adebiotech Innovation-Proteines-Prod

Technologies innovantes
en séparation industrielle des protéines

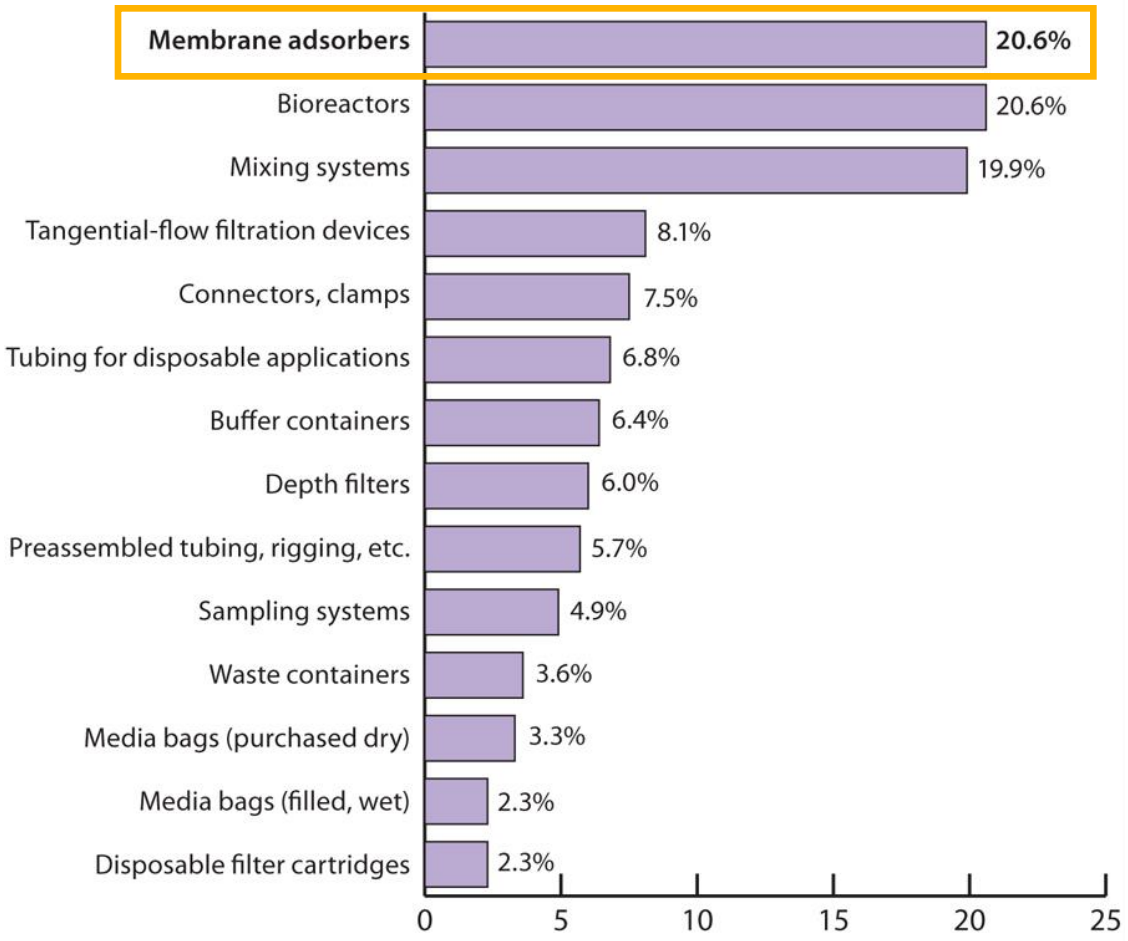
28, 29 et 30 octobre 2013

sartorius stedim biotech

L'élimination de contaminants par chromatographie sur membrane: état des lieux, applications clés et innovations

Amélie Raveneau – Virus and contaminant removal project manager – Sartorius Stedim Biotech

