Eliminating the Downstream Processing Bottleneck with Monoliths and Simulated Moving Bed Chromatography

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Trends in IgG purification

Two major trends have developed in the manufacture of monoclonal antibody-based therapeutics:

1) The transition towards single-use processing materials.

2) The drive for increasing productivity in downstream processing.

This presentation will show how monoliths and simulated moving bed chromatography advance both trends.

Packed particle columns

Fluid flows preferentially between the particles in a packed bed, not through the particles. Flow rate must be slow enough to permit solutes to diffuse into the pores to achieve efficient binding, and out of the pores to achieve efficient elution.

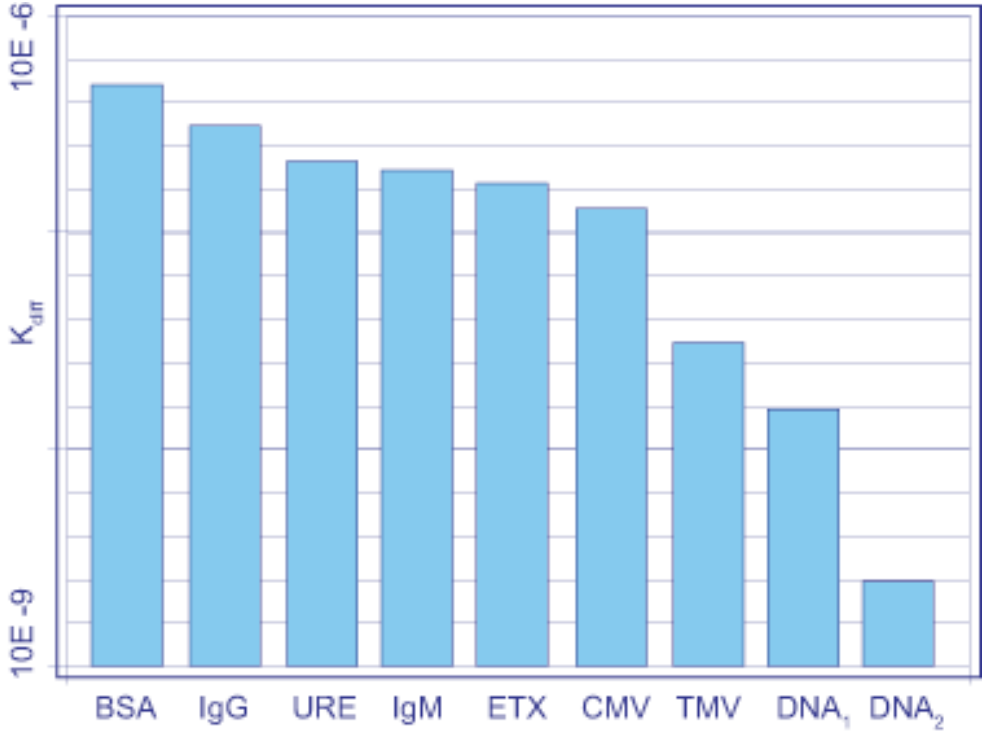
Diffusion is slow, especially for large solutes such as proteins, even slower for DNA, endotoxin, and viral particles.

As a result, both binding capacity and resolution decline significantly with increasing flow rate.

A. Jungbauer, 2005, Chromatographic media for bioseparation, *J. Chromatogr.*, **1065** 3-12. N. Afeyan, N. Gordon, J. Mazaroff, C. Varaday, S. Fulton, Y. Yang, F. Regnier, 1990, Flow through particles for the high performance liquid chromatography separation of biomolecules, *J. Chromatogr.,* **519** 1-29. A. Tongta, A. Liapis, D. Siehr, 2001, Equilibrium and kinetic parameters of the adsorption of a-chymotrypsin A onto hydrophobic porous adsorbent particles*, J. Chromatogr. A*, **686** 21-29

Packed particle columns

Diffusion constants of selected solutes



Solute	Size	K_{diff}
BSA	66 Kd	6.7×10^{-7}
IgG	150 Kd	4.9×10^{-7}
URE	480 Kd	3.5×10^{-7}
IgM	1 Md	2.6×10^{-7}
ETX	2 Md	2.1×10^{-7}
CMV	5 Md	1.2×10^{-7}
TMV	40 Md	5.0×10^{-8}
DNA_1	4.4 kbp	1.9×10^{-8}
DNA_2	33 kbp	4.0×10^{-9}

