Adsorptive Chromatography: the Influence of Stationary Phase Architecture

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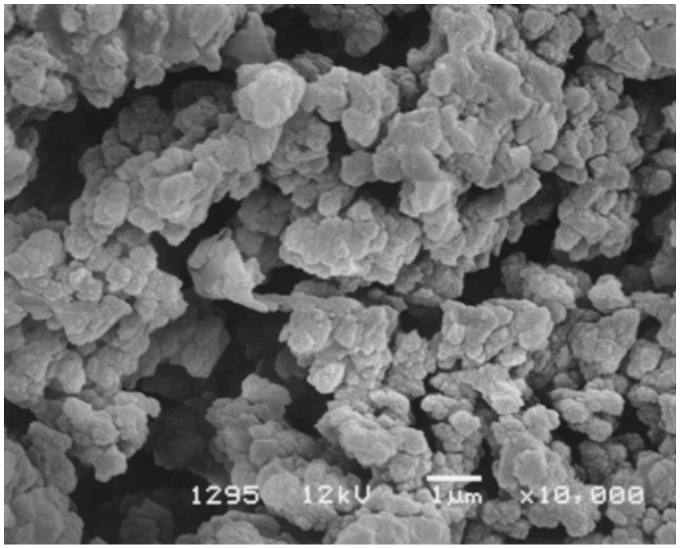
Stationary phase architecture

Adsorption chromatography – which includes methods such as affinity, ion exchange, and hydrophobic interaction chromatography, among others – can be conducted in a variety of physical formats, including porous particles, monoliths, and membranes.

But there is no “One size fits all.”

Each physical format has a distinctive set of strengths and limitations that makes it more or less suited for a particular application.

Continuous vs discontinuous structure

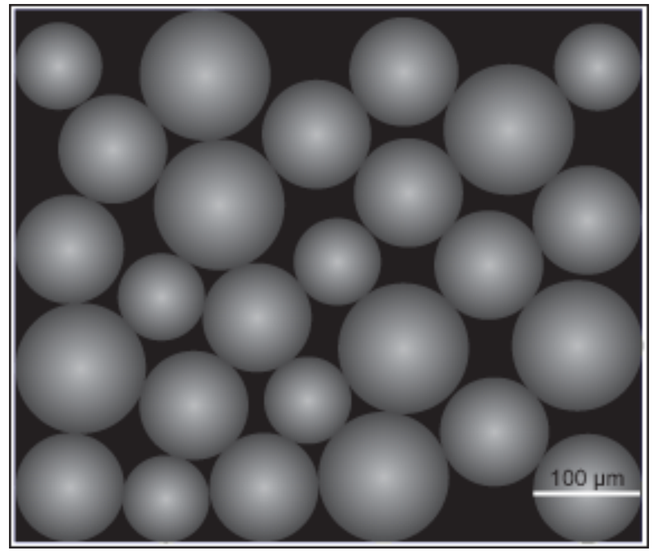


Monoliths

Channel diameter 1-2 µm throughout the entire bed.

Each channel connects to an average of 10 other channels.

Channel volume is about 60% of total monolith volume.



Packed particle column

Mean diameter 40-150 µm, +/- 50%

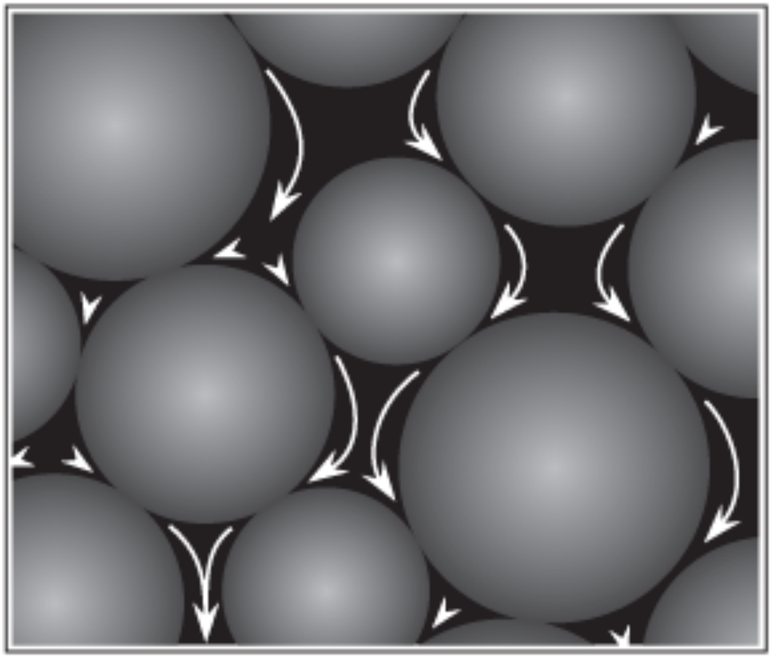
Inter-particle void volume 40% of V_t

Inter-particle distances 10-90% of mean particle diameter.

Different flow regimes between the void volume and particle surfaces.

Consequences of discontinuity

Fluid flows preferentially between particles — not through them.

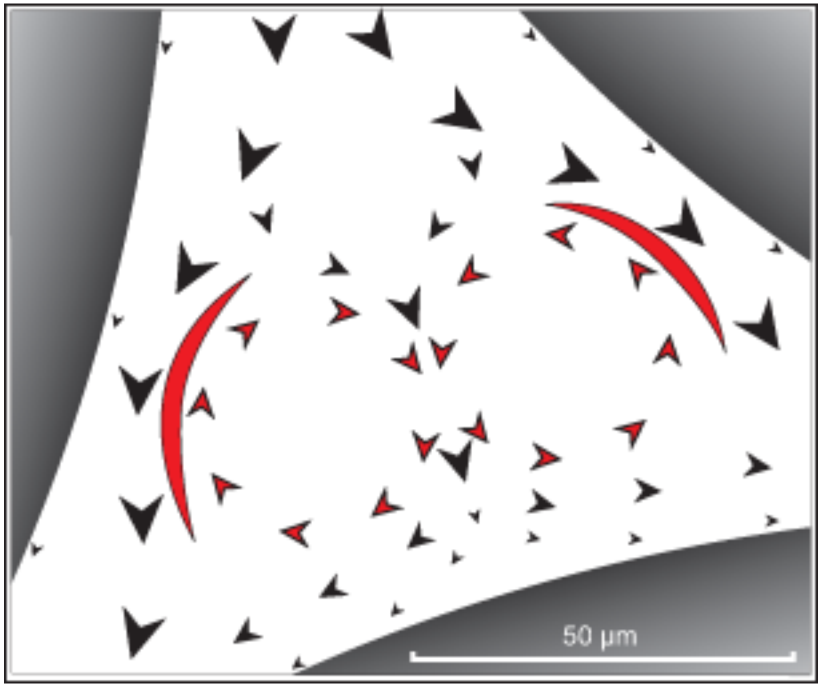


Fluid takes the path of least resistance.

