrophoretic mobility of the charged analytes themselves also contributes to their movement through the column. Depending on the properties of the peptide and pH of the mobile phase, the electrophoretic contribution can be in the same direction as the EOF or in the opposite direction. Another obstacle arises from possible coulombic interaction of the positively charged analytes with the negatively charged surface of the stationary phase; this could lead to permanent adsorption.

El Rassi's group is developing monolithic poly(pentaerythritol diacrylate monostearate) CEC columns that are nonpolar, are free of the undesired electrostatic interactions, and provide significant EOF. This property was ascribed to the ability of the polymer to adsorb sufficient amounts of ions from the mobile-

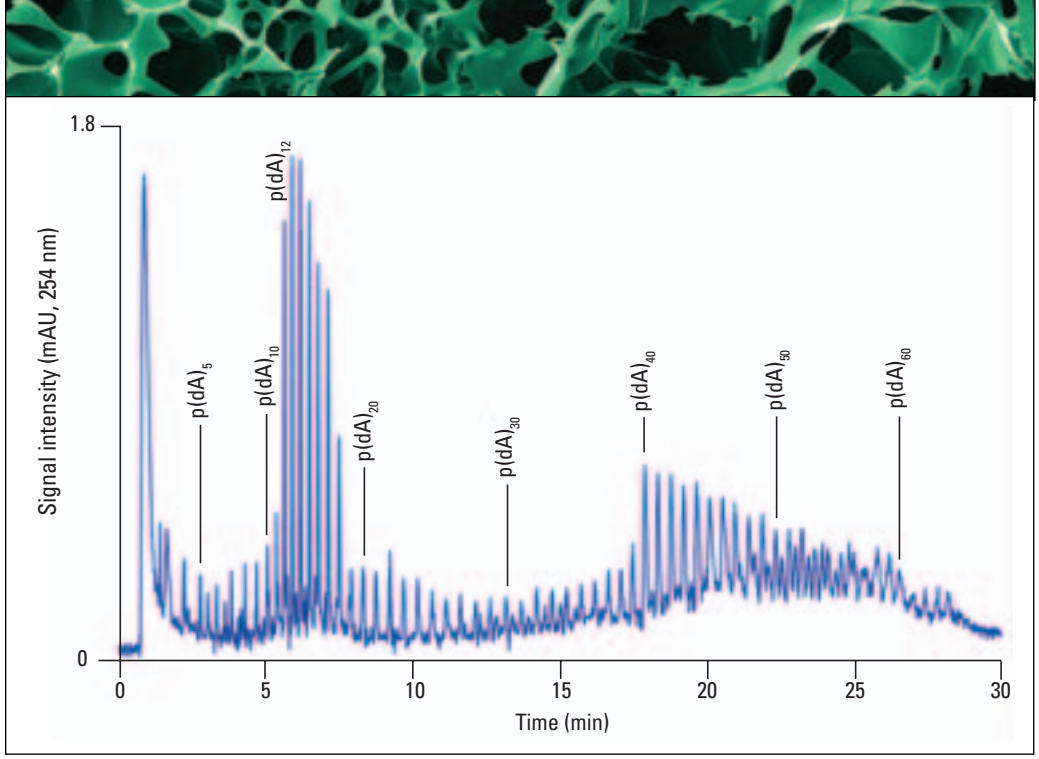


FIGURE 3. High-resolution separation of phosphorylated and non-phosphorylated deoxyadenylic [p(dA)] acids on a 60 × 0.2-mm-i.d. poly(styrene-*co*-divinylbenzene) monolithic capillary column with a mobile-phase gradient of 1–10.4% acetonitrile in water in 100-mM triethylammonium acetate. (Adapted with permission from Ref. 25.)

phase electrolyte. The adsorbed ions imparted to the neutral monolith the zeta potential necessary to support the EOF required for mass transport across the monolithic column. However, the absence of fixed charges allowed the rapid and efficient separations of proteins and peptides at pH 7.0 (*22*).