URE: urease
ETX: endotoxin
CMV: cucumber mosaic virus
TMV: tobacco mosaic virus

Packed particle columns

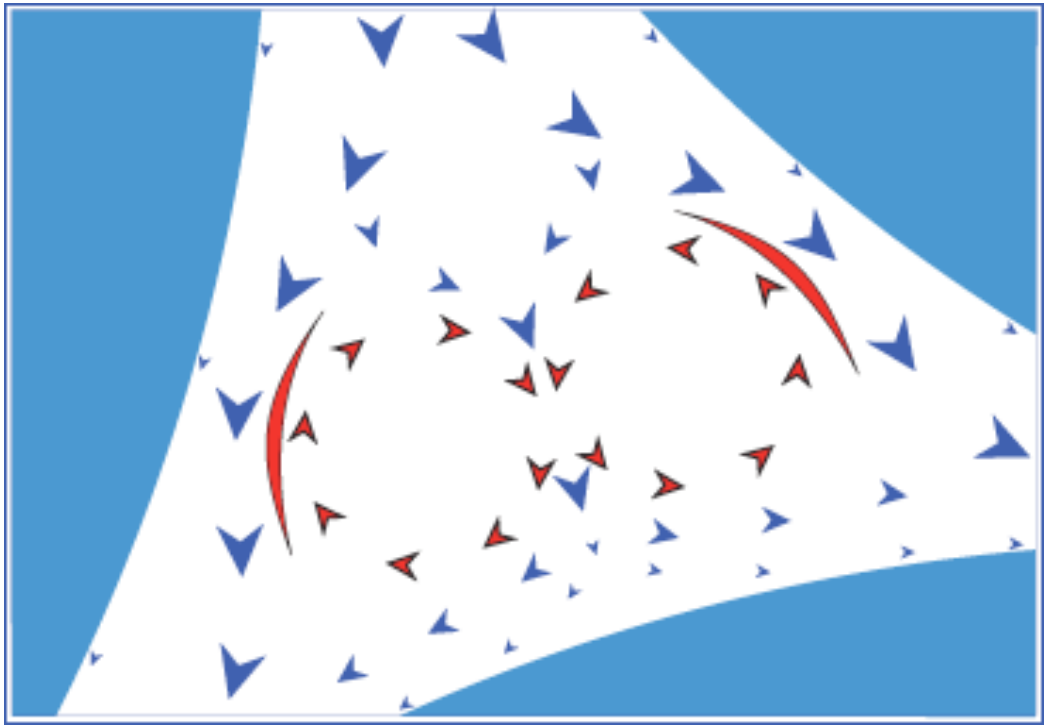
Loss of performance is compounded by the void volume, which constitutes about 40% of a well packed bed.

Differential friction between the particle surfaces and inter-particle void regions creates eddies, vortices that erode resolution and dilute peaks as they flow down the column.

Eddies also create shear forces. Eddy dispersion remains constant with increasing flow rate but shear increases in direct proportion.

Packed particle columns

Inter-particle eddy dispersion and shear



Blue areas indicate chromatography particles. The white area indicates the void space between particles. Arrows indicate the direction of flow. Arrow size indicates relative flow velocity. Blue arrows mark primary flow. Red arrows mark eddy flow. Red crescents mark zones of adjacent countercurrent flow where shear occurs.

Packed particle columns

Intra-particle void and pore connectivity

Although pores penetrate the entire particle, only pores that open to the surface are accessible to solutes, and these only to a shallow depth. The remainder of the particle is unutilized.This limits capacity but conserves resolution and recovery, since solutes that diffuse deeply into the particle would require more time to diffuse back out.

In addition, there is a low degree of pore connectivity within the accessible pore volume. Computer models suggest an average pore connectivity of about 1.5 for Sepharose™. This means that a given solute molecule may diffuse into and out of the same pore, or enter one pore and exit through a connected pore.

J. Meyers, S. Nahar, D. Ludlow, A. Liapis (2001) Determination of the pore connectivity, pore size distribution and pore spatial distribution of porous particles from nitrogen sorption measurements and pore network modeling, J. Chromatogr. A, ***907*** *57-71*

Monoliths

A monolith can be defined as a continuous stationary phase cast as a homogeneous unit. Channel size in monoliths for biomolecule purification mostly ranges from 2-5 microns.

Large channels make the adsorptive surface directly accessible to solutes as they pass through the column.

Mass transport is dominantly convective. Capacity and resolution are relatively unaffected by flow rate.

This makes monoliths less sensitive to variations in flow rate, column configuration, and residence time than porous particles.

Monoliths

Monoliths do not have a void volume.

