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Membrane Chromatography:
Not Just for Viral Clearance Anymore?

WPI Bioengineering Institute Symposium:
New Developments in Biomanufacturing
February 20, 2007

Lisa Crossley, Ph.D., P.Eng.
CEO, Nysa Membrane Technologies

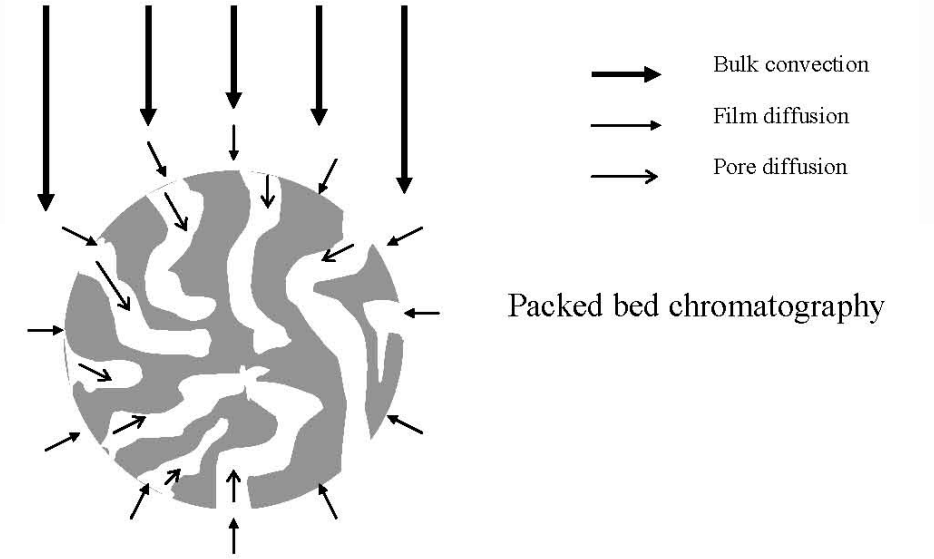
Outline

- Overview of Membrane Chromatography
  - Evolution
  - Current Technology
  - Recent Advances
- Applications
  - Viral Clearance
  - Disposable Capture Chromatography
- Constraints
- Future possibilities

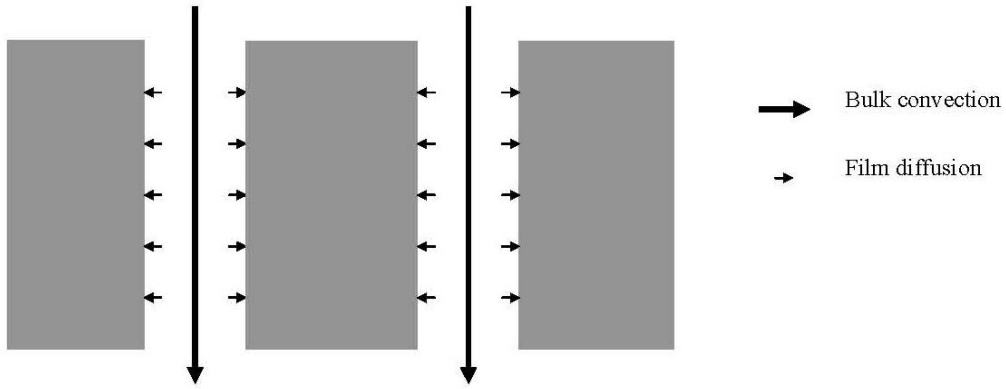
Evolution of the Technology

- Membrane chromatography technology evolved from the bioprocessing industry's need to overcome the mass transfer limitations associated with conventional resin-based chromatography
- Transport phenomena associated with resin bead chromatography are complex
  - Fluid stream must be dispersed through the resin bed
  - Solute must diffuse into the pores within the beads in order to reach the available binding sites
  - Complex pore geometry provides additional resistance to solute transport to the binding sites

Mass Transfer Advantages



Packed bed chromatography



Membrane chromatography

Solute Transport in Membrane vs Resin Bead Chromatography Systems:

