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Monoliths Seen to Revitalize Bioseparations

New Research Will Broaden the Range of Applications

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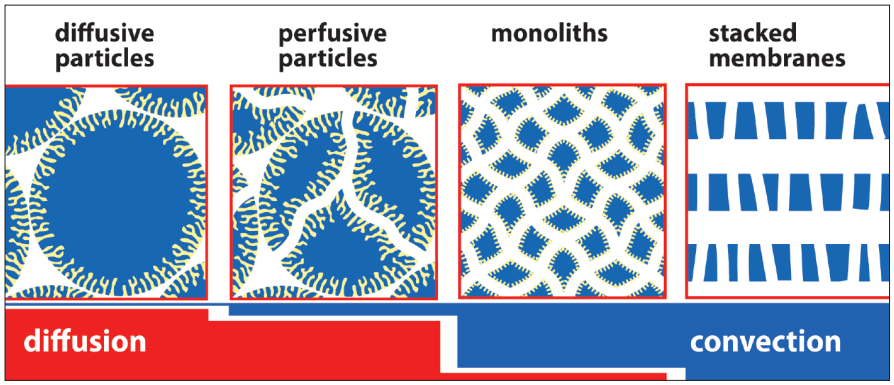
The early 1980s marked a major renaissance for chromatography. An unprecedented diversity of new analytical and preparative products was introduced within an exciting period of a few years, creating the foundation for the products currently serving the industry.

Advances in capacity and flow rate have led to valuable improvements in throughput since that time, but they have not been able to keep pace with the production capacity of the burgeoning biopharma industry.

One of the most promising evolutionary steps was the introduction of perfusion chromatography in the late 1980s. Perfusion technology showed, for the first time, that chromatography media could support high flow rates without sacrificing resolution. Now, having demonstrated the ability to maintain both resolution and capacity independently from flow rate, monolithic chromatography supports are offering new hope to close the gap between cell culture production capacity and downstream processing.

In parallel, research into new polymers, configurations, and surface chemistries promises to bring the benefits of monoliths to an increasingly broad range of applications.

The potential of monoliths to propel bioseparations into the next generation was evident during discussions at the second Monolith Summer School in Portoroz, Slovenia, earlier this year. Monoliths are chromatographic stationary phases that are polymerized directly in a column as a single unit. They were introduced independently by Hjertén's, Svec's, and Tennikova's groups in the late 1980s.



Mass transport in contemporary chromatography formats. Areas of diffusive transport are indicated in yellow, convective transport in white, and support matrix in blue. Mass transport in traditional porous particles occurs almost exclusively through diffusion. A small component of convective flow occurs at the particle surface. Perfusive particles employ convective channels to gain access to the interior of the particle, but transport is still dominated by diffusion. Monoliths are almost exclusively convective, with a small diffusive component contributed by so-called mesopores. Stacked membranes are purely convective.

Analytical Applications

Early work demonstrated excellent potential for analytical applications and this has come to fruition. Silica-based reverse-phase monoliths introduced by Tanaka in 1996 are now offered commercially by **Merck** (www.merck.de) and **Phenomenex** (www.phenomenex.com).

Compressed gel-based analytical ion exchange monoliths (UNO™) are provided by **Bio-Rad Laboratories** (www.bio-rad.com). **Dionex** (www.dionex.com) sells rigid porous polymer-based monolithic columns (SwiftPro®) in various formats for reversed-phase and ion-exchange chromatography. A complete line of ion exchange, reversed phase, hydrophobic interaction, and affinity monoliths (CIM®) are available from **BIA Separations** (www.biaseparations.com).

The performance of commercial analytical monoliths already equals the best of their particle-based counterparts, and the technology endows capabilities that have yet to be exploited. Capillary monoliths presented at the meeting demonstrated efficiencies in excess of 100,000 plates per meter and complete separations in less than 10 seconds. These columns also minimize consumption of expensive organic solvents, minimize sample volume, then compound overall savings by minimizing used solvent disposal costs.