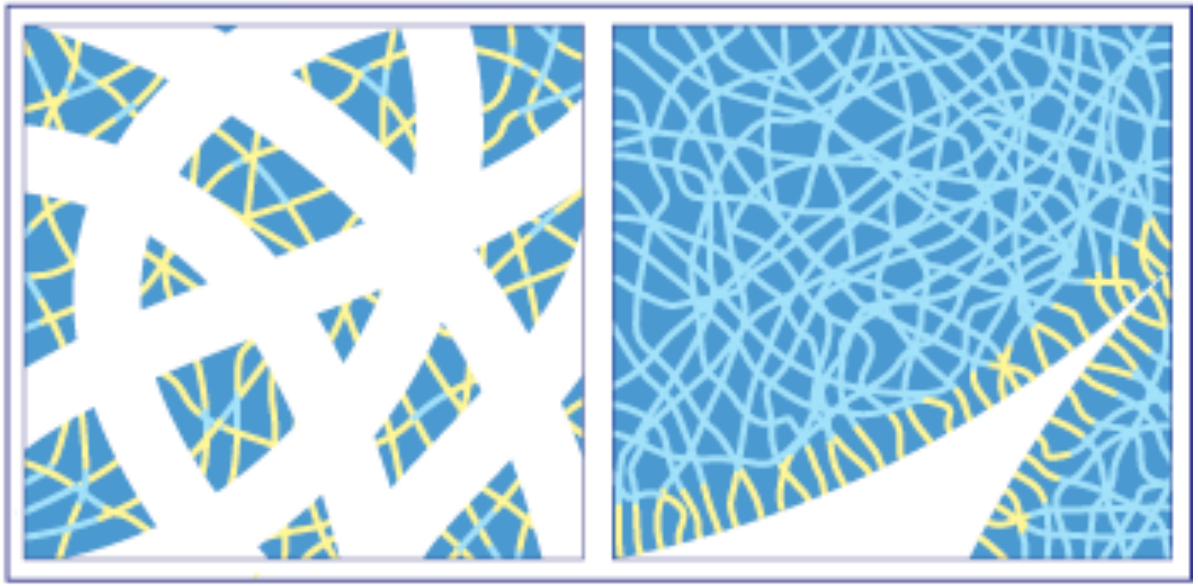
Channels are highly interconnected. Computer models indicate connectivity values ranging from 6 to greater than 10, meaning that a solute that enters one channel may exit any of more than 10 others. This permits effective utilization of the entire monolith.

Flow is laminar. Laminar flow means lack of eddies, which eliminates the major source of peak broadening. This improves resolution, increases the concentration of eluted peaks, and eliminates eddy-associated shear.

A. Jungbauer, 2005, Chromatographic media for bioseparation, *J. Chromatogr. A*, **1065** 3-12. R. Hahn, M. Panzer, E. Hansen, J. Mollerup, A. Jungbauer, 2002, Mass transfer properties of monoliths, *Sep. Sci. Technol.,* **37**(7) 1545-1565. A. Strancar, A. Podgornik, M. Barut, R. Necina (2002) Short monolithic columns as stationary phases for biochromatography, *Adv. Biochem. Eng. Biotechnol*, **76** 49-85

