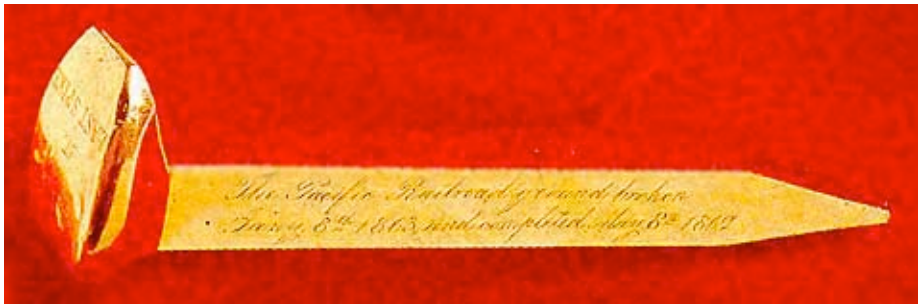
The Promise of Monolith-based Industrial Purification of Biomolecules

Pete Gagnon, Validated Biosystems

3rd International Monolith Symposium, Portoroz, May 30–June 4, 2008

The promise of monoliths

At 2:27 PM on May 10, 1869, a golden spike was driven, uniting the Union Pacific and Central Pacific railroads, and completing the first transcontinental railway system in North America.



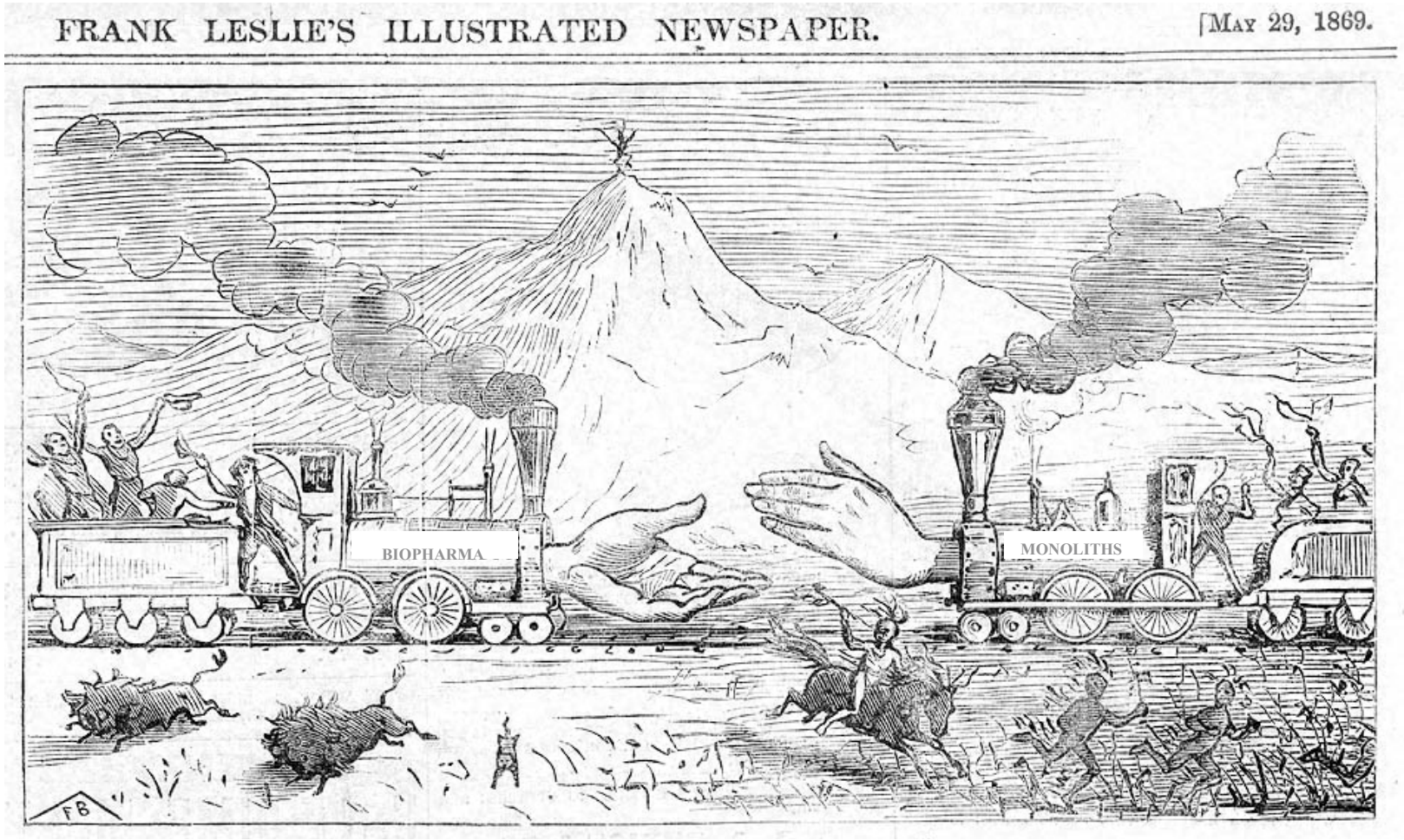
462 g 17k gold, inscribed with the names of the railroads and their directors

The promise of monoliths

The spike symbolized an integration of resources that sparked a new era of industrial expansion.

First Transcontinental Monolith Conference, Promontory, Utah, May 10, 1869

The promise of monoliths



The promise of monoliths

Monoliths offer unique benefits to virtually all chromatography applications, but the greatest beneficiaries are large biomolecules:

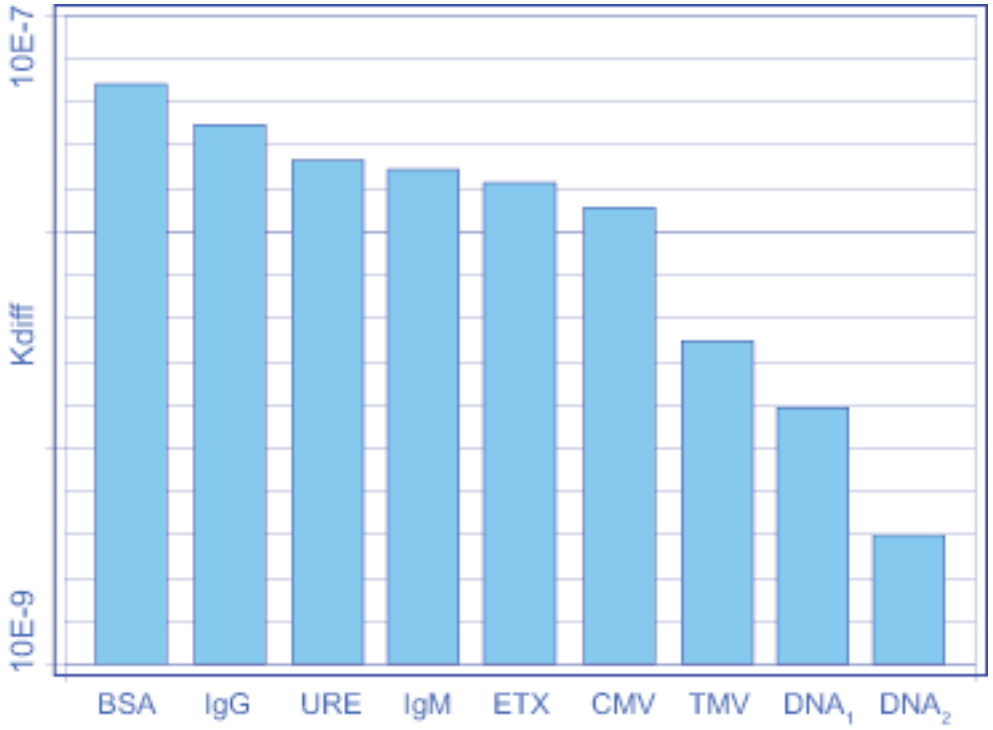
Purification of DNA plasmids, removal of DNA contamination from protein preparations

Purification of Viral vectors and vaccines, removal of virus contamination from protein preparations