This causes target molecules to be swept away from particle surfaces, which affects every aspect of column performance.

Consequences of discontinuity

The frictional differential between particle surfaces and the deep void space creates eddies — areas of persistent countercurrent flow.



Gray areas indicate particles.

The white area indicates the void space between particles.

Black arrowheads indicate primary flow.

Red arrowheads indicate countercurrent (eddy) flow.

Arrowhead size is proportional to local flow velocity

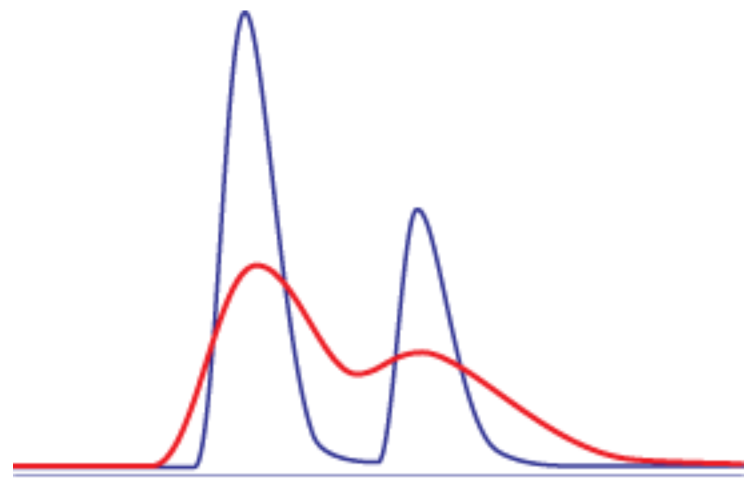
Red crescents indicate areas of adjacent primary and counter-current flow, where shear occurs.

Representative scale for reference.

Modified from Gagnon, P., 2008, The emerging generation of chromatography tools for virus purification, BioProcess International. 6 (Suppl. 6) 24-30. http://validated.com/revalbio/pdffiles/VirPurBPI.pdf

Consequences of discontinuity

Eddies create dispersion. Dispersion degrades separation performance and dilutes the eluted product. The degree of dispersion and dilution are independent of flow rate.



Eddies also create shear forces that damage labile biomolecules. Eddy-generated shear is proportional to flow rate.

Eddies also fulfill the essential function of mixing in the void space, without which solute transport to particle pores would be hopelessly inefficient.

Discontinuity of mass transport

Mass transport *refers to the way solutes (proteins, DNA, virus particles) move through a chromatography column.*

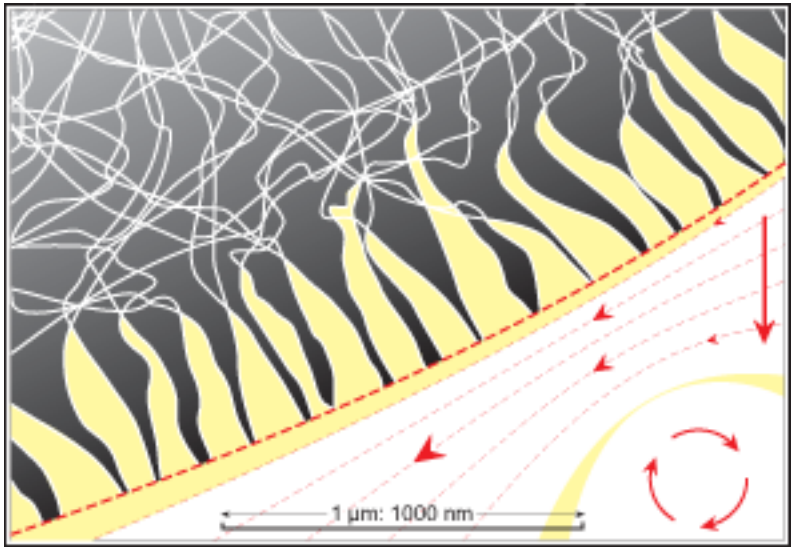
Convection *can be defined as movement induced by an external force, such as the flow of buffer, induced by gravity or a pump.*

Diffusion *can be defined as random thermal movement from an area of high concentration to an area of low concentration.*

Mass transport in packed porous particle columns is multimodal*; a combination of laminar and turbulent convective transport through the void volume, and diffusive transport from the particle exterior into the pores.*

Multimodal mass transport

Multimodal mass transport in packed particle columns



Gray area: particle body
White lines: pore boundaries
Heavy dashed line: particle boundary
Yellow: areas of diffusive transport
White: areas of convective transport
Light dashed lines: laminar flow contours
Redheads: direction/rate of laminar flow
Vertical arrow: main axis of column flow
Circular arrows: turbulent (eddy) flow

Friction at the particle surface creates a fluid layer with a flow rate approaching zero. There is no flow within the pores. This permits solutes to enter/exit the pores by diffusion. Flow rate increases with distance from the particle surface, partly due to laminar flow, partly due to preferential flow through the void volume, making diffusive pore-entry less efficient. Note that laminar flow at the particle surface may be tangential, even perpendicular, to the main axis of flow through the adjacent void.

Consequences of diffusive transport

The larger the solute, the more slowly it diffuses.

The more slowly it diffuses, the longer the time required for it to enter or exit from a pore.

Column flow rate can be reduced to compensate for the low diffusion constants of large solutes. This is uneconomical from a manufacturing perspective, but the alternative is to sacrifice binding capacity and resolution.