- Dominance of convective vs diffusive mass transfer

- Convection is a significantly faster form of mass transfer than diffusion

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Membranes vs Resins

- Flow distribution is more of a challenge in columns than in typical membrane configurations
- Dominance of convective mass transfer in membranes translates to higher throughputs, resulting in reduced processing time and less time in the suite
- Faster rate of mass transfer in membrane chromatography systems means they are able to handle more concentrated feed streams, reducing the total buffer volume required for a given purification step
- Binding sites in macroporous chromatography membranes are significantly more accessible for large biomolecules (viruses, plasmids, large proteins), dramatically increasing binding capacities for these targets as compared to an equivalent resin bed where >95% of binding sites are sequestered within the beads

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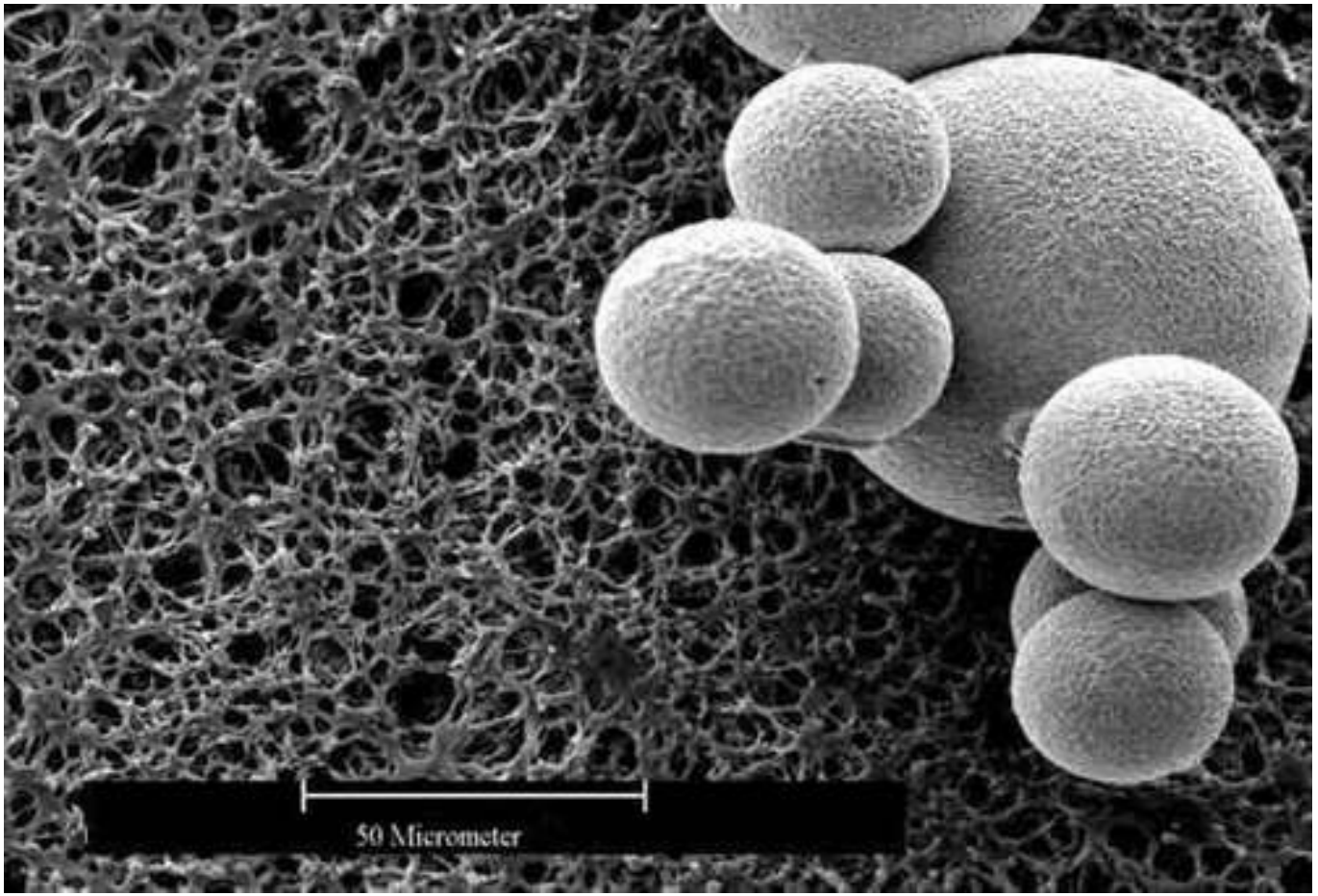
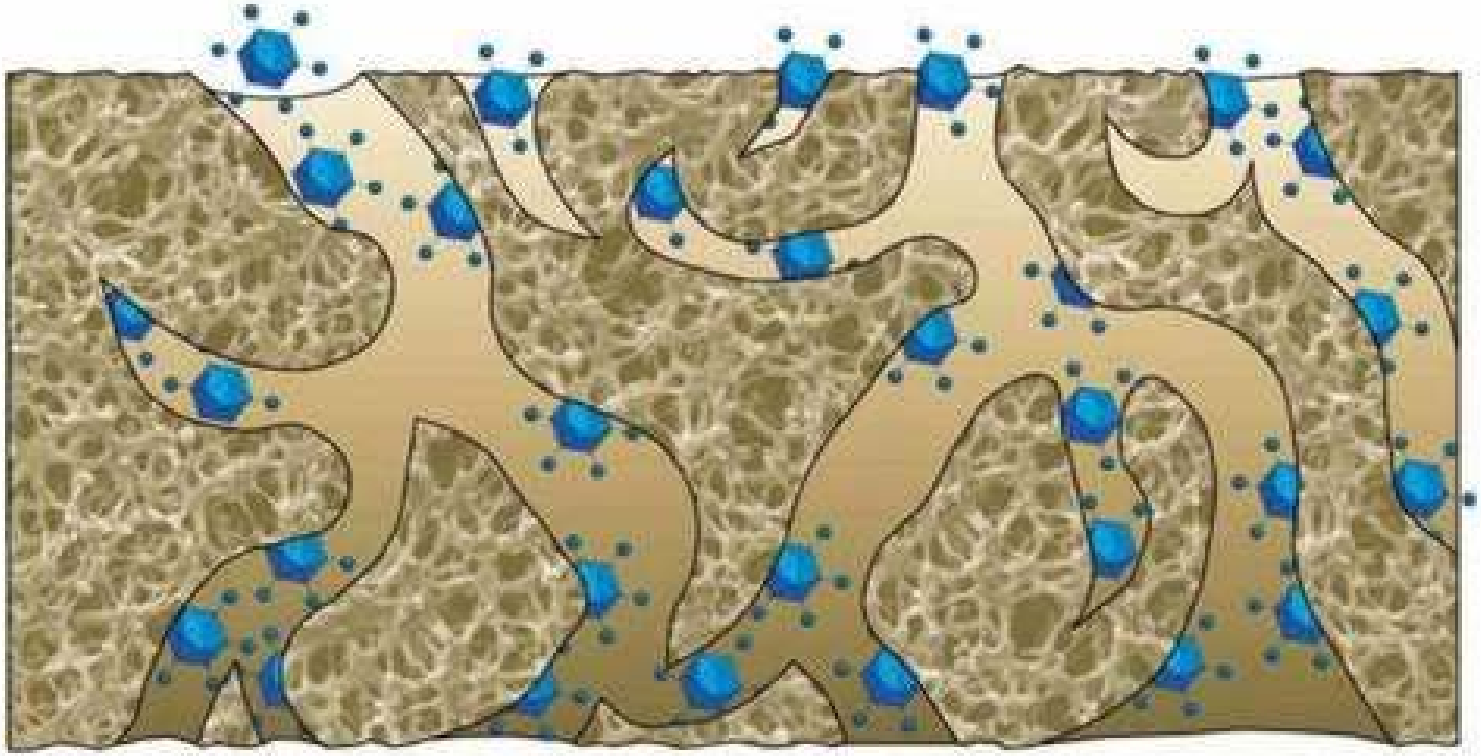
Membranes vs Resins



