Bringing Downstream Productivity into Phase with Upstream Antibody Production

Pete Gagnon, Validated Biosystems

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

The need for speed

When monoclonal antibodies were first beginning to be commercialized, expression levels over 100 mg/L were considered outstanding, and cell culture was viewed as the bottleneck in manufacturing productivity.

Antibody expression levels now commonly exceed 1 g/L and reports of 10 and 15 g/L have been recently announced.

Downstream processing is now considered the bottleneck.

The need for speed

In one sense, the bottleneck is artificial. Cell culture production takes about two weeks (not counting preparation of seed stock) and purification takes about a week.

In another sense, the bottleneck is real, and a genuine concern. Process time for the protein A capture step from 20,000 L of cell culture supernatant (CCS) commonly requires 72-96 hours. This represents multiple cycles.

The long hold time for IgG produced in the early cycles increases the risk of degradation by proteolysis, deamidation, etc. It also increases the risk of contamination.

The need for speed

The bottleneck is also real from an economic perspective. Long process times tie up expensive manufacturing space and limit overall facility capacity. They also inflate labor costs per unit of finished product.

Anion exchange membranes have accelerated flow-through polishing purification of IgG and established the industrial value of convective mass transport, but they have not proven competitive for bind-elute applications.

The need for speed

The inability of traditional chromatography media to keep up with cell culture production has led many companies to pursue an ABC approach to antibody purification:
Anything But Chromatography.

ABC suggestions to date have concentrated on variations of precipitation methods that were previously used for plasma fractionation. Amgen has developed and presented results from an undisclosed method capable of achieving 99% antibody purity with a precipitating agent that can be recycled.

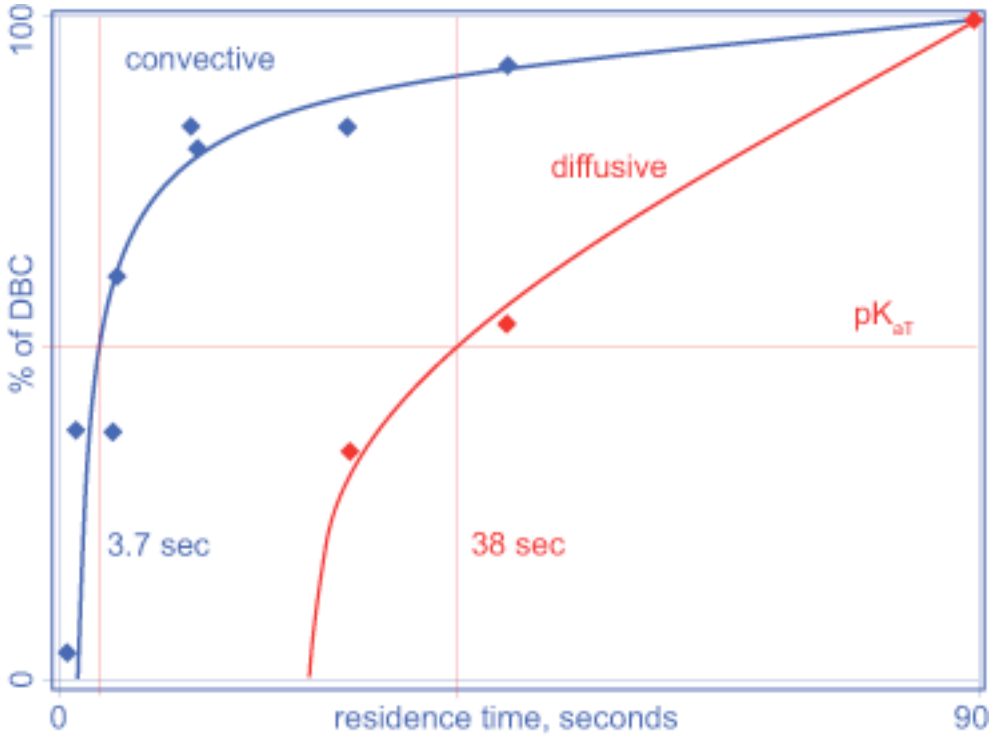
The need for speed

This presentation models a monolith-based IgG purification process of protein A, cation exchange, and anion exchange chromatography, and compares it with a parallel procedure on traditional media, to determine if monoliths can significantly improve throughput of chromatographic processes.

Input data are derived from small scale process models: 1 mL columns for porous particle-based media, 1 mL membranes, and 0.34 - 2.5 mL monoliths.

Affinity capture on protein A

IgG binding efficiency



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 the residence times.

