Productivity improvements in the capture and initial purification of monoclonal antibodies

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Efficiency = Productivity

Productivity of chromatography processes results from optimizing numerous contributory factors, including the architecture of the chromatography media itself.

This presentation will focus on the contribution of mass transport efficiency to overall productivity in protein A affinity capture of monoclonal antibodies.

Chromatographic efficiency

A 1990 publication by Afeyan *et al** was the first in the popular literature to emphasize mass transport as a primary determinant of chromatographic performance.

The authors suggested that both resolution and capacity could be enhanced by optimizing the pore architecture of the chromatography media.

* N. Afeyan, N. Gordon, J. Mazaroff, C. Varaday, S. Fulton, Y. Yang, and F. Regnier, 1990, Flow through particles for the high performance liquid chromatography separation of biomolecules, *J. Chrom.,* **519** 1-29

Chromatographic efficiency

Afeyan *et al* described pores in traditional porous media as stagnant pools in which mass transport could occur only by diffusion, placing an artificial limitation on performance.

Flow velocity needs to be kept low with diffusive pores to allow molecules with slow diffusion constants to reach the binding surface. This applies especially to large molecules such as proteins.

The practical significance is that diffusive transport causes both capacity and resolution to decline dramatically with increasing flow rate.

Chromatographic efficiency

Diffusion constants for selected proteins

Protein	**Mass**	**K_{diff} cm^2/sec**
IgM	960 kD	2.6×10^{-7}
IgA	335 kD	3.7×10^{-7}
IgG	150 kD	4.9×10^{-7}
Albumin	67 kD	6.7×10^{-7}
Light chain	23 kD	9.1×10^{-7}

An IgG molecule would diffuse about 15.5 cm^2/year at this rate.

Chromatographic efficiency

The breakthrough described by Afeyan *et al* was the notion that convection could contribute to overall efficiency of mass transport.

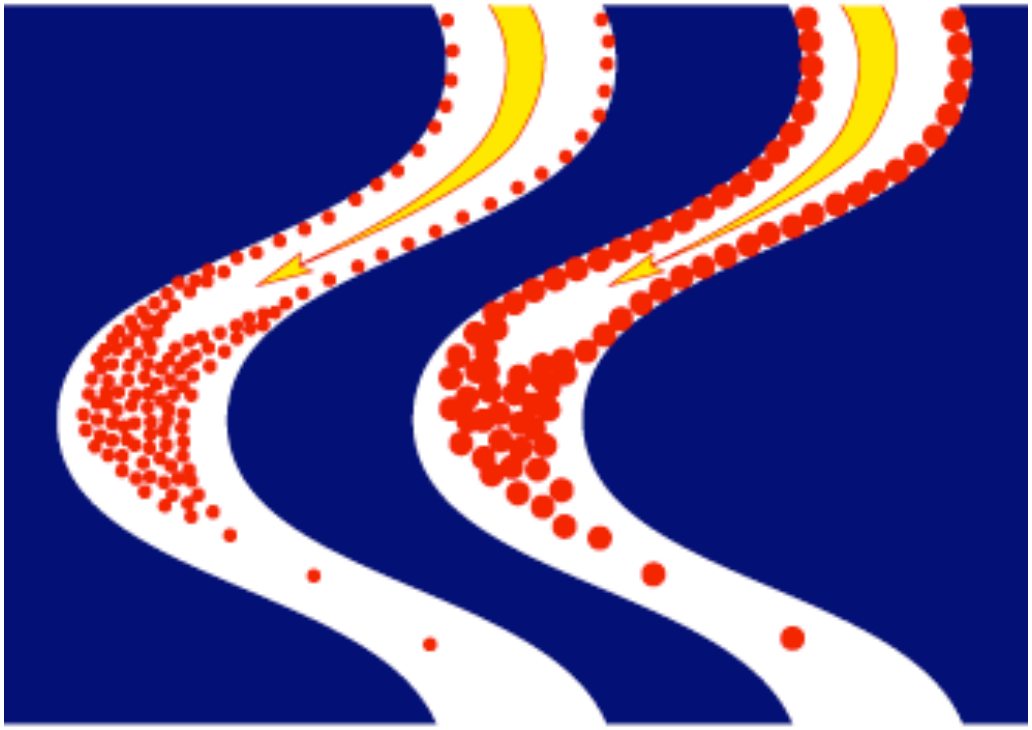
Convective transport is independent of flow rate.

The practical benefit is that both capacity and resolution are independent of flow rate.

Even a modest convective contribution was shown to significantly improve performance.

Chromatographic efficiency

The “River” model of convective mass transport



As described by Ales Podgornik, BIA Separations. As suggested by the figure, higher mass capacities are achieved with larger solutes.