Novotny's team prepared a hydrophilic monolithic stationary phase by copolymerization of acrylamide, methylene bisacrylamide, 2-cyanoethyl acrylate, and vinylsulfonic acid and used it to separate complex glycan mixtures by CEC. The eluent and a matrix solution were co-deposited on a standard MALDI plate with a sample deposition device they developed for interfacing CEC and MS. This deposition device enabled the generation of 2500 discrete, highly homogeneous dots containing separated glycans, the masses of which were subsequently determined with MALDI MS. They also demonstrated the versatility of this monolithic device for separating a tryptic digest of ovalbumin in gradient microHPLC mode (*23*).

Micro- and nano-HPLC

The availability of monolithic capillary columns initially developed for CEC stimulated transfer of this format into the capillary-HPLC arena. Horváth's group prepared monolithic capillary columns based on a styrene–divinylbenzene copolymer for the separation of proteins and peptides (*24*). At the minima of the van Deemter curves, the chromatographic performance of these columns under hydraulic flow conditions approached that of the same columns operated in electrochromatographic mode. Nevertheless, the 15–20-µm plate heights achieved for a small, unretained analyte were still significantly higher than those obtained with microparticulate stationary phases.

A different porogenic mixture and polymerization conditions were used to form a monolith with large pores suitable for convective mass transfer; this brought about a significant improvement in the performance of polymer-based monolithic columns. These columns were first used for nucleic acid separations, for which plate heights as low as 5 µm were obtained in isocratic mode. Figure 3 shows the rapid separation of a ladder of adenosine oligonucleotides in a monolithic poly(styrene-*co*-divinylbenzene) capillary column with an i.d. of 200 µm (*25*). The whole series of phosphorylated oligomers, ranging in size from 5 to 60 mers, was baseline-separated. Moreover, peaks attributable to the 5′-dephosphorylated species were distinguishable as a second series in the chromatogram. In addition to being highly efficient and robust, these columns are adaptable and enable the separation of many classes of biopolymers, such as double-stranded DNA, single-stranded oligodeoxyribonucleotides, oligoribonucleotides, proteins, and peptides.

In a direct comparison of a 2-µm, nonporous, poly(styrene-*co*-divinylbenzene) stationary phase with a poly(styrene-*co*-divinylbenzene) monolith in the separation of nucleic acids, the monolith performed 30–40% better than the microparticulate stationary phase (*26*). This improvement was attributed to the high interconnectivity of the pores in the monolith and to the structure that lacked any micropores accessible to biopolymers. When monolithic capillary columns were combined with tandem MS, a significant improvement in identifications of complex peptide mixtures occurred (*27*). The peptides from a tryptic digest of a mixture of 10 proteins were analyzed by tandem MS followed by automated peptide fragment fingerprinting via database searching; 69% and 59% of all peptides were identified in all 3 runs of triplicate analyses on the monolithic and microparticulate columns, respectively. Moreover, the total number of peptides identified was ~2× higher with the 100-µm-i.d. monolithic column than with the 75-µm-i.d. microparticulate column.

Silica-based monolithic columns in capillary format were obtained first by solidification of potassium silicate in a fused silica capillary (*28*) and only later by an in situ preparation of the monolithic structure by the sol–gel process (*29*). The typical plate heights achievable with these columns are 10–20 µm. Because of their very high permeability, columns as long as 1 m or more could be used; they offer >60,000 theoretical plates per column. Most applications of capillary silica monoliths are in the separation of small molecules, whereas organic monolithic capillary columns excel in the separation of macromolecules.

Karger's group used 20-µm-i.d. monolithic poly(styrene-*co*-divinylbenzene) capillary columns for the identification of proteins extracted from ~1000 cells of breast cancer tissue and demonstrated detection limits for peptides in the 1–10-amol

range (*30*). Because of their chemical stability at extreme pH values, monolithic poly(styrene-*co*-divinylbenzene) columns were also applicable to 2D separations of complex peptide mixtures by reversed-phase HPLC at high and low pH values. Although neither separation mode was perfectly orthogonal, this 2D separation offered peak capacities superior to those observed with the more-common ion exchange combined with reversed-phase HPLC. Reaching the extremes, Luo et al. recently reported the application of a 70-cm-long, 20-μm-i.d. monolithic silica column combined with quadrupole ion trap MS for the analysis of a bacterial proteome (*31*). They identified 2367 different peptides that covered 855 distinct *Shewanella oneidensis* proteins by using a very long gradient and just 2.5 μg of tryptic digest.