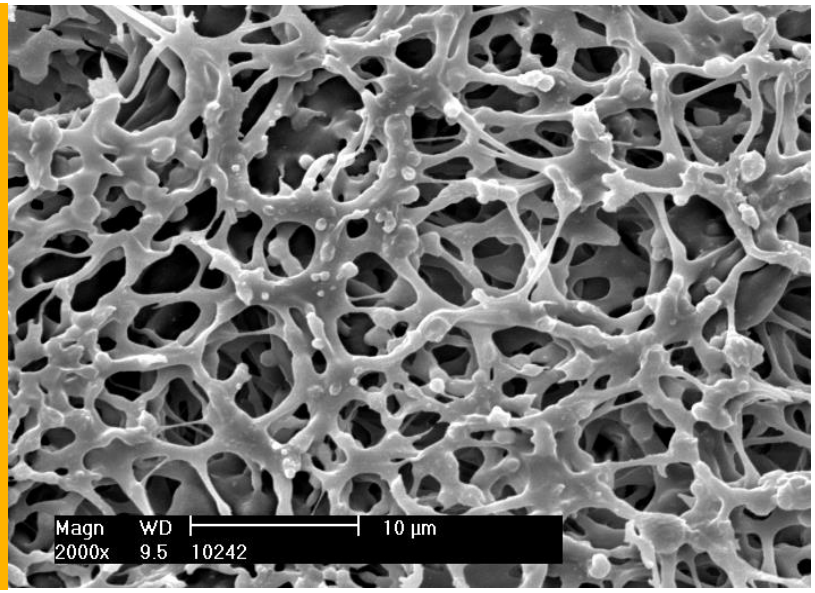
Introduction



Average annual growth rate for disposable components, 2006–2013

Langer, ES, BioProcess International, Vol. 11, No. 9, October 2013, pp. 16–19

Agenda



- **Membranes vs. resins**
- Current applications
- Latest innovation

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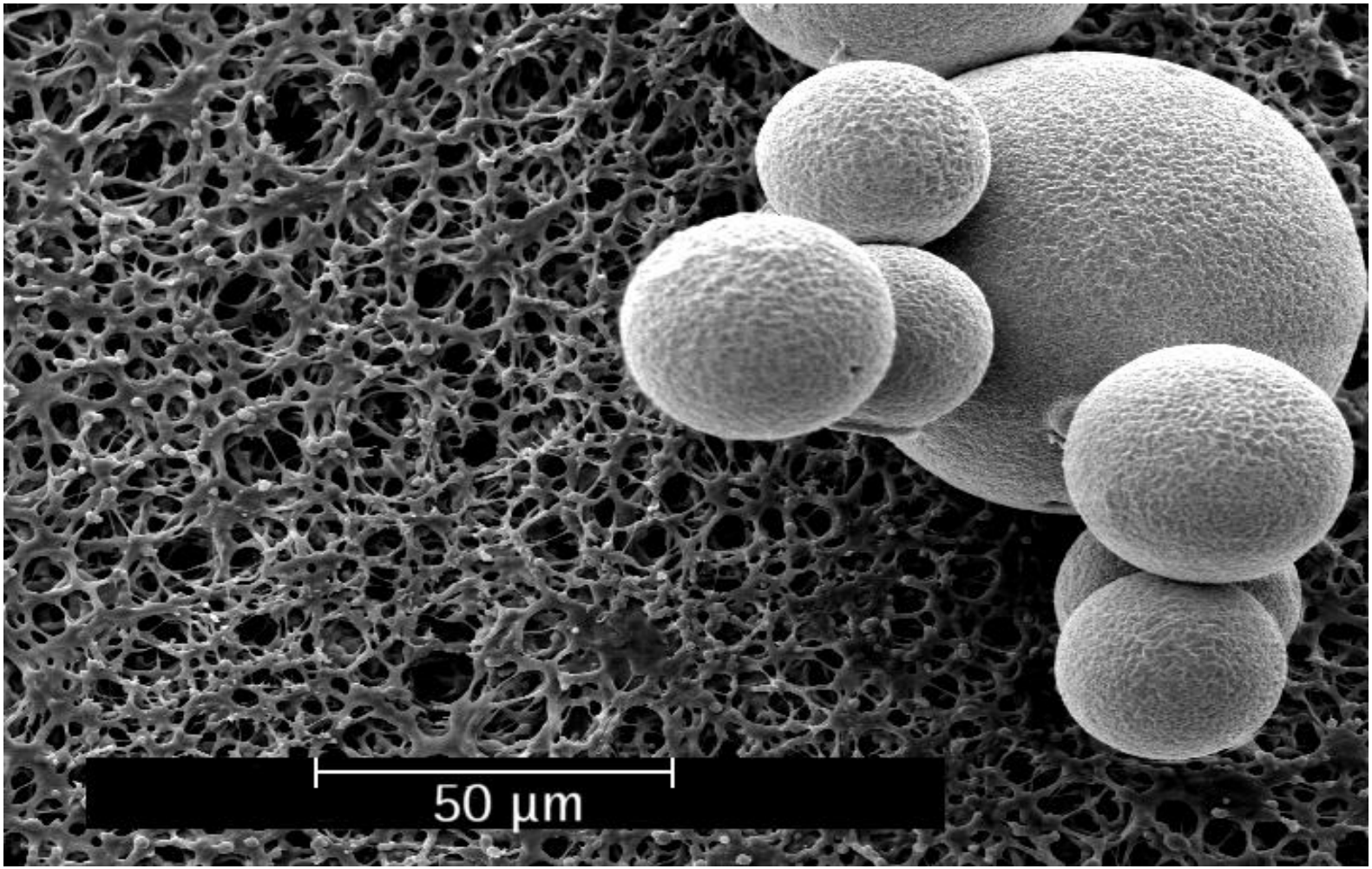
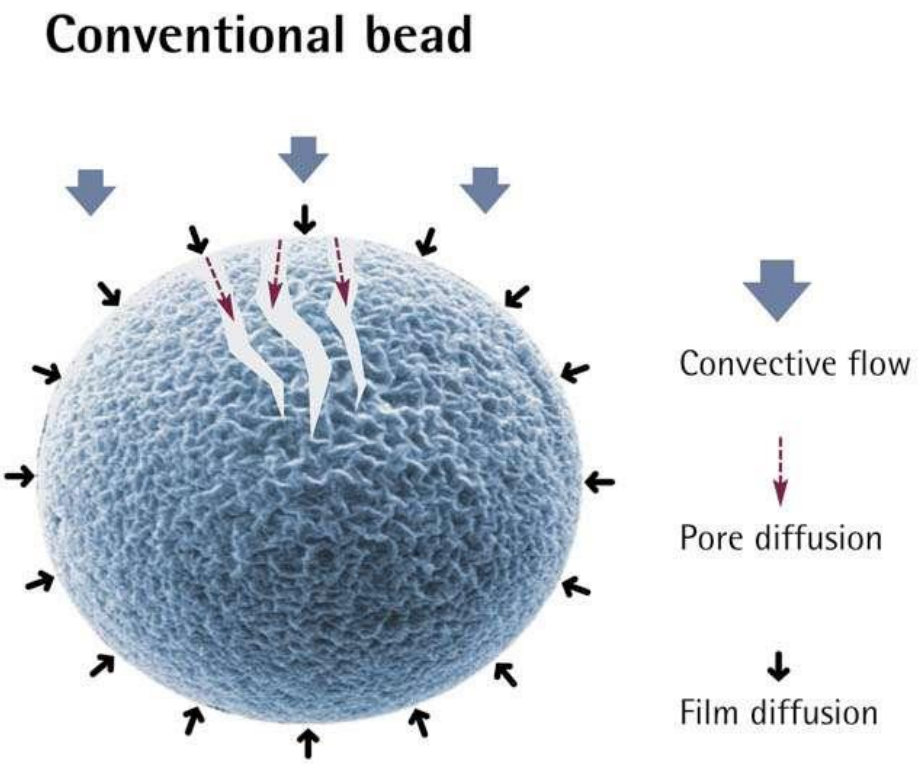
2 different media for chromatography



