

New technologies to increase the efficiency of downstream processing

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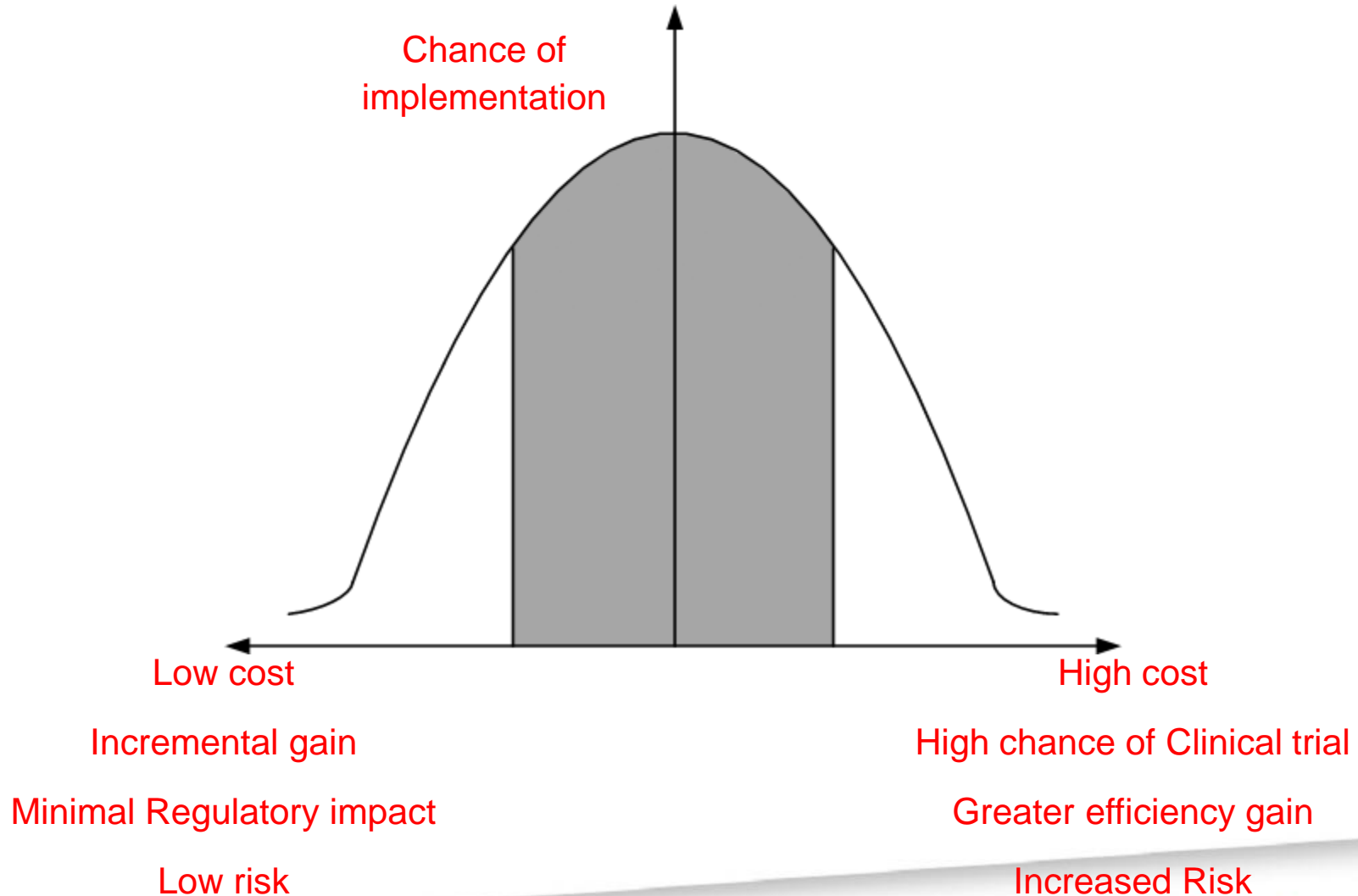
Pressures to increase efficiency

- Increasing manufacturing costs (raw materials, consumables, wages etc)
- Increasing competition
- Decreasing margins
- Simplification of processes
- Unmet market demand (particularly in developing countries)

Pressures to maintain status quo

- Requirements for Process Development
- Requirements for Process validation
- Requirements for product registration