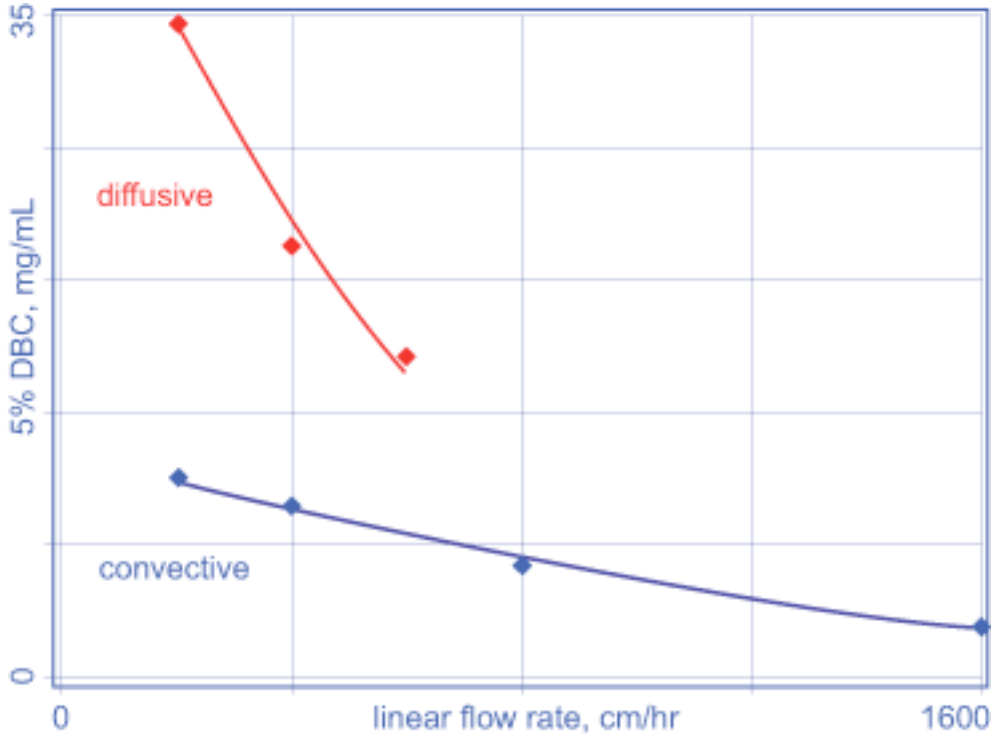
All values expressed as % of the 5% breakthrough value at a residence time of 90 seconds.

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

Affinity capture on protein A

Dynamic capacity, monoclonal IgG



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

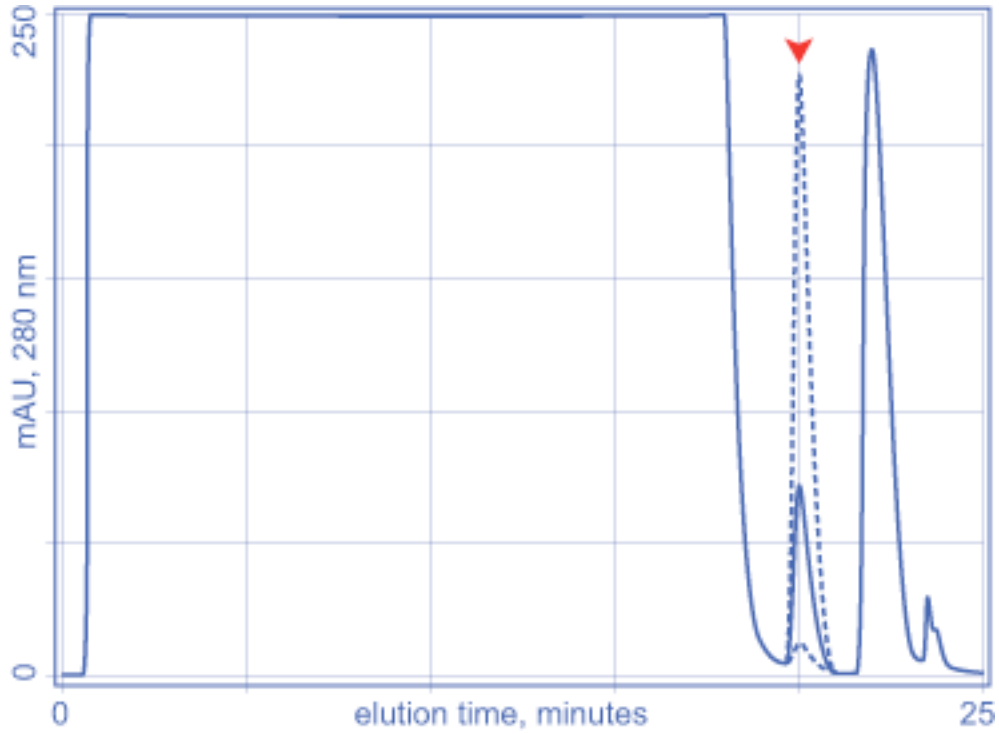
Diffusive: MabSelect Xtra, 1 mL (5 x 50 mm)

Monoclonal IgG_1 chimera

The higher binding efficiency of the monolith is also apparent in the shallower slope of the capacity curve versus flow rate, but its capacity in mg/mL is substantially inferior.