Membranes are fast

Mass transfer effects conventional bead versus membrane adsorber



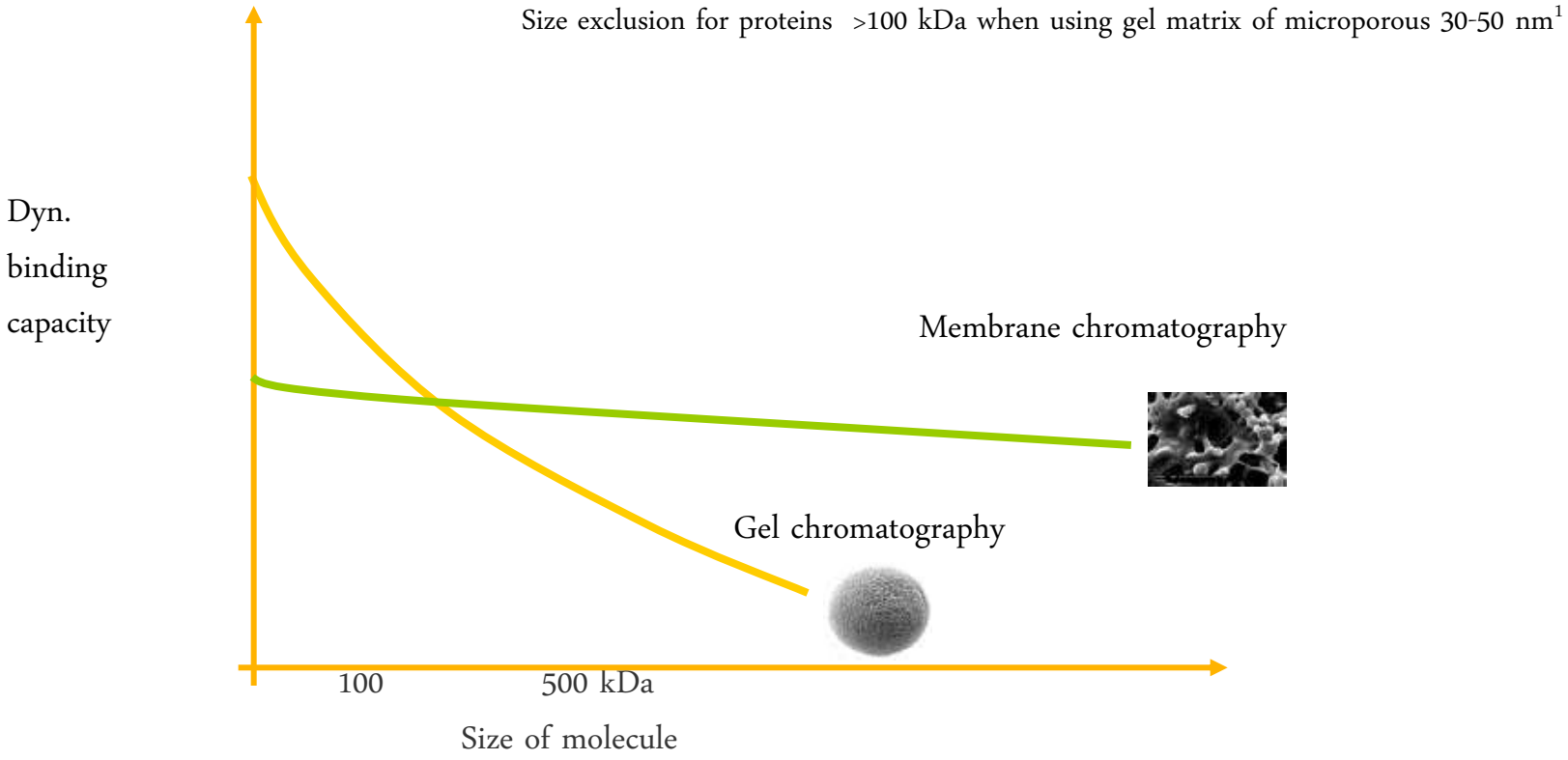
Diffusion limited

Convection limited

Limited size exclusion effect with membrane adsorbers

Ideal for capture of big molecules

Size exclusion for proteins >100 kDa when using gel matrix of microporous 30-50 nm[1]



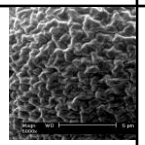
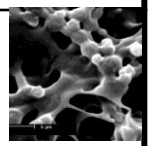
1: E. Karlsson *et al.*, Protein Purification, Principles (1989) Jan-Christer Janson, Lars Rydén Eds., VCH Weinheim, pp 123.