Considerations for Process changes to an existing process

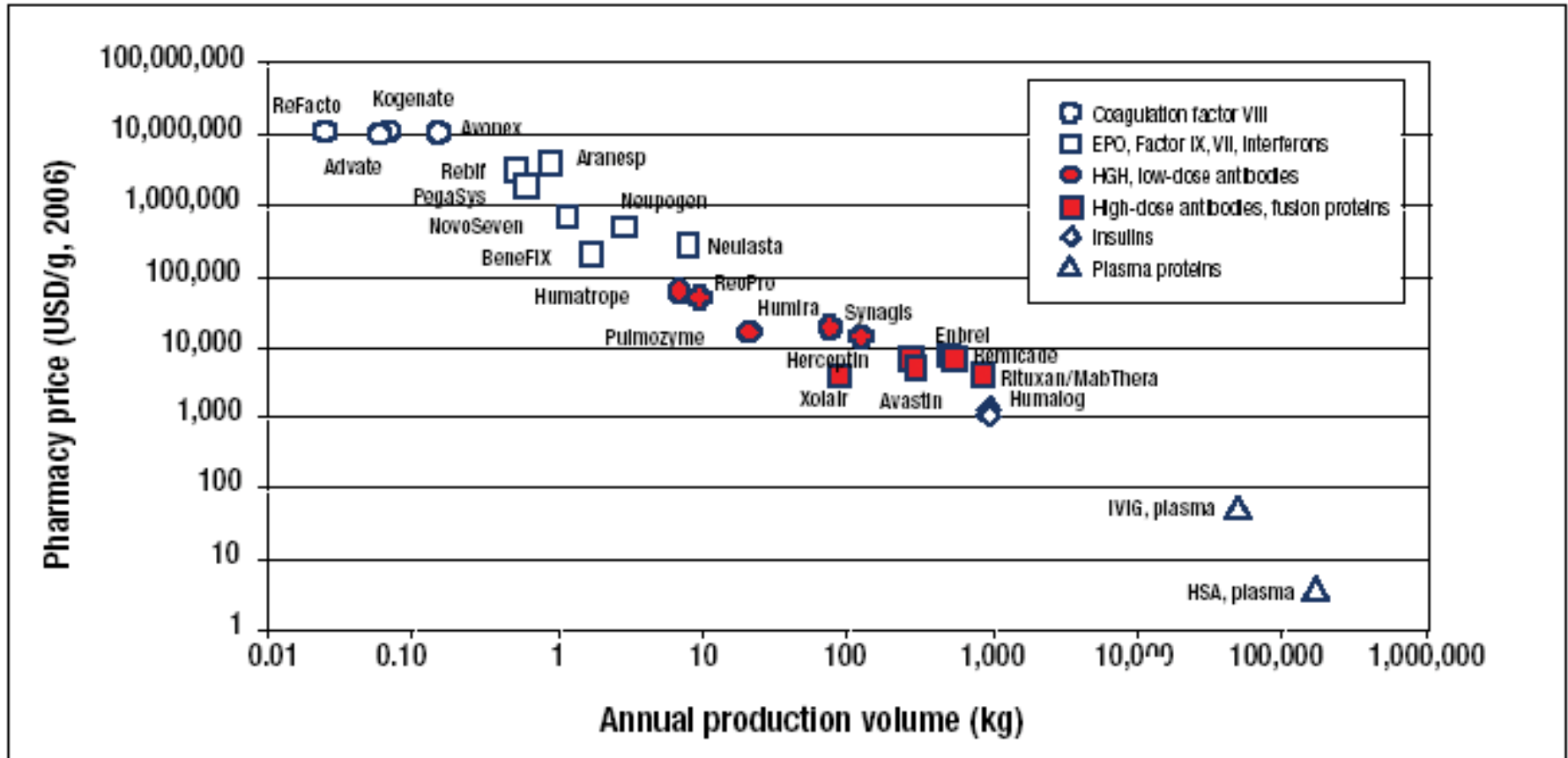


Plasma fractionation industry

- Large batch sizes (10 – 15 tonnes)
- Plasma: High protein concentration (60-70 mg/mL)
- Manufacturing plant operated continuously



Price & Production of Protein Therapeutics (2006 data)



Efficient and Profitable Plasma Fractionation

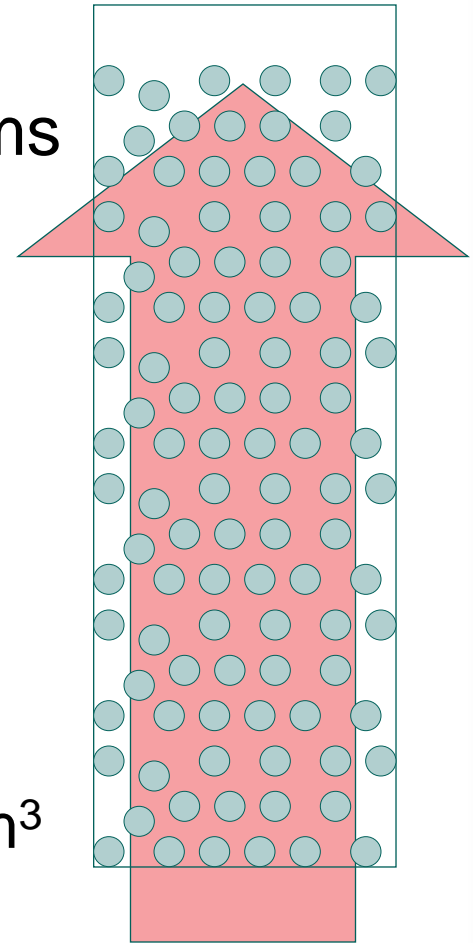
- Profitability dependent on:
 - Maximising number of different proteins extracted
 - Maximising yield
 - Efficient downstream processing
- Efficient downstream processes result in:
 - Lower CAPEX
 - Smaller footprint
 - Reduced cycle times
- Efficiency improvement programs: on existing processes

