



Applications of membrane adsorbers in biopharmaceutical companies

Dr. Andreas Kocourek, Sartorius Stedim Biotech, Application Specialist, Purification
Australia (andreas.kocourek@sartorius-stedim.com)

Agenda

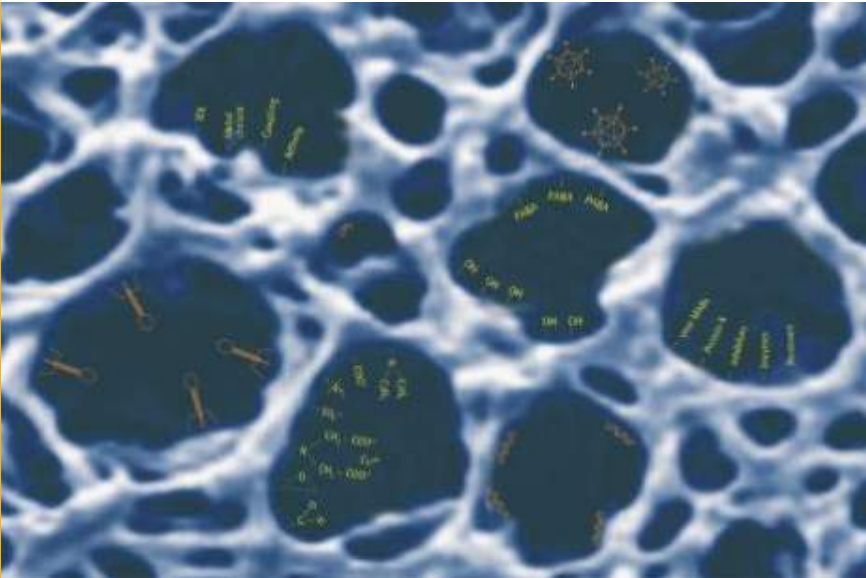


1. Membranes

2. Formats

3. Applications

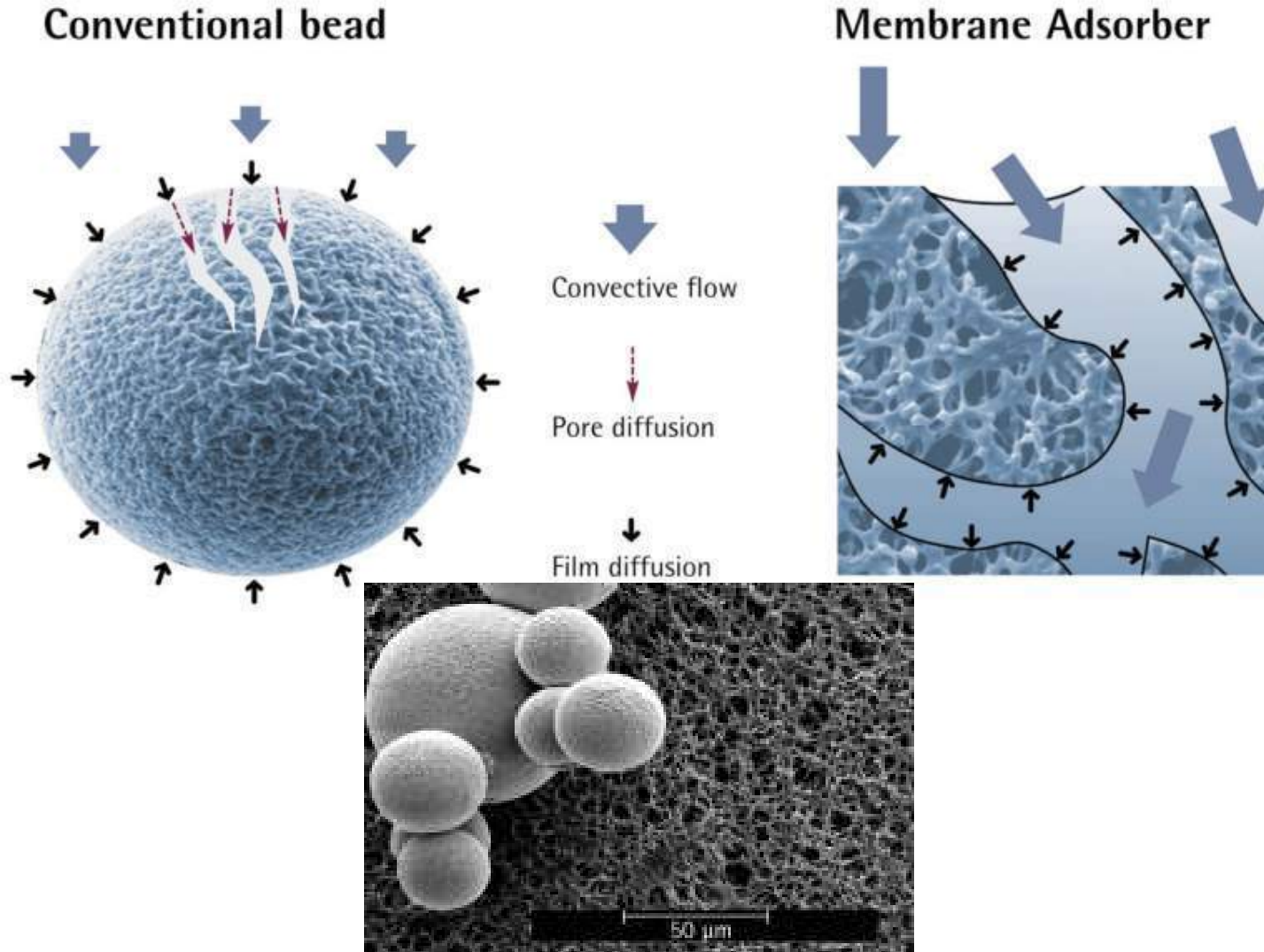
Membrane Adsorbers are formatted ready for use