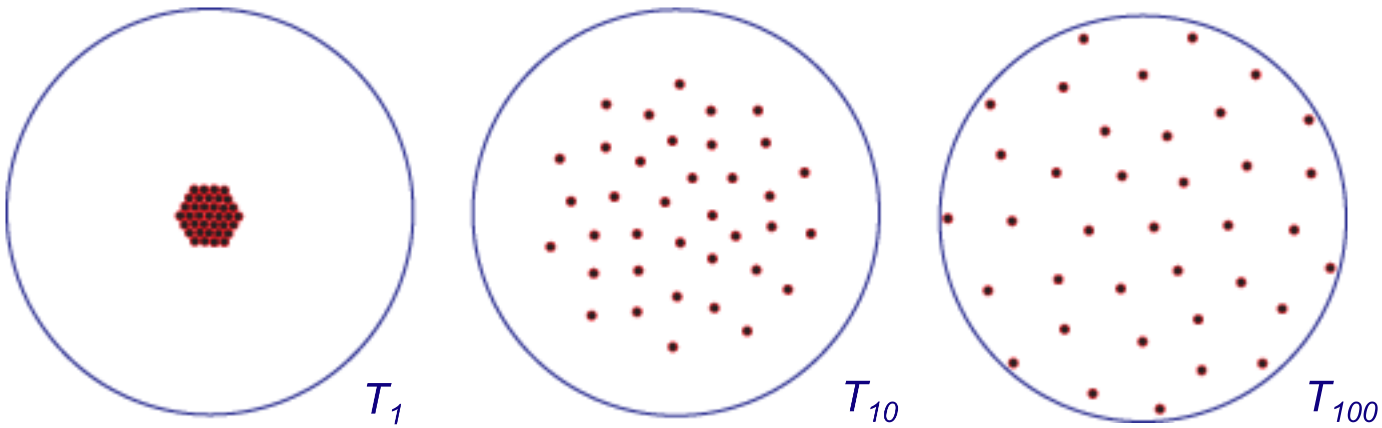
Diffusion constants of selected solutes

Solute	Size	K_{diff}		$Delta_{BSA}$
Sodium	53* Da	1.4×10^{-5}	<	478.6x
BSA	66 kDa	6.7×10^{-7}	–	1.0x
IgG	150 kDa	4.9×10^{-7}	>	1.4x
URE	480 kDa	3.5×10^{-7}	>	1.9x
IgM	1 MDa	2.6×10^{-7}	>	2.6x
ETX	2 Mda	2.1×10^{-7}	>	3.2x
CMV	5 Mda	1.2×10^{-7}	>	5.6x
TMV	40 Mda	5.0×10^{-8}	>	13.4x
DNA_1	4.4 kbp	1.9×10^{-8}	>	35.3x
DNA_2	33 kbp	4.0×10^{-9}	>	167.5x

*Monohydrated ion. URE: urease. ETX: endotoxin.
CMV: cucumber mosaic virus. TMV: tobacco mosaic virus

Consequences of diffusive transport

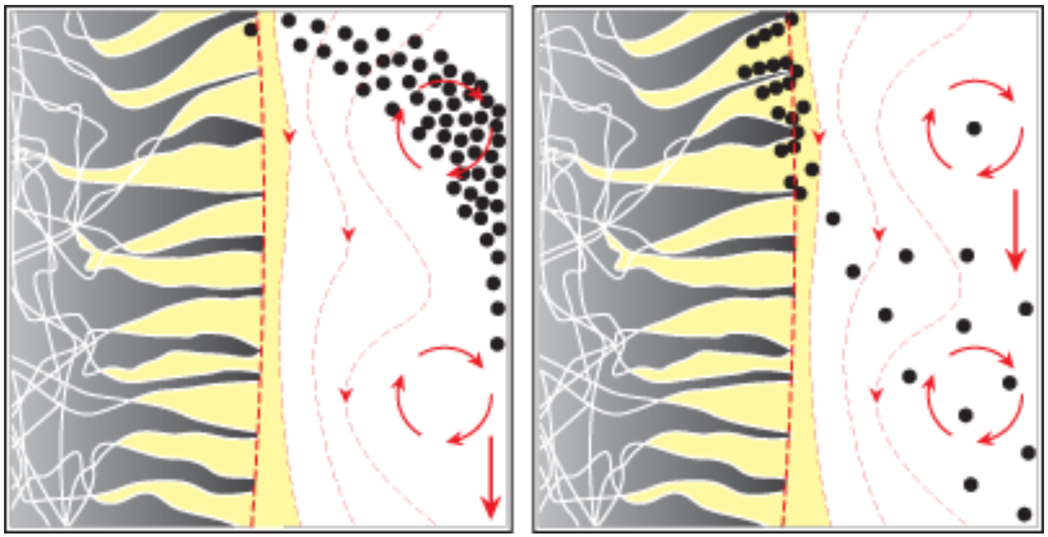
Diffusion is an equilibrium process.



The diffusion constant for a given solute represents the maximum velocity at which an individual molecule can migrate, but the direction and rate at which equilibrium is achieved depends on the orientation and steepness of the solute distribution gradient. As solute molecules disperse, gradient steepness is reduced. Thus migration is in a continuous state of deceleration, ultimately reaching a net rate of zero. For porous particle columns, this translates into decreasing efficiency of diffusive mass transport during binding and elution.

Consequences of multimodal transport

Solute binding in a packed particle column

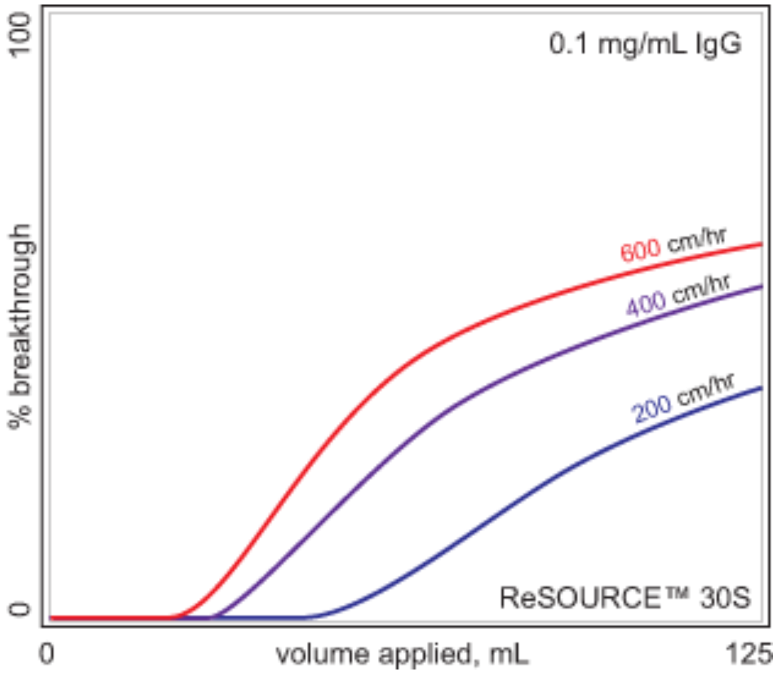


Left panel: binding, T_1
Right panel: binding, T_2
Gray: particle body
Heavy dash: particle boundary
Black circles: solute molecules
Vertical arrows: main axis of flow
Circular arrows: eddy flow
Yellow: areas of diffusive transport
White: convective transport
Light dash: laminar flow contours

Solute molecules are concentrated in a horizontal zone when first introduced but move faster through the void space than near the particle surfaces –except in eddies where they lag behind. The slowness of diffusion hinders the access of solute molecules to binding surface within the pores, increasing the lag behind the sample front. As the sample flows down the column, attrition of solute and eddy dispersion reduce the local solute concentration and further hinder diffusive transport. Increasing flow rate reduces solute residence time near a given pore and further restricts diffusive entry.

Consequences of multimodal transport

Effect of flow rate on dynamic capacity in a packed particle column



These curves illustrate the process of saturating the binding surface of a column of packed particles (cation exchange).

100% efficiency would be indicated by a vertical line at the point of saturation.