Electron micrograph of a Sartobind® Q membrane and standard chromatographic beads

Membranes vs Resins



Schematic view of viruses binding to functional groups in Sartobind® Q membrane pores

Current Technology

- Conventional chromatographic membranes:
  - Functional groups are covalently linked to porous polymer microfiltration or ultrafiltration membranes
  - Large pore sizes of 0.45 – 3.0 µm permit feed streams to traverse the membranes in convective flow resulting in favorable mass transfer rates
  - High flow rates , throughputs and wide flow rate ranges can be accommodated
  - Very stable binding capacities and excellent resolution across a wide range of flow rates
  - Loading capacity is typically limited (up to an order of magnitude lower as compared with packed bed chromatography)

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Existing Products

- Pall Mustang®, Sartorius Sartobind® lines
  - Cation and Anion Exchange functionalities, Protein A Affinity
  - 96 well plates, disk filters, capsules, cartridges

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Recent Advances

- Process-Scale Disposable Products
  - Pall Mustang® XT5000
- New Membrane Formats
  - Sartorius Sartobind® Direct
- Novel Composite Technologies
  - 3M Empore® Membranes
  - Nysa Structured Gel Membranes

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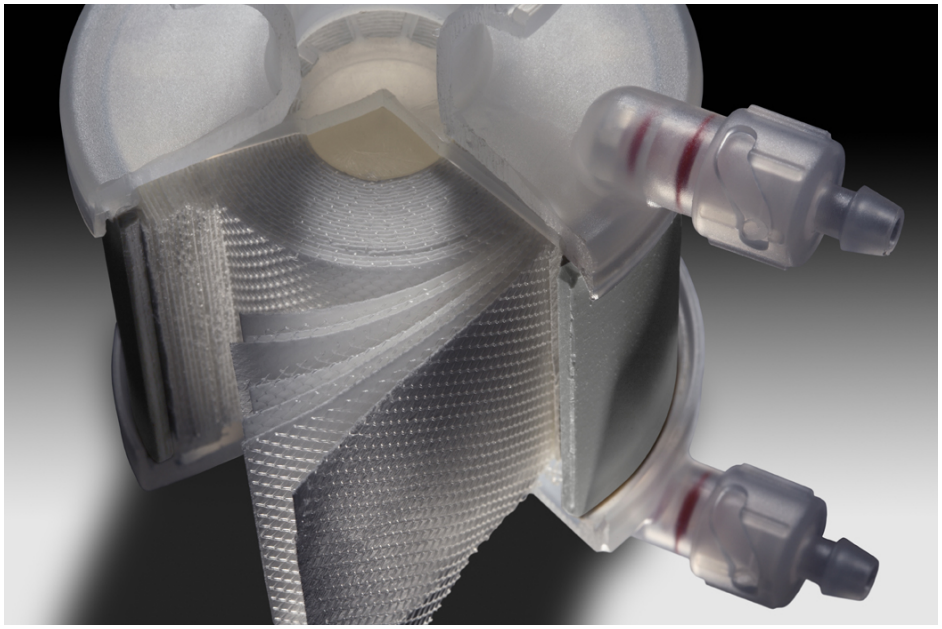
Process Scale Products

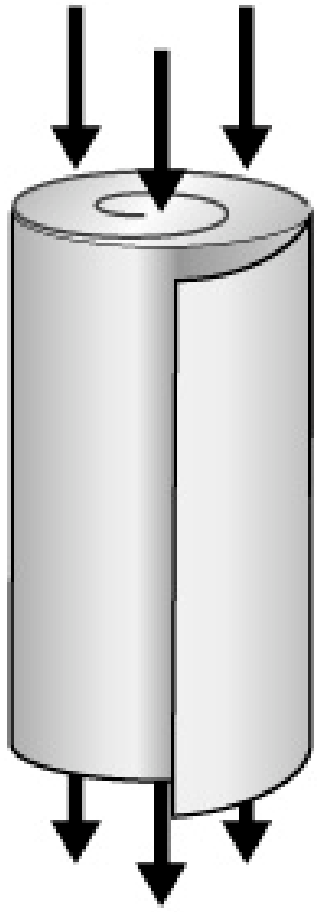
- Pall Mustang® XT5000 5L disposable
  - Largest chromatography capsule available
  - Single-use format

5515 North Service Road, Suite 102, Burlington, ON, Canada L7L 6G4 Tel: 905.319.2682 Fax: 905.319.0430

New Formats

- Sartobind® Direct
  - Tangential flow
  - Intended for direct protein capture





Novel Technologies

- 3M Empore® line:
  - Resin particles embedded in polymeric fibrils
  - 90% sorbent : 10% PTFE by weight
  - Two densities available
  - Standard Density
    - Contains 47 μm particles, high throughput
  - High Density
    - Contains 12 μm particles, lower throughput
    - Very low elution volumes
    - Clean sample matrices required