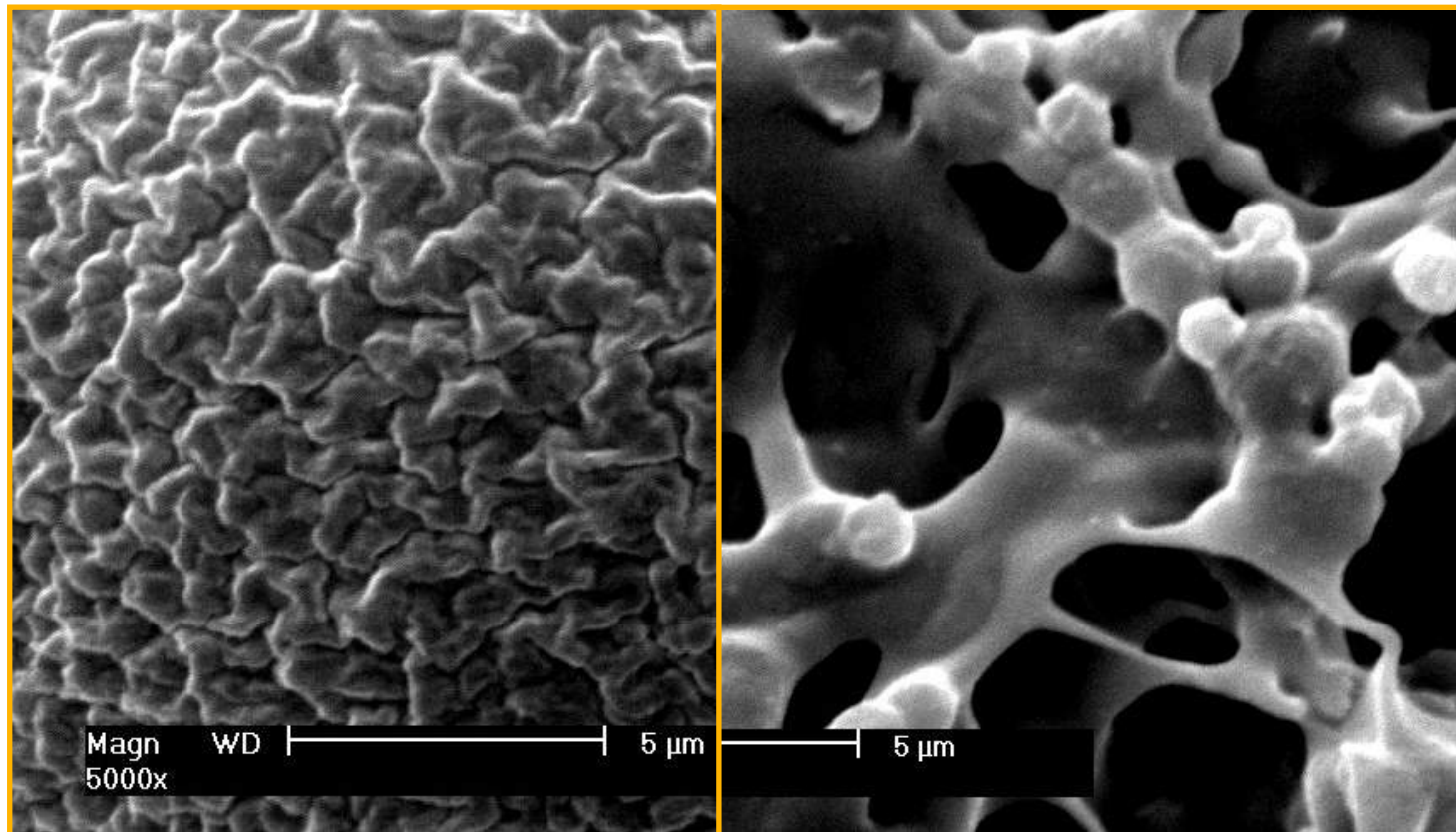
- PES, PE or regenerated cellulose
- Surface modified or grafted
 - » IEX (e.g. Q, S)
 - » HIC (Phenyl)
 - » STIC (primary amine)
 - » IMAC
 - » Conventional ligands (Protein A)



Diffusion limited gels (time) versus convection limited (flow rate)



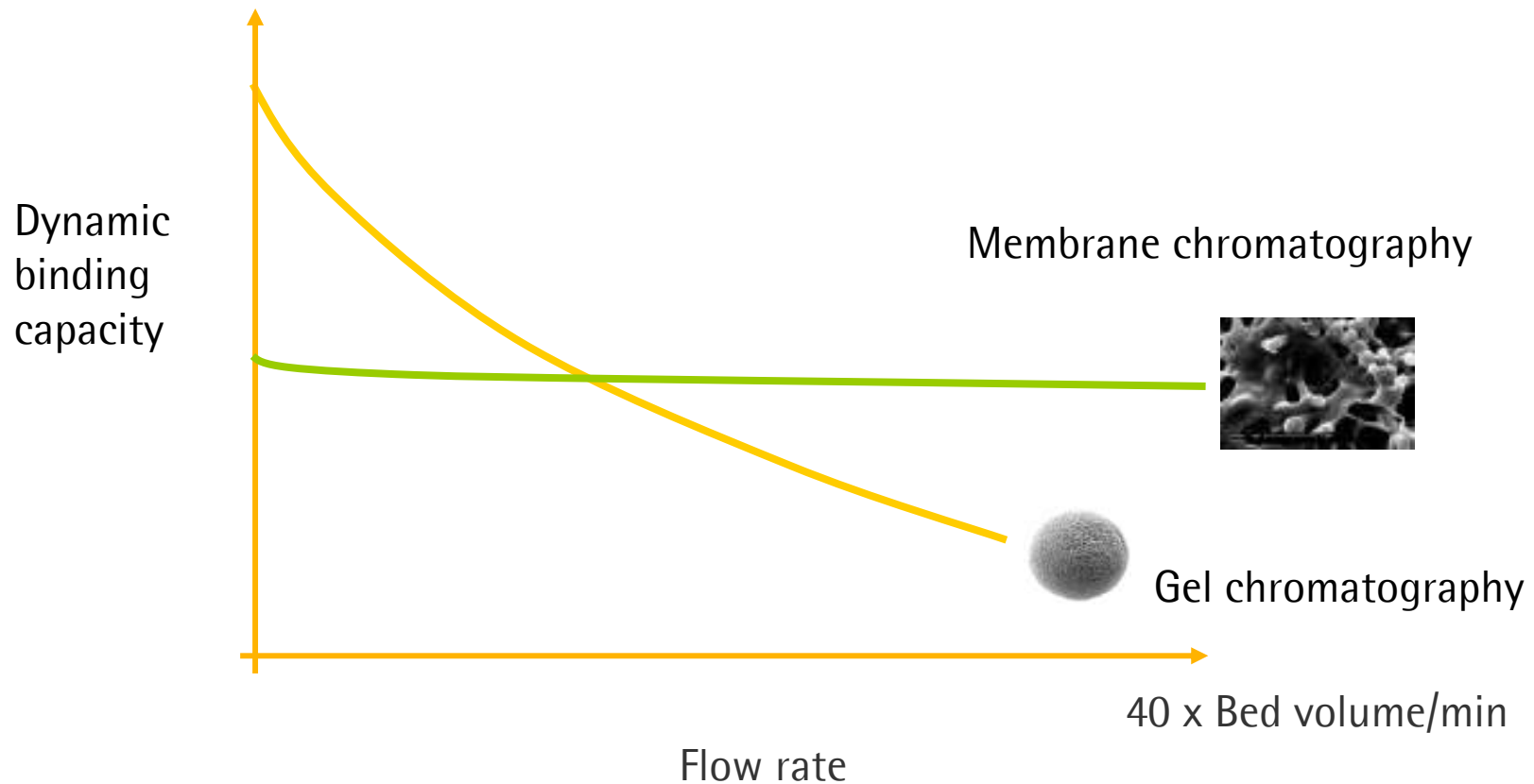
Diffusion limited gels (time) versus convection limited (flow rate)



Average pore size
15 - 40 nm

Average pore size
0.3 - 5 μm

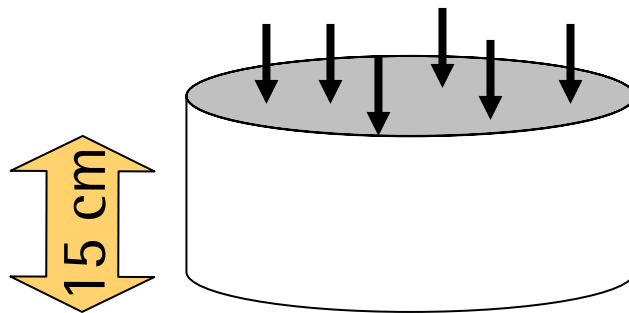
Dynamic binding capacity ./ Flow rate -> Contaminant removal



Flow rate: 0.5 bed volumes/min for the column
 30 bed volumes/min for Membrane Adsorber