R&D at CSL Biotherapies

- Main activities:
 - Support & optimise existing downstream processes for plasma fractionation (IgG & Albumin)
 - Evaluate new technologies – particularly process steps that are inefficient or adversely impact on product quality
 - Expanded bed adsorption chromatography
 - Single pass Tangential Flow Filtration
 - Membrane chromatography

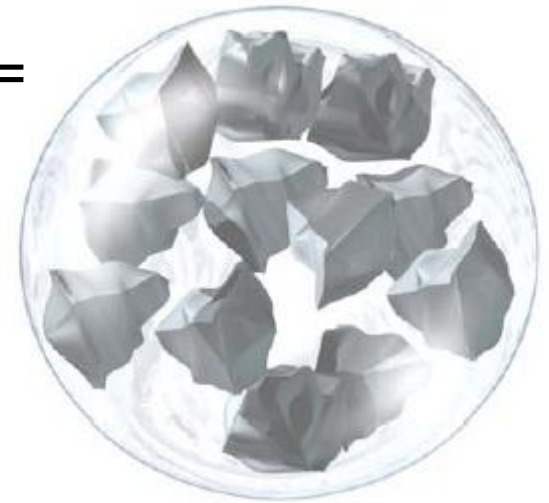
Expanded bed adsorption (EBA) chromatography

- Capture of proteins from crude feed streams
- Non-clarified samples
- Limited adoption of technology
- STREAMLINE media
 - Quartz inner core – resin density = 1.2 g/cm^3
 - Flow rates restricted by density



Expanded bed adsorption (EBA) chromatography

- Newer generation media (Fastline media)
 - Tungsten carbide inner core – density = 3.0 g/cm^3
 - Flow rates of 300 – 600 cm/h can be achieved



► Isolation of IgG from plasma

Current method for Albumin & IgG separation

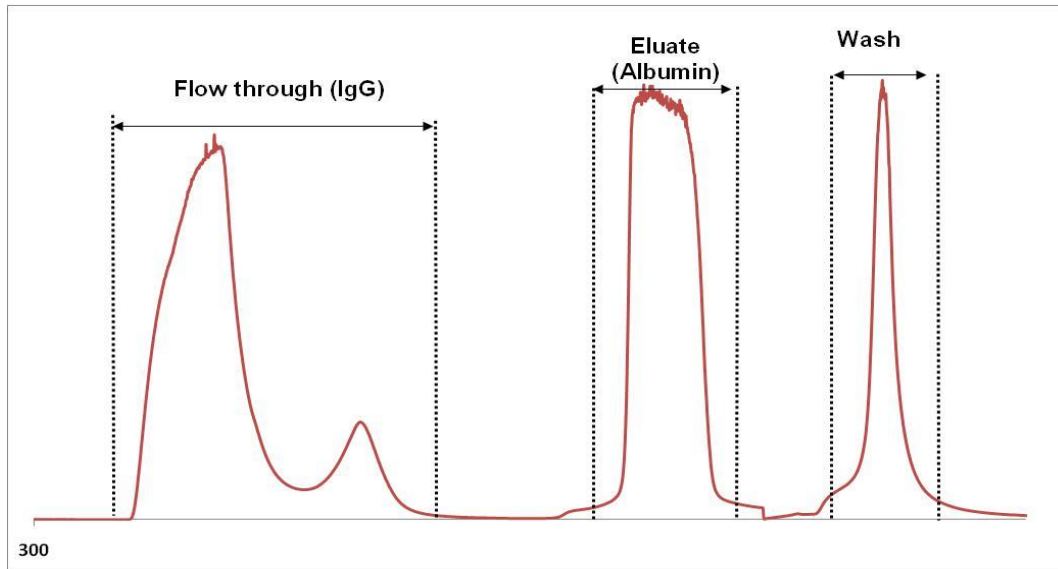
- Packed bed chromatography – DEAE Sepharose-FF
 - Albumin binds
 - IgG flows through
- Flow rate restricted due to column pressure drop
- Sample: ratio of Albumin:IgG (5:1)

- Significant bottleneck in process
- Significant buffer usage

Development: IgG & Albumin separation using Fastline IVIg

- Identified loading and elution conditions
- Investigated effect of protein loading
- Optimal conditions
 - Protein loading = 9-fold increase
 - Flow rate = 2-fold increase (only marginally higher after accounting for void volume)