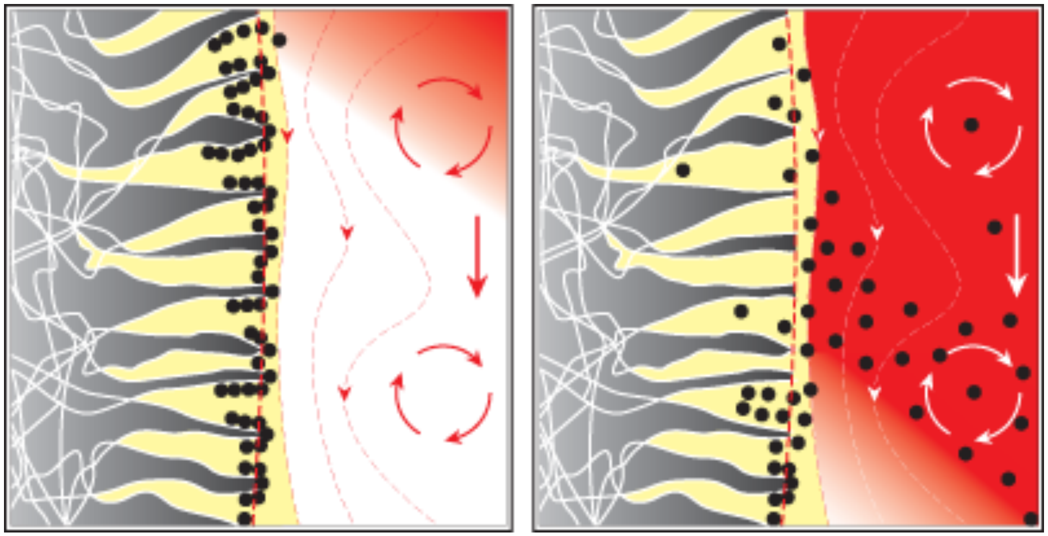
Preferential void flow and eddy dispersion contribute to the gradually sloping breakthrough, but the dominating factor is the low efficiency of diffusive mass transport, which manifests as decreasing capacity with increasing flow rate.

The molecular weight of IgG is about 150 kDa, with a hydrodynamic diameter of about 12 nm. Larger molecules with slower diffusion constants respond less favorably: lower capacity, shallower slope.

*Redrawn from R. Hahn, M. Panzer, E. Hansen, J. Mollerup, and A. Jungbauer, 2002, Mass transfer properties of monoliths, Sep. Sci. Technol., **37**(7) 1545-6, with permission*

Consequences of multimodal transport

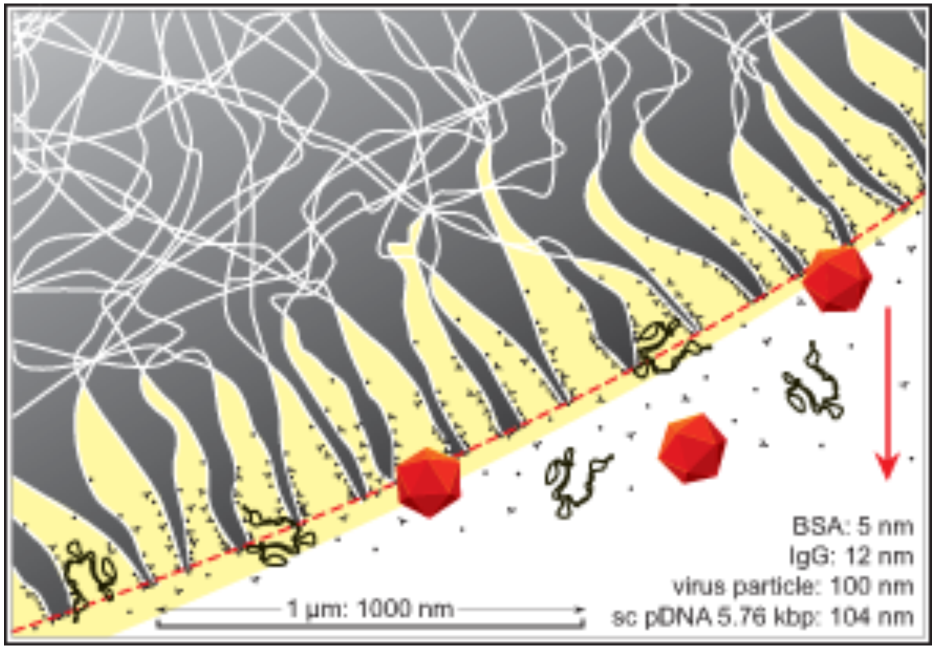
Elution in a packed particle column



Left panel: elution, T_1
Right panel: elution, T_2
Gray: particle body
Heavy dash: particle boundary
Black circles: solute molecules
Vertical arrows: main axis of flow
Circular arrows: eddy flow
Yellow: areas of diffusive transport
White/red: convective transport
Red intensity: eluent concentration

As elution commences, solute molecules bound at the particle surface are released and begin to flow down the column. Solute molecules inside the pores are also released, but take time to diffuse out into the zone of convective flow, and therefore lag behind the solvent front. The lag factor increases with increasing flow rate. Preferential void flow and eddy dispersion further spread the elution zone. The overall effect is to dilute the product and reduce peak resolution from other species.

Consequences of pore size distribution



Gray area: particle body
White lines: pore boundaries
Red dashed line: particle boundary
Yellow: areas of diffusive transport
White: areas of convective transport
Vertical arrow: main axis of flow
Average pore size: ~100 nm
Solute size expressed as hydrodynamic diameter

1. A. Jungbauer, J. Chromatogr. A., (2005) 1065 3-12

Most porous particle chromatography media are optimized for protein applications. Average pore size among different products ranges from about 60 to100 nm. Pore size needs to be about 10x the solute diameter to support unrestricted diffusive transport.[1] Thus most proteins enter easily but most plasmids and virus particles are too large. Since most of the binding surface area resides within the pores, capacity for large biomolecules is reduced dramatically.

Consequences of pore size distribution

Dynamic binding capacity

Solute	Method	Particles	Monoliths		Delta
BSA	AX	75-150	20-25	<	4-6x
IgG	AX/CX	50-125	20-25	<	3-5x
IgM	AX/CX	13-65	13-69	:	1x
ETX	AX	9-15	115-150	>	10-12x
gDNA	AX	0.3-1.5	12-15	>	10-40x
Flu virus	CX	8-9 $\log_{10}$	10 $\log_{10}$	>	10-100x

AX: anion exchange. CX: cation exchange. ETX: endotoxin. gDNA: genomic DNA
All values expressed as mg/mL except influenza virus, expressed as particles/mL.
See slide 9 for solute molecular weights and diffusion constants.