Column 20 L

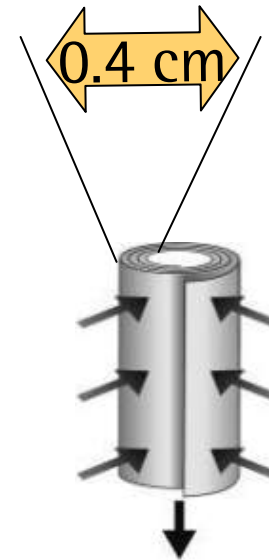
$$\frac{10 \text{ L / min}}{20 \text{ L}}$$



results in a flow rate of 0.5 BV / min

Membrane Adsorber 0.5 L (~2 m²)

$$\frac{14 \text{ L / min}}{0.5 \text{ L}}$$



results in a flow rate of 28 BV / min

Need for Throughput Results in Oversizing Columns

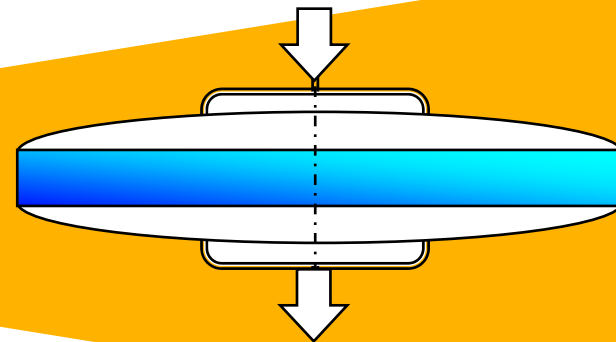
Bottleneck at production scale = flow rate

Membrane based system



- 0.5 L bed volume
- 1000 l/h
- BC 15 g sufficient for contaminant removal

Beads based system

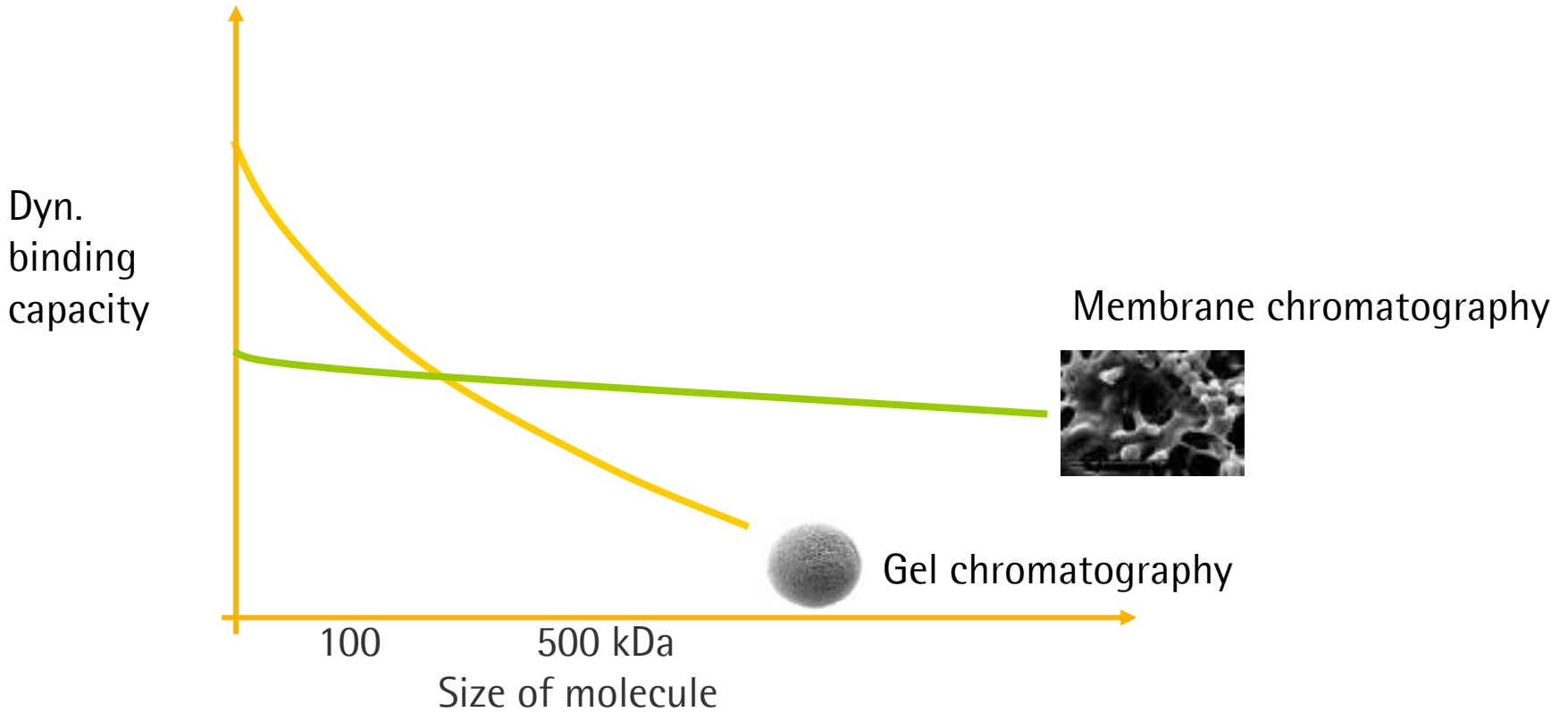


- 50 cm / 30L bed volume
- 1000 l/h
- BC 1500 g oversized

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

Dynamic binding capacity ./ Size -> Capturing large molecules

Size exclusion for proteins >250 kDa when using gel matrix



Gosh R. J Chromatogr A, 2002, 952, 13-27.

Reichert U, Linden T, Belfort G, Kula MR, Thommes J. J Membr Sci, 2002, 199, 161-166.

Agenda



1. Membranes

2. Formats

3. Applications

Sartobind membrane adsorber portfolio process scale

4 mm bed height

8 mm bed height

Q
S
STIC

Size ml	pico	nano	mini	5"	10"	20"	30"	mega
	0.08	1	7	70	180	360	540	1620 ml

Q
S
HIC

	nano	150 ml	Jumbo
	3	150	5000 ml

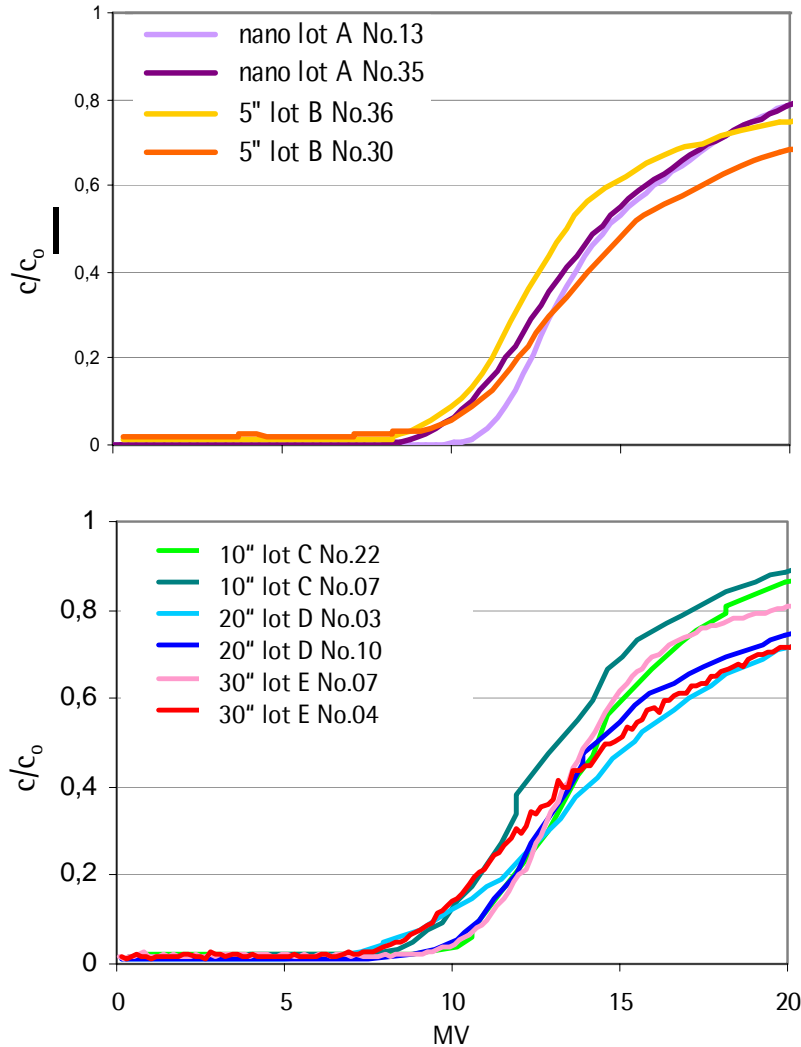
Contaminant removal: flow through mode to remove DNA, Host cell proteins, endotoxins, viruses