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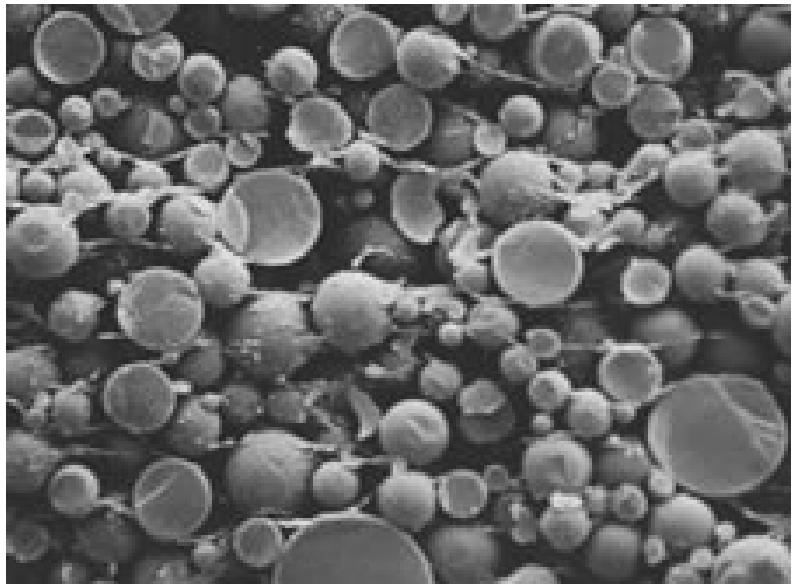
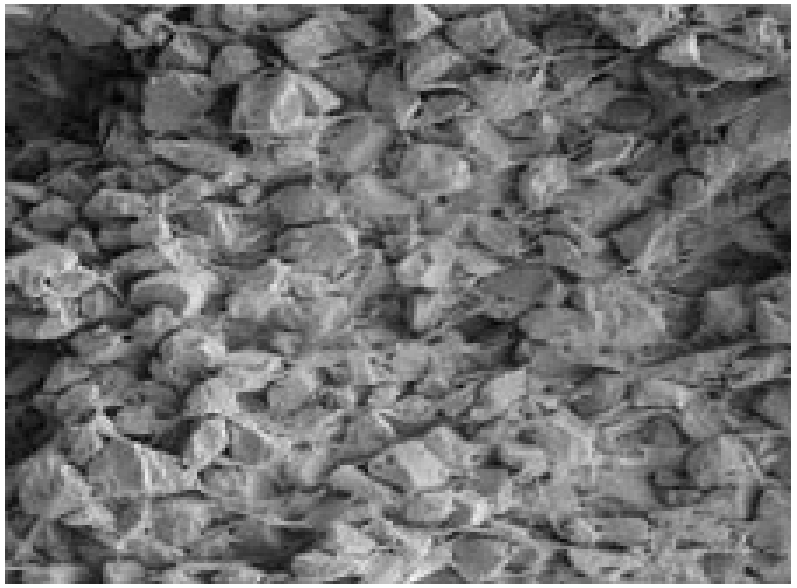
3M Empore®

Empore Membrane Types



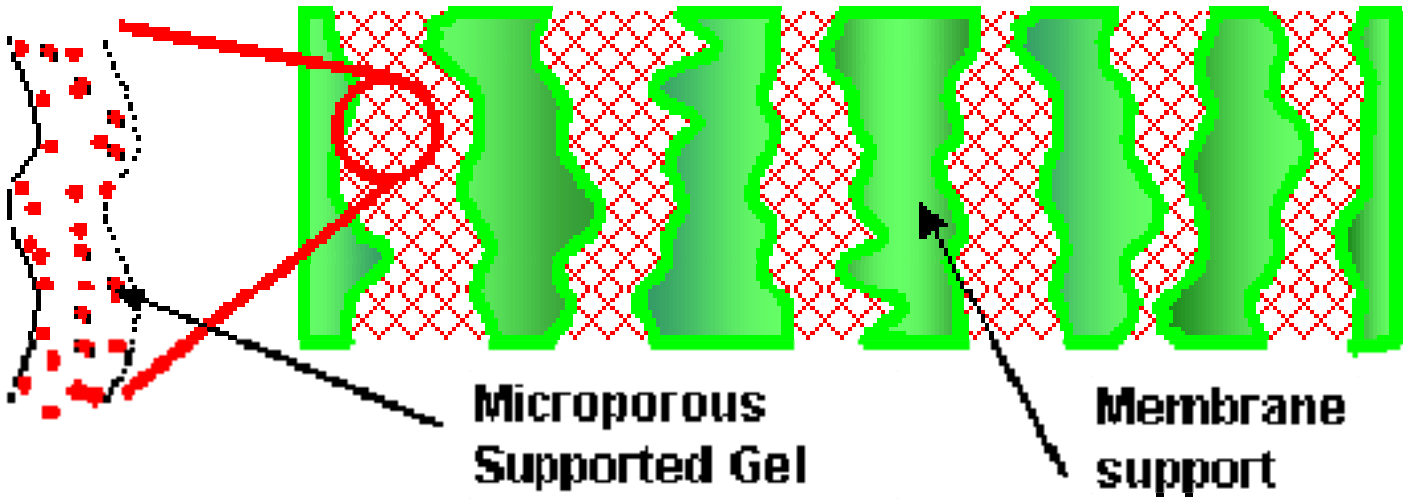
Poly(Styrene Divinylbenzene)