Affinity capture on protein A

Secondary washes



CIM Protein A HLD, 3 x 0.34 mL

4 mL (CV)/min

EQ: 5 CV PBS

Load: 12 CV IgG1 CCS

Wash 1: 5CV PBS

Wash 2: 5 CV 25 mM NaPO4, 5 mM EDTA, 200 mM arginine, 1.5 M NaCl, pH 7

Elute: 200 mM arginine, 50 mM acetate, pH 3.8

Regen: 0.1M citrate, pH 2.5

Secondary washes have been widely adopted because they significantly enhance removal of host cell proteins, DNA, and virus, some of which bind nonspecifically to the solid phase, some of which bind nonspecifically to the antibody, either directly or though intermediates. This wash enables the successful subsequent use of anion exchange in flow-through mode. The price for this benefit is that secondary washes increase buffer volume and process time. Many different formulations are employed. This one contains components for disruption of non-specific electrostatic, hydrophobic, hydrogen bonding, and metal binding interactions.

Affinity capture on protein A

Diffusive protein A media, capacity 35 g/L

- *100 L filtered CCS at 1 g IgG/L = 100 g*
- *Column volume (CV): 4 L (15 cm diameter, 22.5 cm height)*
- *Linear flow rate 200 cm/hr, 0.11 CV/min, 35.4 L/hr*
- *Process volume: 114 L, 28.5 CV (5 CV equilibration, 3.5 CV load, 5 CV wash1, 5 CV wash2, 5 CV elute, 5 CV regenerate)*
- *Time per cycle: 3.2 hr*
- *Product per cycle: 140 g*
- *Productivity 43.75 g/hr, 1.05 kg/24 hr*

On this basis, processing 20 kg of antibody in 96 hours would require a 19 L column (35 x 20 cm bed).

Affinity capture on protein A

Monolithic protein A media, capacity 10 g/L

- *100 L filtered CCS at 1 g IgG/L = 100 g*
- *Monolith volume (CV): 8 L, radial flow unit*
- *Flow rate 4 CV/min; 32 L/min, 1920 L/hr*
- *Process volume: 208 L, 26 CV (5 CV equilibration, 1 CV load, 5 CV wash1, 5 CV wash2, 5 CV elute, 5 CV regenerate)*
- *Time per cycle: 6.5 minutes*
- *Production per cycle: 80 g*
- *Productivity: 738 g/hr, 17.7 kg/24 hours*

20 kg of antibody could be processed on this 8 L unit in 27 hours.

Affinity capture on protein A

Comparison, 20 kg	Convective	Diffusive	Delta
Binding capacity	10 g/L	35 g/L	1/3.5
Media volume	8 L radial	19 L (35x20)	1/2.4
Volumetric flow	1920 L/hr	192 L/hr	10/1
Buffer vol./cycle	208 L	542 L	1/2.7
Cycle time	6.5 min	169.4 min	1/26.0
Product/cycle	80 g	665 g	1/8.3
Cycles/20 kg	250	30	8.3/1
Process time/20 kg	27 hr	85 hr	1/3.1
Buffer/20 kg	52,000 L	16,260 L	3.2/1

Affinity capture on protein A

35 cycles is the frequently used interval at which the price of conventional protein A is said to become negligible relative to other material costs. The monolith process uses 42% of the media volume used by the diffusive process, but exhibits 29% of its capacity per unit volume. This would put the amortization point at about 50 cycles, out of the total of 250 cycles to process 20 kg of IgG.

This means that monolithic protein A can be treated as a disposable. This would eliminate expenditures for development and validation of cleaning and sanitization protocols, eliminate material costs associated with their manufacturing use, and eliminate the costs associated with storing and tracking used media.

Affinity capture on protein A

Next to CCS, buffers are the most costly expendable component in downstream processing. 3-fold greater buffer consumption by the monolith represents a substantial increase in material costs.

However, productivity is increased by more than 300% because of the reduction in processing time, and labor costs are reduced by the same increment.

Affinity capture on protein A

Substantial labor savings are also obtained from monoliths eliminating the need to pack columns.

Elimination of packing eliminates the buffers and supporting equipment required to pack columns: slurry tanks, transfer vessels; plus the costs for maintenance, validation, associated labor, and storage space.