Membrane chromatography is complementary to resins

Capture / Bind & elute

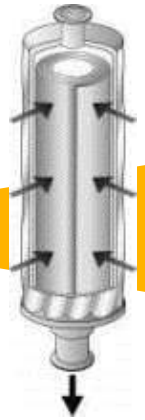
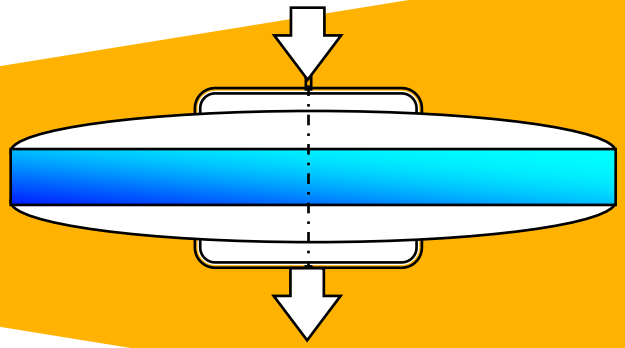
Intermediate

Polishing / Flowthrough

	Conventional resin	Membrane chromatography
Average pore size	15-40nm 	3-5µm 
Mass transfer	Mainly diffusive	Mainly convective
Flow-rate	Low	High
Limitation	Flow-rate	Capacity
Typical applications	- Capture	- Flowthrough mode - Capture of large molecules
Handling	Packing needed	Like a filter
Operation	Mostly re-usable	Mostly single-use

To summarize in one example

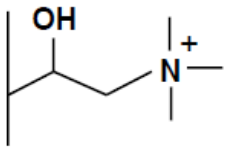
Bottleneck at production scale = flow rate

Membrane based system	Beads based system
	
- 0.5 L bed volume	- 50 cm / 30L bed volume
- 1000 l/h	**- 1000 l/h**
- BC 15 g sufficient for contaminant removal	- BC 1500 g oversized

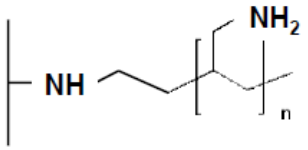
H.L. Knudsen et al., J. Chromatogr. A 907, 145-154, 2001

Ligands available on the market

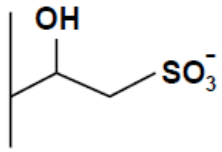
Quaternary ammonium – strong AEX



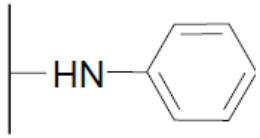
Primary amine – salt tolerant weak AEX



Sulfonic acid – strong CEX



Phenyl – hydrophobic interaction



Typical applications:

DNA removal

virus clearance

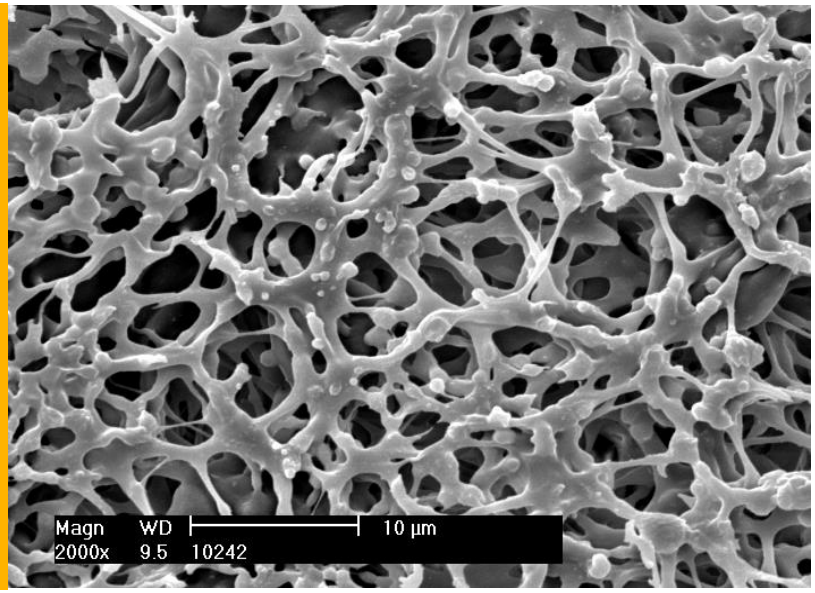
host cell proteins removal

endotoxins removal

aggregates removal

large molecule purification

Agenda



- Membranes vs. resins
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Convective media now part of the design space

Product Development and Realisation Case Study A-Mab

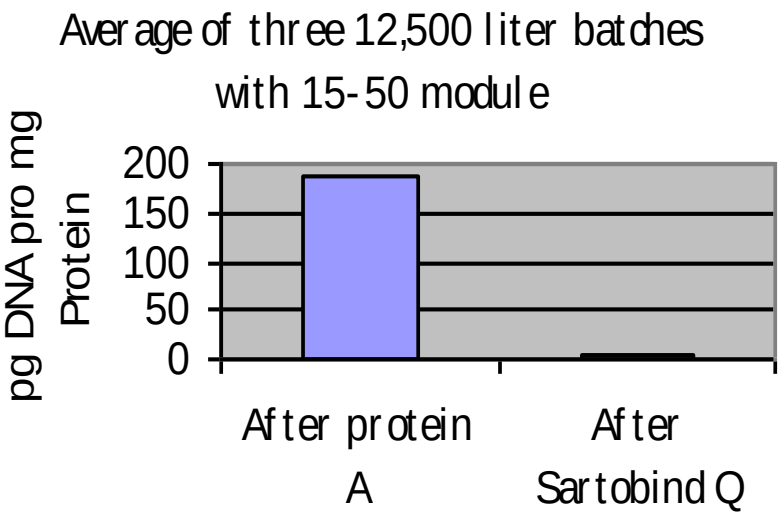
7.5.2 Protocol for Replacement of the Anion Exchange Resin with a Membrane

