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 scale-up 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.

Methodology

Breakthrough curves and Dynamic Binding Capacities (DBC): Chromatography media: ProSep® Ultra Plus and Eshmuno® A are pre-packed in 5 mL columns.

Feeds: Purified Polyclonal IgG (pIgG) in PBS (2 g/L)

Both media 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 in Practice 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. 5 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).

<table>
<thead>
<tr>
<th>Case</th>
<th>Dual Flow Rate Loading</th>
<th>RT (min)</th>
<th>Loading %</th>
<th>Effective RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eshmuno® A 4 min</td>
<td>3</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Eshmuno® A 4 min Dual Flow</td>
<td>3</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

*Loading % is relative to 10% BT at a RT of 4 min (single flow rate).

In this work the use of a dual flow rate for loading to increase the DBC as well as 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.

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.

<table>
<thead>
<tr>
<th>Resin &amp; Load strategy</th>
<th>Target Loading (relative to 10% BT)</th>
<th>Loading (g/L)</th>
<th>Increased utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProSep® Ultra Plus Single Flow</td>
<td>80</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>ProSep® Ultra Plus Single Flow</td>
<td>95</td>
<td>54</td>
<td>20%</td>
</tr>
<tr>
<td>Eshmuno® A Dual Flow</td>
<td>95</td>
<td>53</td>
<td>18%</td>
</tr>
</tbody>
</table>

Process Modelling Case Study

Inputs:

Batch information: 2000 L, 2 g/L titre, 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

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.