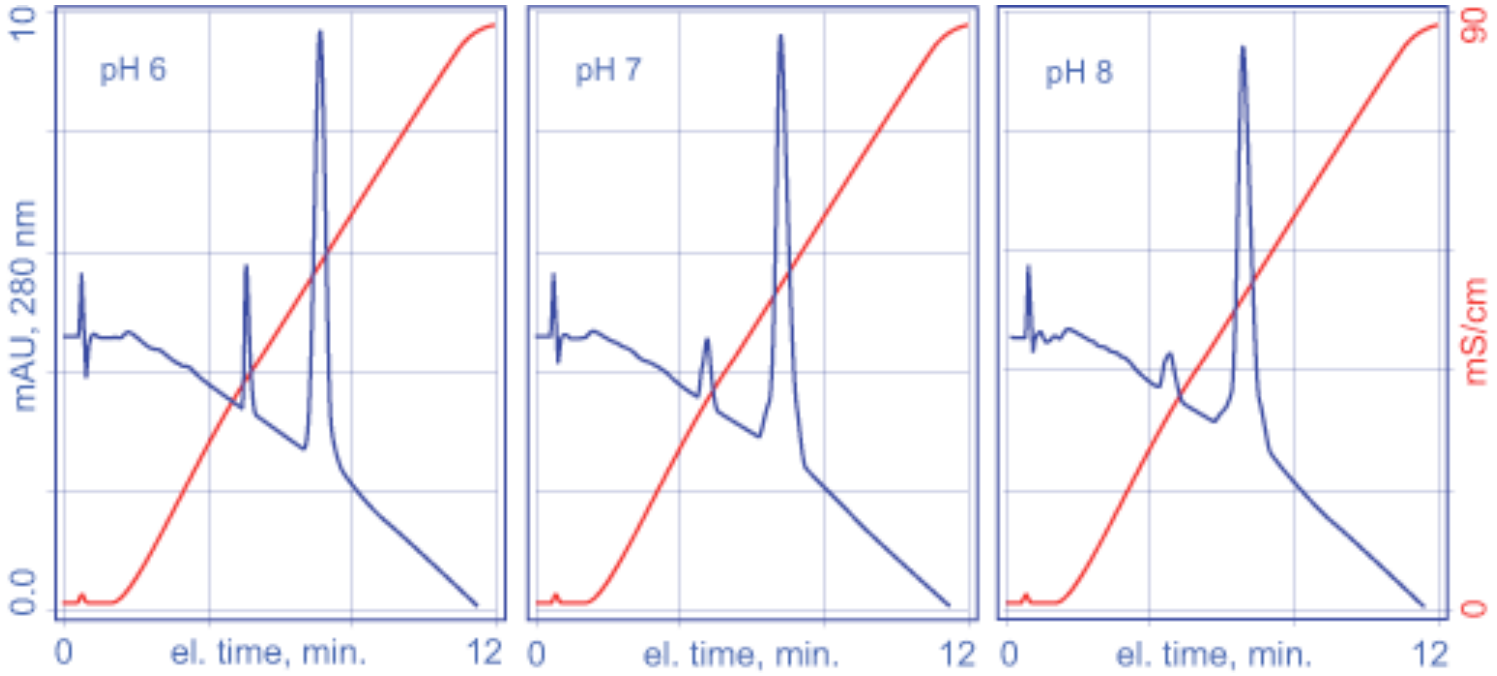
Affinity capture on protein A

Savings in column packing are compounded by the ability of monoliths to tolerate air. It passes through the monolith without disrupting chromatographic performance.

In contrast, the introduction of air into a packed particle column generally causes failure of that production cycle and requires repacking. Manufacturers mostly keep back-up columns on hand to maintain processing continuity in the event of such a failure, but this essentially doubles the cost of media and column hardware to support a given process.

