Membrane Chromatography as a Robust Purification System for Large-Scale Antibody Production

Joe X. Zhou and Tim Tressel

Recombinant monoclonal antibodies (Mabs) have continued to increase in importance as therapeutics for the treatment of cancer and autoimmune diseases. Yet, the large-scale production of these molecules remains a challenge, and requires a manufacturing process that delivers a high yield and reliability as well as an extremely pure product. The manufacturing process must also control costs and unexpected operational issues. Robust manufacturing-scale unit operations are therefore required. In this review, the authors discuss one such example, the membrane adsorber (MA). They also introduce some basic concepts, briefly summarize the history of MA and describe the use of this technology in a late-stage monoclonal antibody purification process.

Downstream Antibody Purification

A typical large-scale purification process is often designed around the use of immobilized Protein A as the primary capture and purification step, in combination with other column operations (Figure 1). Protein A chromatography, in general, delivers a product-related purity of more than 99% — with most process impurities, including proteases, being washed away in the flow-through fraction. Because of this, the ensuing process operation units are considered as polishing steps, responsible for the separation of product-related isomers, and the removal of trace amounts of host cell proteins and recombinant DNA (HCP/rDNA) and viruses (1).

Viral inactivation
Separate product related isomers and remove trace amount of HCP/rDNA. Viral clearance capacity
Polishing steps

Several chromatography models have proven very useful for the removal of trace amounts of impurities and viruses. Among these, flow-through anion exchange (FT-AEX) is perhaps the most powerful tool that can be used to remove a variety of viruses, DNA and endotoxins. When operated at near-neutral pH and a low conductivity, many viruses, DNA, endotoxins and a large percentage of host cell proteins are negatively charged at neutral pH and will bind to the AEX resin. The typically basic (positively charged) protein A chromatography, in general, delivers a product-related purity of more than 99% — with most process impurities, including proteases, being washed away in the flow-through fraction. Because of this, the ensuing process operation units are considered as polishing steps, responsible for the separation of product-related isomers, and the removal of trace amounts of host cell proteins and recombinant DNA (HCP/rDNA) and viruses (1).

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Figure 1: The antibody downstream process.

Table 1. An example of buffer usage for Q packed bed chromatography and Q membrane chromatography.

<table>
<thead>
<tr>
<th>Items</th>
<th>5&quot; Q membrane (70 mL)</th>
<th>40 L column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-equilibration</td>
<td>NA</td>
<td>3 CV = 120 L</td>
</tr>
<tr>
<td>Equilibration</td>
<td>10 DV = 3.5 L</td>
<td>5 CV = 200 L</td>
</tr>
<tr>
<td>Wash</td>
<td>30 DV = 10.5 L</td>
<td>3 CV = 120 L</td>
</tr>
<tr>
<td>Regeneration</td>
<td>NA</td>
<td>3 CV = 120 L</td>
</tr>
<tr>
<td>Storage</td>
<td>NA</td>
<td>680 L</td>
</tr>
<tr>
<td>Total</td>
<td>14 L</td>
<td></td>
</tr>
</tbody>
</table>
than for conventional packed bed chromatography (Table I). The single-use MA is robust and simple to use, with the membrane column packing or subsequent cleaning validation required. Table II summarizes data for an IgG molecule processed with 34 Q membrane compared with a Q column.

A BRIEF HISTORY OF Q MEMBRANE CORROSION

In 2001, a thorough evaluation using a 10-layer Sartorius membrane stack-Swinnny-13 mm-fildder scale-down model for an antibody process was done by Knudsen et al. from Genentech, Inc. (5). The study reported that the Sartorius Q provided a 2.9-LRV reduction (LRV) of MuLV at a flow rate of 620 cm/h, at a capacity of 2000/L, sorbent, and about 550 g/m². The viral log reduction decreased when the antibody load increased. For example, an LRV of 1.0 was calculated when a capacity of 1100 g/m² was reached. A 0.5 LRV was reported for 300 g/m². By contrast, Q Sepharose FF provided a viral clearance power of >5.1 LRV at a capacity of 50 g/L. At approximately the same time, Parrish Galliher and Elizabeth Fowler from Millennium Pharmaceuticals, Inc. used the Sartorius Q capsule module (Q-10 inch) in large-scale antibody production for viral removal (6). The capacity was estimated to be 450 g/L in a cross-sectional flow rate for membrane resistance than Knudsen. Approximately 4.0 VLR was reported for Reso-3, MuLV, and PRV.