Purification of Proteins: IgM, Immunoconjugates

Limitations of porous particles

Low diffusion constants of large biomolecules



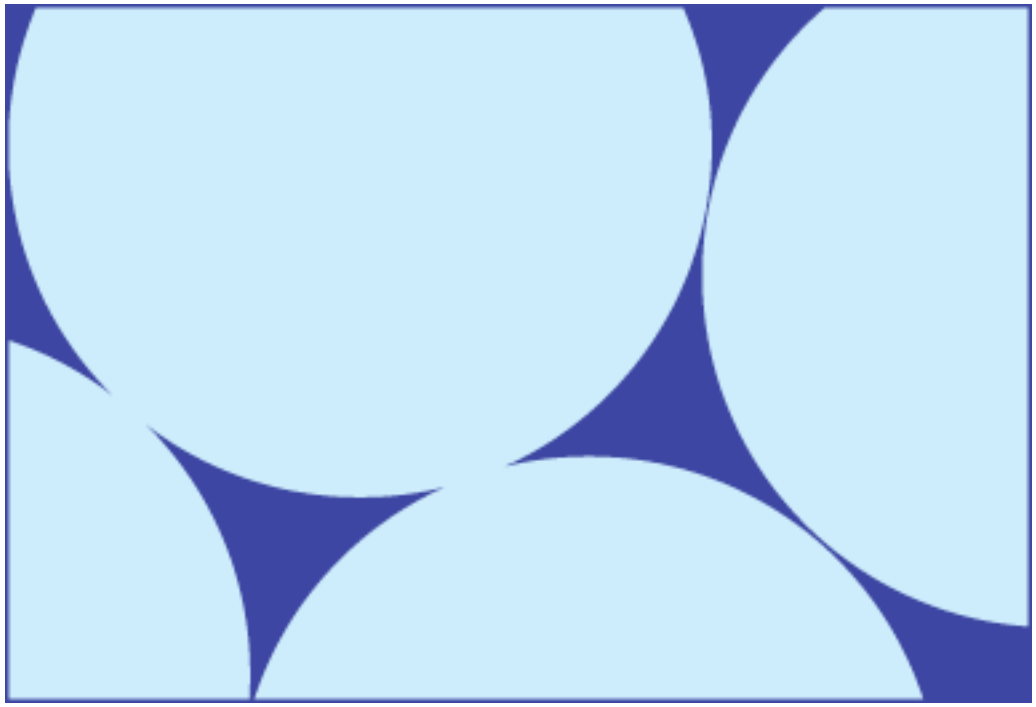
Solute	Size	K_{diff}
BSA	66 Kd	6.7×10^{-7}
IgG	150 Kd	4.9×10^{-7}
Urease	480 Kd	3.5×10^{-7}
IgM	1 Md	2.6×10^{-7}
ETX	2 Md	2.1×10^{-7}
CMV	5 Md	1.2×10^{-7}
TMV	40 Md	5.0×10^{-8}
DNA_1	4.4 kbp	1.9×10^{-8}
DNA_2	33 kbp	4.0×10^{-9}

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

Reproduced from: A comparison of microparticulate, membrane, and monolithic anion exchangers for polishing applications in the purification of IgG monoclonal antibodies, Pete Gagnon, Richard Richieri, Simin Zaidi, Franics Aolin, IBC International Conference and Exposition, October 1-4, 2007, Boston, MA, USA

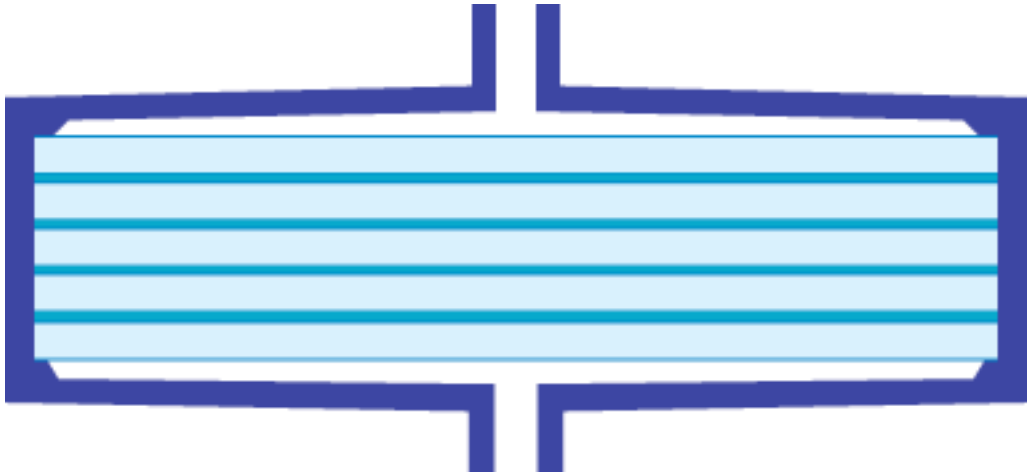
Limitations of porous particles

Interparticle void space



Limitations of membranes

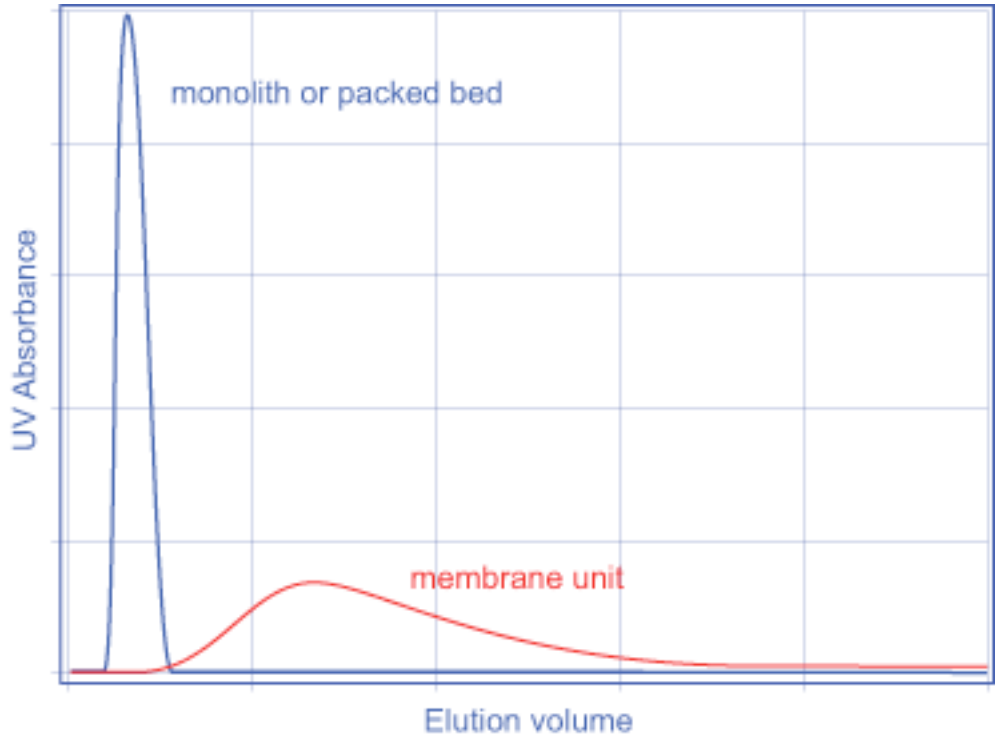
Flow distribution



Uncontrolled distribution and turbulent mixing occur in the housing.
Turbulent mixing occurs between layers of filtration media.

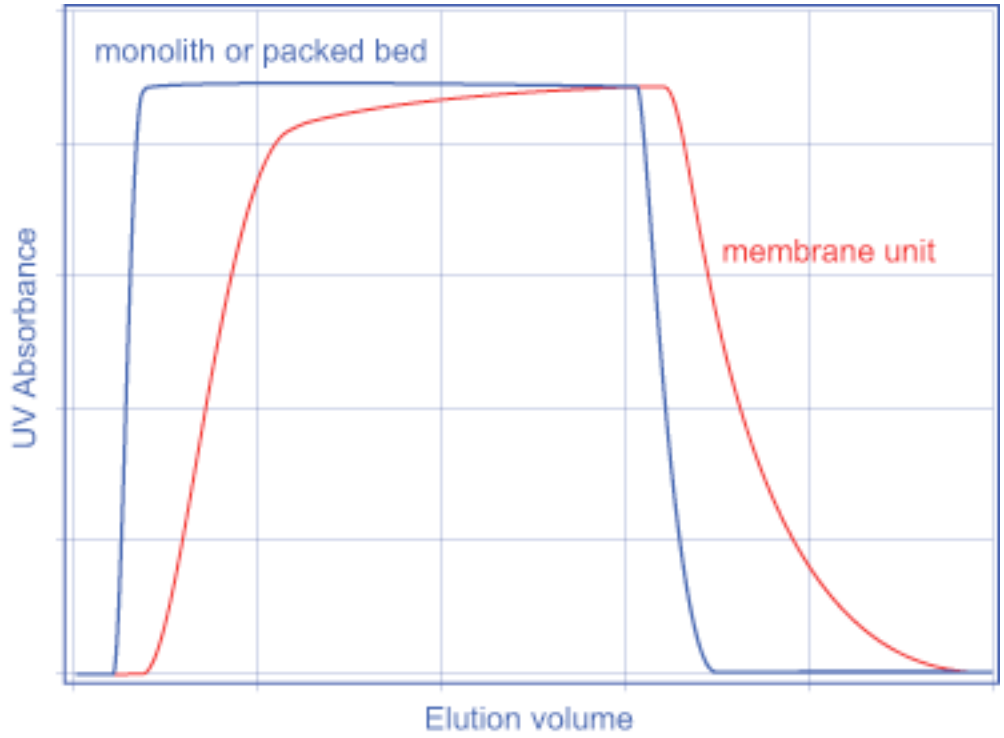
Limitations of membranes

Quality of flow distribution correlates with dispersion.