Cation exchange

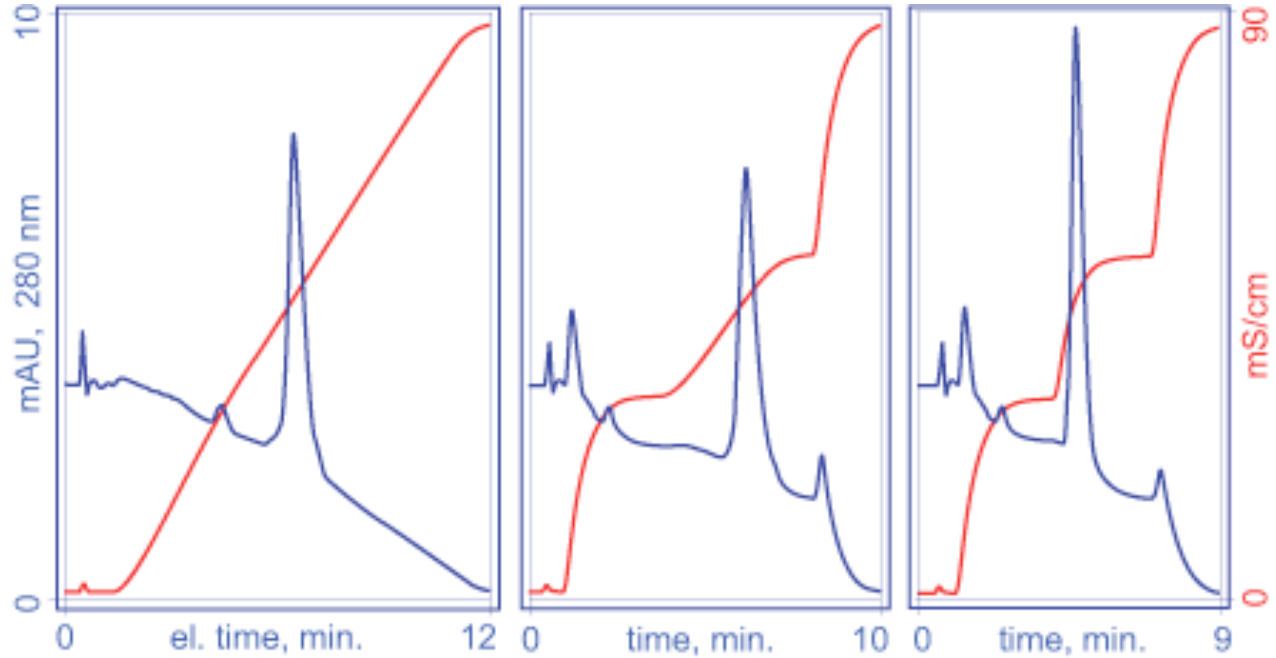
Screening pH



The first peak contains antibody fragments that co-purify on protein A. The leading and trailing shoulders on the main peak are glycosylation isoforms. Isoform separation is generally more apparent the closer the operating pH is to an antibody's pI, which in this case is about 9.5. The screening buffers at pH 6, 7, and 8 are MES, Hepes, and Tris (50 mM). Gradients to 1 M NaCl. CIM SO3, 0.34 mL, 4 mL/min. Most IgGs bind fairly well at pH 6. Few do so at pH 7, and IgG monoclonals that bind well at pH 8 are uncommon.

Cation exchange

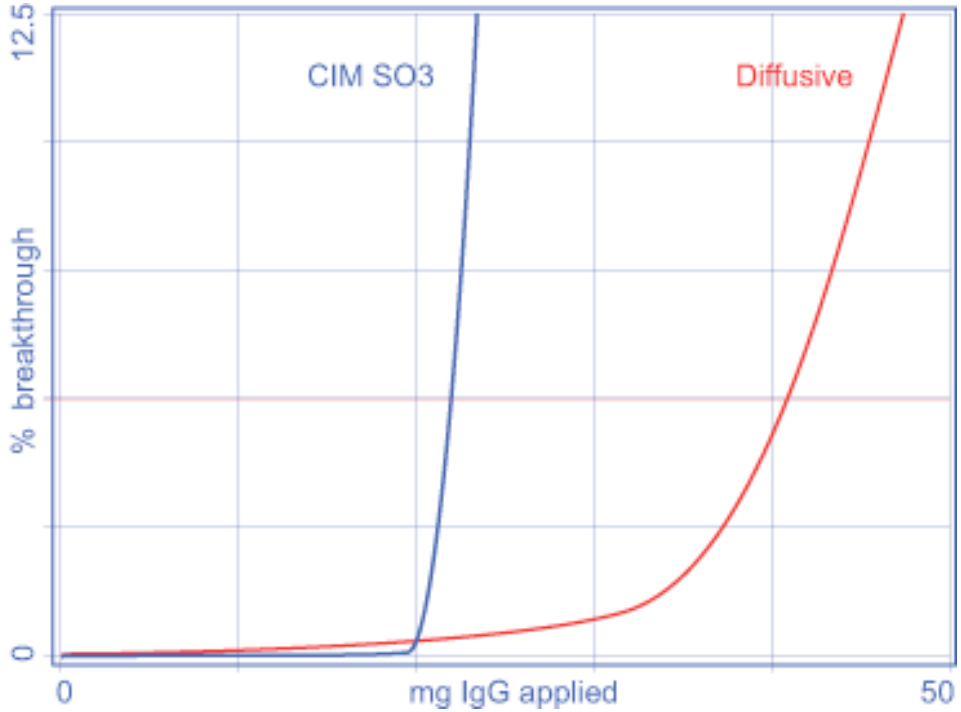
Development of gradient intervals



Industrial ion exchange methods employ step gradients almost exclusively, but linear gradients are helpful for identifying step intervals. The first profile shows the screening gradient at pH 8. The second shows the effects of a preliminary step to remove early eluting contaminants, with the remaining linear gradient truncated by another step. Eluting with a linear gradient permits visual confirmation that the antibody has not been partially eliminated. The slope of the steps is from precolumn dispersion caused running 0.34 mL columns on a chromatograph configured for larger columns.