The following is an example of a change to be included in an Expanded Change Protocol (ECP) and/or Regulatory Agreement/Post Marketing Plan and will be used in conjunction with the internal change management system to introduce a new membrane technology into the A-Mab manufacturing process. ***

CMC Biotech Working Group

Pfizer
Genentech
A Member of the Roche Group
MedImmune
Lilly
ABBOTT
gsk GlaxoSmithKline
AMGEN

DNA Removal from a Therapeutic Antibody Campath 1-H with Sartobind Q



- ...clears the DNA below detection limit
- superior pressure/flow relation
- ...the process time is reduced 23-fold, excluding the benefit in handling for set-up.
- ...diminish a loss of product
- ...installed as in-line filters and can be disposed after use[1]

FDA approval March 2001

MILLENNIUM

Boehringer Ingelheim

1: Galliher P. *et al.*, Validation of Impurity Removal by the CAMPATH-1H Biomanufacturing Process (2001) IBC's Biopharmaceutical Production Week, Paradise Point Resort – San Diego, CA, November 12-15.

Virus Removal with Sartobind Q

Viruses	Size (nm)	LRV Run1	LRV Run 2	Virus Recovery (%)
MVM	16 - 25	6.03 ± 0.21	6.03 ± 0.20	100
Reo-3	75 - 80	7.00 ± 0.31	6.94 ± 0.24	100
MuLV	80 - 110	≥ 5.35 ± 0.23	≥ 5.52 ± 0.27	> 70
PRV	150 - 250	≥ 5.58 ± 0.28	≥ 5.58 ± 0.22	100

Monoclonal Antibody solution at 4,3mg/ml, pH 7,22 at 3,95mS/cm

Zhou JX et al J. Chromatogr A. 2006

Polishing with Membrane Chromatography: Clearance of Endotoxin

Endotoxins were removed from GST-protease from **26.900 EU/mg to 0.13 EU/mg**. Protein dissolved in TRIS / NaCl buffer, pH 8. Sartobind® Q

	Run 1	Run 2
Process volume (start)	50 L	47 L
Process volume (end)	60L	59 L
Process time	~10 – 20 min	~ 10 – 20 min
Protein concentration	4.10 g/l	4.14 g/l
Conductivity	16.63 mS/cm	17.41 mS/cm
Pre use endotoxin level	26,900 EU/mg	10,100 EU/mg
Post use endotoxin level	0.13 EU/mg	0.18 EU/mg
Protein recovery	81.4 %	84.6 %
LRV endotoxins	5	5

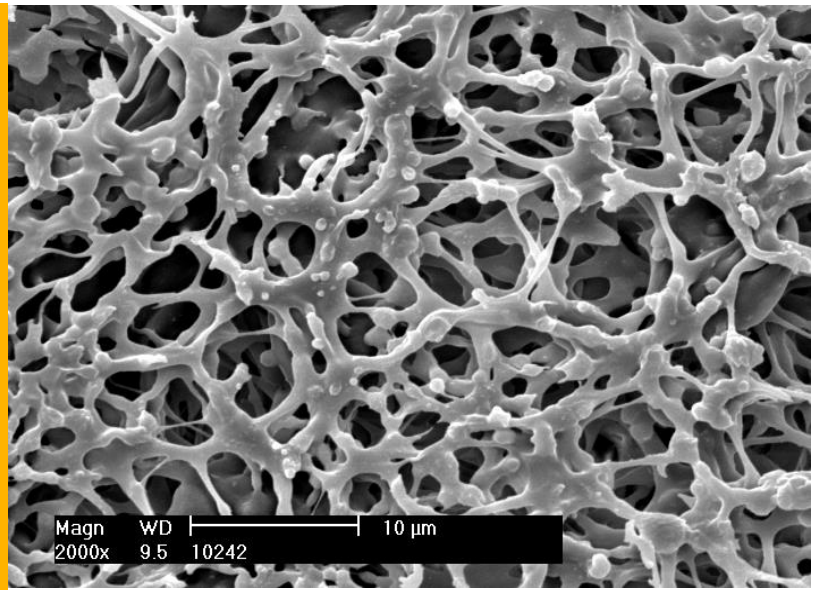
Clutterbuck A, BioPharm Int. 2007

Summary Process Optimisation with Membrane Chromatography

Criteria	Q Sepharose Fast Flow	Q membrane
Flux/Flow Rate	100 – 150 cm/h	450 – 600 cm/h
Capacity	50 – 70 g/L	>3000 g/m^2 (>10.7 Kg/L)
Buffer consumption	100%	5%
Operation time	8 – 9 hours	2 – 2.5 hours
Cleaning Validation	Yes	Single use
Virus Clearance	Good	Good

Li, Feng; Zhou, Joe, X.; Yang, Xioaming; Tressel, Tim and Lee, Brian. Current Therapeutic Antibody Production and Process Optimization. Bioprocessing Journal, September/October 2005