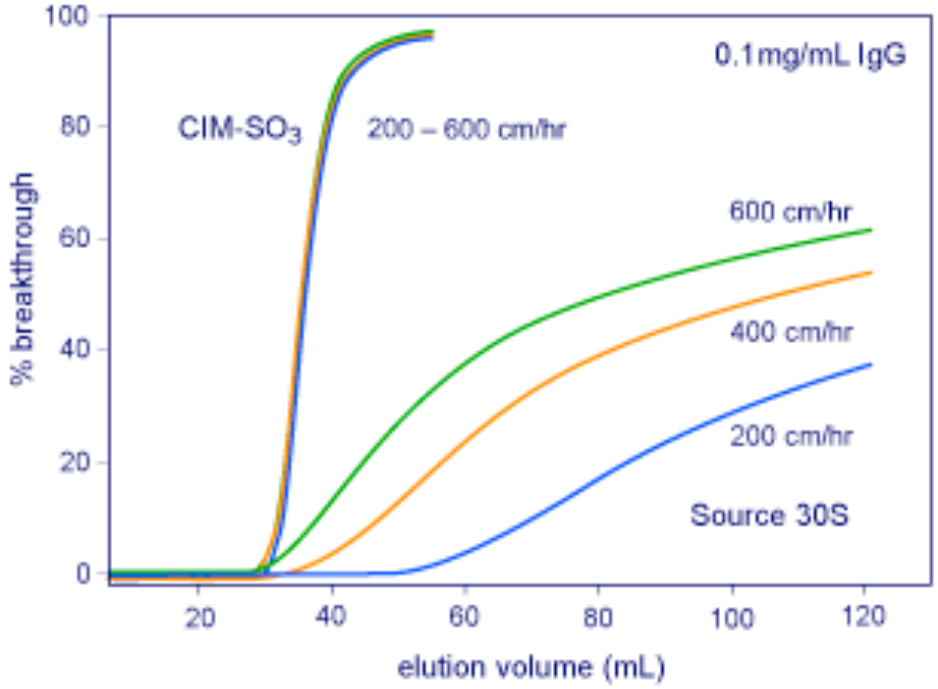
Chromatographic efficiency

The “Delta” extension” to the River model

Connectivity is very high between the channels, producing turbulent flow that carries solutes to the binding surfaces.

Chromatographic efficiency

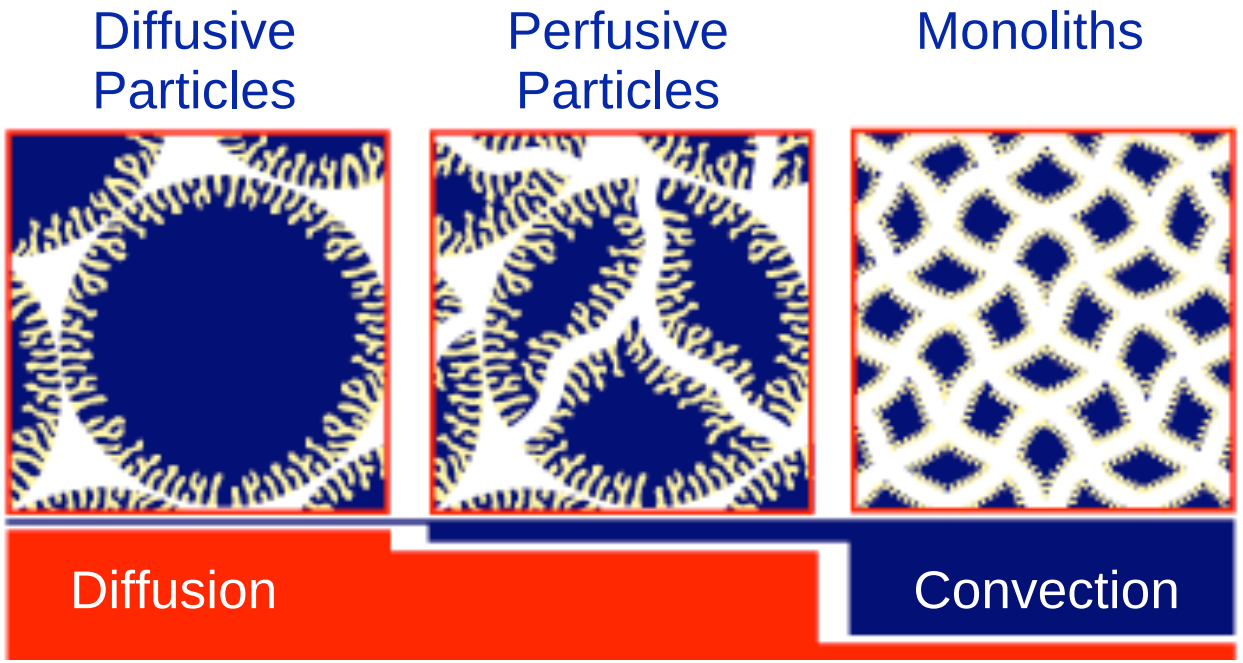
Breakthrough on a convective vs a diffusive cation exchanger



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.

Chromatographic efficiency

Diffusion and convection in different pore architectures



Blue: support matrix. Yellow: areas of diffusive flow. White: areas of convective flow

Experimental design

Characterize dynamic binding characteristics of protein A affinity media representing different proportions of diffusive and convective mass transport. 1 mg/mL monoclonal human IgG1 at linear flow velocities from 200–1600 cm/hr.

Dominantly convective: CIM® Analytical Protein A HLD, 1 mL (12 x 9 mm*), BIA Separations.

Perfusive (mixed convective/diffusive): POROS® MabCapture A™ (late stage beta), 1 mL (5 x 50), Applied Biosystems.

Dominantly diffusive: MabSelect Xtra™ 1mL (5 x 50), GE Healthcare.

*three x 3 mm disks were stacked to achieve a combined bed volume of 1mL.