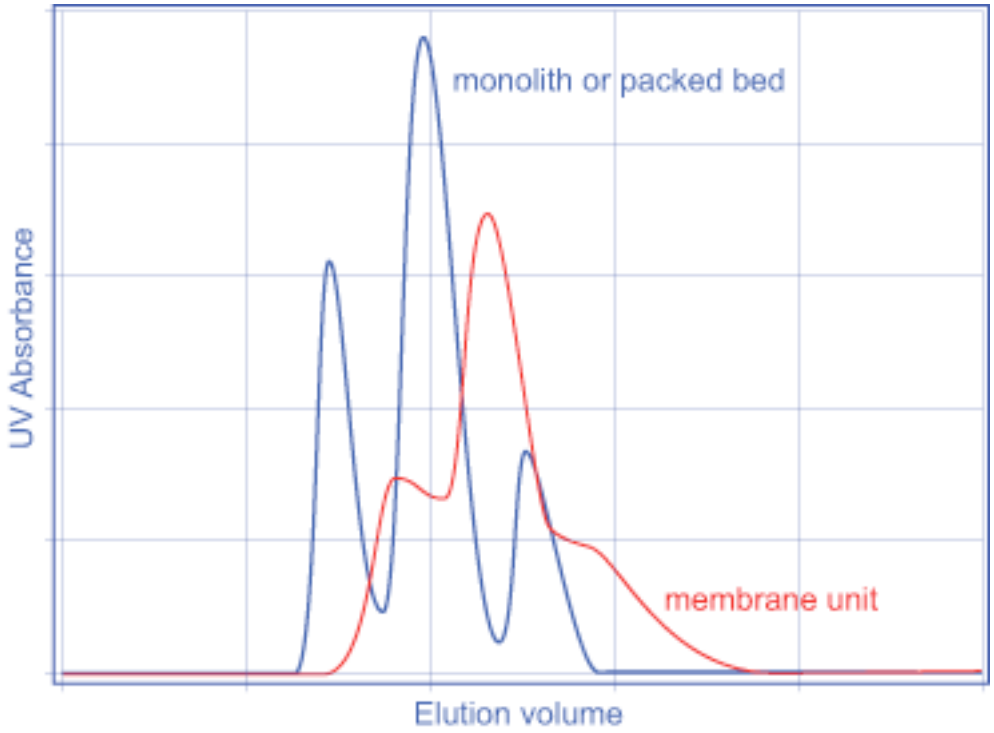
Limitations of membranes

Dispersion increases process volumes in flow-through applications



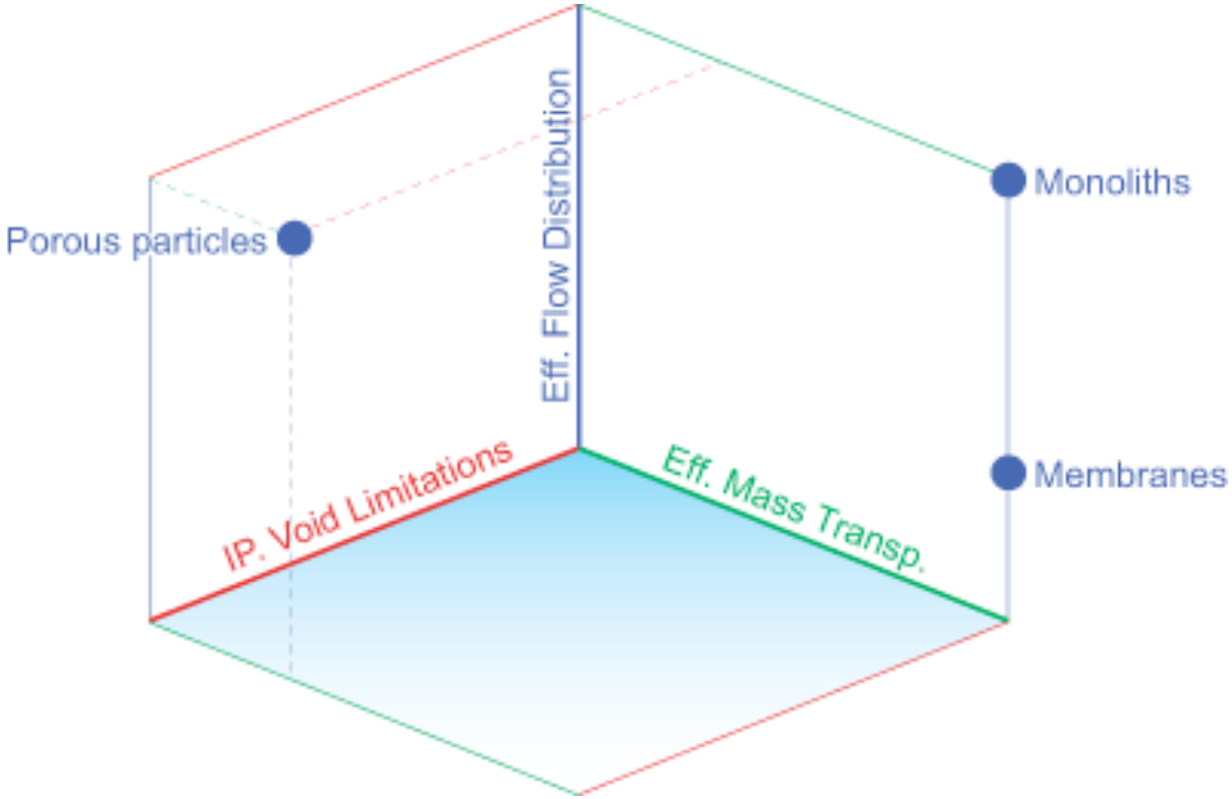
Limitations of membranes

Dispersion degrades fractionation in bind-elute applications



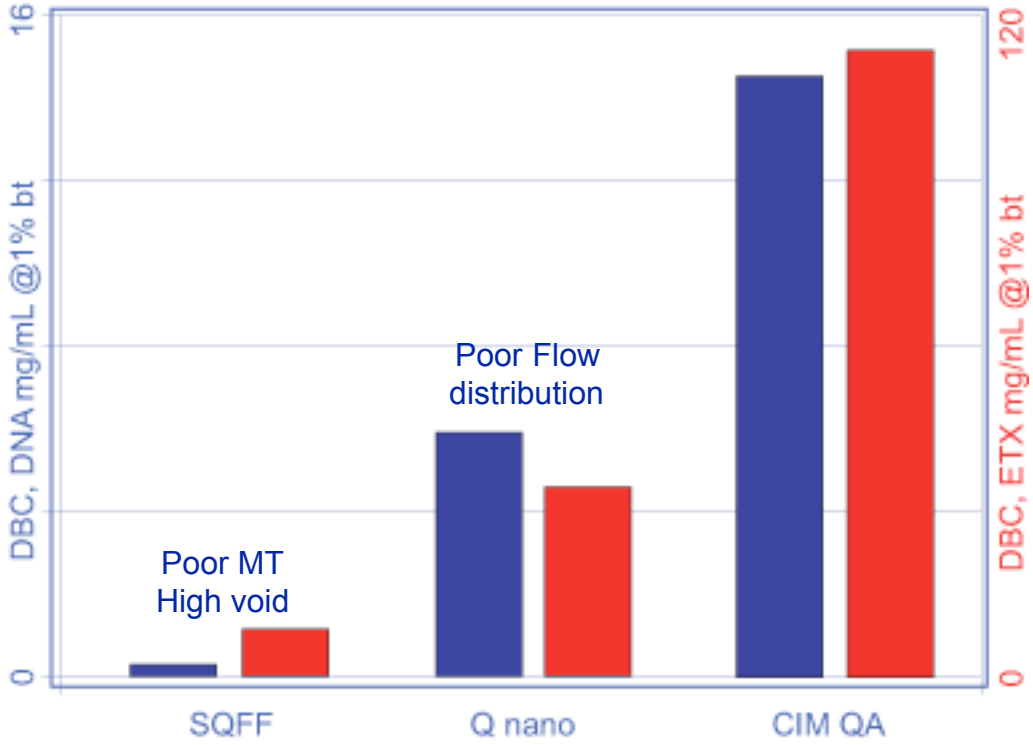
Chromatographic efficiency

Overall chromatographic efficiency



Chromatographic efficiency

Overall chromatographic efficiency, dynamic binding capacity



Q Sepharose™ Fast Flow, 1 mL HiTrap™

Sartobind™ Q nano, 1 mL

CIM® QA, 3 x 0.34 mL (1 mL)