Cation exchange

Dynamic capacity, IgG



CIM SO3, 3 x 0.34 mL (1 mL)
4 mL/min

Fractogel® EMD SE HiCap
5 x 50 mm (1 mL), 1 mL/min

A: 50 mM Tris, pH 8.0

EQ: A

Load: protein A eluate

Cation exchange

Diffusive cation exchanger, capacity 40 g/L

- *2000 L protein A eluate at 10 g IgG/L = 20 kg*
- *Column volume: 39 L (50 cm diameter, 20 cm height)*
- *Linear flow rate 200 cm/hr, 0.17 CV/min, 393 L/hr*
- *Process volume: 1131 L, 29 CV (10 CV equilibration, 4 CV load, 5 CV wash, 5 CV step, 5 CV elute)*
- *Time per cycle: 2.9 hr*
- *Product per cycle: 1.56 kg*
- *Productivity: 538 g/hr, 20 kg/38 hours*

Cation exchange

Monolithic cation exchanger, capacity 20 g/L

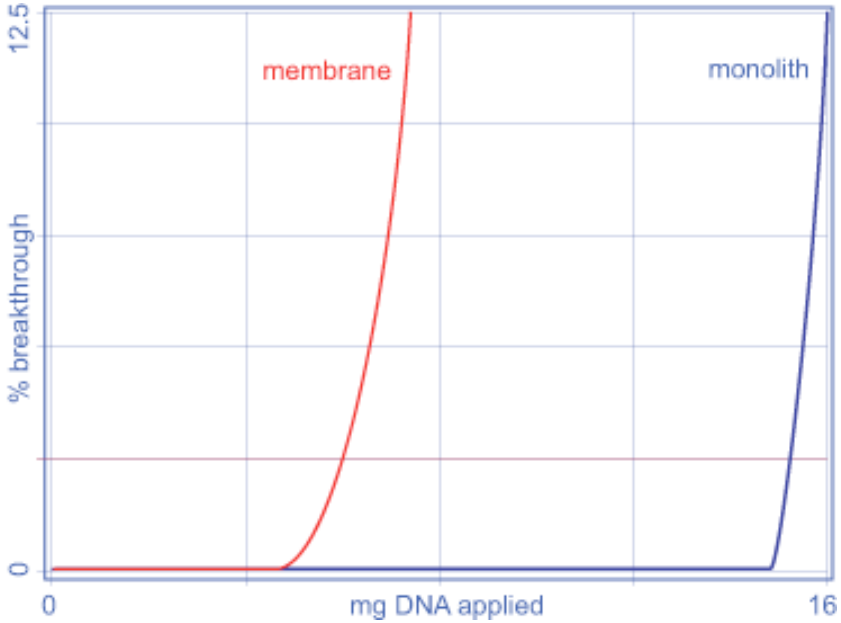
- *2000 L protein A eluate at 10 g IgG/L = 20 kg*
- *Monolith volume: 8 L radial unit*
- *Flow rate: 4 CV/min, 32 L/min , 1920 L/hr*
- *Process volume: 216 L, 27 CV (10 CV equilibration, 2 CV load, 5 CV wash, 5 CV step, 5 CV elute)*
- *Time per cycle: 6.75 minutes*
- *Product per cycle: 160 g*
- *Productivity: 1.4 kg/hr = 20 kg/14.3 hours*

Cation exchange

Comparison, 20 kg	Convective	Diffusive	Delta
Binding capacity	20 g/L	40 g/L	1/2
Media volume	8 L radial	25 L (20x20)	1/3.1
Volumetric flow	1920 L/hr	393 L/hr	4.9/1
Buffer vol./cycle	216 L	1520 L	1/7.0
Cycle time	7.5 min	174 min	1/23.2
Product/cycle	160 g	1.56 kg	1/9.75
Cycles/20 kg	125	13	9.6/1
Process time/20 kg	14.3 hr	38 hr	1/2.7
Buffer/20 kg	27,000 L	19,760 L	1.4/1

Anion exchange

Dynamic capacity, DNA



Sartobind™ Q nano, 1 mL

CIM QA, 3 x 0.34 mL (1 mL)