The relationship of monoliths to other contemporary separation media is illustrated in the *Figure*. Professor Alois Jungbauer, department of biotechnology, University of Natural Resources and Applied Life Sciences (Vienna), emphasized the most prominent feature of monoliths—they exploit convective mass transport through large channels (1–5 µm) while porous particles must rely on diffusion through relatively narrow pores (600–1000 Å).

Diffusion is slow and increasingly so for larger solutes (*Table*). This is the reason why both capacity and resolution decline with increasing flow rate and solute size on traditional chromatography media.

Ales Podgornik, Ph.D., head of R&D for BIA Separations, likens convective flow to a river: the mass of the objects flowing down the river doesn't matter. A tree trunk or a cork flow equally with the current, however fast it might be. Access to the river banks—the binding surface—is unrestricted, so capacity and resolution are both independent of flow rate, regardless of solute size.

Although the kinetics of binding and elution are independent of solute size, there is an important difference with respect to absolute capacity. Prof. Jungbauer and Prof. Shuichi Yamamoto, of Yamaguchi University (Japan), both emphasized that in contrast to porous particles, binding capacity on monoliths increases with the size of the molecule. This suggests that the number of chemical binding sites may be the limiting factor and highlights a valuable application feature for large proteins, plasmids, and viruses.

Another important distinction from microparticles is that the channels in monoliths are highly interconnected. This creates high surface accessibility and uniform frontal migration throughout the support. The combination of convective flow with high interconnectivity allows rapid separations to be carried out with extremely short beds.

CIM disks sold by BIA Separations for analytical applications and preparative method scouting have a bed height of 3 mm. Scale-up products have bed heights ranging up to 46 mm. Interconnected channels and short beds translate into low back pressure, allowing high throughput without requiring costly high pressure pumps.

One other distinction is that up to 40% of packed particle beds are occupied by wasted space: the void volume between the particles. This represents not only lost capacity but also lost resolution because turbulent mixing in the void erodes the separation achieved by the surface chemistry.

Monoliths have no void volume. The entire bed, less the volume occupied by the support matrix, is functional. In this

Representative Diffusion Constants for Selected Solutes

Solute	Size	D_e (cm^2/sec)
H^+	1 Da	4.5×10^{-5}
NaCl	58 Da	1.4×10^{-5}
BSA	66 kDa	6.7×10^{-7}
IgG	150 kDa	4.9×10^{-7}
Urease	480 kDa	3.5×10^{-7}
IgM	960 kDa	2.6×10^{-7}
Cucumber mosaic virus	6 MDa	1.2×10^{-7}
Tobaccco mosaic virus	40 MDa	5.0×10^{-8}
DNA	4.4 kbp	1.9×10^{-8}
DNA	33.0 kbp	4.0×10^{-9}

respect, monoliths have an advantage over membrane chromatography as well. Mass transport on membranes is convective as it is with monoliths, but flow aberrations between layers and dead volumes within the housings contribute to turbulent mixing and sacrifice some of the resolution achieved by the surface chemistry.

Finally, monoliths do not require packing. Packing, process development, validation, and labor are eliminated. Differences in packing skills among operators become a non-issue. Scale-up scale-down variations in packing quality become a non-issue. Channeling from inadvertent introduction of air and the subsequent need to repack a column are eliminated.

Enhancing Bioseparations

Some of the most compelling applications for monoliths to date have been in areas where the mass-transport limitations of conventional chromatography media reduce their effectiveness. Wolfgang Buchinger, of **Boehringer Ingelheim Austria** (www.boehringer-ingelheim.at), discussed the use of monoliths for production of pharmaceutical-grade DNA plasmids. A monolithic anion exchanger gave far better throughput than conventional media, enabling the processing of 200-L batches of fermentation broth on a CIM monolith of only 800 mL. The system was fully validated and is now in routine commercial cGMP production.

The potential for monoliths to make substantial improvements in the field of virus purification was introduced by BIA Separations' Petra Kramberger, who presented a study comparing a monolith-based procedure with the published standard method for purification of tomato mosaic virus. The standard technique requires five days of manual intensive work. The monolith-based approach gives equivalent purity and better recovery in two hours.

