Simple process strategies to increase the utilization of Protein A media in clinical manufacturing

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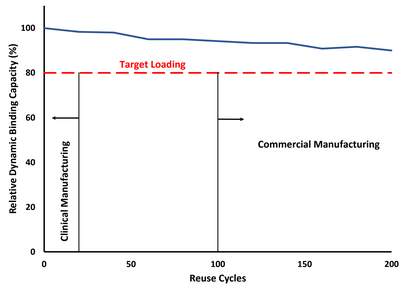
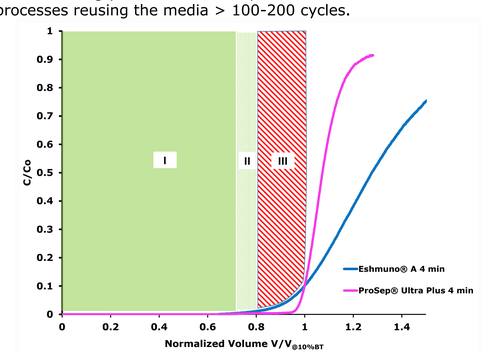
Background

Protein A chromatography media are one of the major contributors to the cost of production of monoclonal antibodies (mAbs), particularly for pilot plant runs and clinical manufacturing. In these cases one column is used to purify a single mAb to prevent cross product contamination. However, the resin is only used to approximately 10% of its potential lifetime. In addition, the safety factor used during the loading step at this scale is the same as for commercial manufacturing (e.g. 80% of 5-10% breakthrough (BT)). This safety factor accounts for potential reduction in binding capacity over the lifetime of the resin although this change generally does not occur until the column has been cycled > 50 times. These two factors combined result in a significant underuse of the resin and a concomitant increase in processing costs.



(Above) Protein A resin is used less than 20 cycles in clinical manufacturing yet the same safety factor is used as for commercial processes reusing the media > 100-200 cycles.



I + II: Target loading with typical safety factor (80% of 10% BT).

I: Actual loading (~90% of target loading) when multiple cycles are run to process a batch due to discrete column sizes.

II + III: Unused capacity in a typical process cycle.

In this work the use of a dual flow rate for loading to increase the DBC as well as an increase in the target loading per cycle (i.e. reduced safety factor) were evaluated. The impact of these strategies on breakthrough curves, DBC and utilization were determined experimentally. A model was used to evaluate the effect of these strategies on processing time and resin costs for a clinical manufacturing case study.

Methodology

Breakthrough curves and Dynamic Binding Capacities (DBC):
Chromatography media: ProSep® Ultra Plus and Eshmuno® A pre-packed in 5 mL columns
Feeds: Purified Polyclonal IgG (pIgG) in PBS (2 g/L)
Purified mAbs A, B, C: 2-4 g/L in PBS

DBC were determined from UV BT curves using frontal experiments conducted with a chromatography system.

Process Model Case Studies:
A Microsoft® Excel based process model (Wang and Mann, Bioprocess International 7(5) May 2009) was used as basis for calculations in the case studies.

Strategy 1. Dual flow rate loading

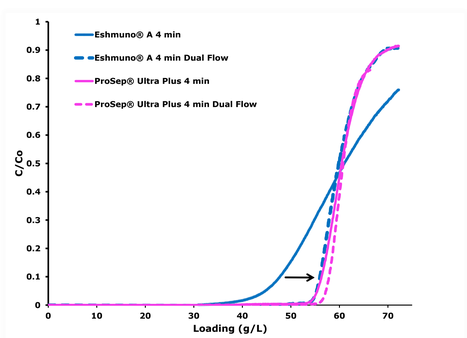
A processing strategy consisting of reducing the flow rate during a portion of the loading has been previously reported to increase the DBC of agarose media by 10% at a relatively long effective residence time, i.e. 9 min (Ghose et al., Biotechnol. Prog. 30 (6) 2014).

Both Eshmuno® A and ProSep® Ultra Plus approach a plateau in DBC at approximately a residence time (RT) < 6 min (data not shown). The rigid base matrix of these media allow for processing at low residence times (e.g. 3 min) for 20 cm bed heights. Two dual flow rate loading strategies, at a relatively short effective residence time, were evaluated for both media (Table below).