All media flowed at 1 mL/min

DNA: 1 mg/mL

Endotoxin: 1 mg/mL

All samples and media equilibrated to 0.05 M Hepes, pH 7.0

The industrial value of monoliths

The value of low dispersion:

- *Low dispersion yields narrower peaks*
- *Narrower peaks contribute to higher resolution*
- *Higher resolution supports more effective fractionation*
- *More effective fractionation permits fewer process steps*
- *Higher resolution also supports better reproducibility*

- *Narrower peaks correspond with higher product concentration*
- *Higher product concentration means lower process volume in preparative applications.*
- *Lower process volumes improve material handling efficiency.*
- *Higher concentration also means higher sensitivity in analytical applications.*

The industrial value of monoliths

The value of faster processing:

- *Faster processing means faster process development*
- *Faster processing means faster assays*
- *Faster processing means faster process validation*
- *Faster processing means faster entry into clinical trials*
- *Faster processing means shorter time to market*

- *Faster processing means minimal product exposure to extreme chemical environments.*

- *Faster processing means more efficient utilization of expensive manufacturing facilities. More cycles per unit time increases plant capacity and accelerates ROI on fixed facility costs.*

The industrial value of monoliths

The value of high binding capacity

- *Higher capacity means lower media costs per unit of productivity.*
- *Smaller columns consume less buffer.*
- *Smaller columns elute product at higher concentration, which corresponds with lower volumes that further improve handling efficiency.*
- *Higher capacity corresponds with a smaller process footprint, which means more effective utilization of expensive manufacturing space.*

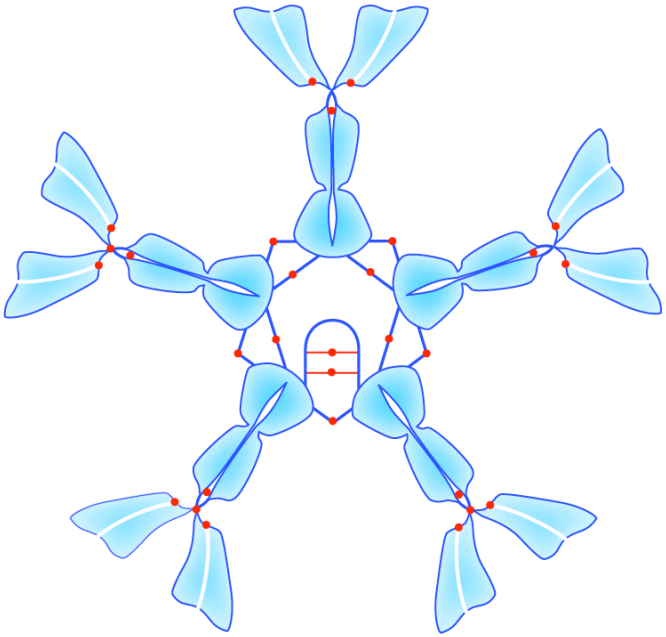
The industrial value of monoliths

The value of convective mass transport

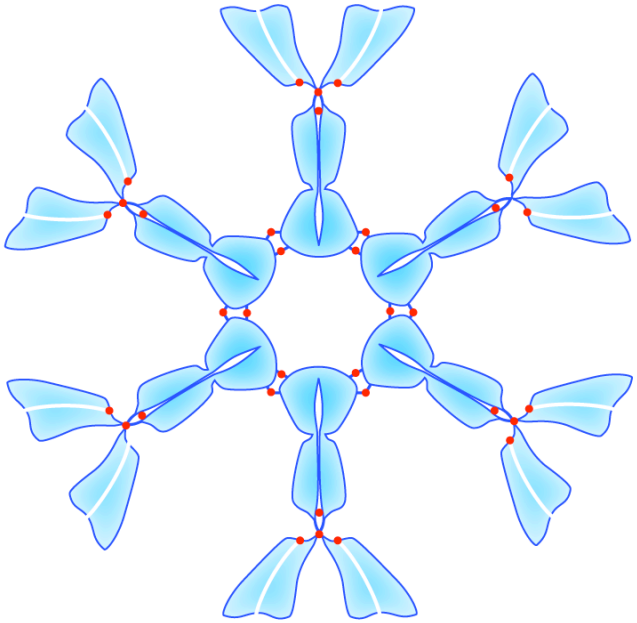
- *Convective mass transport means that you can have high resolution and high capacity at high flow rate – without compromise – no matter how big the molecule.*