Purification: bind & elute of viruses and virus like particles, large proteins

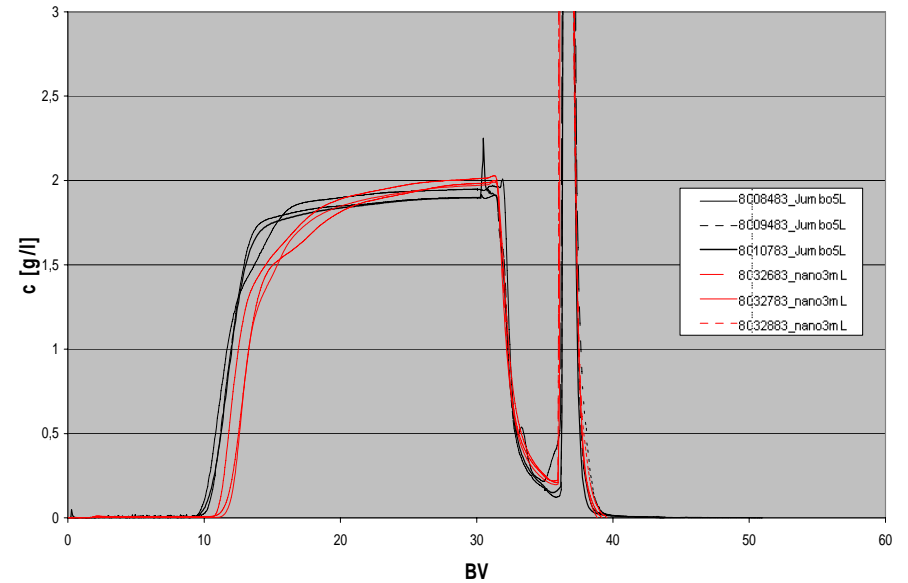
Single-use

Single-use / intra-batch use

Scale-up performance



Q Jumbo 5 liter lots vs. Q nano 3 ml



Agenda

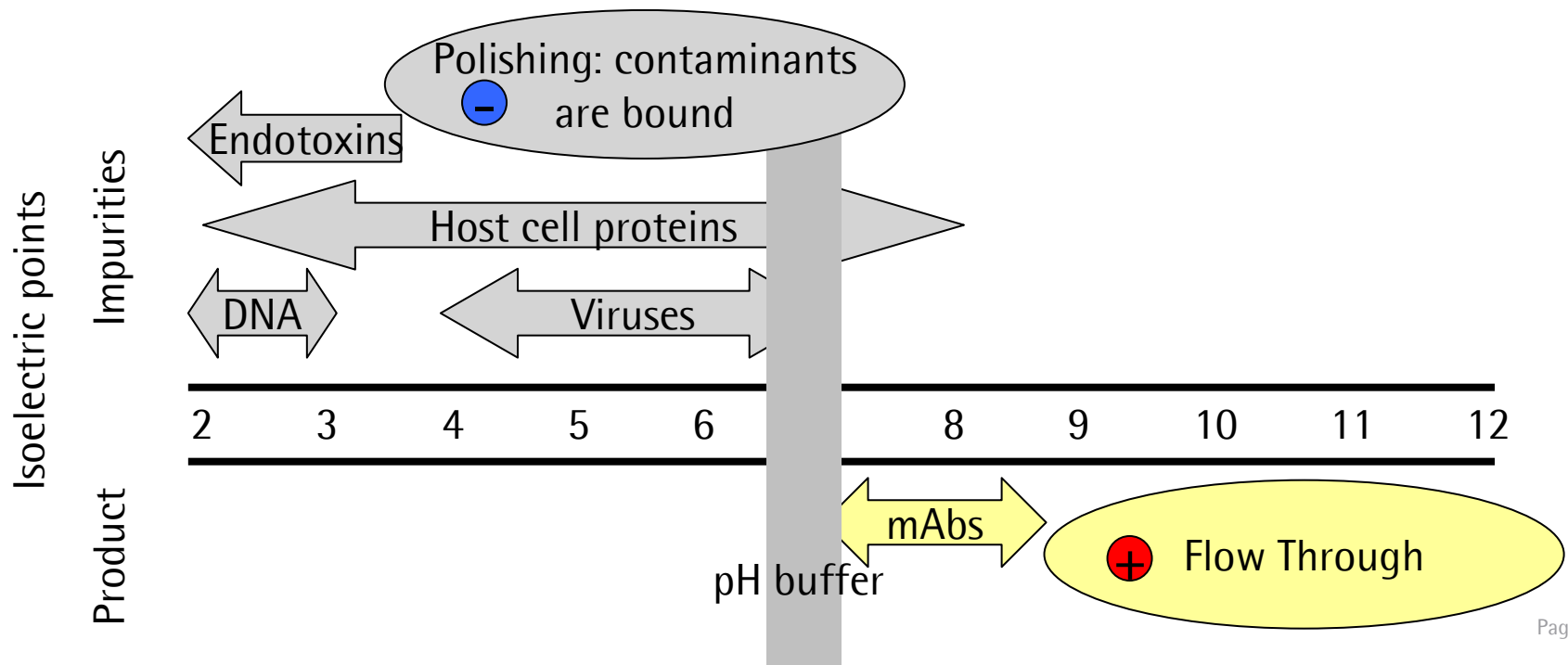
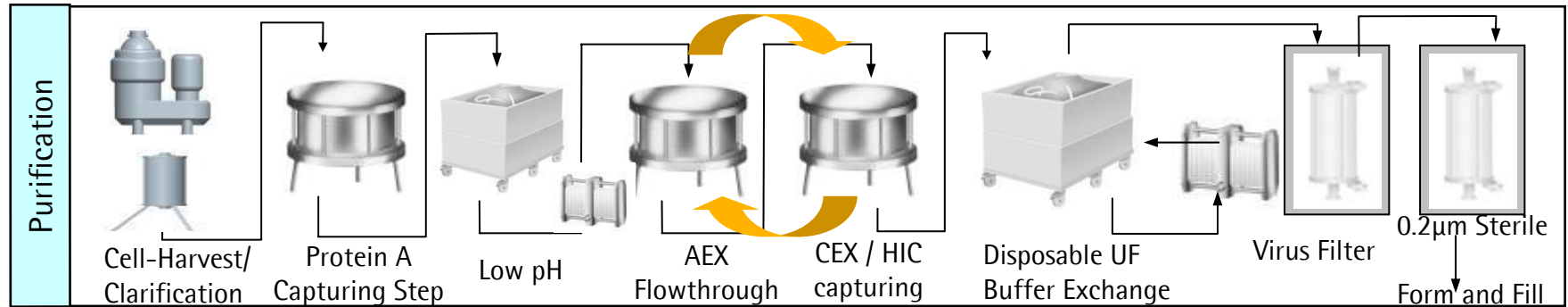