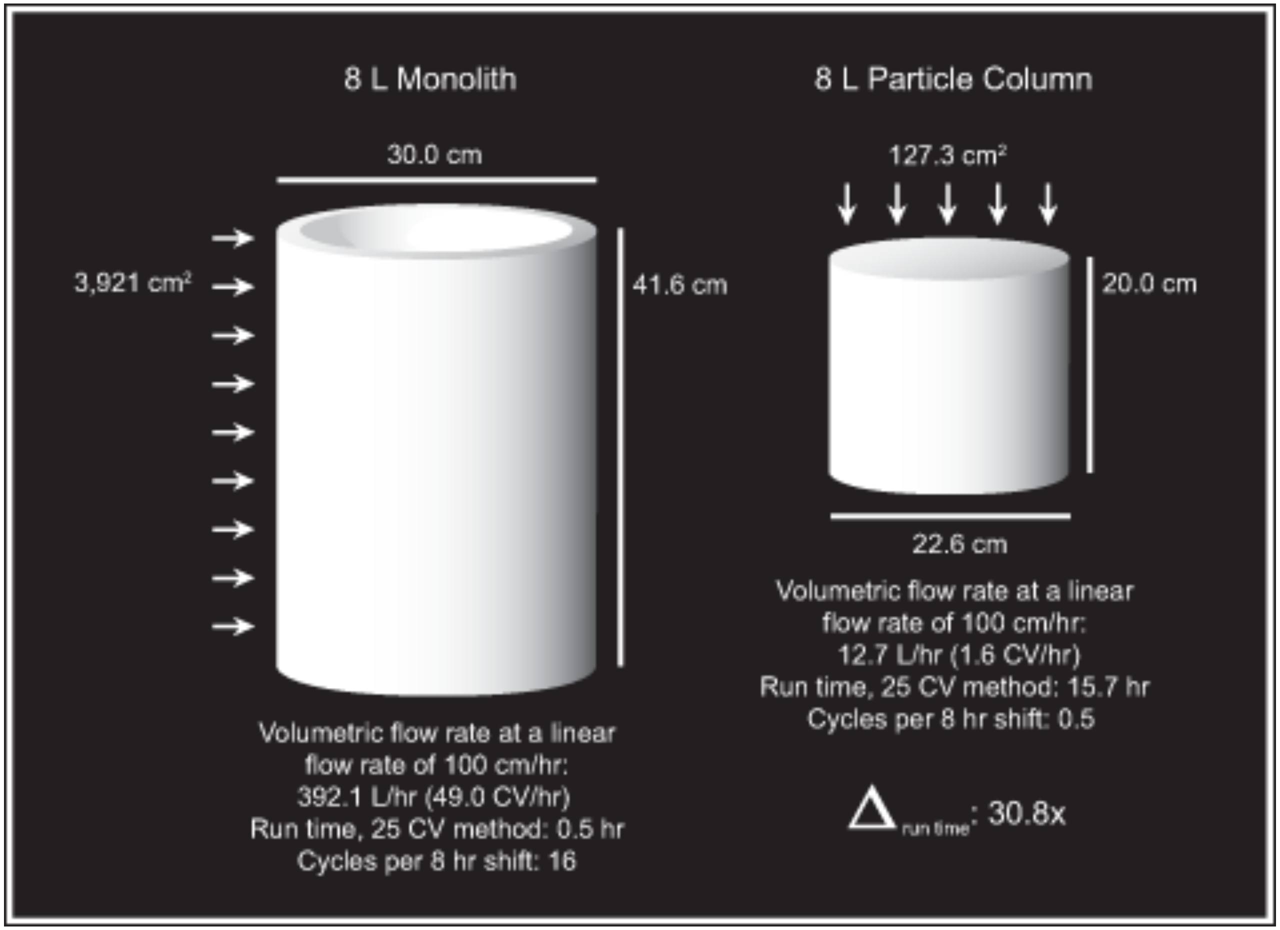
Porous particles support higher capacity for small molecules. Monoliths support higher capacity for large molecules. Experimental data suggest that the crossover occurs at about 1 MDa for globular proteins, or a hydrodynamic diameter of about 22 nm (all solute types).

Consequences of pore size distribution

How important is a 10x capacity differential?

- *10x higher capacity means 10x lower media volume to accomplish the same fractionation: 10x lower material cost.*
- *Eluted product volume is 10x smaller, 10x more concentrated, requires 10x less storage space, and 10x less time to load at the next purification step.*
- *The process requires 10x less buffer: 10x less water; 10x less chemical consumption.*
- *The process requires 10x smaller hardware (buffer preparation, column, chromatograph).*
- *The process occupies a 10x smaller manufacturing foot print, which translates into 10x greater productivity per square meter of manufacturing space.*

Consequences of bed configuration



Faster run time compounds the benefits of higher capacity.

Mass transport in monoliths

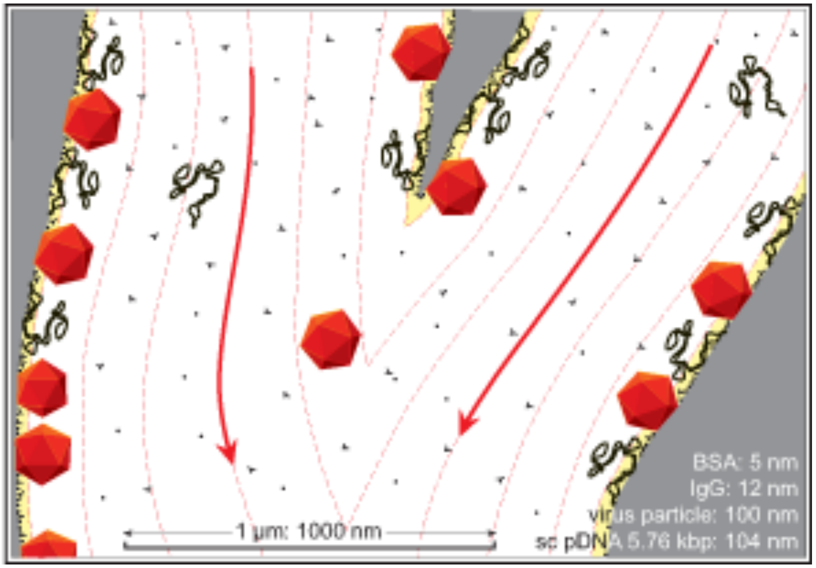
***Mass transport in monoliths is convective**. Capacity and quality of fractionation are independent of flow rate and molecular size (diffusion constant).*

***Binding and elution occur at channel surfaces**. This supports instantaneous transfer kinetics, contributing to efficient surface utilization (high binding capacity), high resolution, and high eluted product concentration.*

***Absence of a void volume avoids dispersion and shear**. This further contributes to high resolution and product concentration, and ensures the highest recovery of labile biomolecules.*

Mass transport in monoliths

Mass transport in monoliths is convective, and flow is laminar.