IgM as an example

Pentameric, 0.96 Md

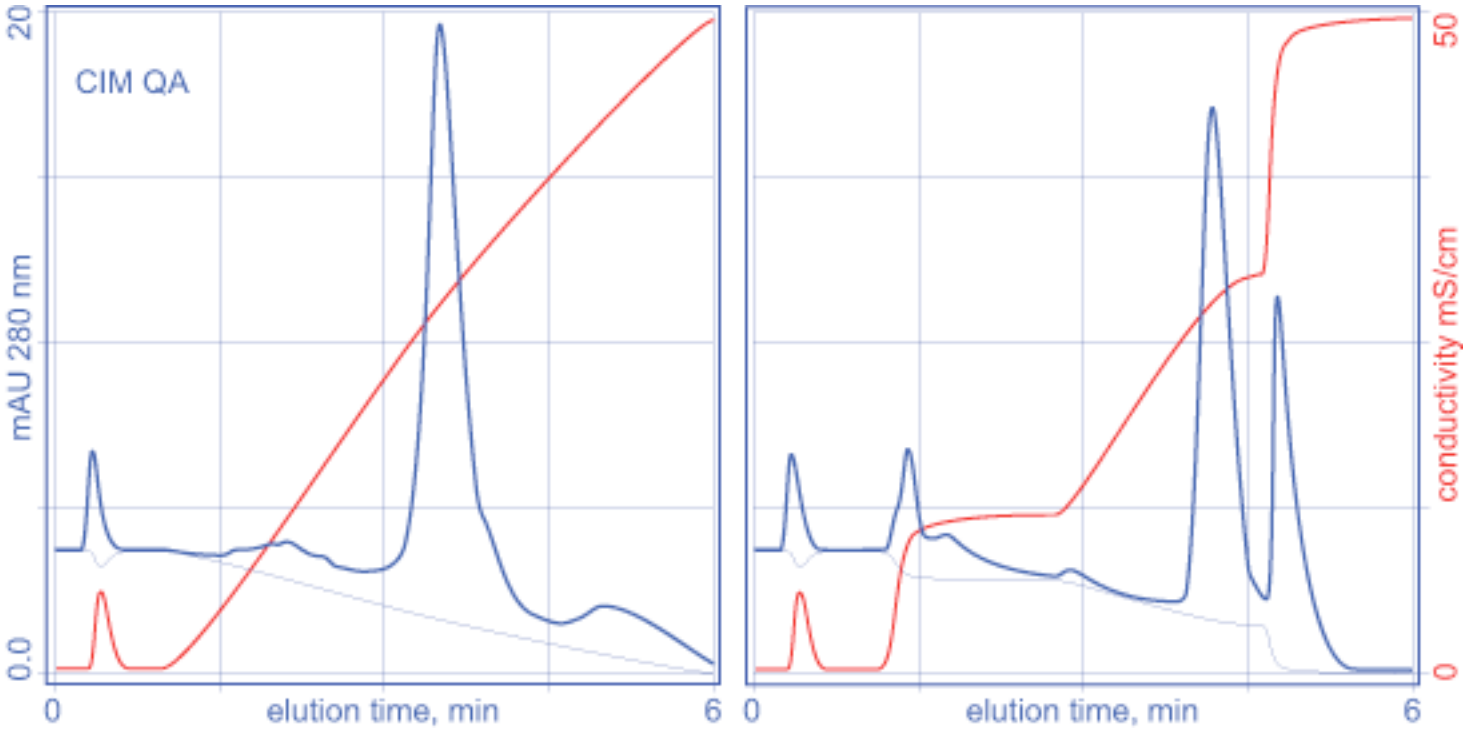


Hexameric, 1.15 Md



IgM as an example

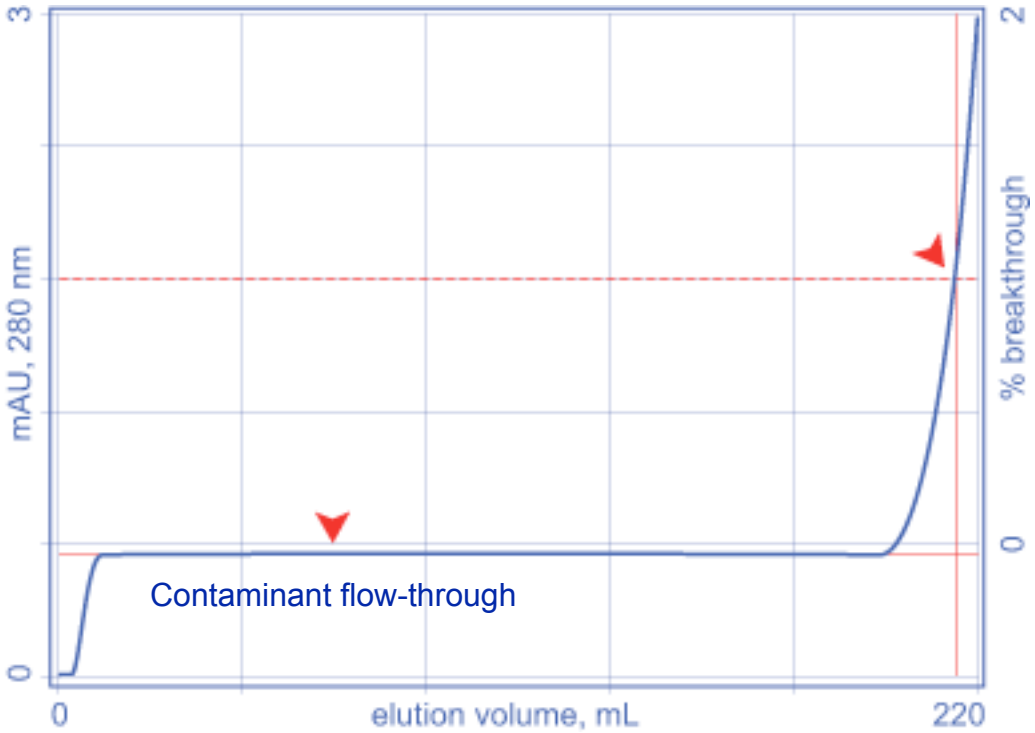
Rapid method development



Hydroxyapatite-purified monoclonal IgM applied to CIM QA at 12 CV (4 mL/min)/min in 0.05 M MES, pH 6; eluted with gradients to 0.5 M NaCl. Complete run times of about 10 minutes support rapid surveys of different chromatography media, different buffers, and optimization of gradient set points.

IgM as an example

High binding capacity



CIM QA 0.34 mL

5 mL/min

CHT-purified human IgM

Sample prep: titrate pH, dil. in-line

Load: 220 mL at 0.15 mg/mL

DBC at 1% bt: **>38 mg/mL**

CIM SO_3 0.34 mL

Same sample, flow rate, loading.

DBC at 1% bt: **>41 mg/mL**

High flow rates also speed up tedious capacity determinations, and the ability to conduct them on 0.34 mL disks conserves sample while further accelerating the process. Figure reproduced from Recent advances in the purification of IgM monoclonal antibodies, P. Gagnon, F. Hensel, P. Andrews, R. Richieri, Third Wilbio Conference on Purification of Biological Products, September 24-26, 2007, Waltham, MA USA

IgM as an example

Increased productivity from rapid throughput

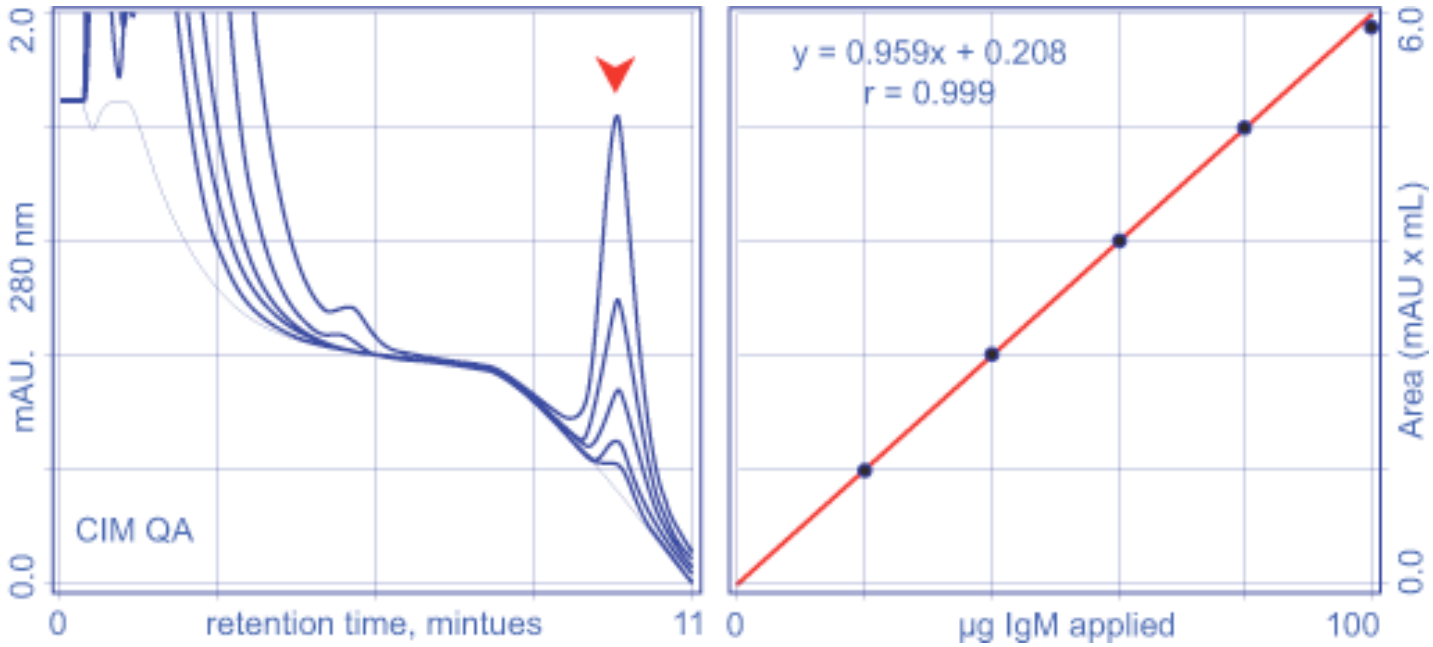
Summary	Volume	mgs	Recovery	Purity	Time
Supernatant	250 mL	50	100/100%	n/a	n/a
CIM SO_3	1250 mL	39	78/78%	90%	70 min
CIM QA	250 mL	33	84/65%	95%	20 min
Hydroxyapatite	250 mL	28	88/57%	99%	140 min

Comparison of the anion exchange monolith and hydroxyapatite steps reveals a net productivity improvement of 700%. An alternative process sequence was run with capture on the hydroxyapatite column. 250 mL undiluted CCS was loaded and eluted in 480 min. This is 7.15 times longer than the cation exchange capture, despite the load volume being 5 times lower for the hydroxyapatite. This demonstrates a net productivity improvement of about 715% for the monolith at the capture step. Time for the alternative process was 518 minutes, more than twice the above process. This illustrates the additional benefit of conducting initial capture – typically the longest step in a process – on a monolith.