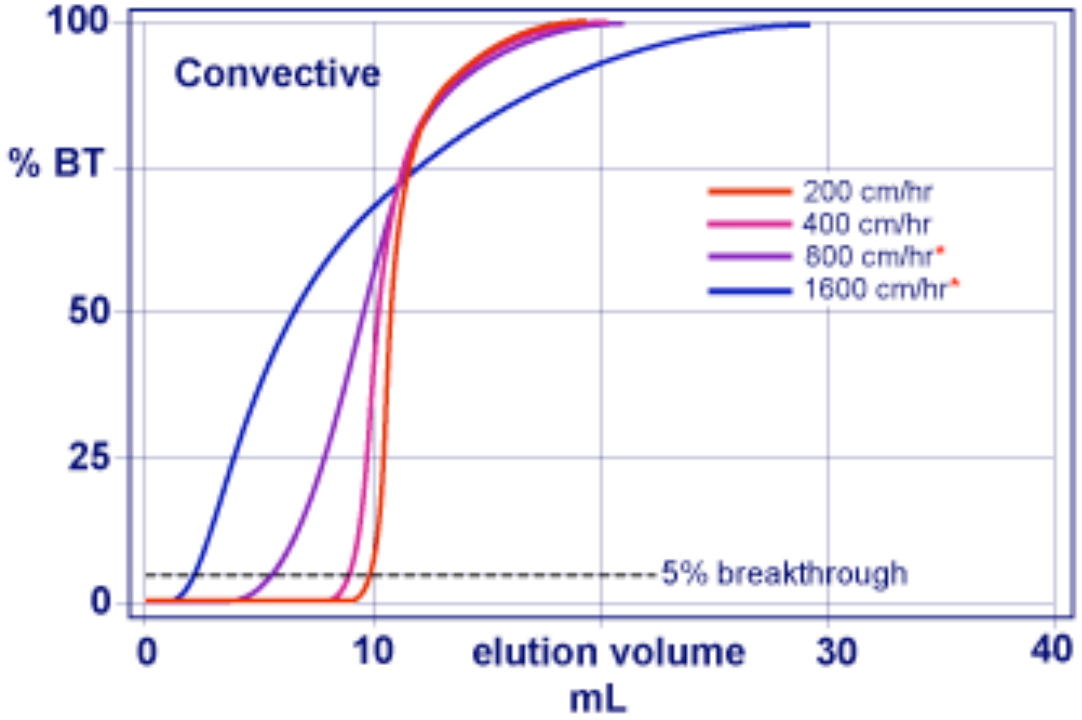
Experimental design

Volumetric flow rates and residence times

LinFV\bed ht	9 mm	50 mm
200 cm/hr	30 mL/min, 16 sec	0.66 mL/min, 90 sec
400 cm/hr	15 mL/min, 8 sec	1.32 mL/min, 45 sec
600 cm/hr	10 mL/min, 6 sec	2.00 mL/min, 30 sec
800 cm/hr	7.5 mL/min, 4 sec	2.64 mL/min, 22.5 sec
1600 cm/hr	3.75 mL/min, 2 sec	5.28 mL/min, 11.2 sec

Experimental results

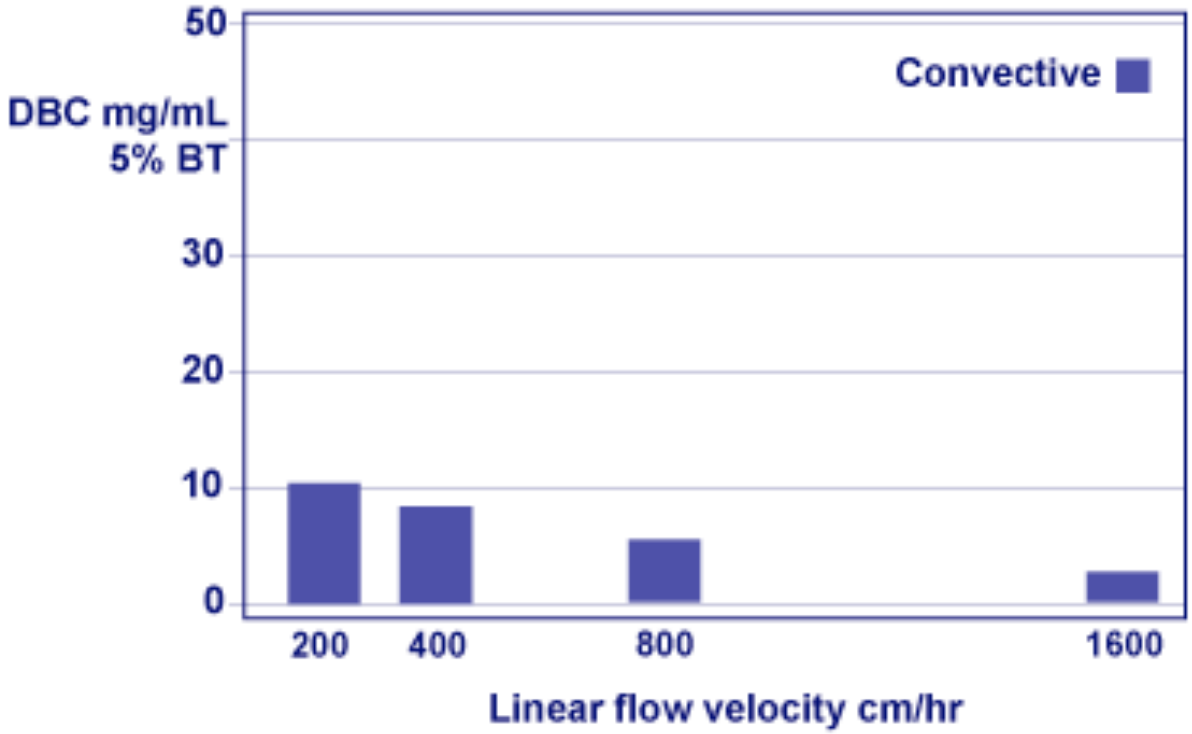
Breakthrough curves for monolithic protein A