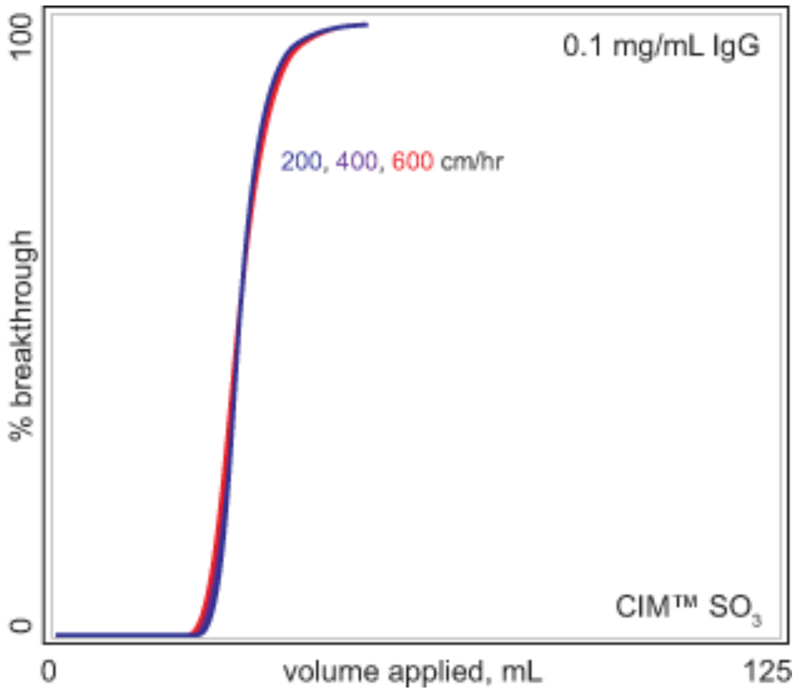


Gray areas: monolith body
Yellow: areas of zero flow rate
White: areas of faster flow rate
Dashed lines: laminar flow contours
Red arrows: main axis of flow.
Average channel size: ~1.5 µm
Solute size expressed as hydrodynamic diameter
Note that average channel size is only about 4% the average void width of a column packed with 100 µm particles.

All flow through a monolith runs parallel to its channel walls. This precludes the eddy formation that causes dispersion and shear in particle columns. Channel convergences and divergences chaotically re-order laminar strata. This promotes efficient solute contact with channel surfaces during loading, and maintains homogenous solute distribution (concentrated, well-resolved peaks) during elution, independent of flow rate.

Practical benefits of convective flow

Effect of flow rate on dynamic binding capacity of a monolith



These curves illustrate the process of saturating the binding surface of a cation exchange monolith.

The near-vertical breakthrough curves illustrate the efficiency of convective mass transport.

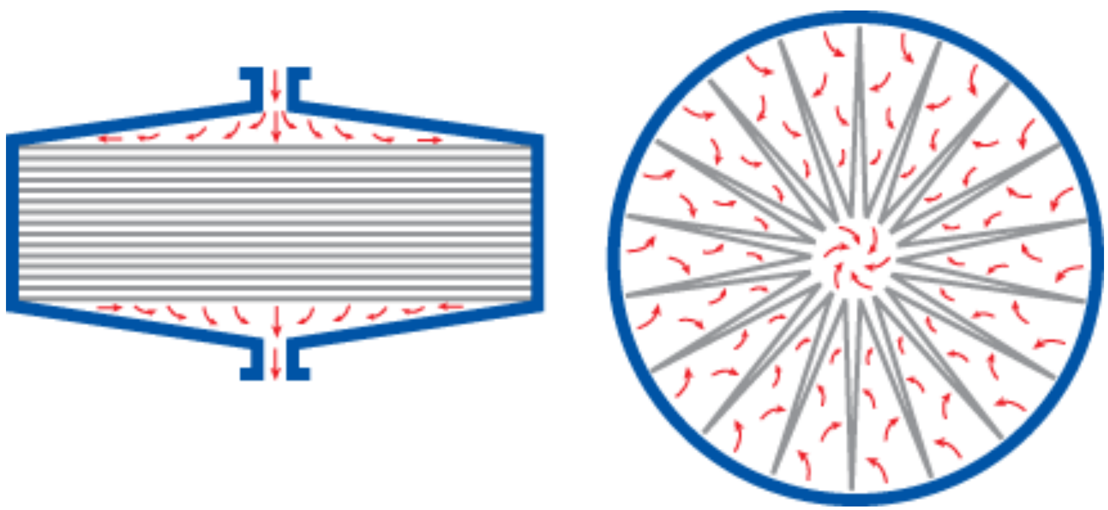
The fact that the curves overlay illustrates independence from flow rate, which translates into better reproducibility across process scales, as well as faster operation.

Dynamic capacity and steepness of the breakthrough curve also remain relatively constant for larger solutes.

Redrawn from R. Hahn, M. Panzer, E. Hansen, J. Mollerup, and A. Jungbauer, 2002, Mass transfer properties of monoliths, Sep. Sci. Technol., ***37****(7) 1545-6, with permission.*

What about membranes?

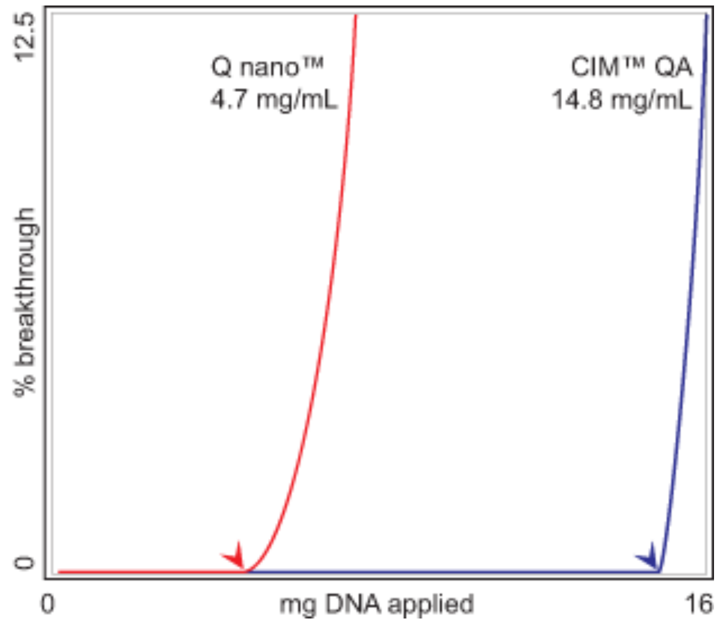
Mass transport in membranes is convective, as in monoliths, but back-pressure is lower. This supports effective operation at faster flow rates, but poorly controlled flow distribution in the housings creates zones of discontinuous/asymmetric flow, reminiscent of the void volume in packed particle columns, and with analogous side-effects.



Left: stacked membrane format. Right: pleated cartridge format. These are two of several strategies to increase capacity by putting as much surface area as possible into a fixed volume housing. Some space nevertheless remains unoccupied, where turbulent mixing can occur, and some membrane surface may be poorly accessible.

What about membranes?

Comparative capacity: membranes and monoliths



Experimental determination of dynamic binding capacity

Q nano: 1 mL stacked membrane

CIM QA: 1 mL monolith

Each loaded with 0.1 mg/mL gDNA in 20 mM Hepes, pH 7, at 1 mL/min.