1 mL/min

DNA: 1 mg/mL

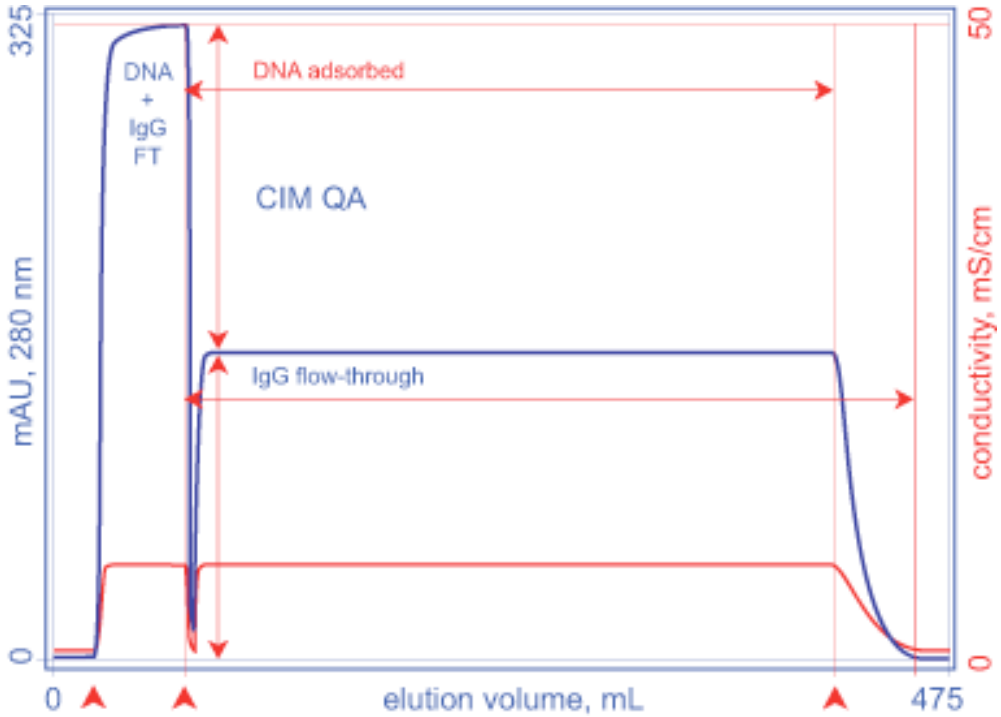
Sample and media equilibrated to 0.05 M Hepes, pH 7.0

Although the membrane supports convective mass transport, the shape of the breakthrough curve indicates lower efficiency than the monolith. This was attributed to dispersion in the housing and between membrane layers. Endotoxin capture follows the same pattern. Higher efficiency and capacity of DNA and endotoxin removal suggest that virus removal should be similarly enhanced but this remains to be demonstrated.

For additional experimental details and results consult: A comparison of microparticulate, membrane, and monolithic anion exchangers for polishing applications in the purification of IgG monoclonal antibodies, Gagnon et al, IBC International Conference and Exposition, October 1-4, Boston, MA, USA

Anion exchange

DNA removal in the presence of IgG



CIM QA, 2.5 mL, radial

6 mL/min

Protein A eluate, diluted to 1 mg/mL and spiked with 0.1mg/mL DNA

EQ: 0.05 M Hepes, pH 7.0

Monolith off line

Saturate system with sample

Monolith in line

Load 335 mL sample

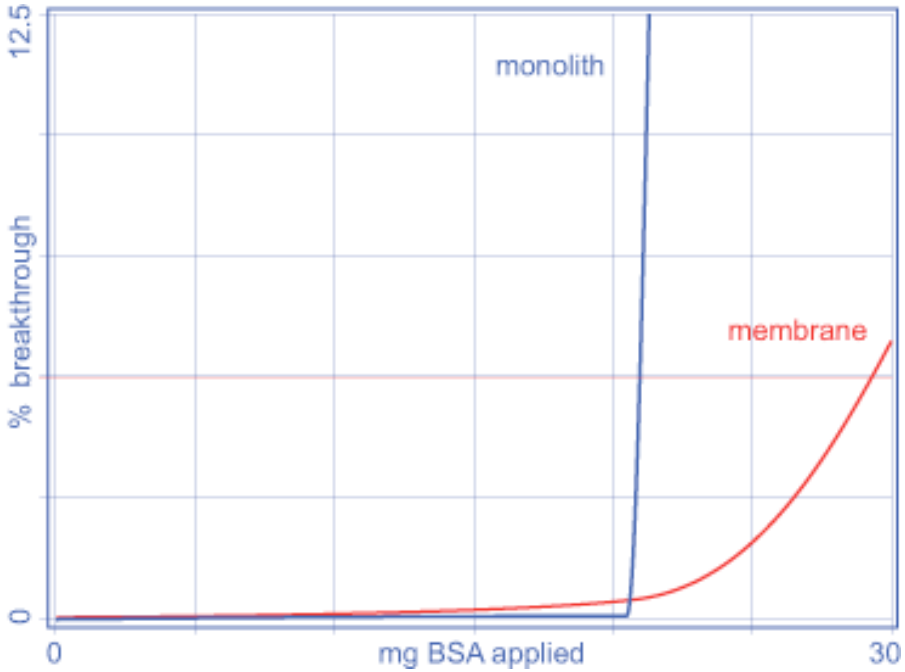
Wash: 0.05 M Hepes, pH 7.0

DNA tested by picogreen

This experiment was conducted to determine the ability of a monolithic anion exchanger to remove DNA when IgG is present in the sample. DNA levels in the eluted IgG were less than 1 ng/mL (limit of linear detection for picogreen) at all sampling points, indicating at least 5 logs of removal. DNA levels in commercial IgG preparations after 2 purification steps are usually less than 50 ng/mL.