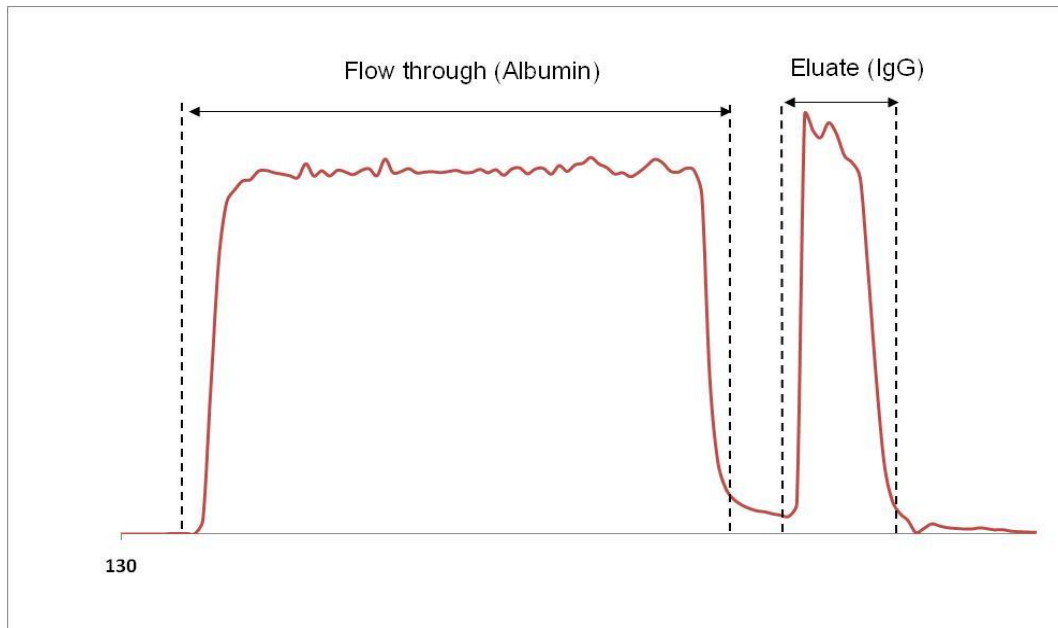




DEAE Sepharose:

IgG purity = 76%

Albumin purity = 98%



Fastline IVIg:

IgG purity = 71%

Albumin purity = 93%

Efficiency gains

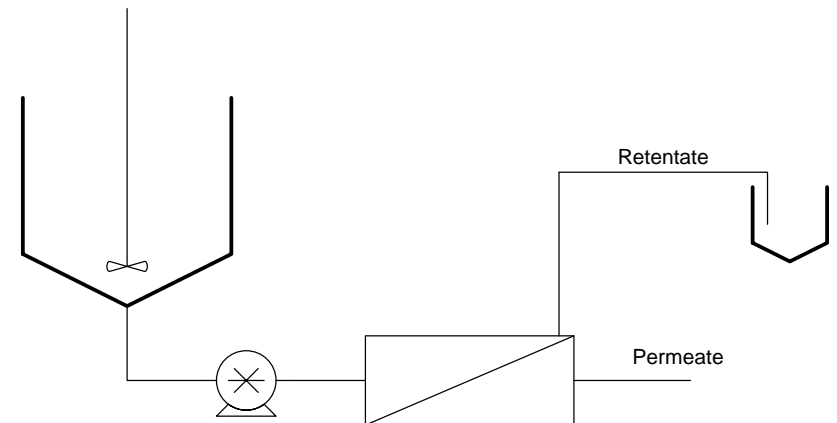
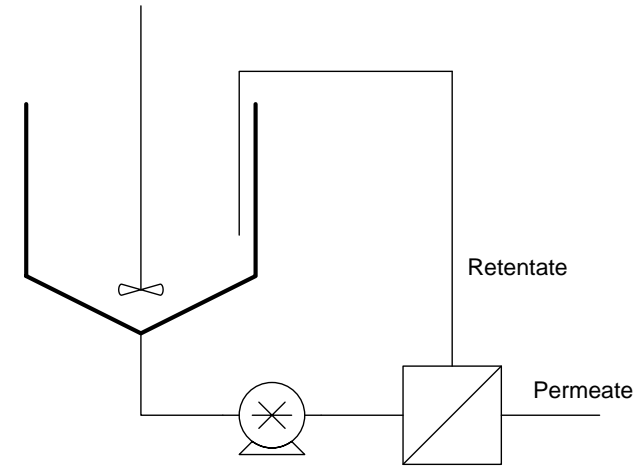
Column	Cycle time	Buffer usage
DEAE Sepharose (200 L column)	100	100
Fastline IVIg (200 L column)	19	22

Project status – EBA chromatography

- Proof-of-principle established
- Evaluation of commercial & regulatory implications of implementing into global business
- EBA using Fastline IVIg – already implemented by Cangene (Canadian Plasma Fractionator)