Agenda



- Membranes vs. resins
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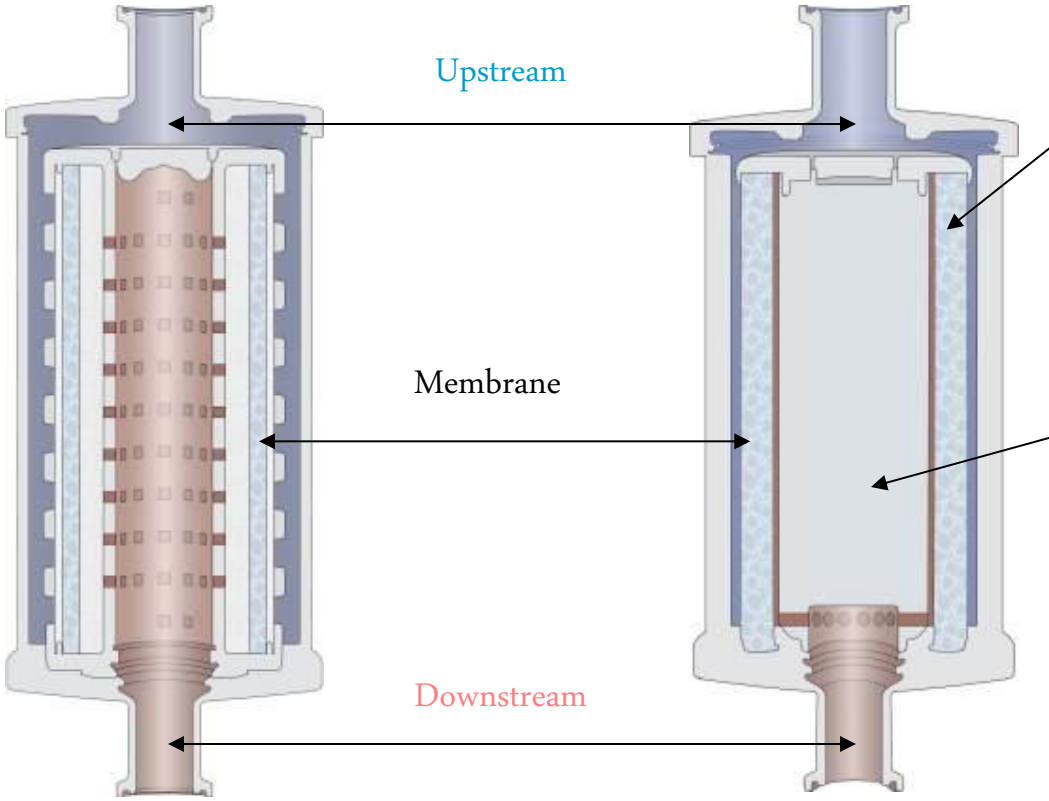
A new design for bind and elute application



8 mm of membrane

- Increased capacity

Central core to reduce the void volume

- Void volume = 1.3 CV
- Sharp elution peak
- Limited dilution effect
- Suitable for bind & elute

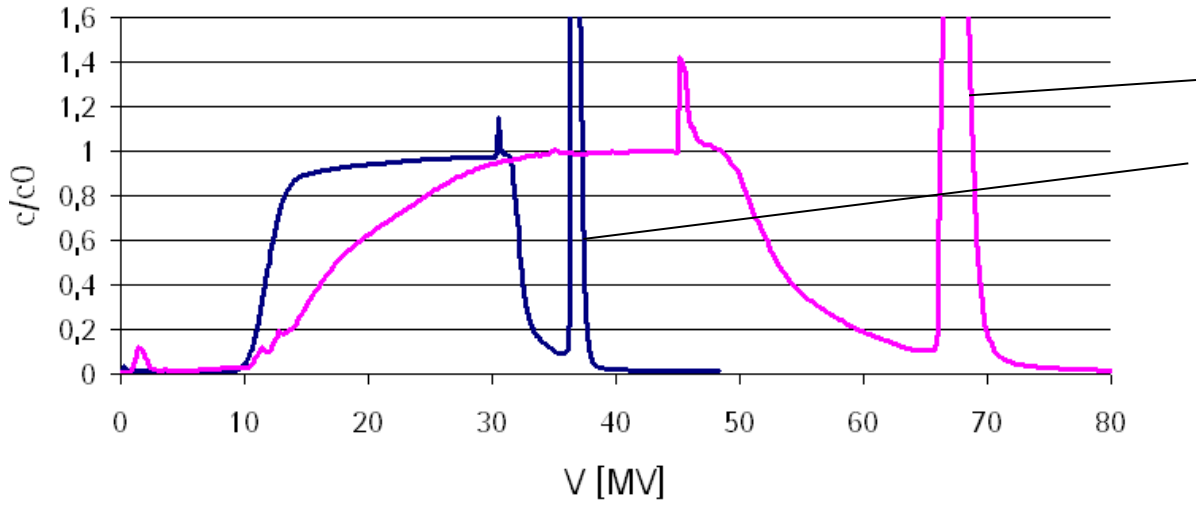
Sartobind 4mm caspule

Not void volume optimised

Sartobind 8mm caspule

Void volume optimised

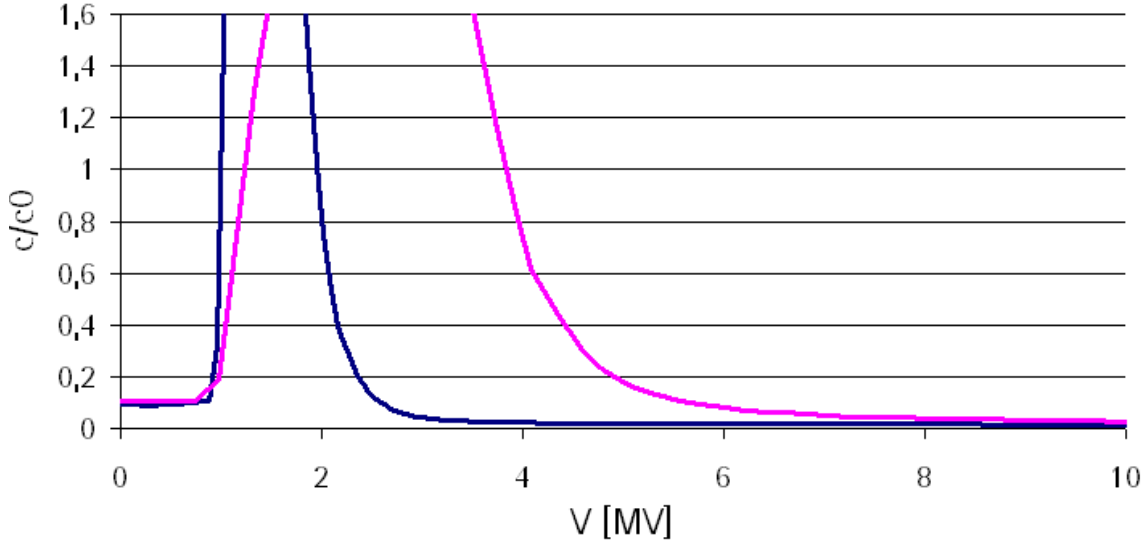
Sartobind 8mm capsules for bind and elute applications