Monoliths vs porous particles

Basic architecture

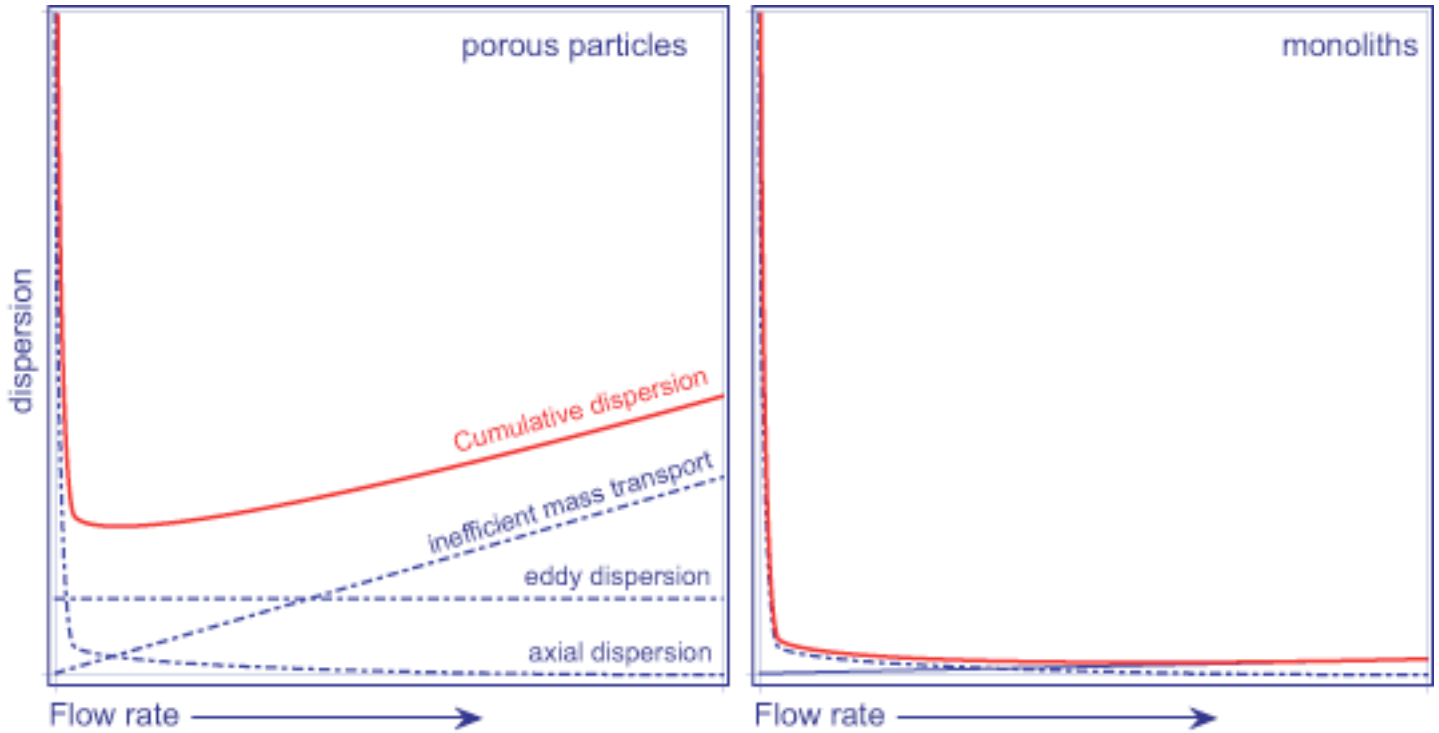


Monoliths | Packed porous particles

Dark blue indicates structural material. White indicates areas of convective mass transport. Yellow indicates areas of diffusive mass transport. Light blue indicates inaccessible pores. As shown, some monoliths have a small percentage of diffusive pores. Convective mass transport occurs in packed particle columns but only through the void volume.

Monoliths vs porous particles

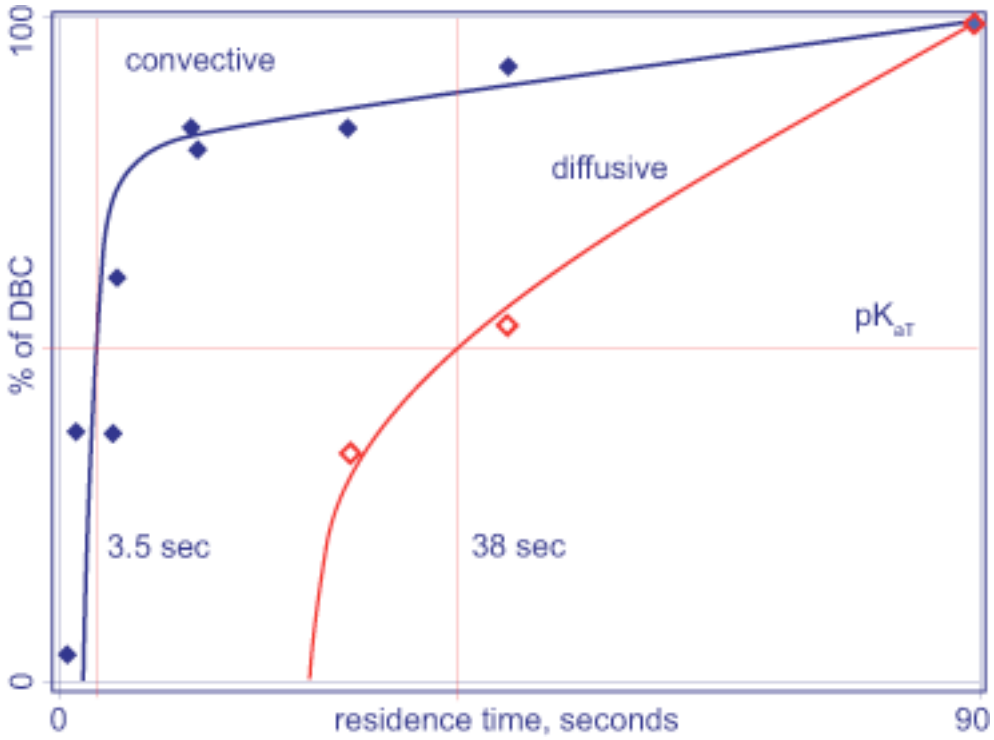
Cumulative dispersion



Cumulative dispersion is dramatically lower in monoliths for two reasons: convective mass transport is independent of flow rate, and eddy dispersion is eliminated.

Monoliths vs porous particles

Efficient mass transport reduces residence time requirements



Convective: CIM® Protein A HLD, 1 mL (3 x 0.34 mL)

Diffusive: MabSelect™ Xtra 1 mL (5 x 50 mm)

Dynamic break-through studies conducted at various flow rates to produce different residence times.