1. Membranes

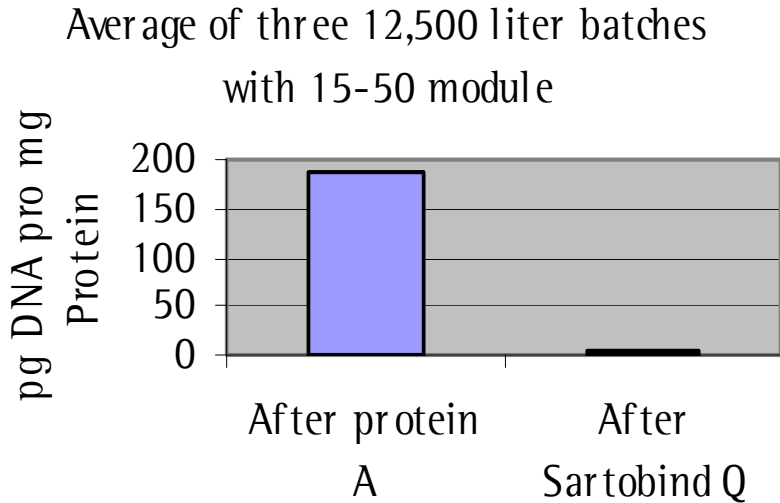
2. Formats

3. Applications

Typical platform for Downstream Processing of mAb



DNA removal from a therapeutic antibody. Campath 1-H



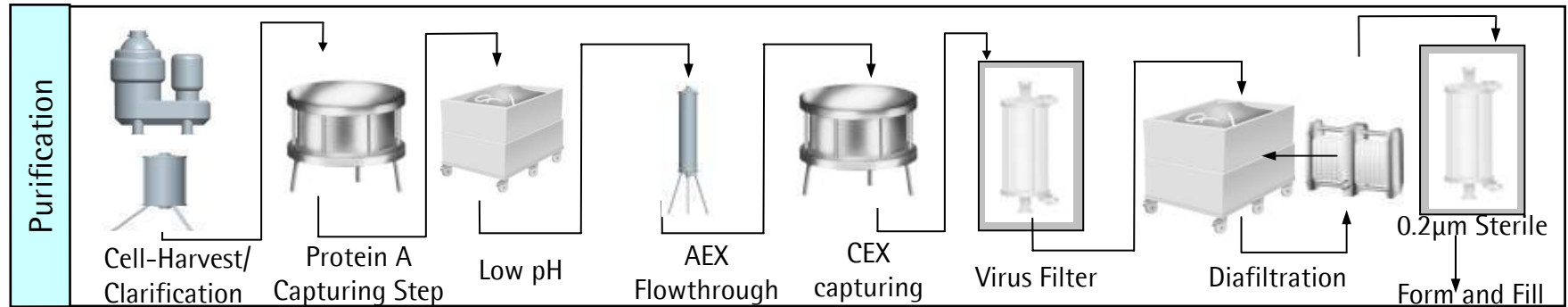
- ...clears the DNA
- below detection limit
- superior pressure/flow relation
- ...the process time is reduced 23-fold, excluding the benefit in handling for set-up.
- ...diminish a loss of product
- ...installed as in-line filters and can be disposed after use.

J. K. Walter, Boehringer Ingelheim Pharma, Bioseparation and Bioprocessing, G. Subramanian (ed.) Wiley VCH, Vol. II p. 447-460, 1998

Gallier P, Fowler E, Millennium Pharmaceuticals Inc.: Validation of Impurity Removal by the CAMPATH-1H Biomanufacturing Process. IBC's Biopharmaceutical Production Week, Paradise Point Resort – San Diego, CA, November 12-15, 2001



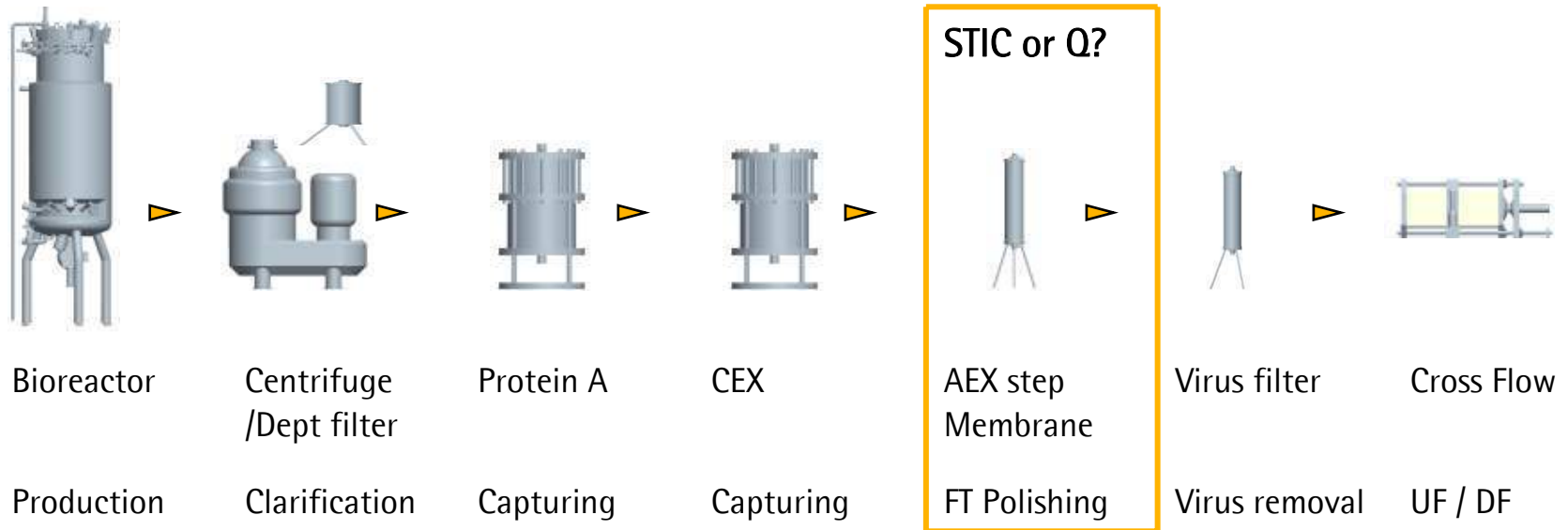
Membrane adsorber implemented in purification platform



Main driver => increased load capacity
 Overall reduced timeline for purification development