In 2004, John Pieracci et al. from Biogen Idec, Inc. presented their antibody process with a Q membrane, the making a rate of 4 m³/cm² at pH 8.0 with a fluid velocity of 40 MV/min as the last polishing step (7). The load capacity was estimated to be 587 g/m² or 2.7 mL/membrane volume. An antibody recovery of about 100% was reported for three lots of 8.7 kg, 5.6 kg and 4.2 kg, respectively. The monitored outputs were antibody recovery (90–99%), membrane pressure drop (5–9 psig) and host cell protein removal (12–23 ppm in the flow-through pool). A viral clearance of 5.4 to 6.5 logs for MuLV was reported.

Recently, Zhang et al. from Abgenix described the use of a Sartobind Q in an antibody pilot plant run to produce material for a toxicity study. The scale-down model used the Sartobind Q75. The detailed study demonstrated that Sartobind Q75 has enough power to remove host cell (HCP) and viruses at a neutral pH and a conductivity below 3 mS/cm. At these conditions, excellent viral removal data were reported in their large-scale antibody process: 5.57 LRV for MuLV, 7.28 LRV for Reto-3, 6.77 LRV for MMV and 5.67 LRV for PRV. The process capacity was estimated to be 480 g/m² or 1750 g/L membrane volume (8). However, with a higher conductivity in the feed solution, the HCP clearance decreased (9).

In summarizing the output from different antibody processes using a Q membrane chromatography, there are several questions that still need to be addressed.

• What is the best description of flow rate for membrane chromatography?
• What is the process capacity needed for a critical economic comparison between the two models in antibody process scale?
• What are the key parameters when using Q membrane for process-scale antibody production?

These concepts and parameters have been successfully applied to purify four lots of recombinant human antibody at the 2000 L scale. The typical feed material has total impurities — such as DNA and HCP — of less than 1%. Thus data from the viral clearance scale-down model should be used to determine the process capacity. Scale-down studies with defined operating conditions (flow rate, buffers, feed stock, temperature) are performed to determine this capacity. The use of at least two viruses is suggested for membrane capacity determination. Ideally, one is small, such as the enveloped virus MVM with a 20 nm particle size, and another is larger, such as the enveloped virus MuLV with a 100 Q membrane.

Theoretically, a membrane volume should be used to estimate the membrane’s capacity for impurities removal. However, the multiple layers of a membrane configuration provide excess binding capacity to reduce the trace impurities, thereby still lead to the MA in a flow-through mode during an antibody polishing step. It really does not matter if the membrane thickness of 25 cm or 0.01375 cm is used. This concept, the membrane is similar to a single sheet cross flow filter. In addition, total surface area can easily be used to compare the performance of membranes from different vendors. Furthermore, the membrane pricing for different module sizes is based on the total surface area used for a process.

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Format. Thus, linear velocity as a flow unit should be used as a common term in membrane chromatography.

Cross-sectional area and total surface area: Linear velocity for membrane chromatography is calculated according to the cross-sectional area surface area only. Total membrane surface should not be used directly for a flow rate. Each membrane module has its own cross-sectional surface area provided by vendors.

mL/min = Linear velocity (cm/h) x cross-sectional surface area (cm²)/60 min

Linear velocity (cm/h) = mL/min x 60 min/cross-sectional surface area (cm²)

Q membrane process capacity and its unit: The main purpose of the AEX step is efficient viral reduction. The typical feed material has total impurities — such as DNA and HCP — of less than 1%. Thus data from the viral clearance scale-down model should be used to determine the process capacity. Scale-down studies with defined operating conditions (flow rate, buffers, feed stock, temperature) are performed to determine this capacity. The use of at least two viruses is suggested for membrane capacity determination. Ideally, one is small, such as the enveloped virus MVM with a 20 nm particle size, and another is larger, such as the enveloped virus MuLV with a 100 Q membrane.

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A team from Amgen Inc. and Sartorius, Inc. redesigned a scale-down model for the Q membrane, the Q-125 (10–12). With the new scale-down model, the Amgen Inc. team was able to achieve a process capacity of >8000 g/m² (total membrane protein mass processed per square meter of membrane) or >10.7 kg/L (membrane volume) with a MVM viral log reduction of >6.0 and a MuLV viral log reduction of >5.0. These concepts and parameters have been successfully applied to purify four lots of recombinant human antibody at the 2000 L scale. The typical feed material has total impurities — such as DNA and HCP — of less than 1%. Thus data from the viral clearance scale-down model should be used to determine the process capacity. Scale-down studies with defined operating conditions (flow rate, buffers, feed stock, temperature) are performed to determine this capacity. The use of at least two viruses is suggested for membrane capacity determination. Ideally, one is small, such as the enveloped virus MVM with a 20 nm particle size, and another is larger, such as the enveloped virus MuLV with a 100 Q membrane.

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are extremely encouraging when considering the replacement of a Q packed bed column with a membrane adsorber as a polishing step in a flow-through chromatography model for process-scale antibody production.

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References