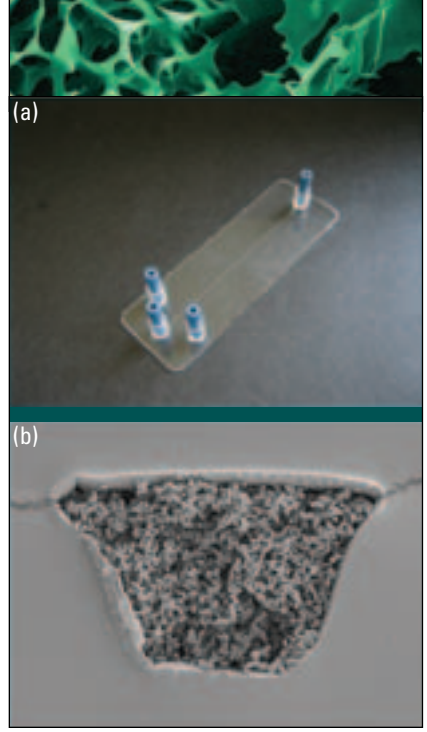


FIGURE 4. (a) Plastic microchip with integrated ports prepared by injection molding of cyclic olefin copolymer and (b) scanning electron micrograph of the porous polymer monolith inside the chip. (Photos courtesy of D. A. Mair.)

New capillary column technologies and chemistries

Organic polymers feature a rich selection of preparation methods and chemistries. For example, monoliths with living functionalities that allow grafting of surfaces with functional polymer chains were prepared via ring-opening metathesis polymerization of substituted norbornene monomers (*32*) or polymerization initiated by stable free radicals (*33*). However, these somewhat exotic methods did not attract widespread attention because of their limitations. In contrast, UV-initiated polymerization now appears to be more viable than its traditional thermally initiated counterpart. The real growth of photoinitiated polymerizations, which were first demonstrated in 1997 (*34*), occurred because this process enables patterning of monoliths within microfluidic devices. A microfluidic channel or capillary filled with a liquid polymerization mixture is irradiated through a mask, and the monolith is created only in the area accessible to UV light. In contrast to thermal initiation, the photoinitiated polymerization technique is much faster, and the monolith is ready in several minutes (*35*).

Given the inherent difficulties frequently encountered in preparing monoliths from monomers with very large differences in polarity, a two-step process has been developed to obtain excellent flow properties and a highly tunable surface chemistry (*35*). A generic monolith with optimized pore structure is prepared from a simple mixture of monomers and porogens. Polymer chains containing monomer units with the desired surface functionalities are then UV-grafted onto the surface through a mask. This approach is extremely versatile, because it allows independent control of the pore structure in the first step and the pore surface chemistry in the second step. The locations and quantity of functional groups can also be controlled. Chain growth can be adjusted, because it is a direct function of exposure. Multiple chemistries and functions can be combined in a single monolith.

For example, a combination of solid-phase extraction (SPE) and enzymatic digestion was demonstrated in a single dual-function microdevice for protein mapping (*36*). Because this device was prepared directly in the nanoelectrospray emitter, interfacing it to a mass spectrometer was very simple. The photolithographic approach also enabled the preparation of capillaries for on-line adsorption of selected proteins, on the basis of their affinity for immobilized copper ions or ion exchange functionalities, followed by electrophoretic separation (*37*). In a similar approach, a moving shutter or a stepped-density mask was used to graft the pore surface with longitudinal gradients of chemistries (*38*). Layers of different chemistries were achieved by consecutive grafting of different monomers on top of each other. These layers were invaluable for creating monolithic columns that can separate basic peptides and proteins in CEC mode (*39*).

Zare's group also adopted the process to prepare photopolymerized sol–gel monoliths in a single step (*40*). Addition polymerization and polycondensation of [3-(trimethoxysilyl)propyl] methacrylate proceeded simultaneously in the presence of a porogen. The resulting material exhibited an amazingly high mechanical strength. Perhaps the major advantage of this approach is that it eliminates the need for drying at the high temperatures that may lead to cracking of standard silica-based monoliths. This photochemical route also facilitates the exact placement of the monolith within the device.