*cartridge leakage

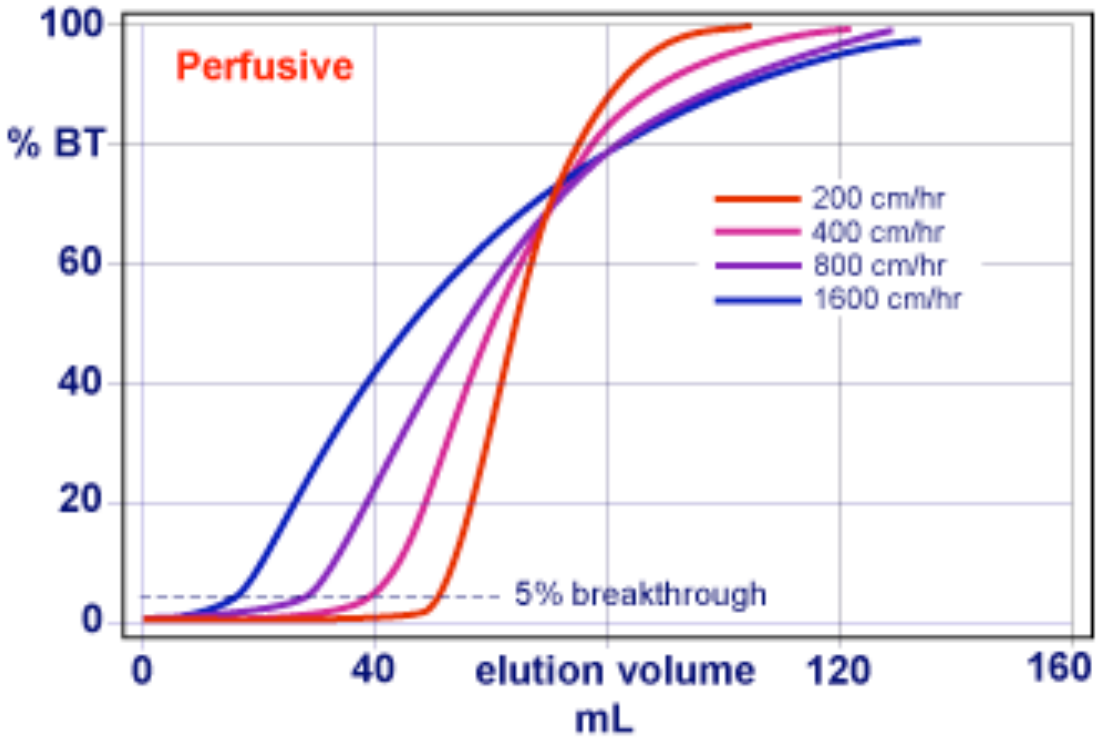
Experimental results

Dynamic binding capacities for monolithic protein A



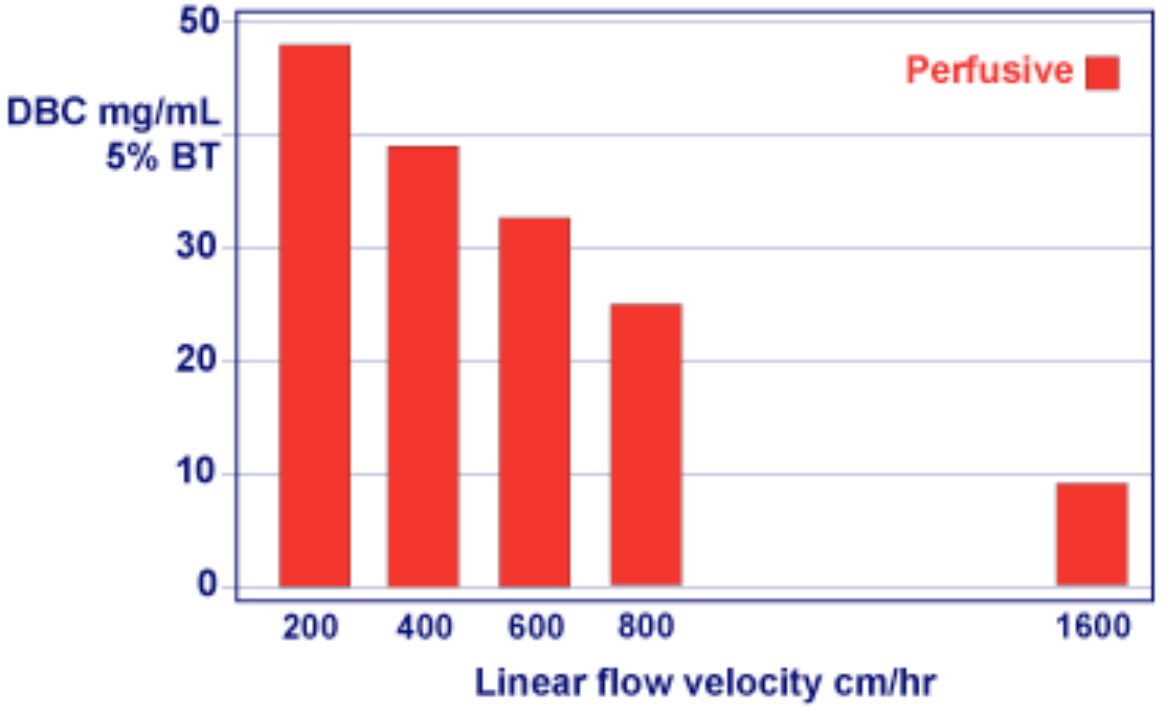
Experimental results

Breakthrough curves for perfusive particles



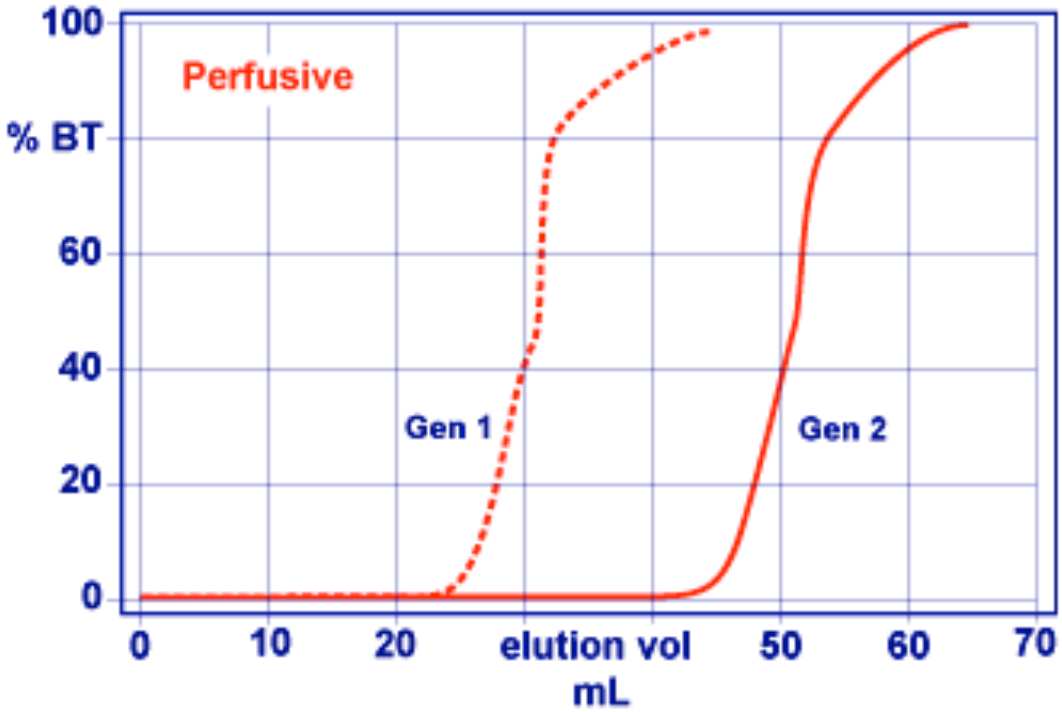