Single Pass Tangential Flow Filtration

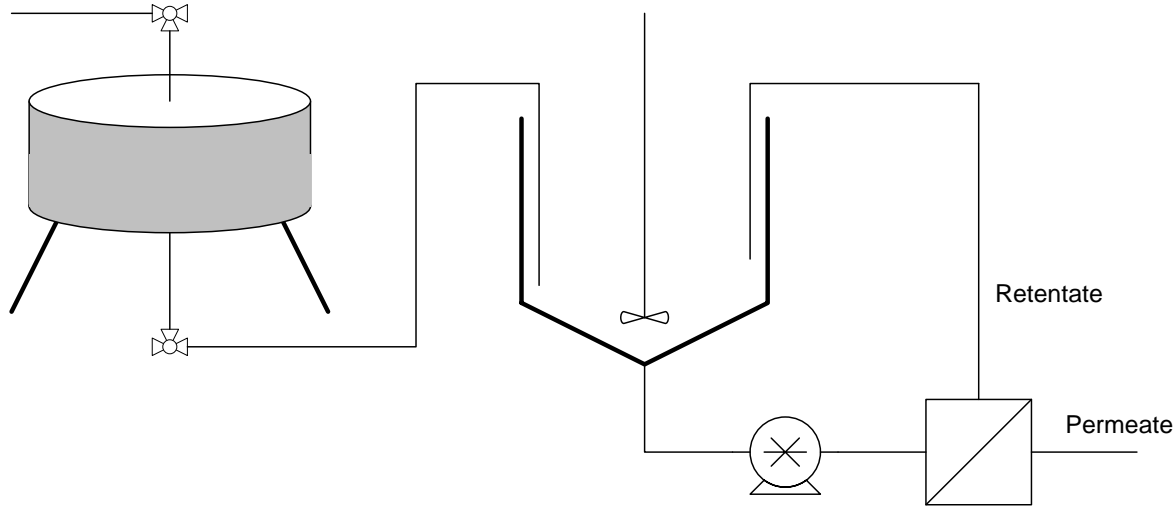
- TFF – used extensively for concentrating protein solutions: product recirculated over membrane until target protein is achieved
- Cadence™ Single Pass TFF – Membrane path is sufficiently long enough to achieve desired protein target in a single pass



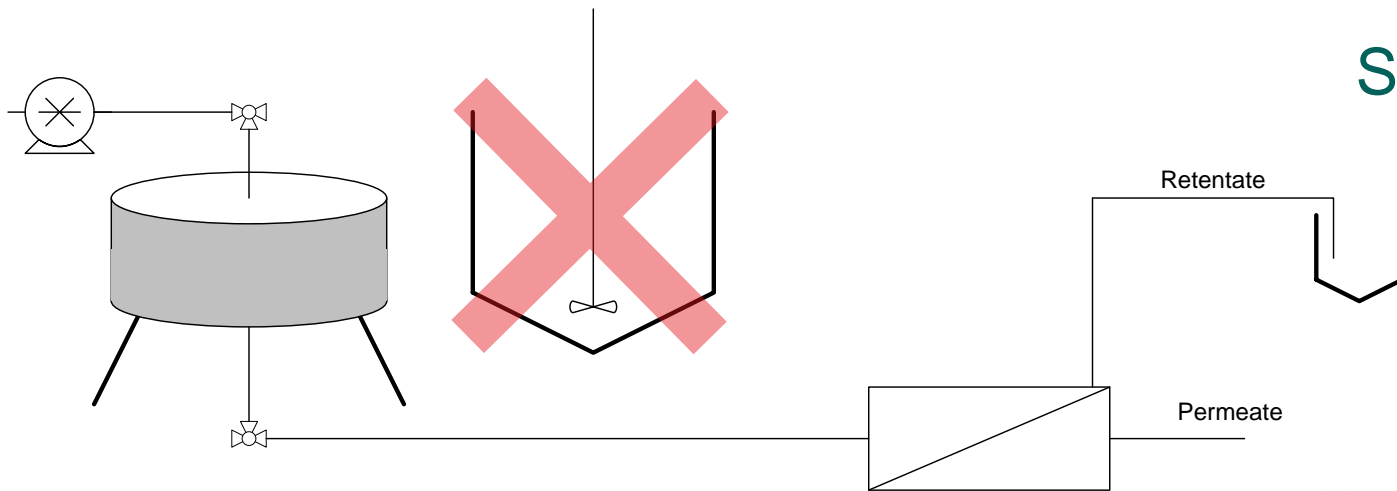
Initial evaluation of Cadence™ TFF

- Pressure excursion testing – Flux monitored at various pressures and protein concentrations
- Identification of path length for IgG and Albumin experiments
- 3% IgG solution concentrated by a factor of 4.5X:
Stable flux & > 99% recovery
- 1.4% Albumin solution concentrated by a factor of 11X:
Stable flux & > 99% recovery