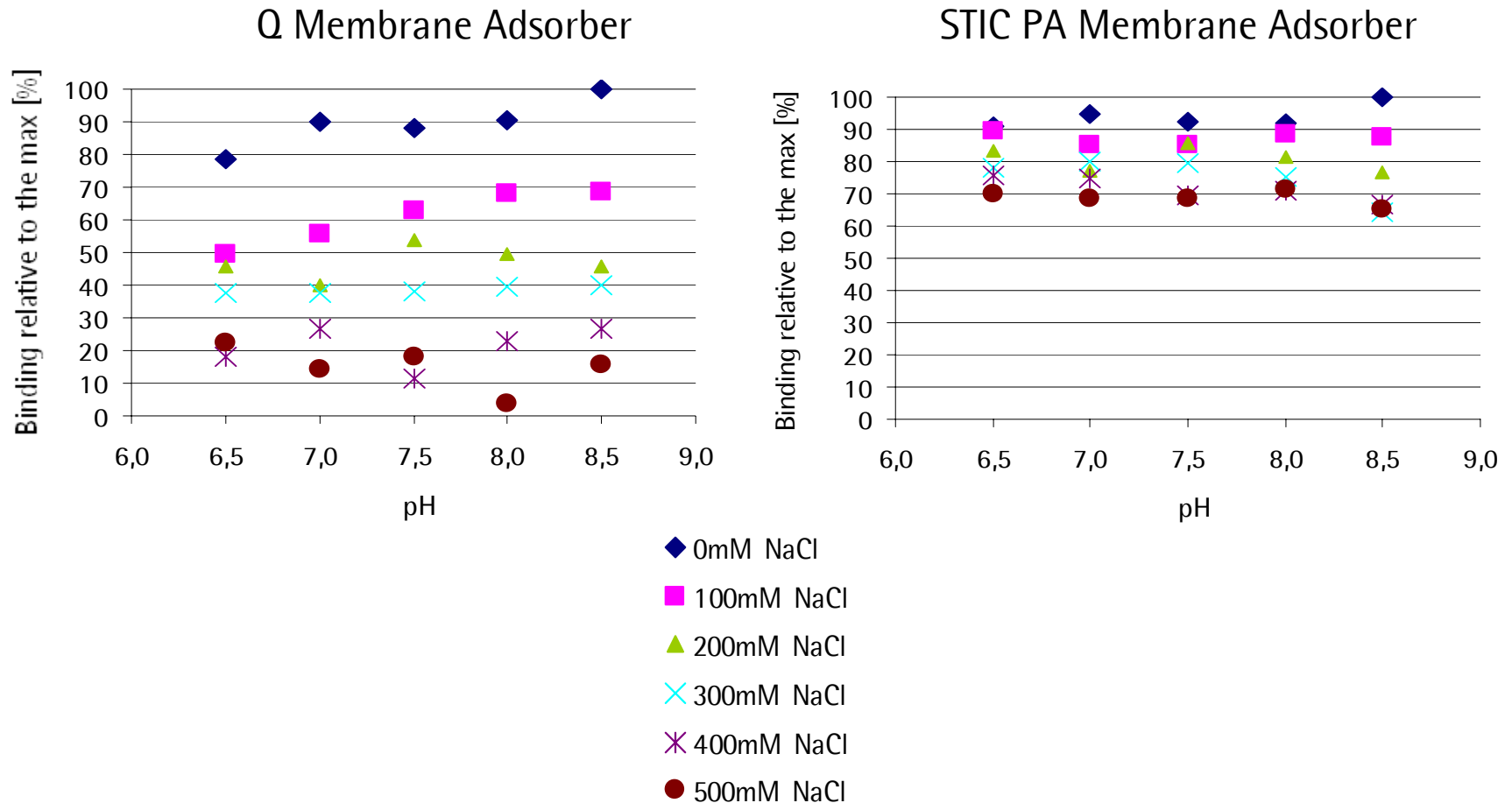
Antibody	Process	HCP (ng/mg)	DNA (pg/mg)	% monomer	Residual protein A (ng/mg)
1	old	< 2	< 2.1	99.4	< 0.3
2	old	1.3	< 12.5	93.0	0.4
3	old	1.2	< 0.7	99.5	0.8
4	new	1.6	< 1.6	99.2	0.5
5	new	2.8	< 2.1	99.0	< 0.3
6	new	7.5	< 0.9	97.7	1.4

Anion exchange step in flowthrough polishing 3rd step: Position for STIC Membrane Adsorbers



Reduces:
Viruses
DNA
HCP
Leached ligand
Endotoxins

HCP binding on Q vs. STIC PA at different salt concentrations:
 Salt tolerant STIC less dependent on salt->large window of operation



Purification bottleneck – Facility / Tank size limitation at high mAb titers:



5 g/L mAb

10000 Liters



200 L Protein A 30 g/L, 8 cycles, 2.5 CV Pool

4000 Liters



500 L CEX 100 g/L, 6 CV Pool, 12-15 mS/cm

3000 Liters



Q needs 4-7 mS/cm, dilution 1:1

6000 Liters

AEX 10 kg/L FT



Purification bottleneck – Facility / Tank size limitation at high titers, e.g.:



5 g/L mAb

10000 Liters



200 L Protein A 30 g/L, 8 cycles, 2.5 CV Pool

4000 Liters



500 L CEX 100 g/L, 6 CV Pool, 12-15 mS/cm

3000 Liters

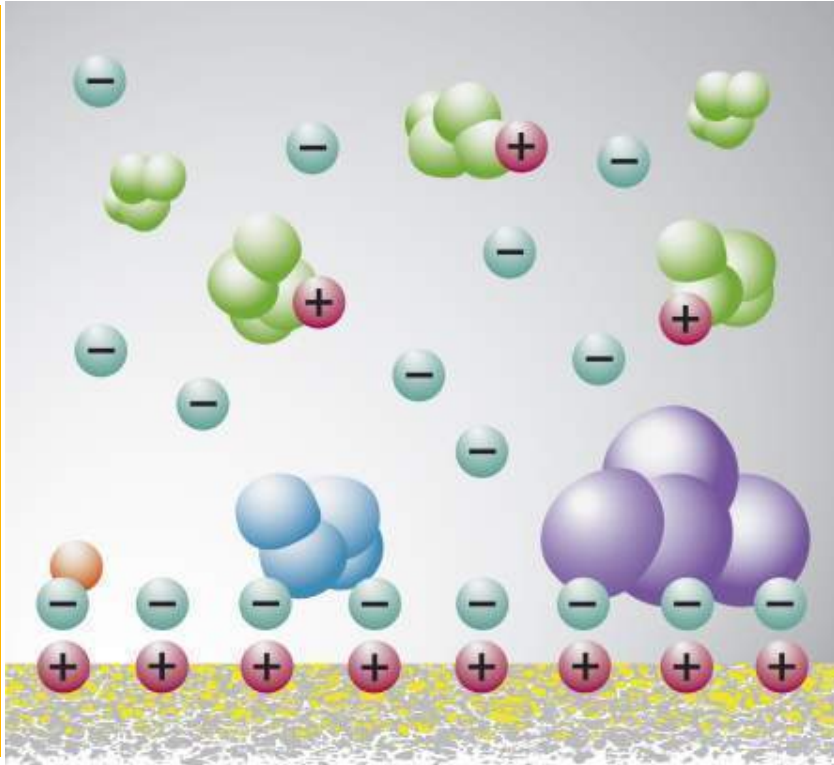


Sartobind STIC AEX 10 kg/L FT

no need for 6000 Liter tank



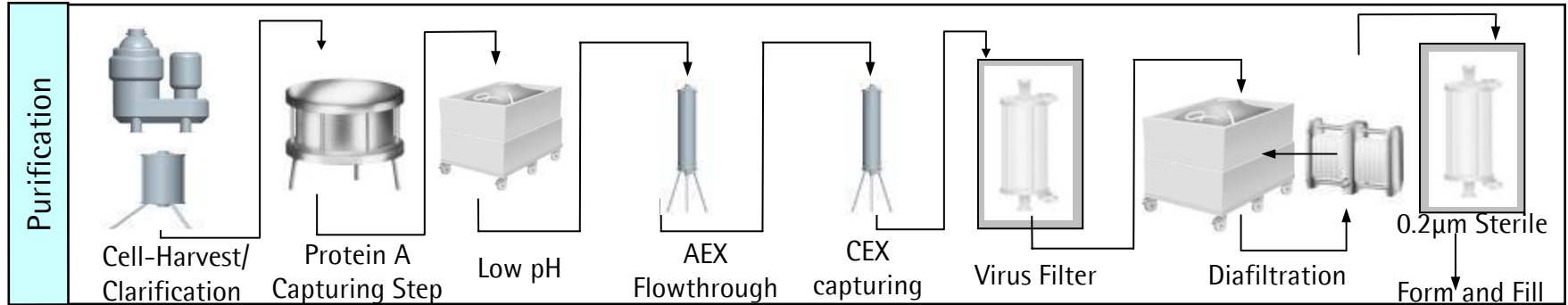
Anion exchange with STIC overcomes limitations at high salt conditions