Experimental results

Dynamic binding capacities for perfusive particles



Experimental results

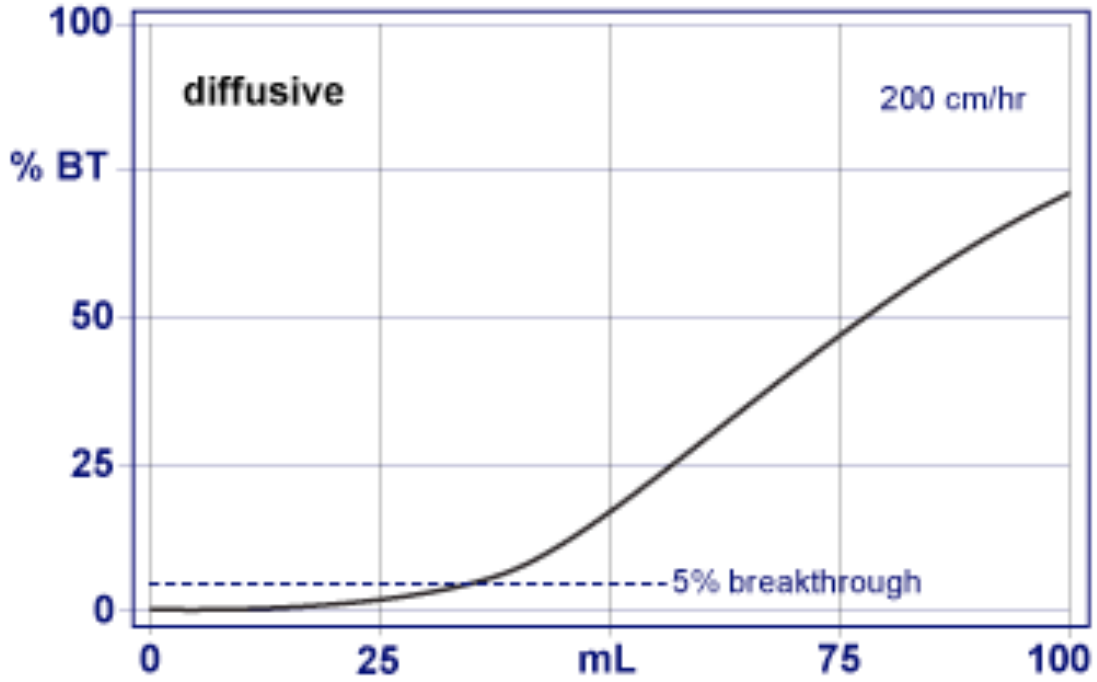
First and second generation perfusive particles



Polyclonal Human IgG.
Gen 1. POROS A 50™
Gen 2. POROS MabCapture A
Data provided by Applied Biosystems.