Tom Ouellette, of the biopharmaceutical development program at SAIC/NCI-Frederick, presented a rapid HPLC quantitation method for adenovirus with a strong anion exchange monolith (CIM QA). He went on to talk about data on purification of herpes virus on CIM DEAE, achieving excellent fractionation and 100% activity recovery of this extremely labile product. He emphasized that the apparent low shear characteristics of monoliths are especially important for virus purification due to the vulnerability of the proteins and carbohydrates extending from the capsid.

During questions following her presentation on monolith-based affinity applications, Prof. Tatiana Tennikova of the Russian Academy of Sciences (St. Petersburg) noted that monoliths are free from the residence time limitations of porous particle supports. Residence time is an artifact of poor mass transport in particle based media. Convection avoids this limitation. This was proven dramatically in a presentation by Prof. Igor Galaev, of the University of Lund (Sweden), demonstrating rapid affinity capture of whole cells on acrylamide monoliths.

Mojca Bencina, of the National Institute of Chemistry (Slovenia), presented work on monolith-based bioreactors, showing that DNAse immobilized on monoliths closely approaches the catalytic rate of free enzyme. She explained that the open channel network allows unrestricted access of DNA to the enzyme.

Manuela Bartolini's group from the University of Bologna, Italy, described a monolith-based acetylcholinesterase bioreactor for high-speed screening of drug candidates in the treatment of Alzheimer's.

Prof. Djuro Josic, from the COBRE Center for Cancer Research Development (www.rih.cobre-cares.org) stated that the unique capabilities of monoliths make them almost ideal for proteomic applications, commenting in particular on the potential for miniaturized ion exchange monoliths coupled with reversed-phase nano-HPLC, leading to MS and 2-D electrophoresis.

Implementation Challenges

Monoliths are currently available off the shelf in radial flow columns up to 8 L. BIA Separations is confident that the key engineering issues in scale up have been addressed, but this is a fundamentally different situation from chromatography with porous particles, where columns with bed volumes greater than 100 L have been used for decades.

Another challenge resides in synthesizing monoliths optimized for protein applications. The current generation has been optimized for large proteins, plasmids, and virus particles. Resolution and capacity for small to midsized proteins exhibit the characteristic independence from flow rate but their absolute capacities are lower on current CIM monoliths than on the latest particle media.

The founder of BIA Separations, Ales Strancar, Ph.D., explained that the initial choice to optimize for larger solutes was strategic—to extend the benefits of chromatography into fields where the diffusional limitations of conventional media dramatically reduce their utility. Creating monoliths optimized for protein applica-

tions is equally a matter of strategic focus.

A third challenge applies equally to all chromatography media, as emphasized by Prof. Klaus Unger, of Johannes Gutenberg-Universität (Germany), the selection of surface chemistries on chromatographic supports has remained essentially static for nearly a quarter century. The research community and chromatography media manufacturers urgently need to develop this unexploited resource if the field of bioseparations is to realize its full potential.

Prof. Frantisek Svec, of the University of California (Berkeley), addressed the future of monoliths in his keynote address. He discussed a new photografting process of monolith synthesis, allowing modification of pore surface independent of the control of porous properties. He also remarked on the successful migration of monolithic technologies from their original applications in liquid chromatography to new fields such as capillary electrochromatography, sample preparation, enzymatic digestion, sensors, and complex devices.

"Monolithic columns went a long way from the initial demonstrations of simple bioseparations in the late 1980s to current large radial flow columns, superfast separation units, and microfluidic systems," he said.

What will the role of monoliths be in the rapidly growing sphere of bioseparations? Monoliths represent an important addition to the capabilities of the field. They have already demonstrated a unique and compelling set of features for analytical applications with solutes of all sizes and equally in process purification of DNA plasmids and viruses. Future products can be expected to extend more deeply into the domain of protein purification, where their potential to increase throughput promises to be an important advance for the entire industry.

As they continue to evolve, monoliths will establish their natural place in the overall continuum of monoliths, membranes, and microparticles. GEN

Pete Gagnon is a member of *GEN*'s editorial advisory board on process chromatography. For an electronic copy of the Conference Book of Abstracts, please contact Damjan Nemec at BIA Separations. E-mail: damjan.nemec@monoliths.com.