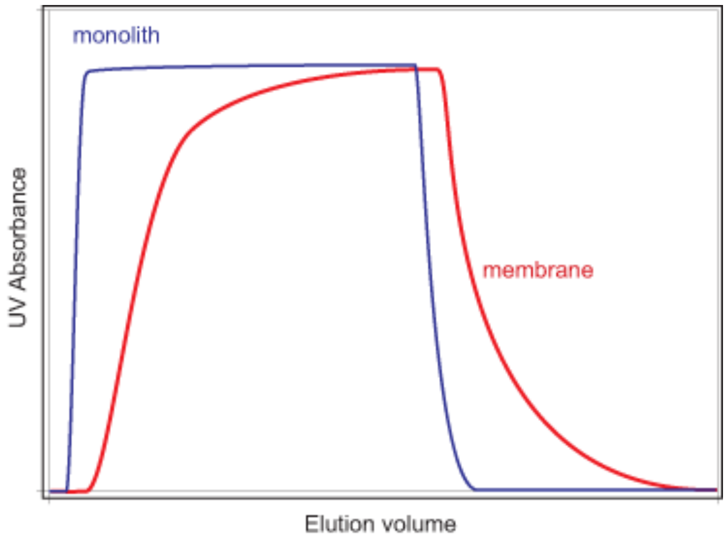
Arrowheads mark the point at which breakthrough begins.

See Gagnon et al 2007 for details: http://www.validated.com/revalbio/pdffiles/IBCBOS07a.pdf

Note that the monolith curve breaks at a sharp angle while the membrane curve breaks gradually. Since mass transport is convective in both media, the gradual break in the membrane curve is understood to reflect dispersion from uncontrolled flow distribution in the housing. The monolith also provides 3x more capacity, indicating 3x more accessible surface area per unit of media volume.

What about membranes?

Effects of housing-related dispersion: carryover

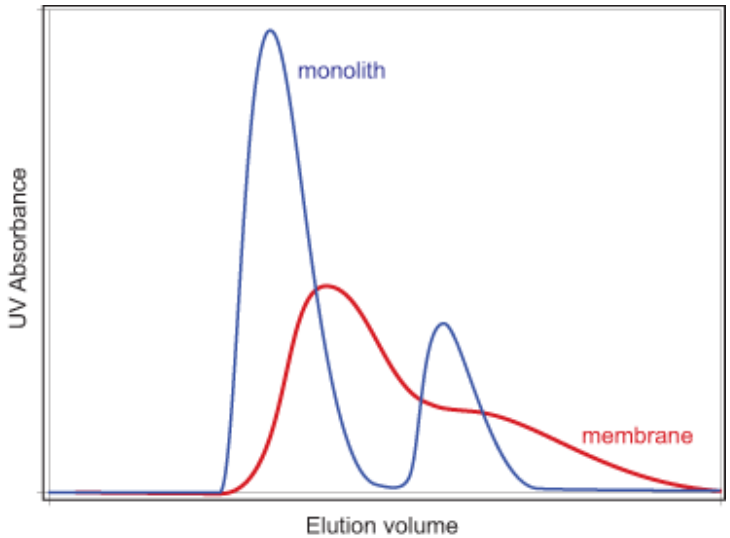


Internal mixing retards clearance of sample after loading. A prolonged wash is required. Otherwise, product elution may occur while significant contaminant levels are still being applied to the media. This is a particular concern when the objective is to reduce highly regulated contaminant classes to very low levels.

Note that the degree of carryover is highly dependent on housing design and media format (stacked, pleated, radial tangential flow). Carryover also affects cleaning and sanitization, but this is usually moot since membranes are typically discarded after a single use.

What about membranes?

Effects of housing-related dispersion: loss of resolution

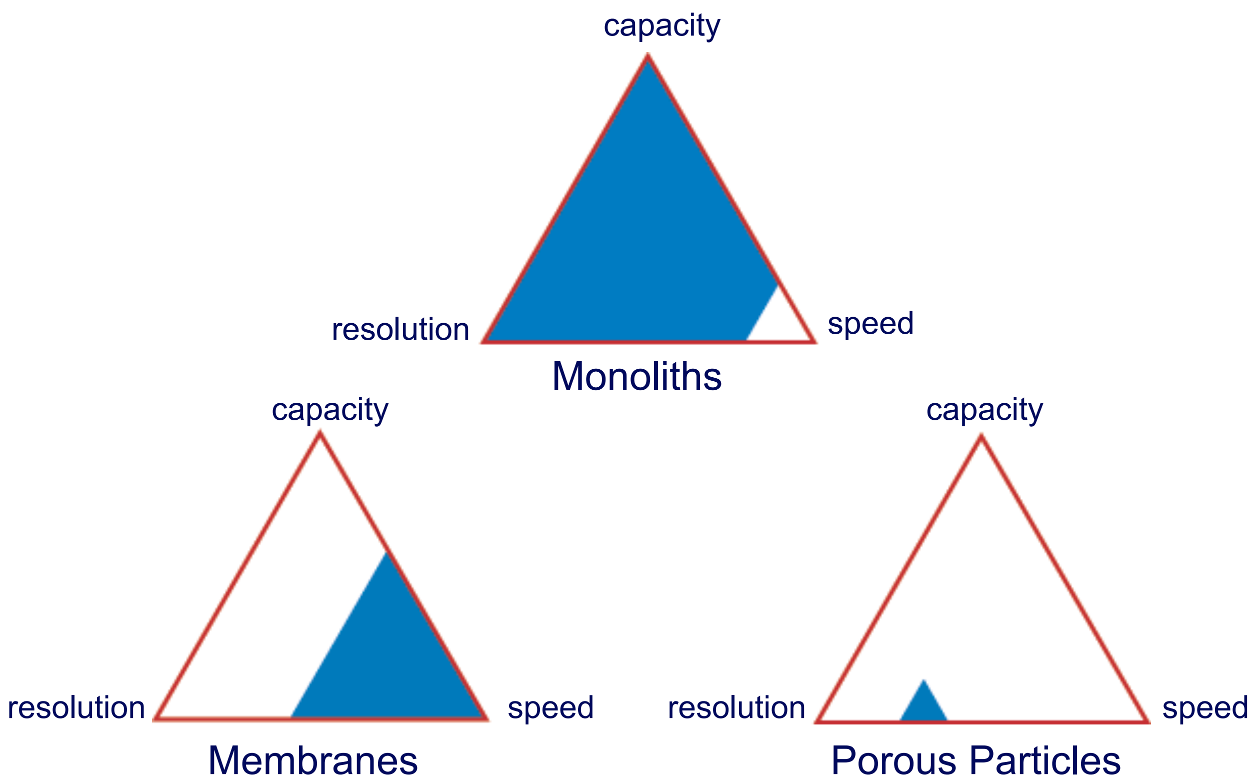


Internal mixing erodes peak boundaries, reduces resolution, and dilutes the eluted product. Additional steps are required to achieve the desired purification; or it becomes necessary to take narrow fractions and discard the contaminated tails, which reduces product recovery. Increased elution volume increases loading time at the next step.

