Bench top continuous chromatography: an enabling platform for highly productive mAb purification

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Abstract
Simulated moving bed (SMB) chromatography and its variant multilolumn continuous chromatography (MCC) have the potential to elevate the industrial chromatographic platform by conversion of conventional batch processes to more efficient continuous processes. The high productivity, recovery and purity achieved by SMB chromatography on an industrial scale for small molecules hold promise for biomolecule manufacture.

We used a lab-scale SMB device to perform continuous Protein A Capture (PAC) for the purification of mAbs. Rather than using one large adsorbent column and a sequential batch protocol, the gains afforded by Step-SMB for processing high titer mAb, per column residence times must be decreased, flow rates and productivity increase. To take full advantage of the potential productivity of conventional batch processes to more efficient continuous processes.

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Unlike batch, Step-SMB is primarily a volume-driven process in which a given volume of feed material can be processed with the same amount of adsorbent, irrespective of its size. As mAb titers increase, flow rates and productivity increase. To take full advantage of the potential productivity gains afforded by Step-SMB for processing high titer mAb, per column residence times must be decreased while maintaining efficient capture.

For this study we purified a humanized IgG1 mAb from CHO culture fluid using the Octave System in a Step-SMB process and an AKTA System in a standard batch process. We compared the performance of an agarose-based Protein A adsorbent with two polyacrylamide-based Protein A adsorbents in both processes.

Step-SMB Chromatography

Figure 1. Octave™ Chromatography System

- Capable of performing SMB/MCC and other continuous chromatography protocols
- Runs up to 8 columns, up to 8 pumps
- Proprietary valve block design, 72 two-way valves, low dead volume; non-metallic flow path
- Scalable from 12 ml/min to 300 ml/min flow rates; grams to kilograms per run

Table 1. mAb purity after single column (SC) and Step-SMB PAC

<table>
<thead>
<tr>
<th>Protocol</th>
<th>MabSelect™ SuRe</th>
<th>TOYOPEARL™ AF-Protein A HC-650F</th>
<th>Am phera™ Protein A</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb load  (g)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Production (g/mL resin/h)</td>
<td>49.5</td>
<td>50.7</td>
<td>46</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>90</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td>Res. Time (min)</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Loading Flow Rate (ml/min)</td>
<td>250</td>
<td>160</td>
<td>343</td>
</tr>
<tr>
<td>mAb Loaded (mg/ml resin)</td>
<td>50</td>
<td>50</td>
<td>57</td>
</tr>
</tbody>
</table>

* Maximum linear velocity possible due to flow rate maximum of 500 cm/h for Equilibrium step

Table 2. Step-SMB PAC productivity with three capture resins at high mAb titters

Conclusions
1. The mAb produced from a Step-SMB PAC process performed on the Octave platform was equivalent or superior in quality to that produced by the analogous batch process. This finding was consistent between three resins: MabSelect SuRe (GE Life Sciences), TOYOPEARL AF-Protein A 650F, and TOYOPEARL AF-Protein A HC-650F (Tosoh Bioscience). See Table 1.
2. Two polyacrylamide Protein A capture resins exhibited high DBC at low residence times. The Step-SMB PAC process with 3-column capture zone configuration allowed a per column residence time of only 0.5 min while loading to capacities only possible with 3 min residence time using single-column capture. Combined with alkaline stability and rigid structure enabling high flow rates, these properties make the resins ideal for processing > 5 g/L mAb titer feedstocks using multilolumn continuous chromatography. See Figure 4.
3. TOYOPEARL AF-Protein A HC-650F demonstrated the best performance in Step-SMB at a mAb titer of 7.5 g/L; productivity was 97% g mAb/L resin/h with 97% yield. See Table 2.