Bonded Silica

Novel Technologies

- Nysa Structured Gel Membranes:
  - A novel membrane construct consisting of a structured hydrogel formed within a suitable support



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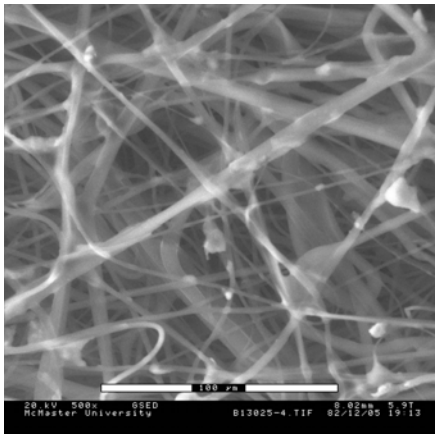
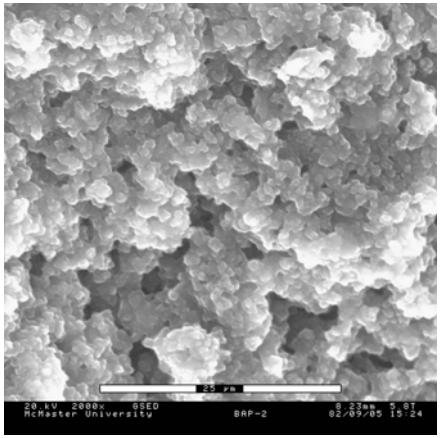
Nysa Membranes

- Two separate components: a flexible porous non-woven plastic and a hydrogel
  - The polymeric support provides mechanical strength to the soft hydrogel
  - The hydrogel determines the properties of the final product (pore size, binding chemistry)



Flexible porous support

Anchored structured gel



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Nysa Membranes

- Key attributes:
  - Manufactured using GRAS raw materials
  - Simple, inexpensive fabrication process yields products that can be cost-effectively deployed as single use disposables
  - Supplied gamma-irradiated or ready for sterilization via autoclave
  - Superior binding capacities (5-10x existing chromatographic resins and membranes)
  - Excellent robustness with respect to mechanical handling, performance (consistency)
  - Inherently hydrophilic, resistant to fouling
  - Stable to cleaning with NaOH, peracetic acid (Minncare)

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M.C. Applications

- Viral Clearance
  - Existing application
  - Accepted by FDA / EMEA
- Disposable Capture Chromatography
  - Chromatographic membranes have not yet gained widespread acceptance for use in capture purification applications (i.e. purification of target molecule from a given feed stream), particularly at process scale

Viral Clearance

- Both the FDA and the EMEA require the use of at least two orthogonal methods for viral clearance in every mammalian cell-based biopharmaceutical manufacturing process
  - Based on the expectation that different mechanisms of purification will provide cumulative performance and therefore better ensure product purity and safety
  - In 2004, BioMarin Pharmaceutical Inc. (Novato, CA) became the first company to receive licensure from both the FDA and the EMEA for a drug product (Aldurazyme®) produced using a sequential dual-membrane process for the removal of DNA and viral contaminants
    - Mustang® Q and Pall Ultipor® (nanofiltration membrane)
- Anion Exchange membranes that provide an LRV of ≥6 are accepted for use in Viral Clearance applications

Disposable Chromatography

Three Key drivers for the adoption of disposable chromatography products:

1. Increasing regulatory scrutiny on cleaning procedures for re-useable units
   - Need to reduce risk, limit potential for cross-contamination:
     - Adventitious agents
     - Protein carry-over
     - Cleaning agents
   - Assays with lower limits of detection

Key Drivers (cont'd)