As with carryover, loss of resolution is highly dependent on housing design and media format.

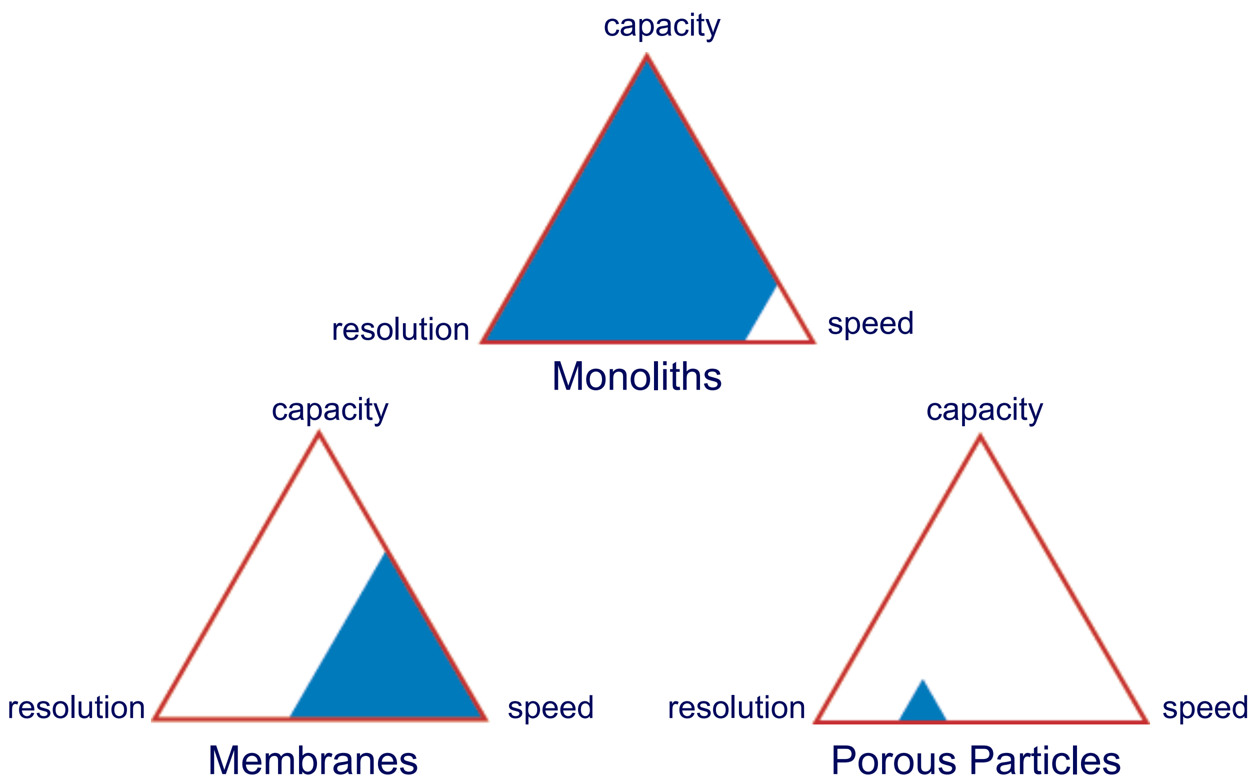
Conclusions

Monoliths are ideally suited to purification of large solutes, such as DNA plasmids, virus, and VLP vaccines.



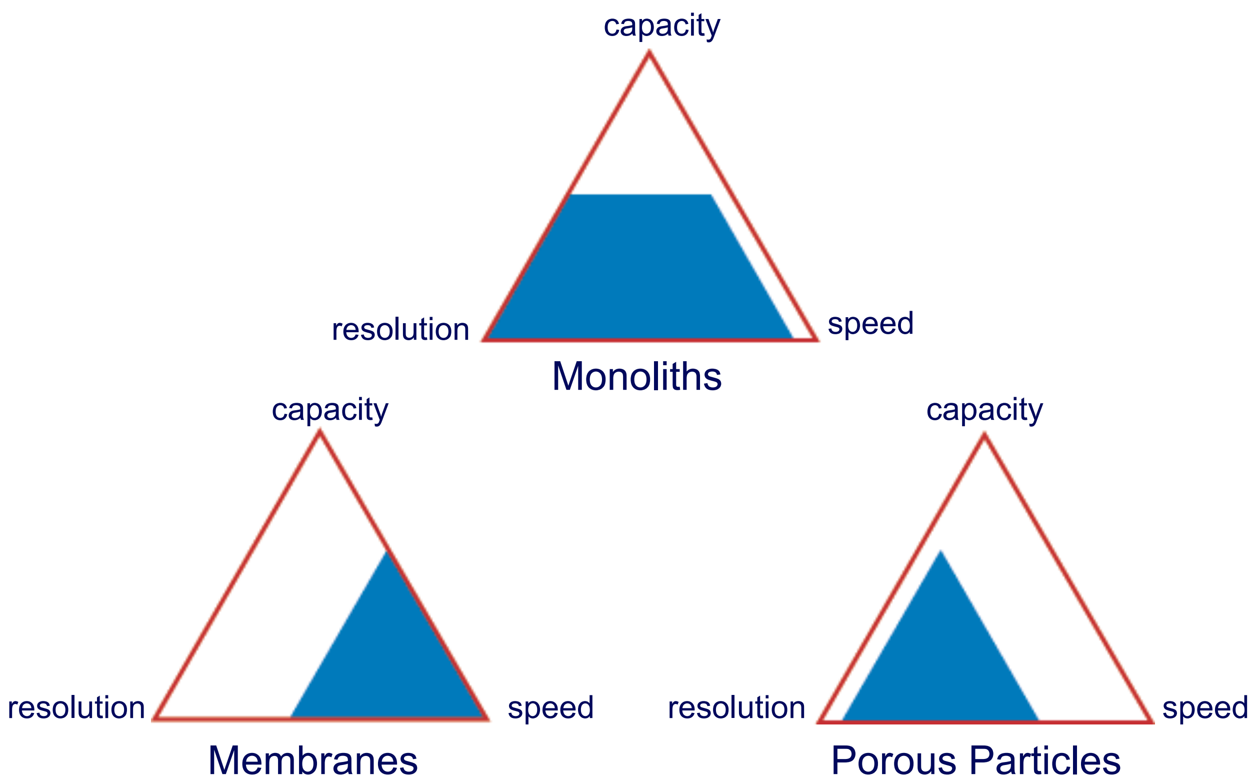
Conclusions

Monoliths are equally suited to removing large solutes, such as DNA, endotoxin, and virus from smaller solutes, such as proteins (IgG).



Conclusions

Monoliths are highly competitive for purification of proteins, protein and PEG conjugates larger than about 500 kDa or D_h > 15 nm.



Conclusions

Monoliths support rapid high resolution fractionation for method scouting and lab scale purification of smaller solutes — and analysis of solutes of all sizes.