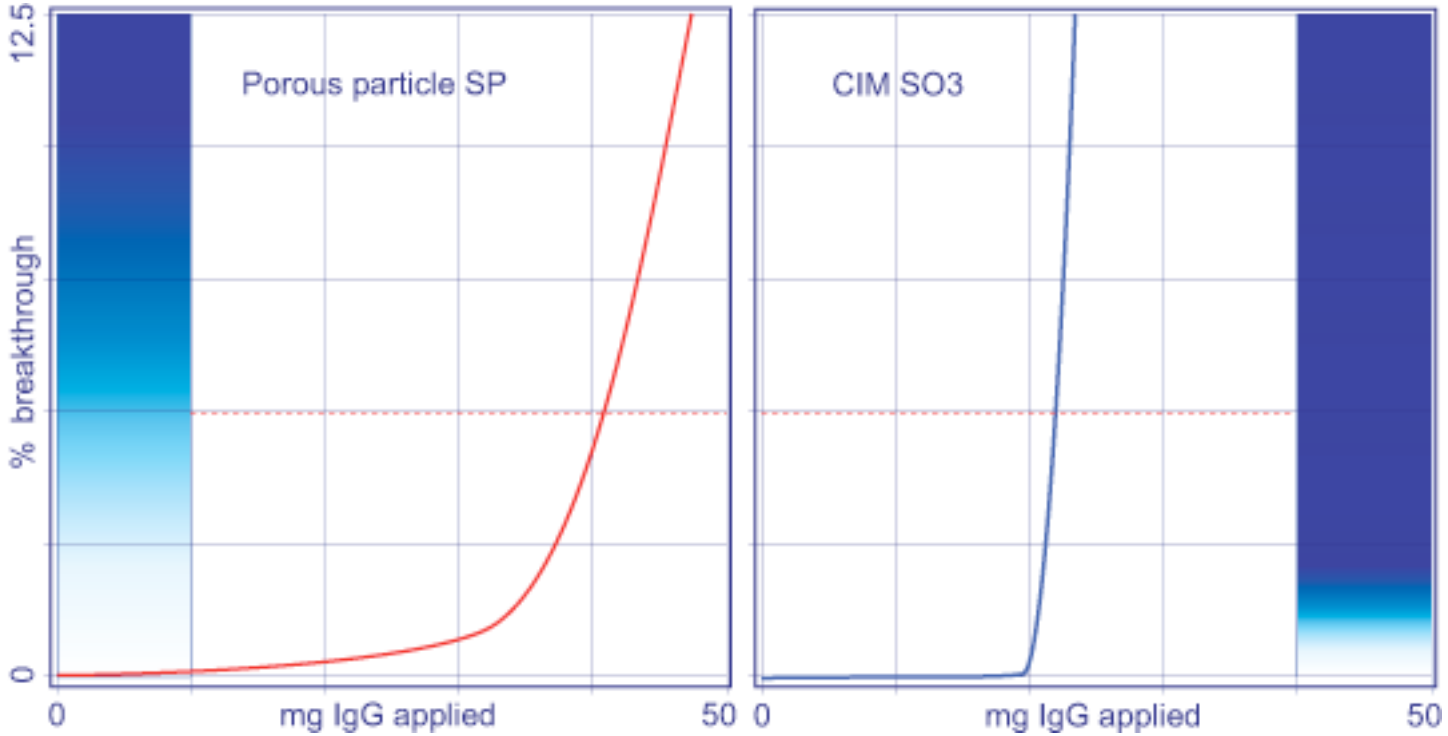
All values expressed as % of the 5% breakthrough value at a residence time of 90 seconds.

The residence “pK” for each product is expressed as the residence time corresponding to 50% of its 5% breakthrough value at 90 seconds.

For additional experimental details and results consult: Productivity improvements in the capture and initial purification of monoclonal antibodies, P. Gagnon and R. Richieri, 2nd Wilbio Conference on Purification of Biological Products, September 2006, Thousand Oaks, CA USA. <http://www.validated.com/revalbio/pdffiles/PUR_MassTrans.pdf>

Monoliths vs porous particles

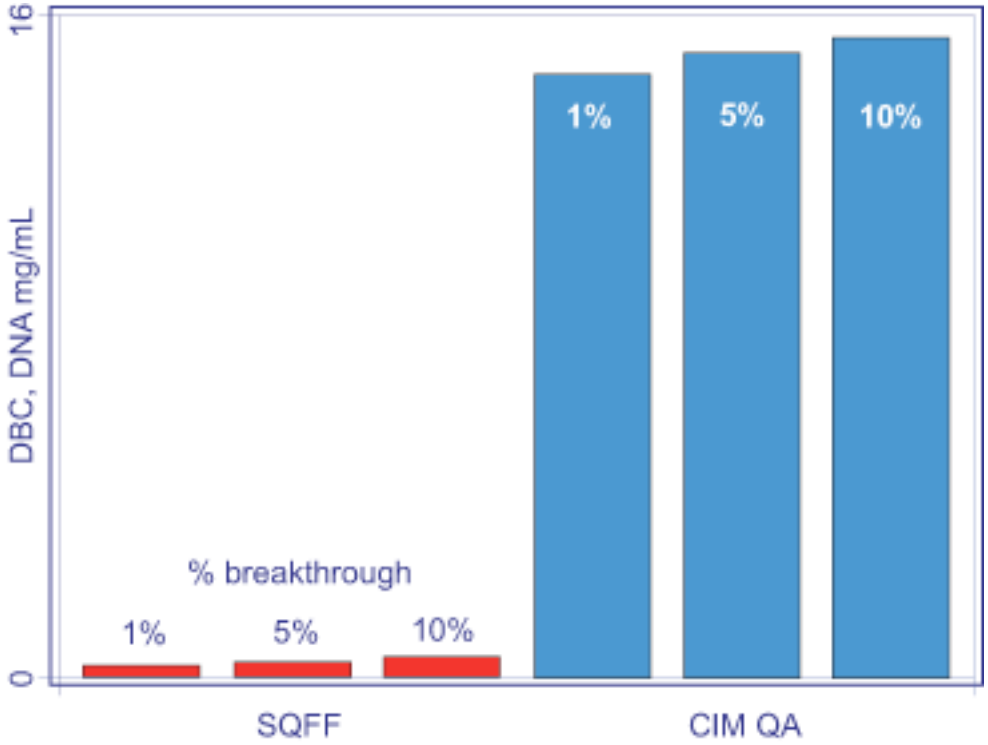
Efficient mass transport permits more complete media utilization.