2. Need for novel approaches to product development and manufacturing for biopharmaceuticals intended to:
   - Limit capital expenditures
   - Enhance flexibility in a given manufacturing plant (existing or new construction)
   - Reduce time to clinic and ultimately to market

Key Drivers (cont'd)

3. Increasing availability of complementary disposable technologies
   - Bioreactors
   - Mixing systems
   - Media and buffer bags
   - Tubing
   - Connectors
   - MF, UF and NF filters
   - Aseptic fill-finish equipment

Constraints

- Regulatory and economic risks associated with the utilization of novel technologies in both existing and new processes
  - "There is no good time to innovate. Significant obstacles to implementation of new technologies exist at every stage of development." - Tom Ransohoff, BioProcess Technology Consultants
- Culture shift
  - Packed bed chromatography has long been the workhorse of the Bioprocessing industry. A switch to membrane chromatography for capture applications represents a significant culture shift

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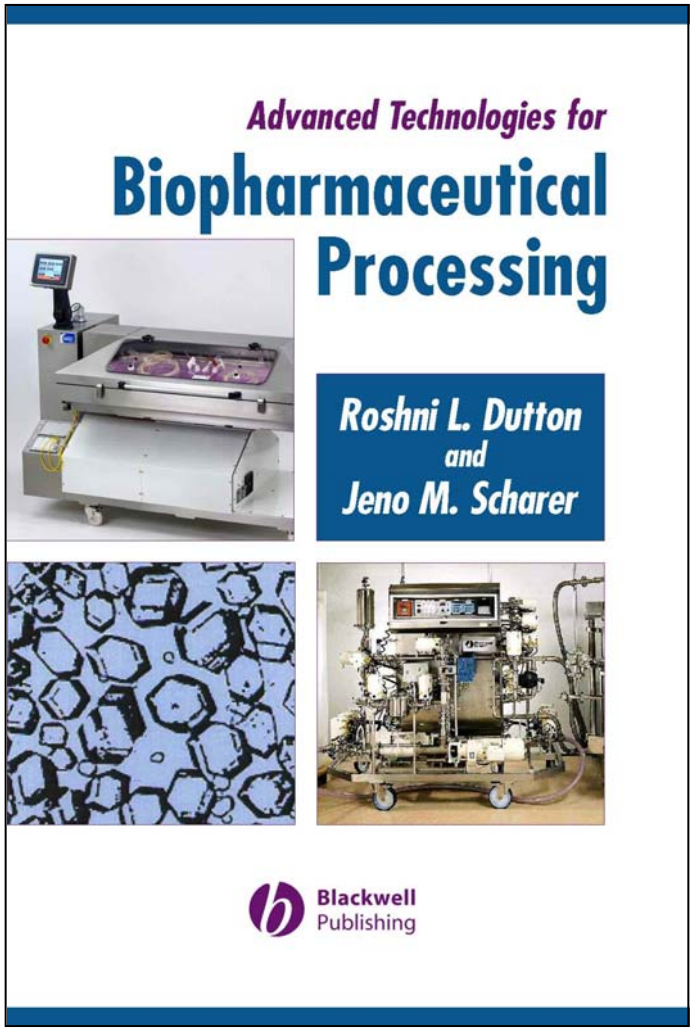
Future Possibilities

- Inexpensive Affinity Ligands
  - Synthetic “Protein A”
  - Camelid antibodies
- Clarification & Capture
  - Novel device designs intended to combine culture harvest and primary capture of the target molecule into one operation
  - i.e. a membrane-based alternative to Expanded Bed Chromatography

Summary

- Membrane chromatography is a valuable technology that has already gained widespread acceptance for select applications such as Viral Clearance
- Advances in membrane chromatography technology and device design have yielded products suitable for use in primary capture applications
- Additional, non-technical obstacles exist to the implementation of novel membrane chromatography products for primary capture applications
- Regulatory, economic and competitive pressures may nonetheless drive the adoption of novel membrane-based capture purification unit operations in lieu of their conventional resin-based counterparts

For more information on Membrane Chromatography and other advances in the field of Biopharmaceutical Processing…



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Thank You!

Lisa Crossley

President & CEO
Nysa Membrane Technologies
5515 North Service Road
Burlington, Ontario, L7L 6G4

Tel: 905-319-2682 x27
Fax: 905-319-0430
E-mail: crossley@nysamembranes.com