Historically, the majority of inorganic stationary phases, including monoliths, consist of silica. Colon's group recently introduced zirconia and hafnia into the collection of chemistries suitable for preparing inorganic monoliths (*41*). Zirconia-based packings recently became a hot topic because of their excellent resistance to high temperatures and extreme pH values. These columns are likely to find their way into applications performed under severe conditions. Guiochon's group prepared a carbon-based monolithic column for HPLC via high-temperature carbonization and graphitization of a phenolic resin rod with embedded sacrificial silica beads as the precursors (*42*). This monolith has a highly interconnected bimodal porous structure and exhibits excellent separation performance and low hydraulic resistance.

Electrostatic attachment of charged latex nanoparticles onto monoliths is a new twist on a concept that has been proven in the functionalization of surfaces for ion-exchange chromatography. Such latex-coated monolithic capillary columns were used to separate saccharides and inorganic ions (*43*, *44*). However, coverage of monolithic surfaces with nanoparticles is less complete than with their microparticulate counterparts. Therefore, further investigations are under way to increase the coverage of the monolithic backbone with latex particles; this coverage seems to be a prerequisite to achieving high separation power.

Monolithic structures are ideally suited for use as components in microchip separation devices.

Enzymatic flow-through reactors

Although monolithic supports were used early in their development for enzyme immobilization, only a few reports were published (*45*). The advent of proteomics has renewed interest in their use for this application. Currently, proteins are digested for several hours with a proteolytic enzyme followed by MS analysis of resulting peptides; this is an indispensable part of most protein mapping protocols. In contrast, the use of enzymes immobilized on monolithic supports in capillary formats dramatically enhances the digestion rate (*46*). For example, a 12-s residence time was sufficient to digest myoglobin and obtain 67% sequence coverage in a monolithic microreactor composed of trypsin immobilized via azlactone functionalities (*47*).

Palm and Novotny developed a process in which acrylamide-derived monomers are polymerized and trypsin is immobilized in a single step (*48*). Their microreactor provided bovine serum albumin sequence coverage of 32% in 52 s. Krenkova et al. immobilized trypsin on a modified glycidyl methacrylate-based monolith and found that <30 s was sufficient to achieve 80% sequence coverage for cytochrome *c* (*49*). Dulay et al. found a 1500-fold increase in the tryptic activity of an enzyme immobilized in a photopolymerized sol–gel monolith compared with its counterpart in solution (*50*). Kato's group developed a technique in which the pore surface of a typical silica monolith is covered with a thin layer of trypsin encapsulated in tetramethoxysilane gel (*29*, *51*).

Although detailed studies have yet to be carried out, the significant acceleration of the reaction rate generally observed for proteolysis with enzymes immobilized on monolithic supports is probably caused by very fast mass transfer of the substrate to the immobilized biocatalyst and the efficient removal of reaction products by convective flow through the pores of the monolith. Because the enzyme moieties are located at the surfaces of large pores, macromolecular substrates do not need to be transported via slow diffusion.

Microfluidics

Because monolithic structures can be formed easily by in situ polymerization, they are ideally suited for use as components in microchip separation devices. Monolithic materials have already been used in modules, such as passive mixers, preconcentrators, valves, and separation units (*52*). Yet, the number of these applications is not what one would expect, given the ease of placing monoliths in microfluidic devices by photoinitiated processes. Moreover, because of the pressure limitations of typical microchips, monoliths with high permeability are the material of choice for transferring chromatographic separations from the column to the microchip.

In due course, silica- and polymer-based monolithic materials were incorporated as stationary phases into microchip devices. Hjertén and his colleagues created a simple capillary in a quartz plate and filled it with an acrylamide monolith for CEC separations (*53*). A silica monolith in a borosilicate glass chip was used for the SPE of nucleic acids that were subsequently amplified via PCR (*54*, *55*). Figure 4 shows a plastic microchip device involving a poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith prepared in situ via photopolymerization initiated with 2,2-dimethoxy-2-phenylacetophenone. Adhesion of the monolith to the microchip was accomplished through photografting of the channel surface with a nanoscopic layer of poly-(ethylene diacrylate) (*56*). The number of separations performed in microchips containing monoliths is rapidly increasing and includes inorganic ions, benzene derivatives, hormones (*57*), peptides, and proteins (*58*).