Dual Flow Rate Loading Cases Evaluated:

Case	RT (min)	Loading* %	RT (min)	Loading %	Effective RT (min)
1	3	75	7	25	4
2	3	25	4.3	25	4

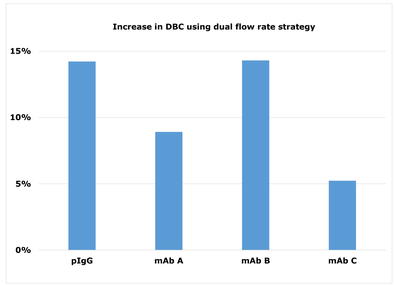
*Loading % is relative to10% BT at a RT of 4 min (single flow rate).



(Above) Dual flow rate loading Case 1 increased the steepness of the BT curve and made it comparable to ProSep® Ultra Plus. As a result the DBC at the same effective residence time increased by ~15%.

Strategy 1 (cont.)

The dual flow rate strategy used in Case 1 (pIgG) was evaluated with 3 monoclonal antibodies. As shown in the chart below the average increase in DBC (@5% BT) for these 3 mAbs was approximately 10%.



Strategy 2. Increasing target loading

The sharp BT curves of ProSep® Ultra Plus and Eshmuno® A with a dual flow rate loading suggest a low safety factor could be used, e.g. setting the target loading to 95% of 10% BT. This simple change could increase the utilization per cycle by 20% (table below). An even higher loading could be targeted (e.g. load to 5% BT) considering the mass of antibody in the flow through at this point is <2% of the total mass loaded (Becerra-Arteaga and Raghunath, ACS BIOT 2012) and the actual loading in multiple cycles would be below the start of the BT.

Resin & Load strategy	Target Loading (relative to 10% BT)	Loading (g/L)	Increased utilization
ProSep® Ultra Plus Single Flow	80	45	-
ProSep® Ultra Plus Single Flow	95	54	20%
Eshmuno® A Dual Flow	95	53	18%

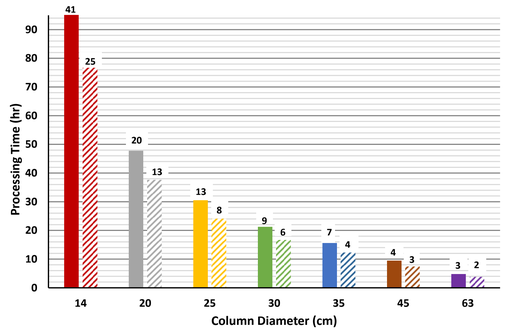
Process Modelling Case Study

Inputs:
Batch information: 2000 L, 2 g/L titer, 1 batch
Column bed height: 20 cm
Residence time: 4 min
Non-loading steps: 20 Column Volumes
Resin cost: $15,000/L

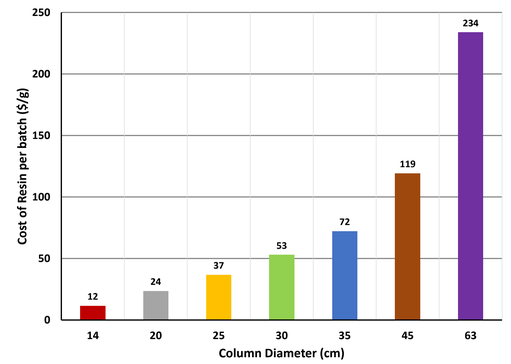
Target Loading:
Case A: 80% of 5% breakthrough (32 g/L)
Case B: 95% of 5% breakthrough dual flow rate loading (52 g/L)
Both loading scenarios are based on DBC data for Eshmuno® A

Outputs:
Processing time
Cycles required to process a batch
Resin cost contribution to production

Process Modelling Results



(Above) Solid and dashed bars represent Cases A and B, respectively. Numbers above the bars represent the cycles required to process the batch.



- The dual flow rate loading strategy combined with a reduced safety factor resulted in lower media volume requirements and process costs.
- In this case study a smaller column is required to meet common constraints for processing time < 24 hours and/or less than 6 cycles per batch.
- Savings in resin cost in either case are ≥ 35%.
- Comparable savings would be achieved for process simulations with 500 and 1000 L batch sizes (data not shown).

Conclusions

Typical safety factors for loading Protein A media in clinical manufacturing result in significant column underutilization.

A dual flow rate loading strategy with an effective residence time of 4 min increased the DBC for Eshmuno® A by up to 15%.

This dual flow strategy also increased the steepness of the BT curve for Eshmuno® A allowing the use of a reduced safety factor without loss of antibody in the flow through.

A reduced safety factor can be used with ProSep® Ultra Plus with a single or dual flow strategy.

These two strategies combined resulted in > 35% savings in resin cost according to the process cost model.

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