Sartobind Q 4mm Mega

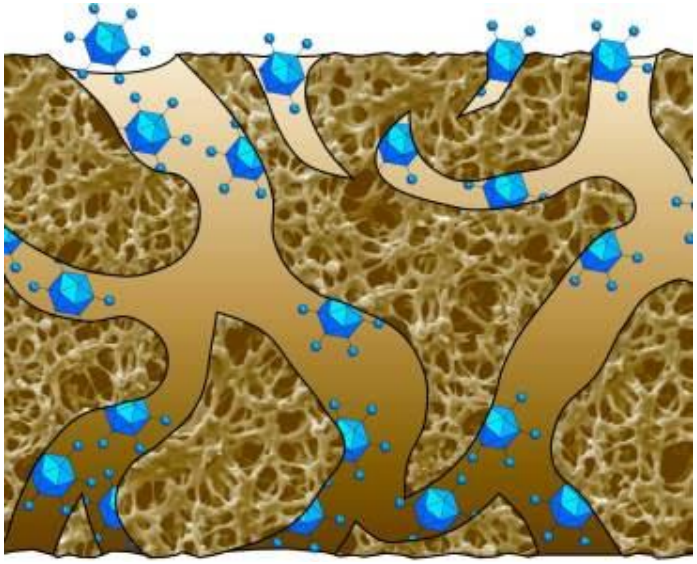
Sartobind Q 8mm Jumbo

Sartobind 4mm capsules are dedicated to contaminant removal in flowthrough mode



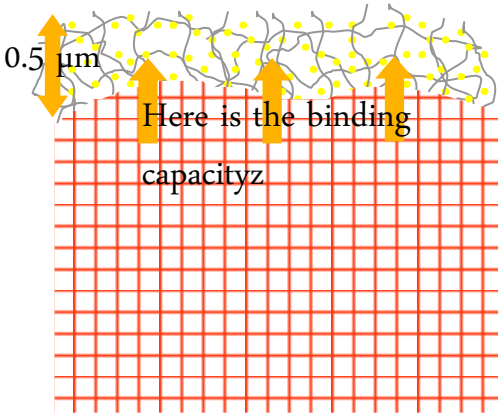
Sartobind 8mm can be used for proteins, DNA, viruses... purification as well

Virus purification with membrane adsorbers

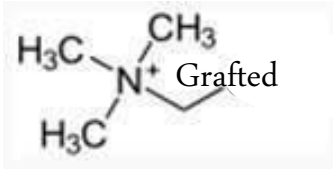


- Influenza virus[2,3]
- Adenovirus[4,5,6]
- Lentivirus[7]
- Adenoassociated virus
- Baculovirus[8]
- Densonucleosis virus[9]
- Pseudorabies virus[10]
- Bovine herpesvirus[1]
- Foot and mouth desease virus[11]
- Rotavirus like particles[12]
- Bacteriophages[13]
- Norovirus (VLP)[14]

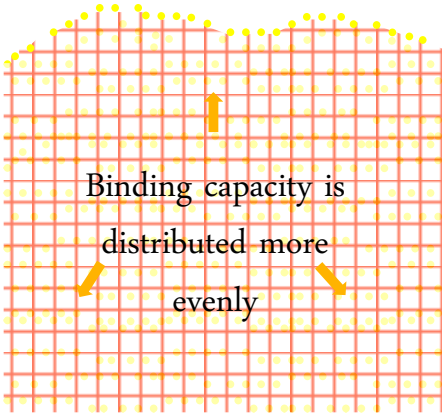
Sartobind STIC® - New generation of membrane adsorbers