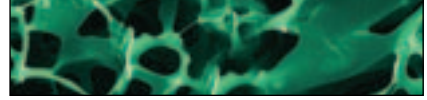
Misconceptions and real problems

Because monolithic columns are a new technology, their potential users logically compare them with classical packed columns. Their first concern is column-to-column reproducibility. Monolithic columns are believed to be produced one column at a time, like Rolls Royce automobiles, whereas standard chromatography columns are packed with stationary phases prepared in large batches. However, this perception may not be completely correct. Although normal packings are produced in larger quantities, each column is then packed individually. Thus, the overall process is not dissimilar, because a larger volume of the polymerization mixture can be prepared and used to produce many monoliths. Several studies have demonstrated that column-to-column reproducibility is not any worse for monolithic columns than for their packed counterparts (*59*).

Clogging of monolithic columns is another fear, because a monolith is believed to work like a dead-end filter. Simple mathematics reveals that the interstitial volumes among spherical particles have a channel size representing ~20% of the bead radius—1 μm for typical 5-μm particles. As discussed earlier, this is just the size of the through pores of typical monoliths. Why, then, should the monolith clog more than the packed column?

Yet another trend is to contrast silica- and polymer-based monolithic columns. The literature indicates that most LC applications of capillary silica monoliths are for small molecules, whereas organic monolithic capillary columns are more broadly used to separate biological and synthetic macromolecules. Nevertheless, this distinction was recently challenged by highly efficient separations of midsized peptides in silica-based monoliths (*60*). Similarly, CEC with polymeric monoliths was used to achieve excellent separation of small molecules (*21*). In the near future, we expect more studies of the optimization and application of silica- and polymer-based monolithic columns for separating small and large molecules.

What then are the real problems preventing monolithic columns from conquering the market? First of all, they compete with the well-established technology of packed columns. The major selling feature of monoliths, which is the ease of high-speed separations, is not very compelling to those who run two experiments a day. However, laboratories where high throughput is the norm may appreciate the properties of monoliths. Another significant problem arises from the limited number of com-



panies producing monolithic columns. Industrial laboratories do not like sole sources, especially not for validated processes. And, last but not least, the current selection of column sizes and stationary chemistries is much more limited than that in the world of packed columns. In particular, longer, 1–2-mm-i.d. columns are not yet commercially available. The only really large-scale column we know of is an 8-L tubular unit that includes a polymeric monolith and is operated in axial-flow mode (BIA Separations).

Conclusions and perspectives

Monoliths are still teenagers. Although much remains to be done, recent achievements open new vistas for this entirely new class of chromatographic columns, also called "stationary phases of the fourth generation". Guiochon recently claimed, "The 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" (*61*). A large body of experimental work has been done, and the commercial availability of various monolithic columns confirms their great potential (*62*).

Their unique properties—in particular, the ease of their preparation, their tolerance to high flow rates, and the rapid speed of chromatographic separations at acceptable back pressures—make monolithic columns superior in some applications to packed columns. Because monoliths are still young, the number of stationary phases, separation mechanisms, and analytical methods remains much smaller than those available for their more mature, packed counterparts. We expect that it is only a question of time until the range covered by monolithic technology is extended and these columns successfully compete with well-established separation technologies.

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Frantisek Svec is the lead scientist at the Molecular Foundry at the Lawrence Berkeley National Laboratory and a visiting professor at the University of Innsbruck (Austria). His research focuses on monoliths and macroporous polymers in different shapes and the use of these materials in LC, electrochromatography, enzyme immobilization, and microfluidics. Christian G. Huber is a professor at Saarland University (Germany). His research interests include the development of high-efficiency stationary phases for the separation of biologically relevant molecules and the implementation of multidimensional separation schemes for complex biological sample mixture analysis. Address correspondence to Svec at fsvec@lbl.gov.

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