Concentration of product immediately from a chromatographic column

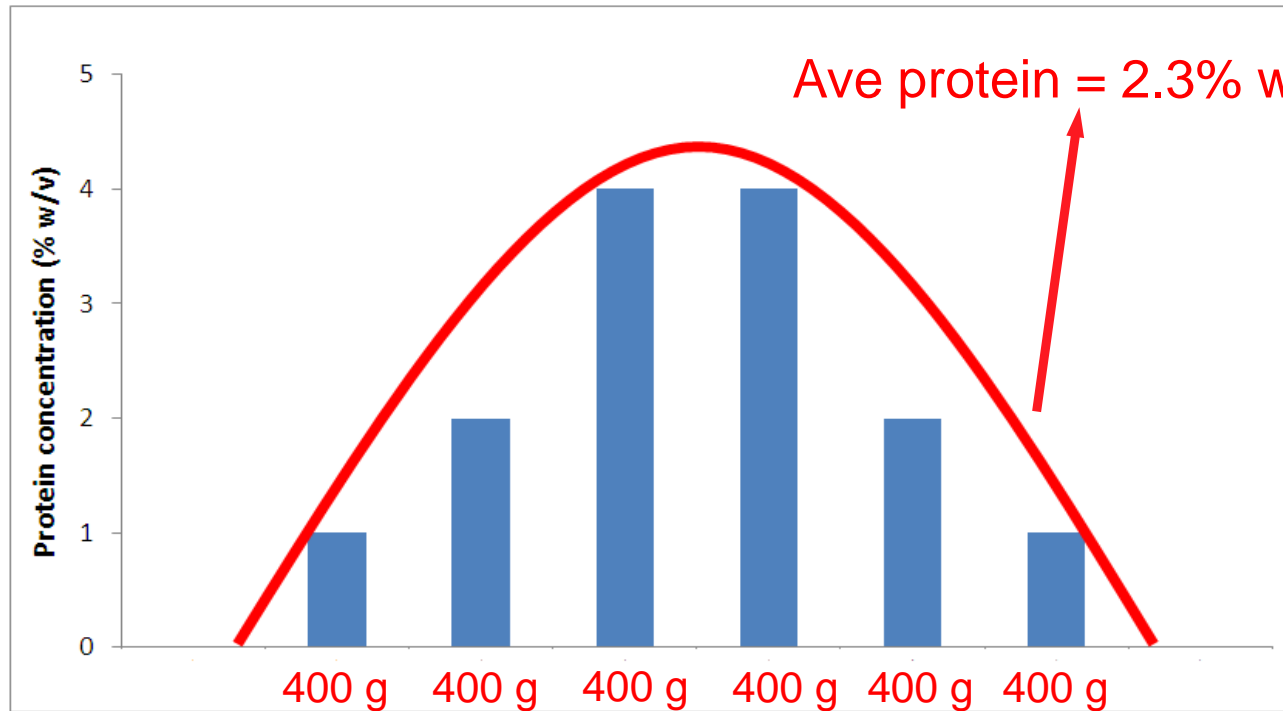


Traditional TFF



Single pass TFF

Albumin elution from gel filtration column

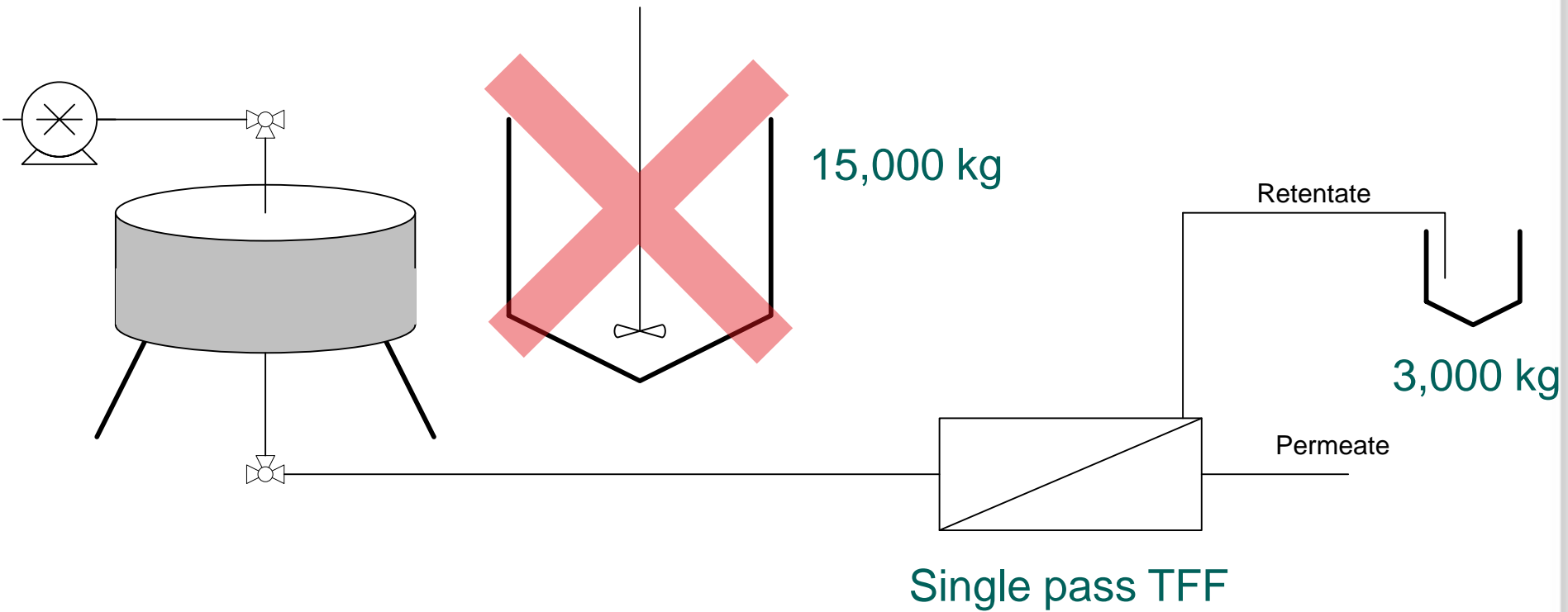


Protein target =
10 – 13% w/v

Final solution = 12.8% w/v protein

Protein recovery = 99.2% recovery

Envisaged optimised process



Project status – Single pass TFF

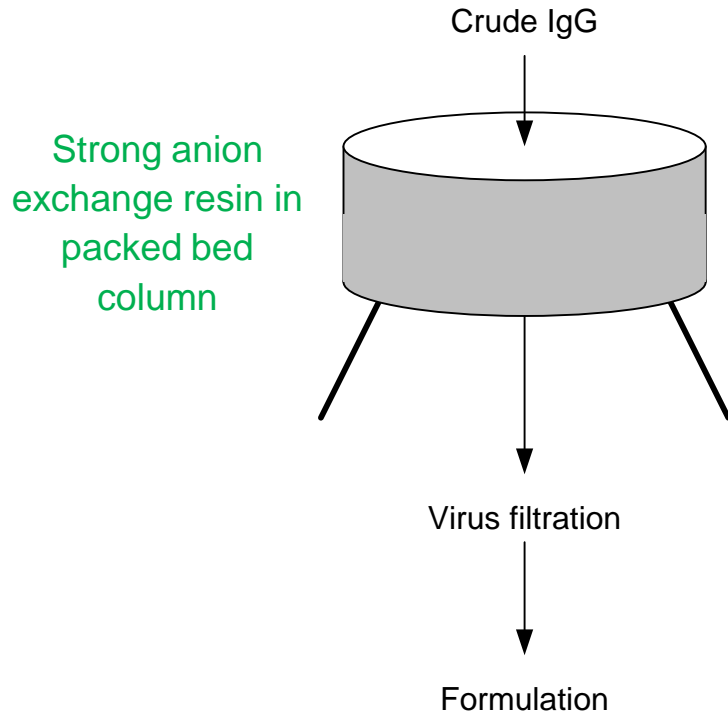
- Benefits of single pass TFF demonstrated