Determination of dynamic binding capacity on strong cation exchangers. Protein A purified IgG_1 chimera at 1 mg/mL. The 1 mL (5 x 50 mm) particle column was operated at 1 mL/min (1 CV/min). Data for the monolith were obtained with a 0.34 mL monolith run at 4 mL/min (12 CV/min), and multiplied by three for comparison to the 1 mL column.

Monoliths vs porous particles

Relative binding efficiency is also apparent in the capacity of large solutes.



Reproduced from: A comparison of microparticulate, membrane, and monolithic anion exchangers for polishing applications in the purification of IgG monoclonal antibodies, P. Gagnon, R. Richieri, S. Zaidi, F. Aolin, IBC International Conference and Exposition, October 1-4, 2007, Boston, MA, USA. <http://www.validated.com/revalbio/pdffiles/IBCBOS07a.pdf>

Monoliths vs porous particles

Dynamic binding capacity

Solute	Method	Monoliths		Particles
BSA	AX	20-25 (81*)	<	75-300
IgG	protein A	10-12	<	25-60
IgG	AX/CX	20-25	<	50-150
IgM	AX/CX	20-50	=	10-50
DNA	AX	12-15	>	0.5-3
Etox	AX	115-150	>	9-15
Flu virus	AX	10-100x**	>	1x

All values in mg/mL except influenza virus.

V. Frankovic (2008) Characterization of a grafted weak anion methacrylate monolith, 3rd International Symposium on Monoliths, Portoroz, May 30-June 4.

** E. Maurer (2008) Purification of live replication deficient influenza virus, 3rd International Symposium on Monoliths, Portoroz, May 30-June 4.*

Monoliths vs porous particles

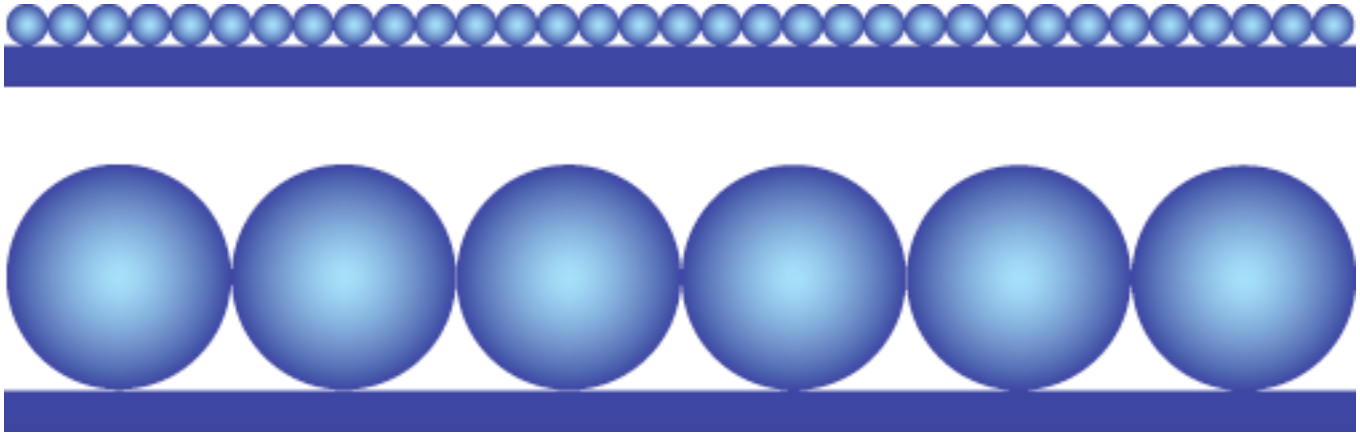
Why is monolith capacity lower for smaller solutes?

Even though the binding surface is immediately accessible, there is less adsorptive surface area per unit volume of media.

This results from their high degree of cross-linking and comparatively large channel size.