Table reproduced from Gagnon et al, 2008, Purification of IgM…, BioPharm International, March suppl., 26-35

IgM as an example

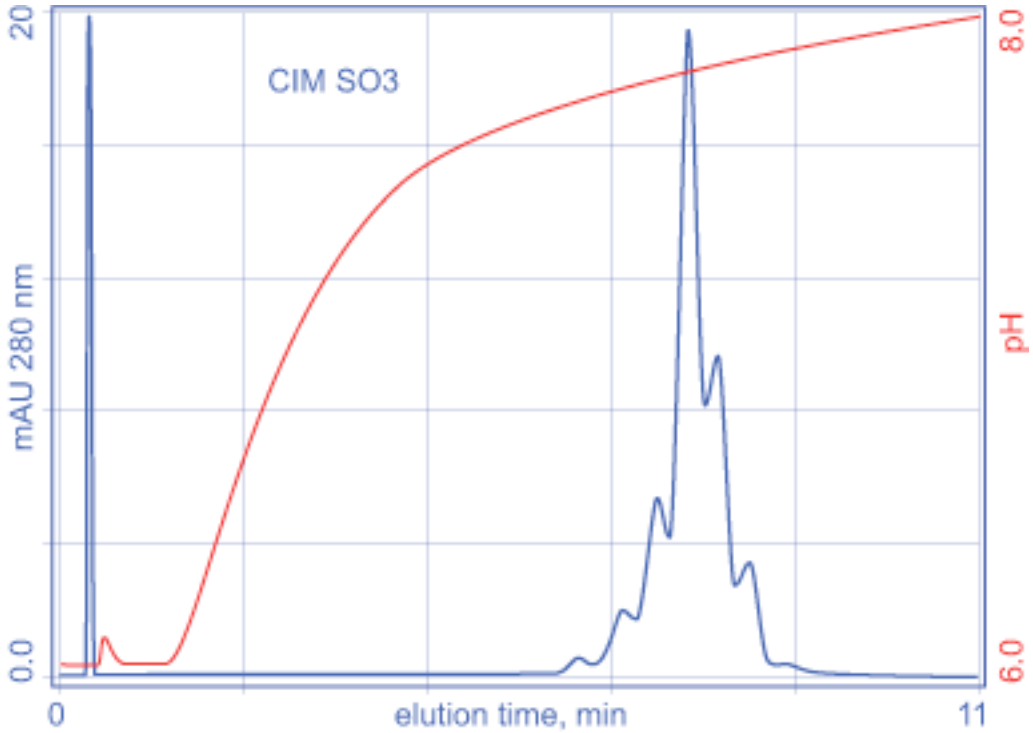
Rapid analysis, IgM content of cell culture supernatant



Reproduced from A high speed monolithic assay for IgM quantitation in cell culture production and purification process monitoring: Pete Gagnon, Richard Richieri, Simin Zaidi, Roy Sevilla, Alexander Brinkman, 3rd Wilbio Conference on Purification of Biological Products, September 24-26, 2007, Waltham, MA USA

IgG as an example

Rapid analysis, fractionation of IgG glycosylation variants



CIM SO3 0.34 mL, 4 mL/min

Purified IgG_1 chimera

A: 0.2% pISep™ (low pH) 1 M glycine, 2 M urea, pH 6.0

B: 1.0% pISep™ (high pH) 1 M glycine, 2 M urea, pH 8.0

EQ: A,

Inject: 100 µL

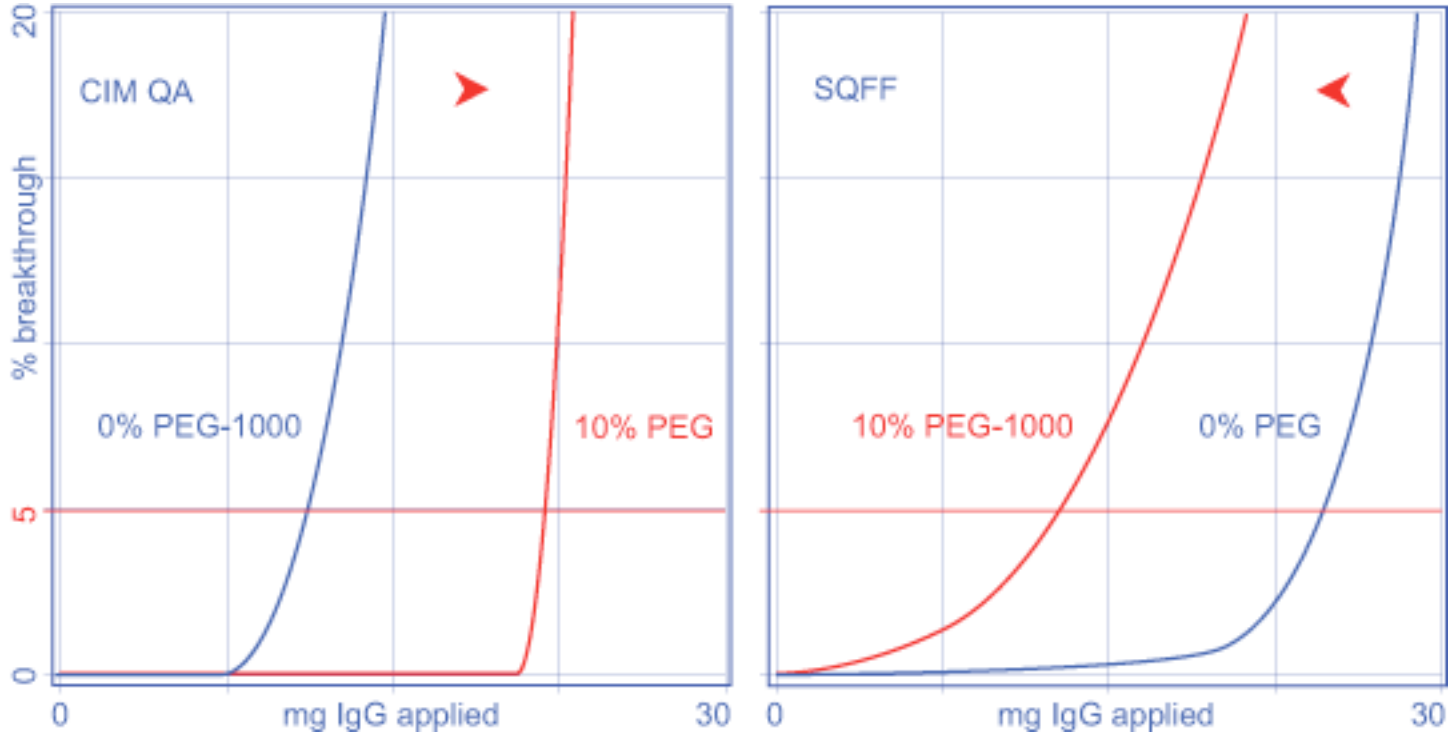
Wash: A

Elute: 130 CVLG to B

Glycine and urea were added to improve antibody solubility at low conductivity. Glycine is zwitterionic within this pH range and urea is non-ionic, so neither interfere with ion exchange binding. This assay can be used to evaluate conservation of glycosylation variants through the course of a purification process, or conditions can be further optimized and scaled up to obtain individual variants for pharmacological or other analyses.