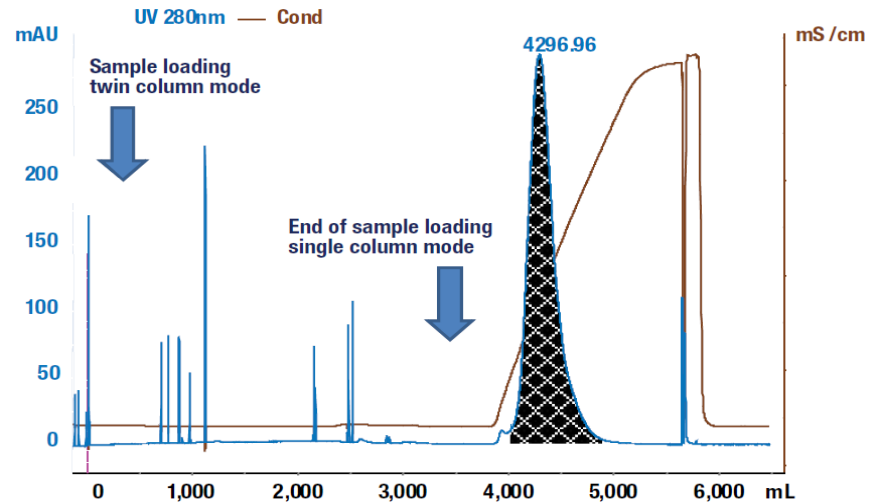
Contaminant removal at 16.8 mS/cm

- Host cell proteins <10 ppm at 10 kg/l
- Virus removal >4 LRV
- DNA >3 LRV
- Endotoxins >4 LRV

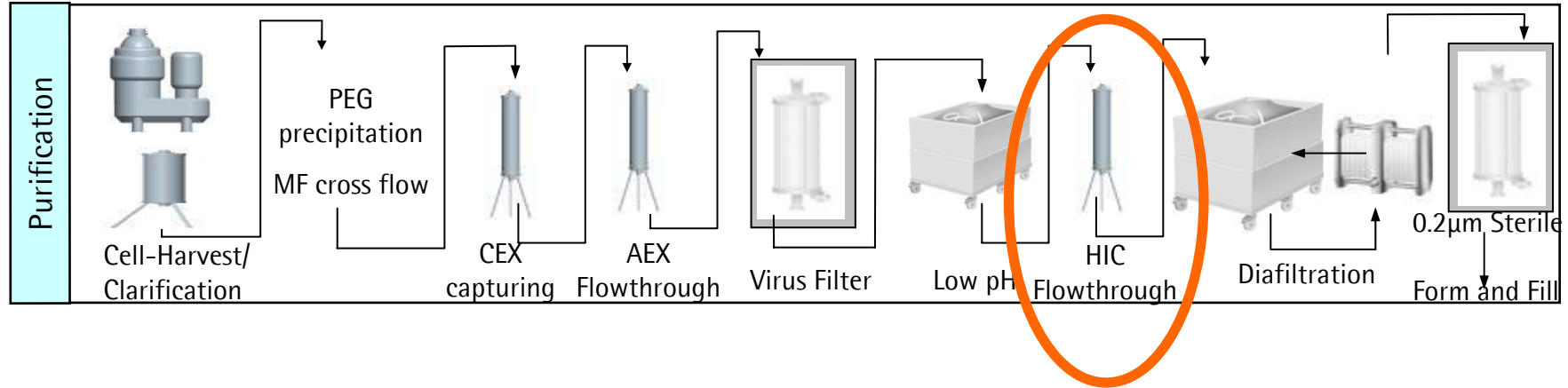
Membrane adsorber implemented in purification platform