Monoliths vs porous particles

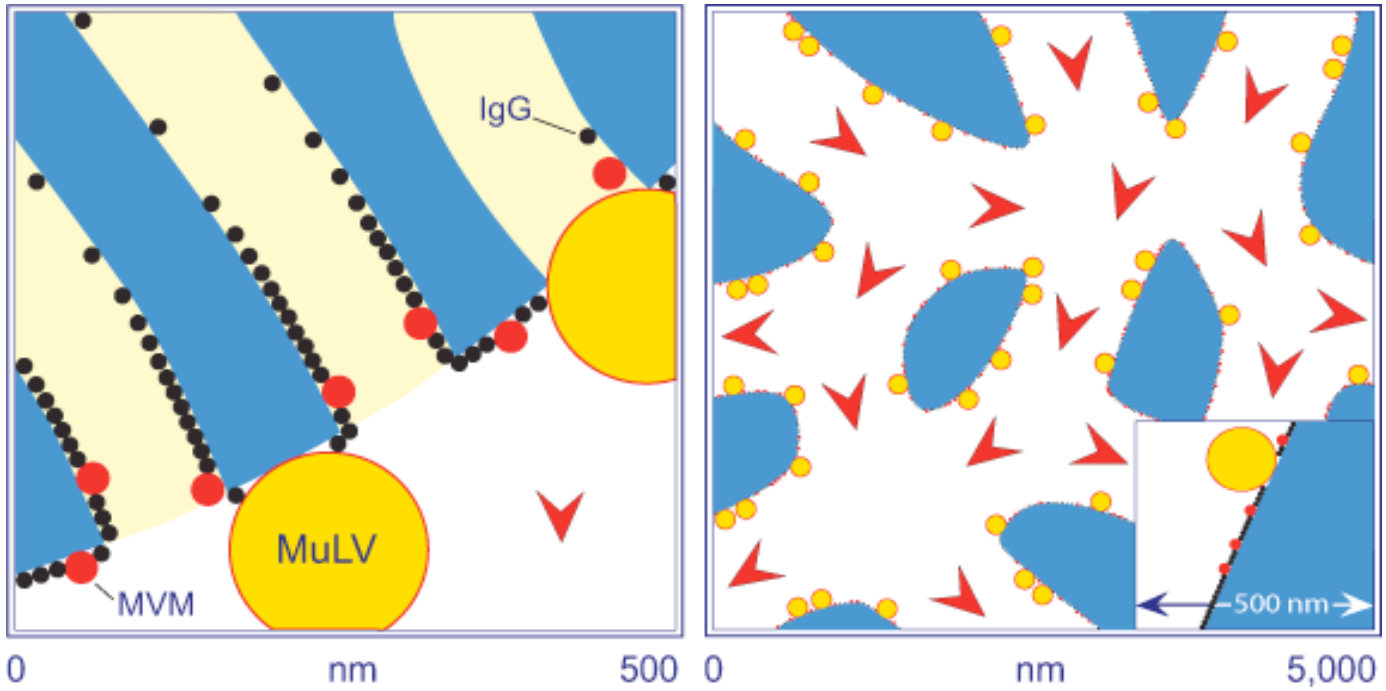
Why is monolith capacity higher for large solutes?



This phenomenon occurs to a degree in porous particle media but is masked by the contrary effects of diffusion and pore exclusion, both of which become more restrictive with increasing solute size.

M. Etzel (2003) in F. Svec and T. Tennikova (eds.) Monolithic materials, Elsevier, Amsterdam, p. 213. S. Yamamoto, A. Kita, (2006) Trans IChemE, part C, 84 72-77. A. Jungbauer, 2005, *J. Chromatogr. A*, **1065** 3-12.

Monoliths vs porous particles



Scale comparison of particle pores versus monolith channels. Illustrated pore size is 100 nm. Channel size is 1 µm (1000 nm). MuLV (diam.150 nm), MVM (diam. 25 nm), and IgG (hydrodynamic diam. 12 nm) are included for reference. Dark blue indicates structural material. White indicates areas of convective mass transport. Yellow indicates areas of diffusive mass transport. Red arrows mark direction of flow. MuLV cannot enter the pores and has access to only the exterior of the particle. Surface access is unrestricted in the monolith.

IgG capture on conventional protein A

8 L protein A column, capacity 25 g/L

- *100 L filtered CCS at 2.5 g IgG/L = 2.5 kg*
- *Column dimensions: 22.5 cm d x 20 cm h*
- *Flow rate: 200 cm/hr, 80 L/hr*
- *Cycle volume: 240 L, 30 CV (5 CV equilibration,10 CV load, 5 CV wash, 5 CV elute, 5 CV regenerate)*
- *Total cycles: 13*
- *Total process time: 39 hr*
- *Total process volume: 3,120 L*

IgG capture on a protein A monolith

800 mL protein A monolith, capacity 11 g/L

- *100 L filtered CCS at 2.5 g IgG/L = 2.5 kg*
- *Flow rate: 3 CV/min, 2.4 L/min, 144 L/hr*
- *Cycle volume: 19.5 L, 24.4 CV (5 CV equilibration, 4.4 CV load, 5 CV wash, 5 CV elute, 5 CV regenerate)*
- *Total cycles: 284*
- *Total process time: 38.5 hr*
- *Total process volume: 5,538 L*

- *Same process time as conventional protein A, 78% higher process volume, 10% the media volume, economics support disposability.*