Indirect value of monoliths

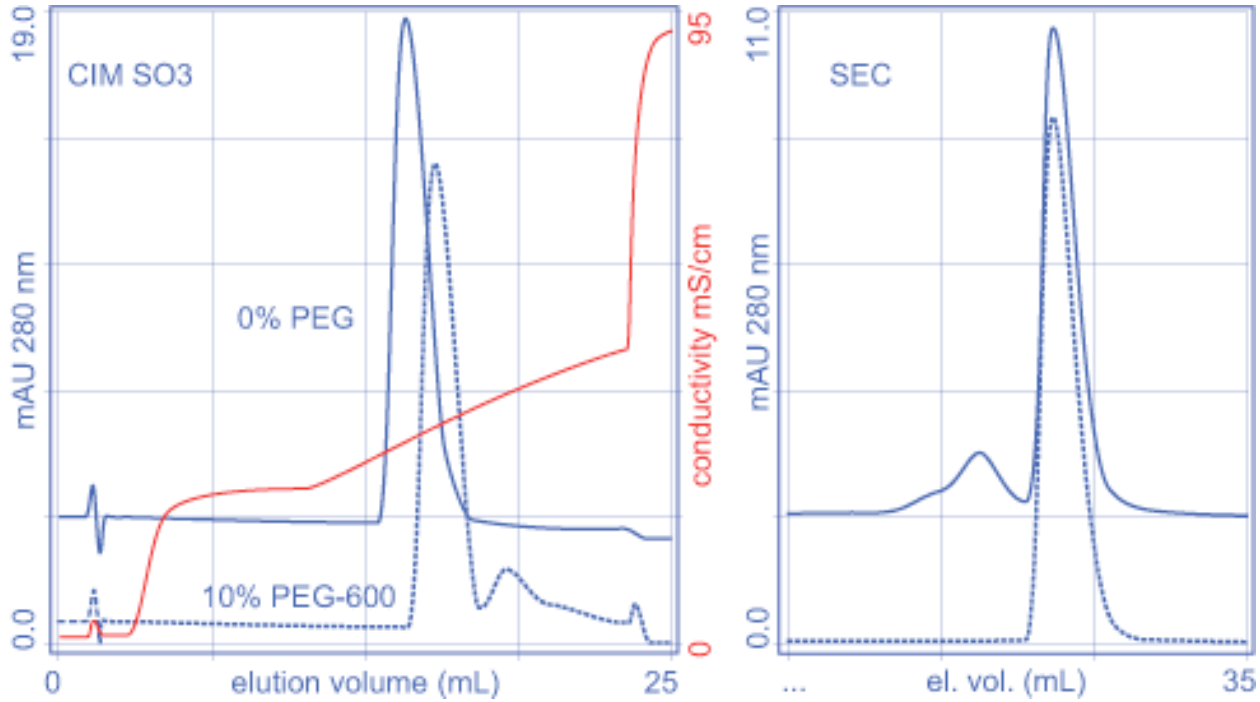
Inertness to viscosity, increased dynamic capacity of IgG in PEG



PEG increases viscosity, which depresses diffusivity. This hinders diffusive mass transport but not convection. PEG does not increase the inherent binding capacity of the solid phase; it enhances utilization. No increase is obtained with solutes that saturate the binding sites in the absence of PEG.

Indirect value of monoliths

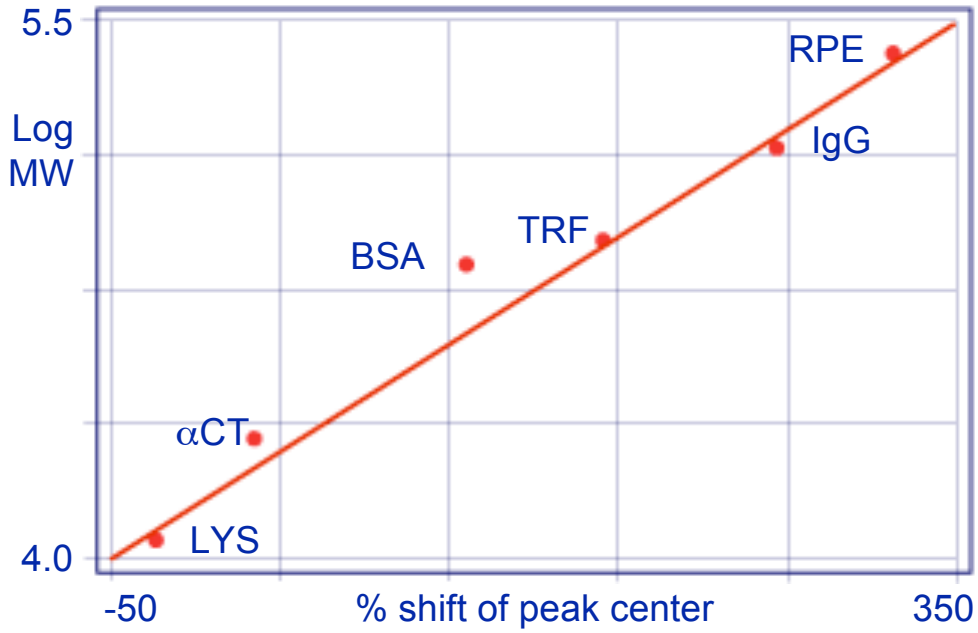
Inertness to viscosity, enhanced removal of IgM aggregates in PEG



PEG preferentially enhances retention of solutes in proportion to their size, providing an effective tool for aggregate removal. It works with porous particle supports but loss of diffusivity contributes to peak broadening that degrades resolution. Analytical SEC was run on a 7.8 x 300 mm TSK-gel® G4000SW$_{XL}$ column at 0.5 mL/min in 0.2 M arginine, 0.05 M MES, pH 6.5.

Indirect value of monoliths

Size dependent enhancement of solute retention by PEG



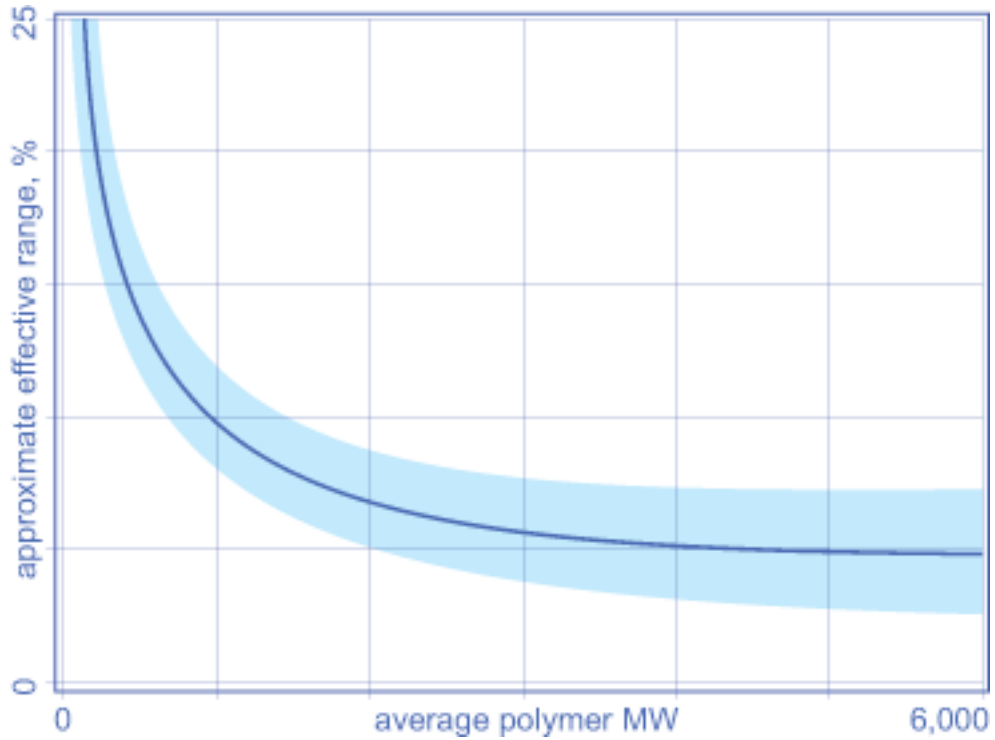
RESOURCE® S/Q
0% PEG versus
10% PEG-6000

RPE: 240 kD
IgG: 155 kD
TRF: 80 kD
BSA: 67 kD
αCT: 23 kD
LYS: 11 kD

Retention of plasmids and viral particles should be enhanced proportionately, permitting more effective removal of smaller cell culture and host cell-derived contaminants, in addition to aggregates. For more information consult: Arakawa and Timasheff (1985) *Biochemistry,* **24** 6756; Arakawa (1985) *Anal. Biochem.* **144** 267; Milby et al (1989) *J. Chromatogr.* **482** 133; Gagnon et al (1996) *J. Chromatogr.* **743** 51