Anion exchange

Dynamic capacity, BSA



CIM QA, 3 x 0.34 mL (1 mL)

Sartobind Q nano, 1 mL

Flow rate: 1 mL/min

BSA: 1 mg/mL

0.05 M Hepes, pH 7.0

Binding capacity for host cell proteins is usually the limiting factor in determining the size of the anion exchanger. In this example, BSA is used as a model. 5% breakthrough values favor the membrane, at about 29 mg/mL versus about 21 for the monolith. However, the monolith passes no BSA until about 20.5 mg have been applied, while the membrane begins to lose efficiency after about 10 mg. Arguably, this makes the monolith the more effective candidate, especially given its higher DNA capacity.

Anion exchange

Assumptions for 20 kg IgG

- *Cation exchange pool diluted 5x to reduce conductivity*
- *IgG concentration in diluted pool, 2.5 mg/mL*
- *8 L monolith, equivalent membrane*
- *200 g IgG per liter of media per cycle = 1.6 IgG kg per cycle*
- *1.6 kg IgG = 640 L at 2.5 g/L*
- *Volume per cycle: 760 L, 95 CV (5 CV EQ, 80 CV load, 5 CV wash, 5 CV regenerate)*
- *Flow rate: 4 CV/min, 1920 L/hr*
- *Time per cycle: 0.4 hr*
- *Cycles to process 20 kg: 13*
- *Total process time: 5.1 hr*
- *Total buffer volume: 9,880 L*

Process summary

Comparison, 20 kg	Monolith	Traditional	Delta
Protein A, volume	8 L	19 L	1/2.4
Process time	27 hr	85 hr	1/3.1
Buffer consumption	52,000 L	16,260 L	3.2/1
Cation exchange, vol.	8 L	39 L	1/4.9
Process time	14.3 hr	38 hr	1/2.7
Buffer consumption	27,000 L	19,760 L	1.4/1
Anion exchange, vol.	8 L	8 L	1/1
Process time	5.1 hr	5.1 hr	1/1
Buffer consumption	9,880 L	9,880 L	1/1
Overall, Media volume	24 L	85 L	1/3.5
Process time	46.4 hr	128.4 hr	1/2.8
Buffer consumption	88,880 L	45,920 L	1.9/1

Conclusions

For the 3-step process modeled in this presentation, monoliths reduce overall process time nearly 3-fold; less than 2 days to process 20 kg of monoclonal IgG with a single 8 L monolith at each step.

Facility capacity triples. Labor costs decrease in proportion. ROI for the facility is accelerated.

Media costs are reduced. The protein A monolith is only 40% the volume of its particle-based counterpart; the cation exchange monolith, only 20%.

Conclusions

Multiple cycles make it economically feasible to dispose of the used monoliths after processing a single antibody production lot. This suspend the need to develop and validate cleaning and sanitization procedures.

Monoliths also eliminate column packing, removing a significant manufacturing expense and source of process variability. This benefit is compounded by the ability of monoliths to pass air without loss of chromatographic performance, and without repacking.

Conclusions

Although the benefits of rapid processing are compelling, higher buffer consumption per unit of finished product is a limitation. Overall buffer consumption for the monolith-based process is nearly twice the volume required for traditional media. This results from the relatively low binding capacities of current generation monoliths for small proteins, such as IgG.

The high volumetric flow rates required to support rapid processing are also an issue. Flow rate for a single 8 L monolith was 10 times higher than conventional media at the protein A step, and 5 times higher at the cation exchange step – and this was at less than half the monolith's volumetric flow capacity. It may be necessary to retrofit existing chromatography skids to accommodate the necessary range of flow rates.

Conclusions

Increasing binding capacity for small proteins would reduce buffer consumption in direct proportion, further accelerate processing, and make implementation of monoliths more attractive to antibody manufacturers.

More extensive modeling should help to reveal the most productive configurations for a particular application: what flow rate, how many monoliths, what size, in series, in parallel…

High efficiency flow configurations, such as Simulated Moving Bed systems have the potential to further reduce buffer consumption and increase productivity.

Conclusions

The speed of monoliths also invites consideration of alternative manufacturing strategies.

An SMB array of 800 mL protein A monoliths could be accommodated with existing industrial skids.
*It could be linked to a cation exchange monolith array via an intermediate viral inactivation loop.**
The cation exchange array could be linked to an anion exchange monolith array.

Such a system would allow continuous processing of more than 2 kg of fully purified antibody per day, without exposing the product to excessive hold times.

**The idea of a viral inactivation loop in conjunction with SMB was suggested by Jorg Thommes.*

Other large scale monoliths…

The Church of Lalibela in Ethiopia. Legend states that it was carved from solid rock by the Knights Templar, with The Ark of the Covenant.

photos from www.sacred-destinations.com

Acknowledgements

Sincere thanks to Avid Bioservices for providing monoclonal antibodies, and to BIA Separations for providing monoliths.

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

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