IgG capture on a protein A monolith

8 L protein A monolith, capacity 11 g/L

- *100 L filtered CCS at 2.5 g IgG/L = 2.5 kg*
- *Flow rate: 3 CV/min, 24 L/min, 1440 L/hr*
- *Cycle volume: 195 L, 24.4 CV (5 CV equilibration, 4.4 CV load, 5 CV wash, 5 CV elute, 5 CV regenerate)*
- *Total cycles: 29*
- *Total process time: 3.9 hr*
- *Total process volume: 5,655 L*

- *10x faster (or 10 times more antibody in the same time) as the same volume of conventional protein A media, 82% higher process volume/kg IgG.*
- *Additional scale-up can be achieved with parallel processing.*

Alternative processing formats

Recent presentations have shown that countercurrent multi-column chromatography systems also have potential to reduce the downstream productivity bottleneck, and improve single-use economics. Such systems are commonly referred to as simulated moving bed (SMB) systems.

In particular, SMB systems have the ability to substantially reduce chromatography media volume and buffer consumption per unit of productivity. Multiple channels allow high cumulative flow rates.

Coordination of monoliths with SMB should therefore compensate for the capacity limitation of monoliths, and offer SMB the benefit of faster throughput, while conserving the option of disposability.

Alternative processing formats

BioSMB™ (Tarpon Biosystems) is a system of software and a disposable flow path that makes counter-current chromatography a plug-and-play processing option.

BioSC™ (Novasep) represents an integrated skid approach.

Both systems can accommodate a variety of media formats and process scales.

Both can accommodate flow-through, capture, and gradient applications.

Conventional protein A

160 L (100 x 20 cm) conventional protein A column

- *20,000 L filtered CCS at 2.5 g IgG/L = 50 kg*
- *Capacity: 25 g/L*
- *Flow rate: 200 cm/hr, 1,571 L/hr*
- *Cycle volume: 4,800 L (equilibrate, 800 L; load 1600 L; wash 800 L; elute 800 L; clean 800 L)*
- *Total cycles: 13*
- *Total process time: 40 hr*
- *Total process volume: 62,400 L*

Monolith-BioSMB system

80 L BioSMB (10 x 8 L protein A monoliths)

- *20,000 L filtered CCS at 2.5 g IgG/L = 50 kg*
- *Capacity: 12.5* g/L*
- *Flow rate: Sample application,1440 L/hr; other operations, 720 L/hr; cumulative flow rate, 4320 L/hr*
- *Cycle volume: 1200 L* (equilibrate, 200 L; load 400 L; wash 200 L; elute 200 L; clean 200 L)*
- *Total cycles: 50*
- *Total process time: 14 hr*
- *Total process volume: 60,480 L*

**Capacity is higher and buffer volume is reduced, compared to the single unit, because of tandem serial column placement within each operation.*

Conventional vs Monolith-SMB

Comparison, 50 kg	Conventional	Monolith-SMB
Binding capacity	25 g/L	12.5/L
Media volume	160 L	80 L
Flow rate	1571 L/hr	4320 L/hr
Cycles	13	50
Process volume	62,400 L	60,480 L
Time	40 hr	14 hr

Despite the lower capacity of the monolith, the SMB system allows it to purify the same amount of antibody, with half the volume of protein A media, in about a third of time, with slightly less buffer — and the number of cycles makes it potentially feasible to dispose of the used media.

Summary

Monoliths offer several options for increasing downstream productivity.

An 8 L monolith can purify the same amount of IgG as an 8 L conventional protein A column, but 10 times faster. Or it can purify 10 times as much IgG in the same amount of time.

An 800 mL monolith can produce the same amount of IgG as an 8 L conventional column in the same amount of time. In addition to requiring only 10% the media volume, the large number of cycles makes it economical to dispose of the monolith after processing a single product lot, thereby avoiding all of the costs associated with cleaning and sanitization.

Summary

The primary liability with processing on a single monolith, or parallel processing on multiple monoliths, is the high buffer consumption, which results from their relatively low capacity.

The ability to reduce manufacturing time by a factor of 10, or increase manufacturing output by a factor of 10 – and dispose of used media after processing a single lot of product – may be economically compelling nevertheless.

Alternatively, the high efficiency of SMB eliminates the buffer volume disadvantage, requires half the protein A media, triples facility output, and still employs a sufficient number of cycles to make single-lot disposability a potential option.

Summary

Elimination of column packing compounds the economy of single-use processing. Monoliths require no packing hardware, no packing labor, and no packing validation. This is especially beneficial in multi-column systems such as SMB.

In addition, monoliths tolerate the passage of air without loss of chromatographic performance. Restoration of fluid flow displaces the air and normal operation can continue. This eliminates the need to repack columns in the event of an air incursion during a manufacturing campaign. It also eliminates the need to keep expensive back-up columns on hand to ensure campaign continuity.

Acknowledgements

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Copies of this presentation can be downloaded at www.validated.com