Indirect value of monoliths

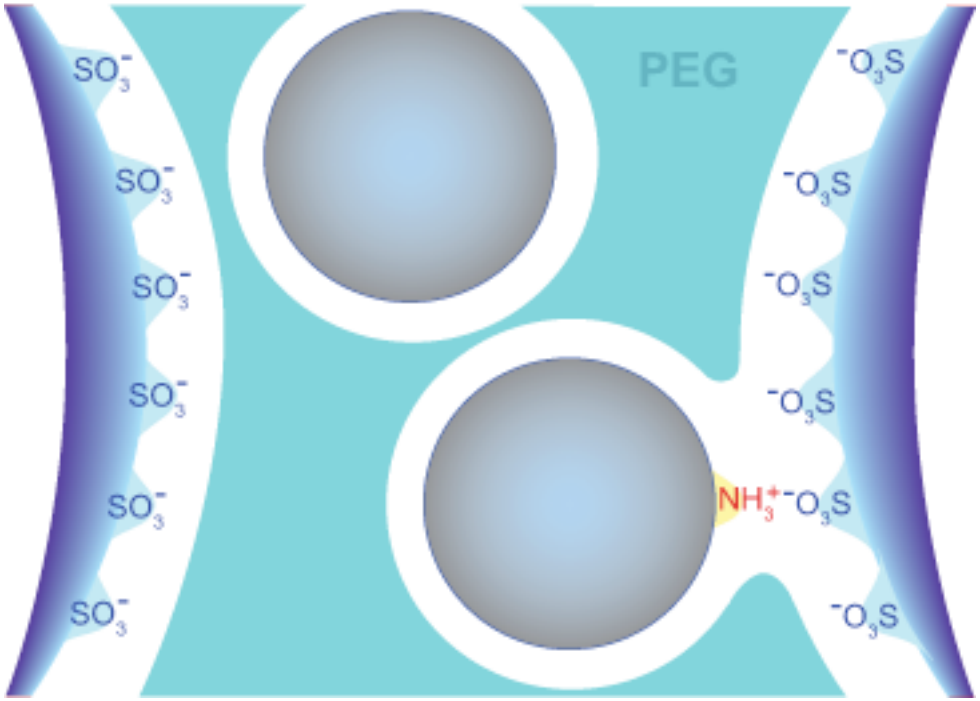
PEG enhances retention in proportion to polymer molecular weight



Polymer molecular weight is highly influential up to about 3000 Daltons. Larger polymers increase viscosity without benefit and complicate removal of residual PEG. 10% PEG-1000 is an effective starting point. PEG is an FDA approved inactive ingredient of many parenteral formulations.

Indirect value of monoliths

Preferential exclusion of PEG



Indirect value of monoliths

PEG is preferentially excluded from protein and solid phase surfaces. The discontinuity between the PEG-free exclusion zone and the high-PEG mobile phase is thermodynamically unfavorable.

When a protein binds to the stationary phase, the two share hydration water, allowing some water to transfer to the mobile phase, thereby lowering the bulk PEG concentration. This reduces the discontinuity between the PEG-free exclusion zone and the high-PEG mobile phase. This is thermodynamically favorable.

Indirect value of monoliths

In addition, the PEG-free surface area of the bound protein is lower than the additive PEG-free surface areas of the protein and stationary phase separately. This is also thermodynamically favorable.

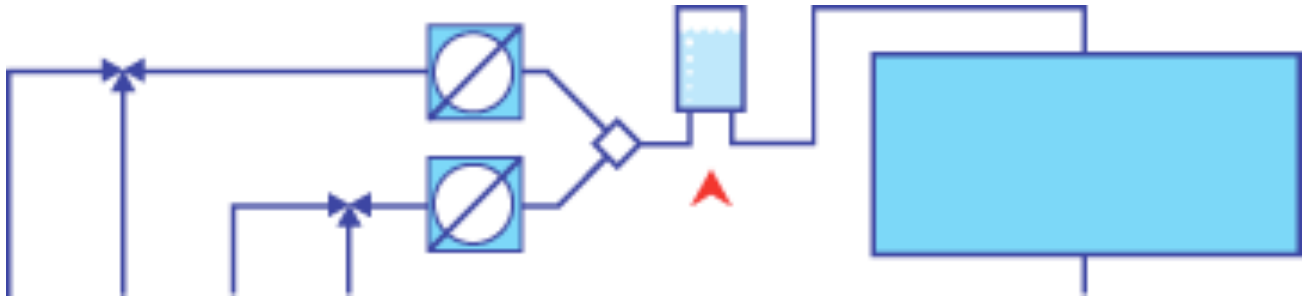
The combination of these effects tends to stabilize the association of the protein with the stationary phase. Proteins consequently elute at higher concentrations of the primary eluting agent than in the absence of PEG.

Indirect value of monoliths

Independence from air inclusions

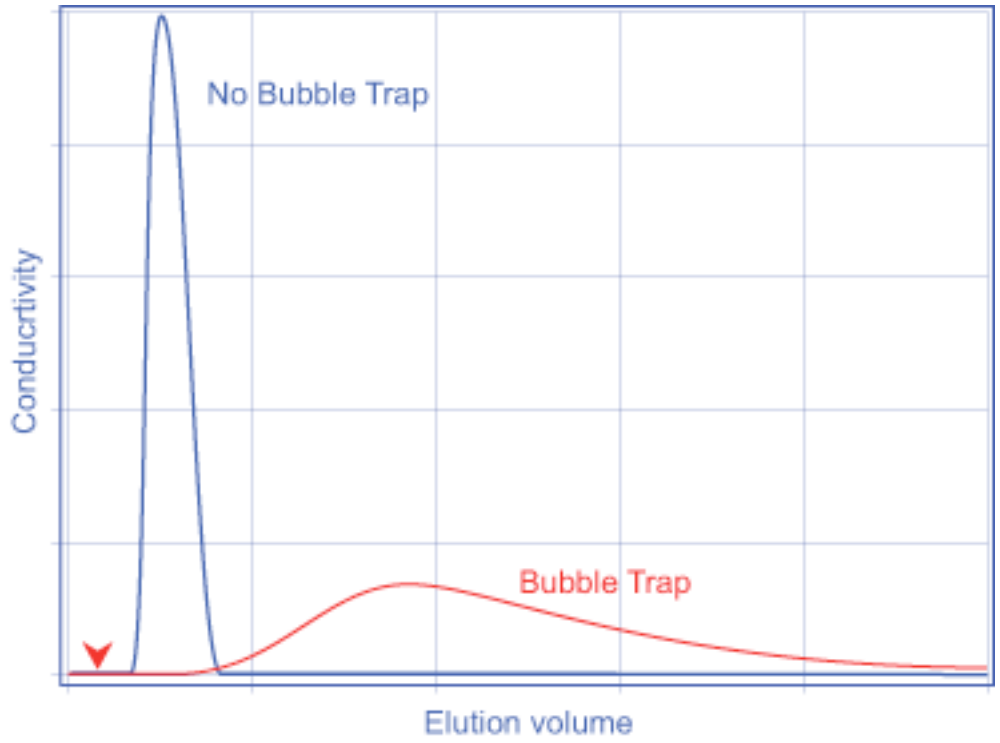
Introduction of air into a packed particle bed causes immediate loss of performance that typically results in failure of that process cycle and requires that the column be repacked.

Bubble-traps are commonly employed to protect the column, but they substantially depress separation performance and reproducibility across scales.



Indirect value of monoliths

Bubble traps increase dispersion in the system



Indirect value of monoliths

Changing the ratio of dispersion volume to column volume affects:

- *Column equilibration volume*
- *Sample loading, contaminant carry-over*
- *Gradient set points*
- *Effectiveness of cleaning and sanitization*
- *Validity of scale-up/scale-down models*

Discrepancies in the ratio of dispersion volume to column volume are a major cause of scale-up/scale-down failure.

For additional discussion and methods for characterizing system dispersion consult Gagnon, 1997, Avoiding instrument-associated aberrations in purification scale-up and scale-down, BioPharm, 10(3) 42-45

Indirect value of monoliths

Monoliths tolerate the passage of air without incident.

This permits omission of bubble traps from skid configurations, which in turn supports better separation performance and better reproducibility across process scales.

This facilitates technology transfer and improves the predictive value of small scale process models, which in turn supports process validation, and facilitates investigation in the event of a process failure.

Conclusions

Like the Golden Spike, monoliths represent an important integration of industrial capabilities.

Monoliths are the only chromatography media that combine convective mass transport and high efficiency flow distribution with the lack of a void volume.

This makes them uniquely qualified to achieve high capacity, high resolution fractionation of large biomolecules.

Conclusions

The ability of monoliths to deliver uncompromised performance at high flow rates offers dramatically higher manufacturing productivity, as well as faster time-to-clinic and time-to-market than can be achieved with porous particle-based media.

Their ability to effectively support enhancements such as improved size discrimination by the use of PEG further increases their value, especially for large biomolecules that are highly responsive to this treatment.

Acknowledgements

Sincere thanks to Avid Bioseparations for providing monoclonal antibodies, to CryoBioPhysica for providing pISep reagents, and to BIA Separations for providing monoliths to support this work.

For more information on monolith applications, antibody purification, the use of PEG, and other process chromatography subjects, please visit www.validated.com.

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