- Can envisage bolting-on to existing process
 - Final concentration of product before formulation
- Identify opportunities for future process development projects

Membrane chromatography

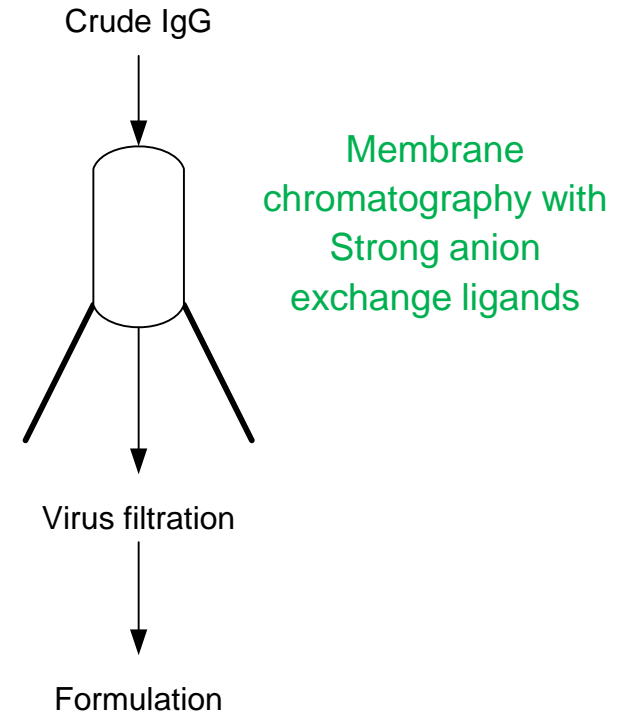
- Packed bed chromatography:
 - Chromatographic resins are expensive – re-use of resins to be economically viable
 - Cleaning validation & Resin life-cycle data required
 - Resin stability data
- Membrane chromatography:
 - Disposable chromatography option
 - High flow rates