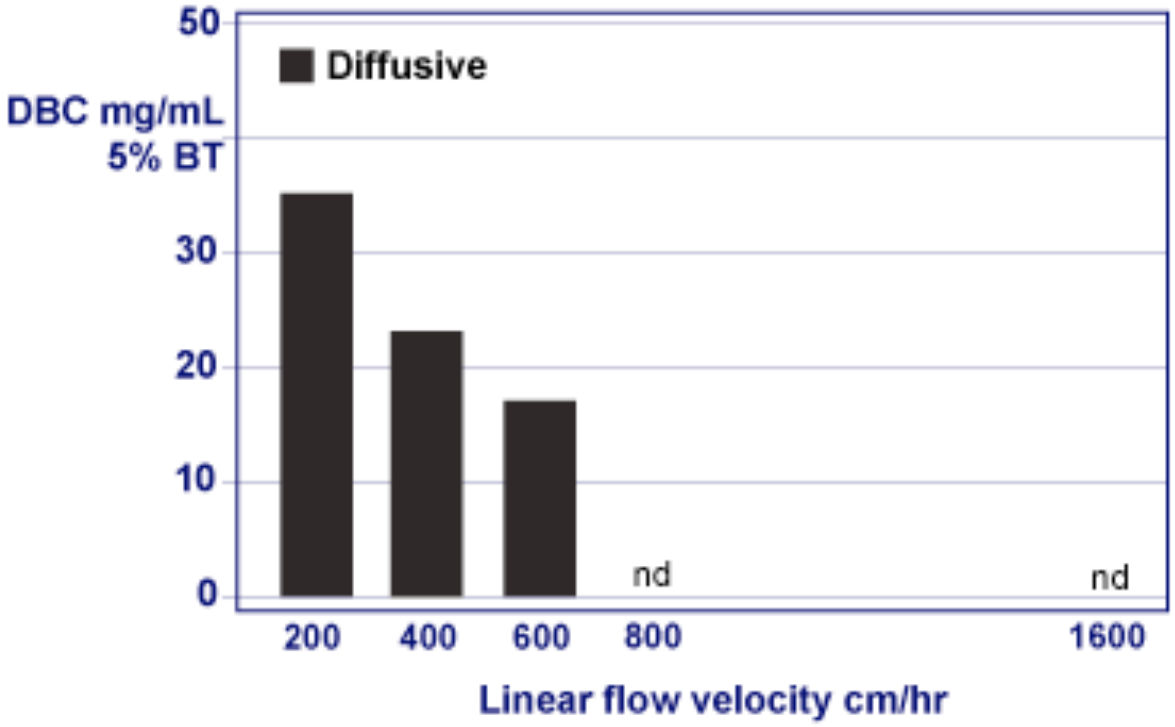
Experimental results

Breakthrough curve for diffusive particles



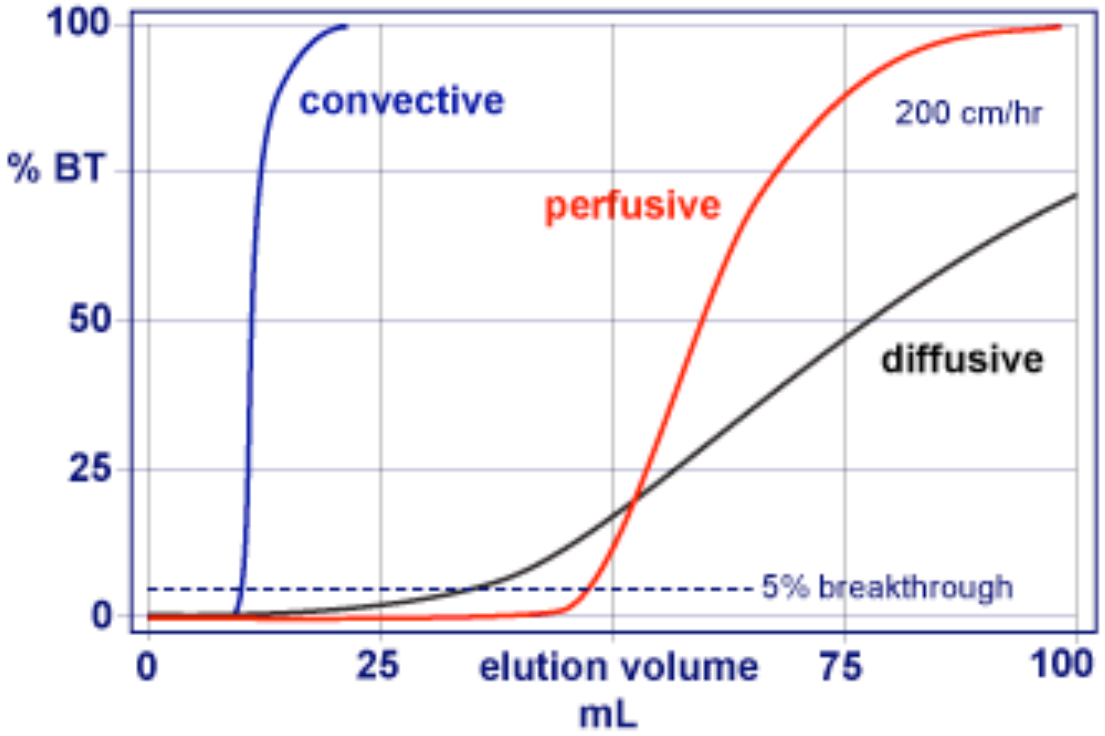
Experimental results

Dynamic binding capacities for diffusive particles



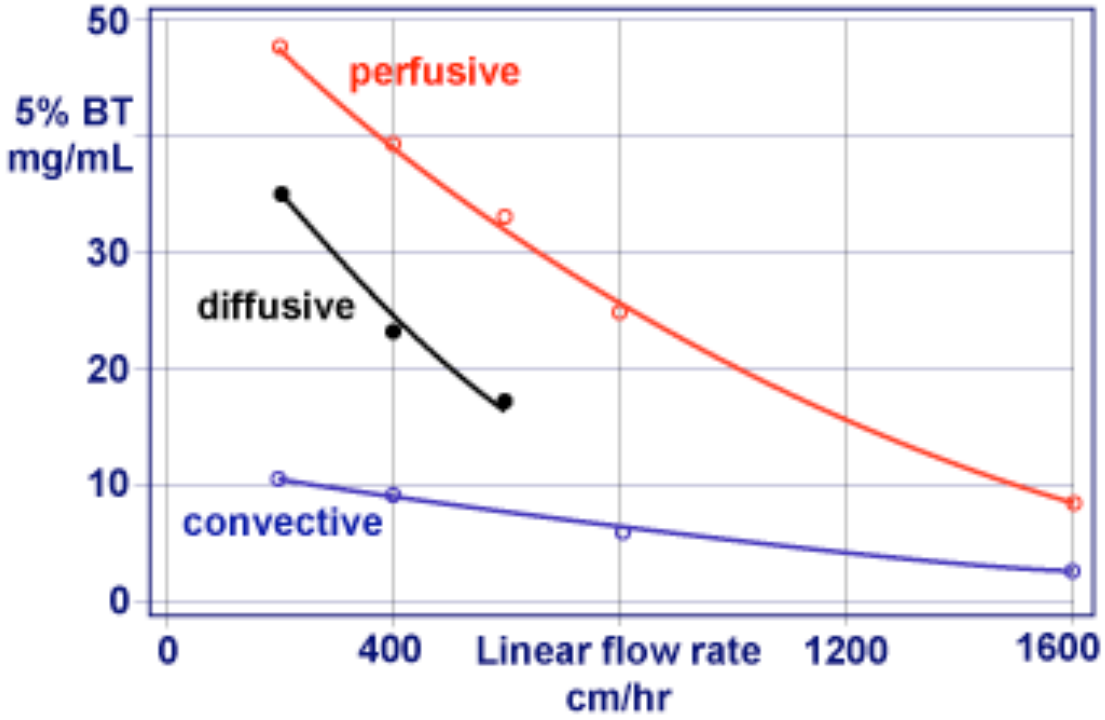
Experimental results

Breakthrough curves at 200 cm/hr, all media



Experimental results

Dynamic capacity comparison, all media



Efficiency = Productivity

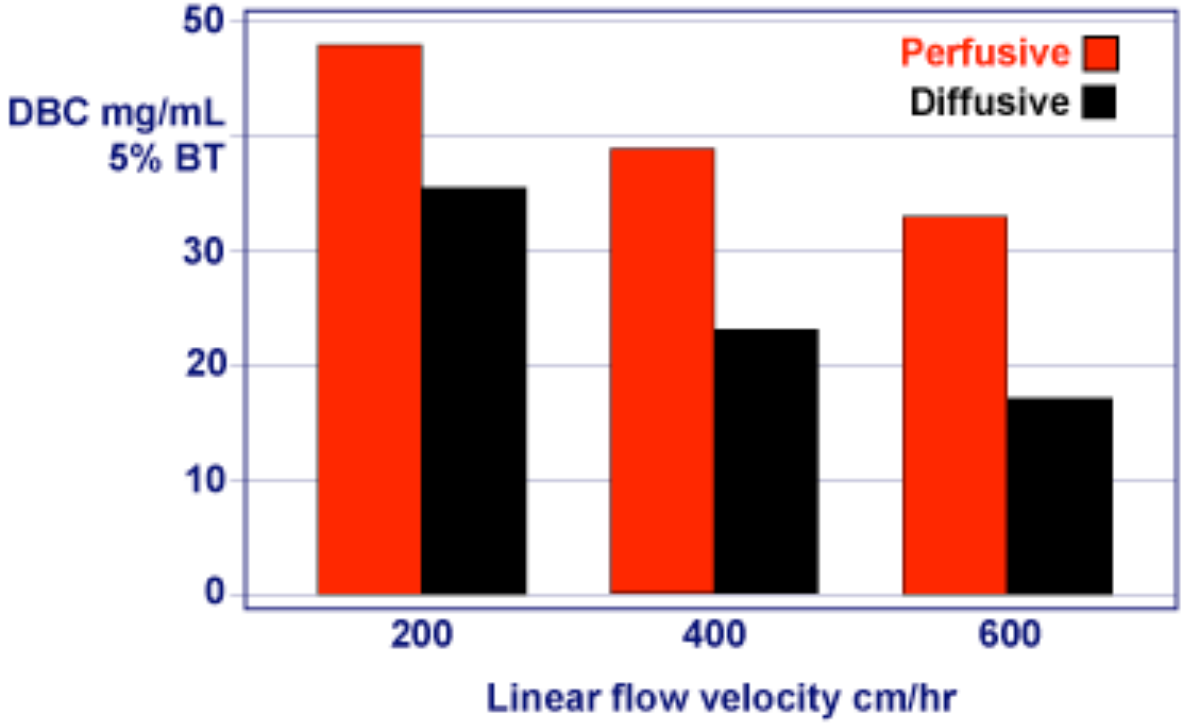
How does the perfusive particle achieve such high dynamic binding capacity?

The breakthrough slope is intermediate between monoliths and diffusive particles. This suggests that it takes the best of diffusive pore architectures, with the best of convective pore architectures, and combines them in proportion to achieve an efficient balance.

The fact that it supports roughly twice the capacity of first generation perfusive media also suggests that a more effective strategy of ligand presentation has been developed.