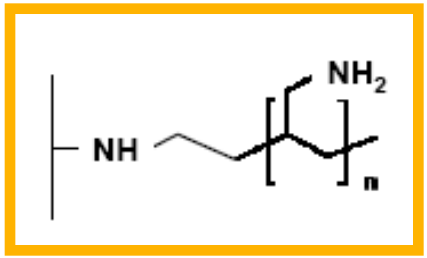
Sartobind Q



Quaternary ammonium



Sartobind STIC



Direct derivatisation

+ ligand density

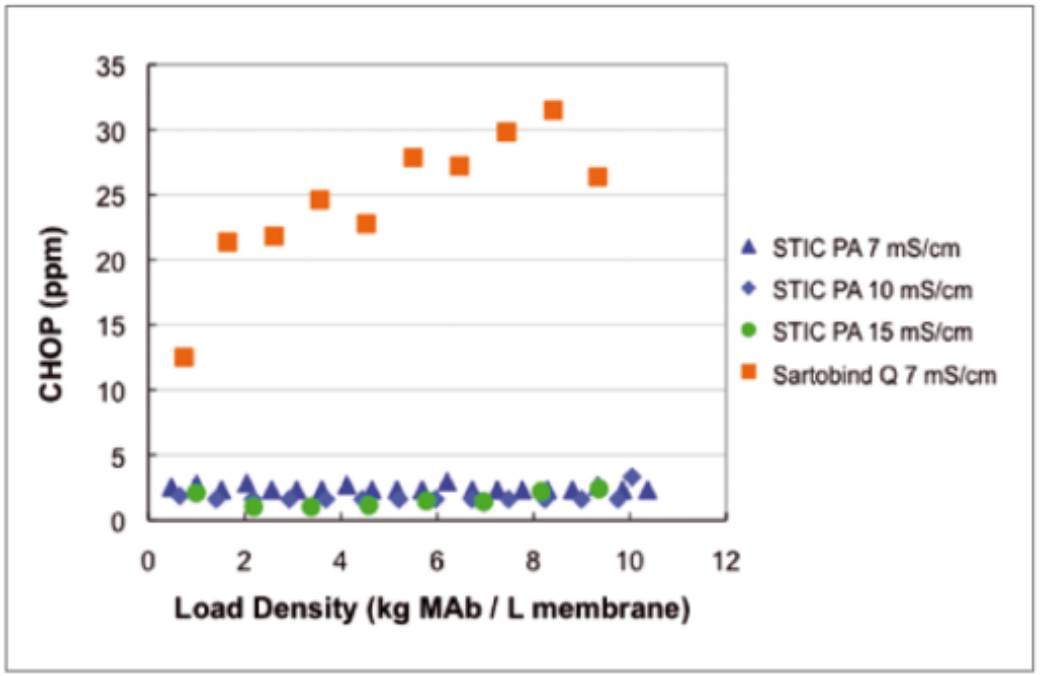
+ pore accessibility

Primary amine (Sartobind STIC PA)

I. Tatárova, I., R. Fáber, R. Denoyel, M. Polakovic, J. Chromatography A 1216 (2009) 941

Host cell protein removal up to 10 kg mAb per L

Comparison of Sartobind Q & STIC PA at pH 8, 500 ppm HCP load, 10 MV/min



References

1. Faber, R., Yang, Y. & Gottschalk, U. Salt tolerant interaction chromatography for large-scale polishing with convective media. BioPharm Int. Suppl. 11–14 (2 October 2009).

2. Fraud, N., Shomglin, K., Faber, R., van Reis, R., Gottschalk, U. & Mehta, A. New membrane adsorber for polishing at high salt concentration. Recovery of Biological Products XIV Conference Lake Tahoe, California, USA, 1–6 August 2010

3. Fraud, N., Faber, R., Mehta, A., Tully, T., Ultra small membrane adsorbers for process development & screening of new membrane adsorbers. 238th ACS Meeting, Washington August 16–20, 2009.

Use of multivalent buffers with salt tolerant membranes

Phosphate inhibits phage binding but DNA contaminant is bound

Ortho phosphate mM	Phage in flowthrough % of start material	DNA in flowthrough % of start material
0	<0.00001	<1
2	<0.0001	<1
10	0.001	<1
30	75 - 83	<1

15 cm² (3 layers) Sartobind STIC PA were loaded with 150 ml ΦX174 1 x 10^7 PFU/ml, salmon sperm DNA 200 ng/ml at 10 ml/min was added.

Comparison of traditional column AIEX and Sartobind STIC

	Traditional AIEX Column Chromatography	STIC Membrane Adsorber
Resin or membrane volume for processing 15kg mAb (FT mode)	>60L	5L
Capital equipment investment	High	Low
Footprint	Large	Small
Operations	Packing/unpacking/cleaning/storage, cleaning validation required	Disposal
Non-value added time	Long	Short
Labor requirement	High	Low
Consumables	High	Low
Buffer tanks	Large	Small
Development requirement	Major development effort	Plug and play
Mass transfer	Pore diffusion	Convective flow
Flow rate	Low	High
Salt tolerant	No	Yes
Process capacity per L	Low	High
Quality control	HETP test	Integrity test

Y. Kang *et al.*, ImClone, BioPharm International (2012) Volume 25, Issue 5, pp. 34-46

Conclusion

Membrane chromatography is implemented in > 100 processes:

- Reduce costs
- Reduce process time
- Reduce process steps

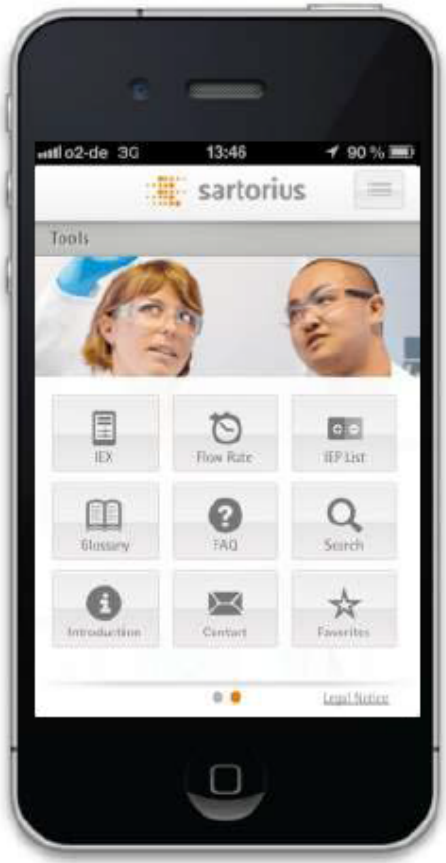
Widely adopted for polishing in the MAb and the vaccine industry

- DNA, viruses, HCP, endotoxins, aggregates removal

What is the next sweet spot?

- Virus purification ?
- Continuous purification ?

Thank you for your attention



Do not hesitate to contact us

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