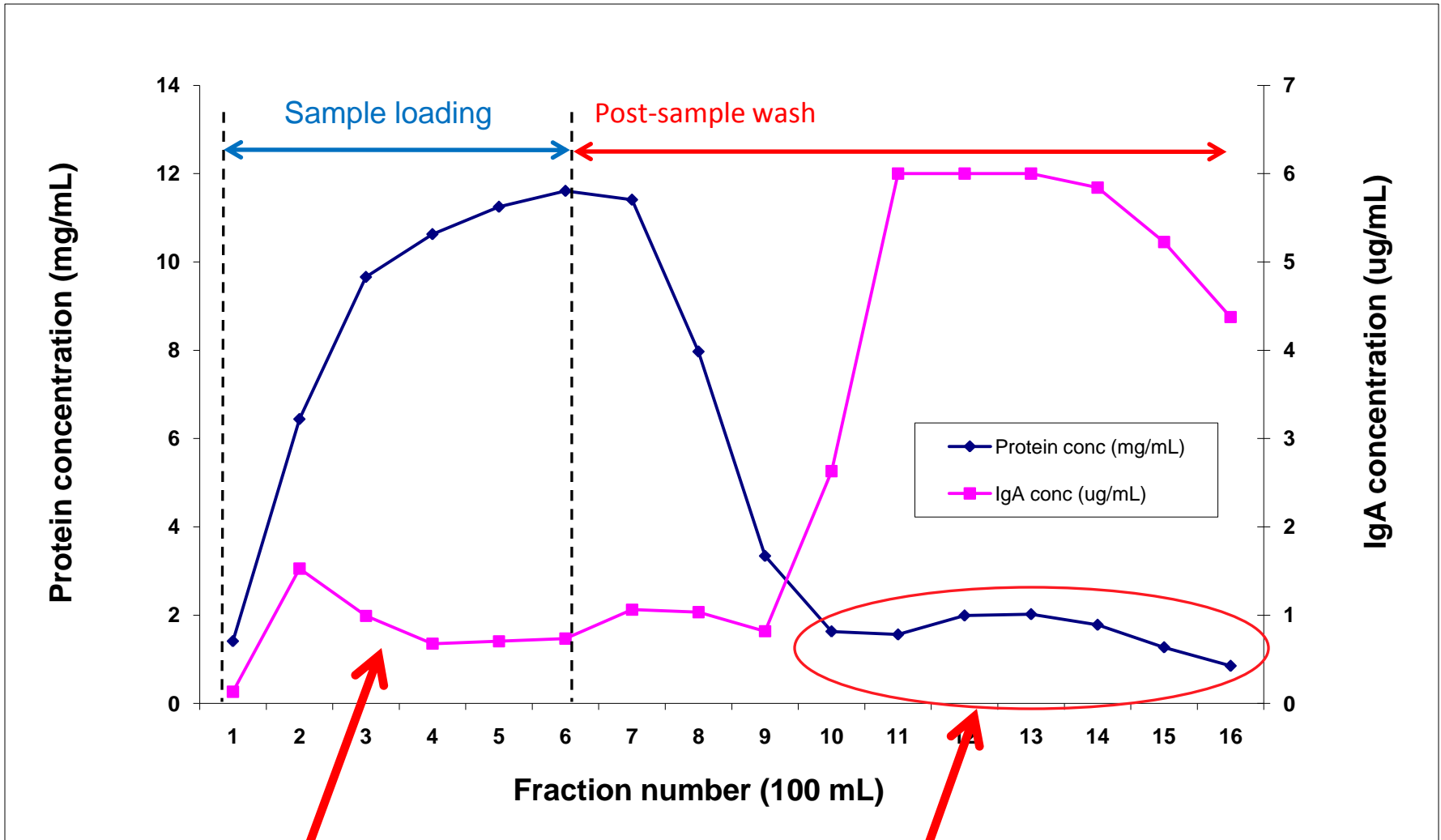
Polishing of Crude IgG



60 mL column volume
Flow rate = 8.6 mL/min



60 mL membrane volume
Flow rate = 513 mL/min



500-fold reduction in IgA levels

10-15% of total IgG

- Will comply with final product limit (< 25 ug/mL)

Evaluation of membrane chromatography trial

- Membrane chromatography:
 - Highly efficient – high flow rates
 - Loss of resolution compared to packed bed chromatography

- Success depends on application:
 - Crude IgG – contains 4% impurities
 - Selectivity between target and impurity

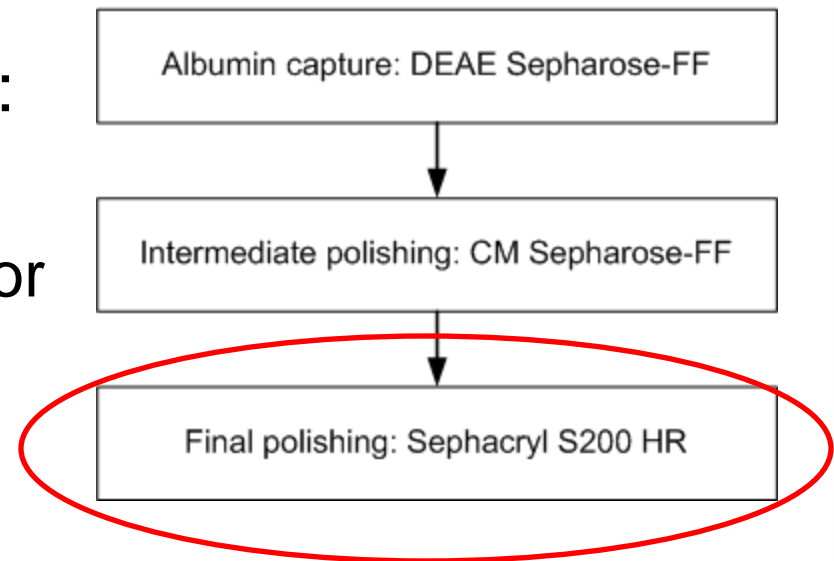
Protein	pI
IgG	4.4 – 10.0
IgA	4.0 – 7.1
IgM	4.0 – 9.1

- Literature for membrane chromatography:
 - Polishing for removal of DNA or endotoxin (< 1% of total)

Project status – Membrane chromatography

- Membrane chromatography has appeal
- Identify opportunities for future process development projects:
 - Custom designed membrane for removal of specific impurities

Albumin purification process



Acknowledgements

- Jennifer Griffin & Esha Pillay (Pall Life Sciences) – Cadence
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Thank you