- mAb fusion protein
- Clinical phase I and II
- Purity 82 – 99%

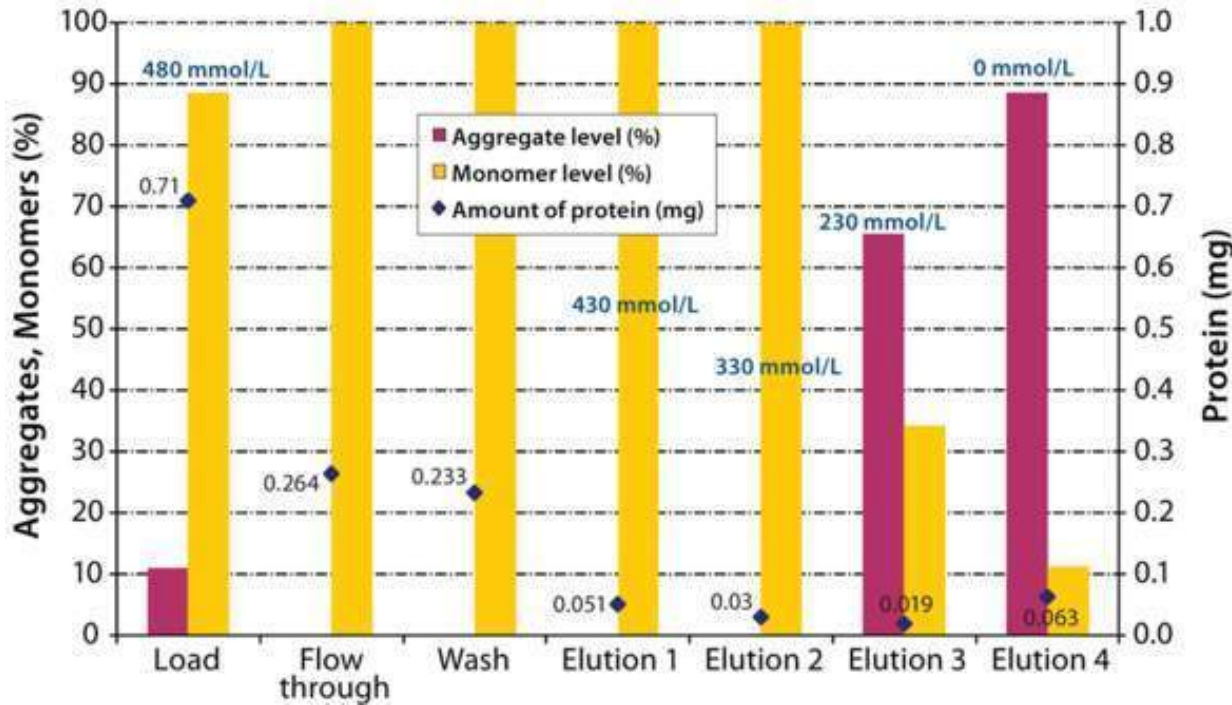


Phenyl Membrane adsorber implemented in purification platform



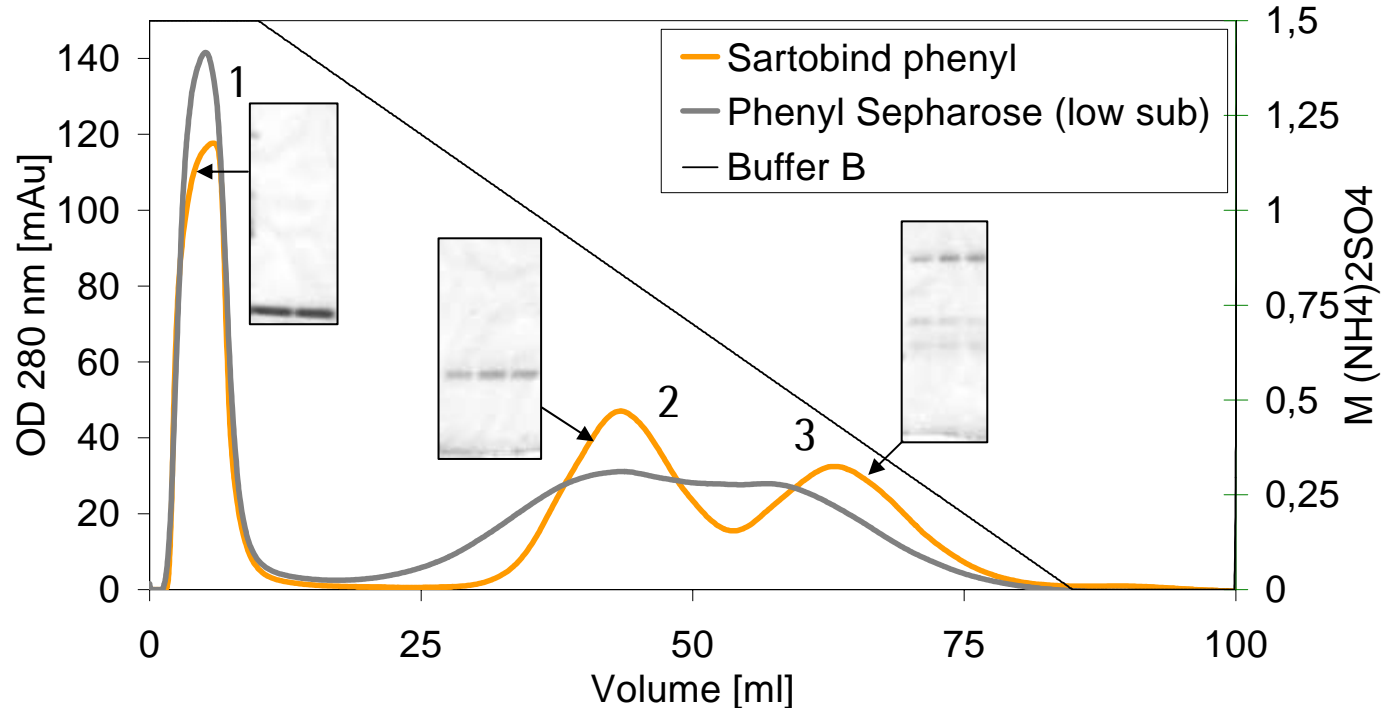
Process		Recovery %	HCP (ppm)	HMW (%)
1 mL CEX	Start	100	115,000	1.5
	End	63	32	0.3
20 mL CEX	Start	100	115,000	1.5
	End	63	9.6	0.3

Aggregate removal from a recombinant protein by Phenyl MA



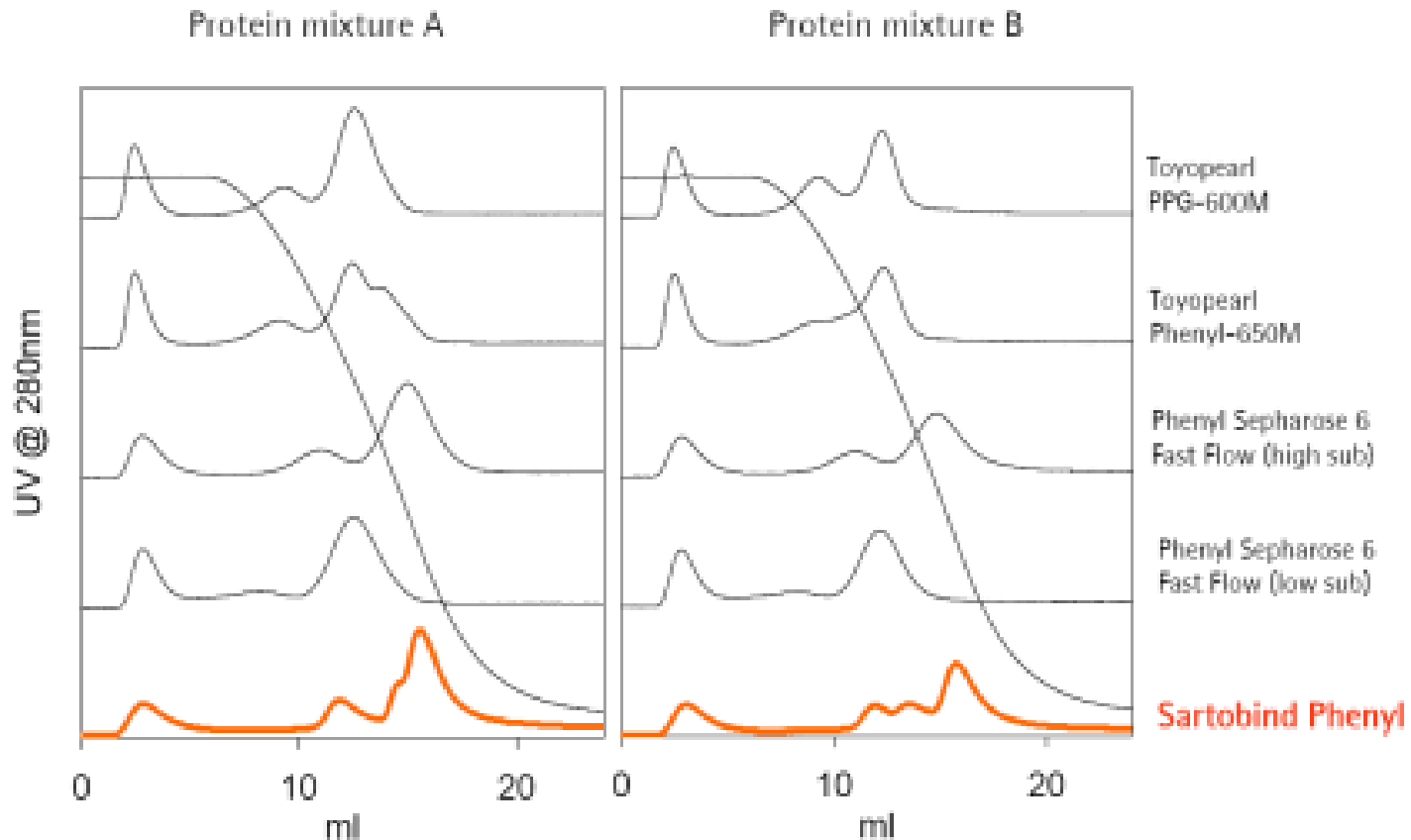
Ebert, S. et al., Efficient Aggregate Removal from Impure Pharmaceutical Active Antibodies, *BioProcess International*, Vol. 9, No. 2, Feb 2011, pp. 36-42.

Phenyl Membrane adsorber for bind and elute application



Gradient elution of cytochrome c (1), trypsinogen (2) and IgG (3) bound on phenyl membrane and resin

Phenyl Membrane adsorber for bind and elute application



Mixture A: 1, cytochrome c; 2, ribonuclease A; 3, lysozyme; 4, α -chymotrypsinogen.

Mixture B: 1, cytochrome c; 2, ribonuclease A; 3, lysozyme; 4, b-lactoglobulin.

Summary

- Advantages of Membrane Adsorbers compared to classical chromatography
 - » High flow rates, large pore sizes
 - » Single Use, no cleaning, no validation, buffer saving up to 95%, less time for set up
- Different chemistries and formats
- Case studies
 - » Flow through, high loading capacity of 10 kg mAb/L to achieve HCP clearance
 - » Bind and Elute
- Several companies use membrane adsorbers in their purification platform



Zhou J X, Tressel T, AMGEN Inc.: Membrane chromatography as a robust purification system for large-scale antibody production. *Bioprocess International*, Sept. 2005, p. 32-37



Thank you for your attention.

Dr. Andreas Kocourek, Sartorius Stedim Biotech, Application Specialist, Purification
Australia (andreas.kocourek@sartorius-stedim.com)