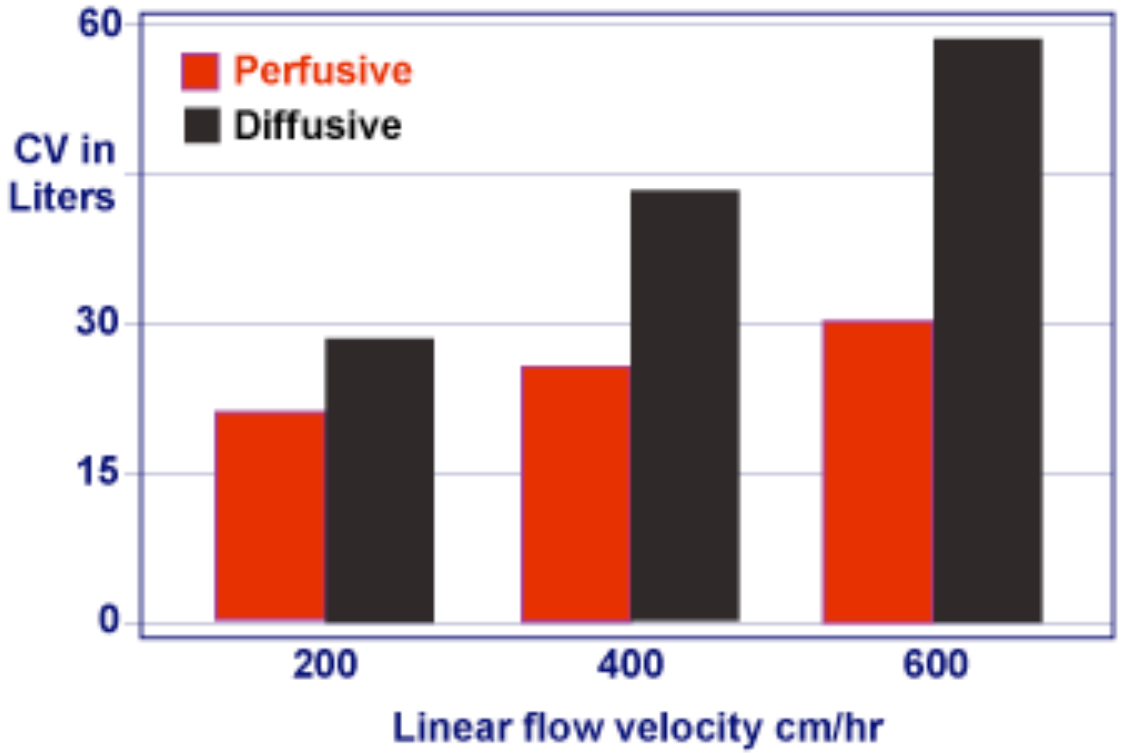
Efficiency = Productivity

Dynamic binding capacity at 5% breakthrough



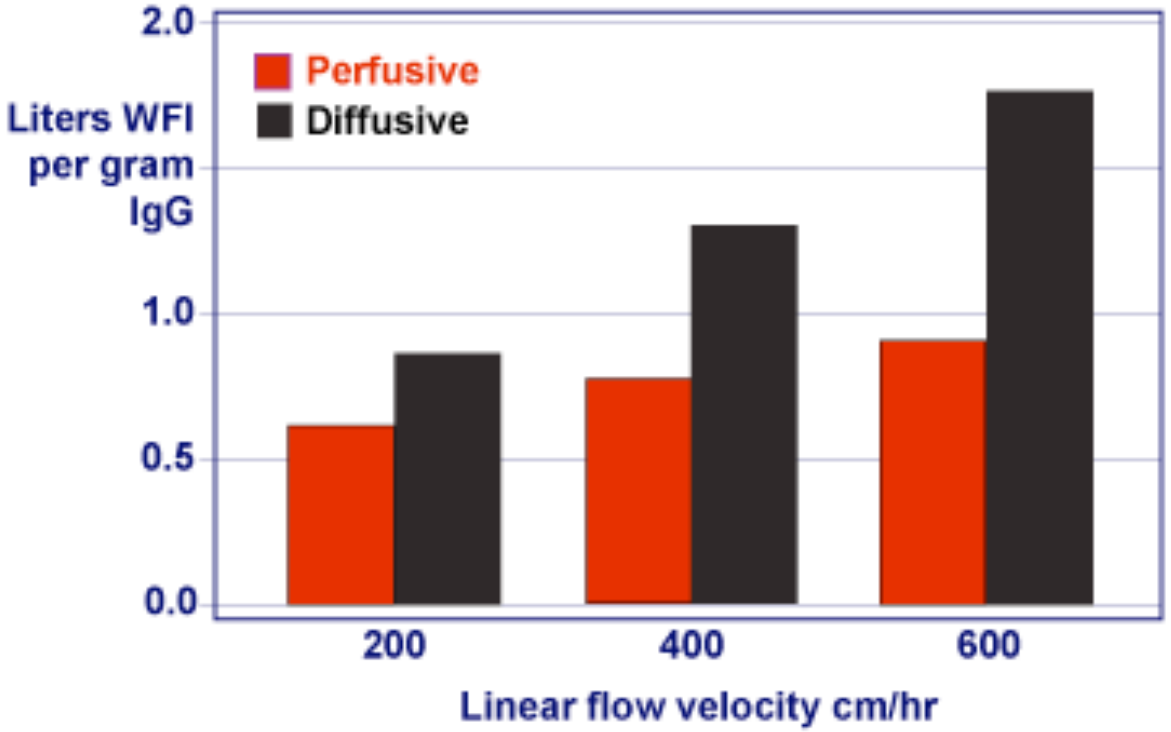
Efficiency = Productivity

Column volume required to capture 1kg of MAb at 1g/L



Efficiency = Productivity

Liters of WFI to process 1 gram of antibody
(10CV equilibration, 10CV wash, 10CV elution)



The immediate future

- Perfusive particles presently offer the most productive combination of diffusive and convective mass transport in a commercial preparative product.
- They can achieve higher capacity, at higher flow rates, in shorter columns, than diffusive media.
- Residence time limitations are still apparent but monoclonal IgG binding capacity still exceeds 30 mg/mL at a flow velocity of 600 cm/hr and a residence time of only 30 seconds.
- This represents an immediate opportunity to increase the productivity of existing antibody purification processes as well as processes currently in development.

The more distant future

- Convection appears to be the most likely source of future advancements in productivity.
- An exclusively convective support might eliminate residence time limitations.
- Increases in productivity would be linear with flow rate.
- This would shift the productivity bottleneck to hardware but leave process and facility configurations essentially unchanged.

The more distant future

Second generation monoliths may find a strategy for increasing surface area without compromising convective flow.

They may also develop ligand presentation strategies that make the best use of the available surface area.

The more distant future

Third generation perfusive particles may permit a larger convective contribution without compromising capacity.

The larger the convective contribution, the lower the residence time effect, and the higher the range of supportable flow velocities.

Equally, the lower the residence time effect, the shorter the bed height required to support effective performance, thereby increasing bed surface area, and further increasing volumetric throughput.

The more distant future

Further optimization of diffusive architectures seems unlikely to create major new advances, but improvements in ligand presentation could elevate capacity for low flow rate applications.

Acknowledgements

The authors would like to thank Applied Biosystems for providing beta test material of POROS MabCapture A and for providing data concerning the relative capacity of POROS MabCapture A and POROS A 50. We also thank BIA Separations for providing analytical CIM Protein A HLD monoliths and GE Healthcare for providing MabSelect Xtra.