Revisiting Protein A Chromatography
Reviewing Next-Generation Capture of Monoclonal Antibodies

Piranavan Thillaivinayagalingam, Keara Reidy, Anna Lindeberg, and Anthony R. Newcombe

Due to the molecular complexity of monoclonal antibodies (MAbs) and potential impurities in cell culture media before purification (host-cell proteins, DNA, media components) (1), subsequent downstream operations must consistently and reproducibly purify products to ensure safety and efficacy.

The latest member of GE Healthcare’s MabSelect family is called MabSelect SuRe LX (2). As Table 1 shows, it has been developed using the same highly cross-linked agarose base matrix and protein A ligand as for other MAb affinity resins (Table 1). Here, we provide an overview of a novel system for MAb capture from a user’s perspective.

**Characteristics**

Interaction involves fragment B of protein A, which establishes a highly selective interaction with IgG and is composed of three alpha helices. As Figure 1 shows, side-chain residues on the binding surfaces of two helices contribute to most of the interactions with IgG (3). Using the same product-contact materials as previous MabSelect resins provides some familiarity to quality control laboratories that have developed, qualified, and validated protein A assays used to evaluate ligand leakage. Extensive analytical validation is unlikely to be required if the new resin is considered as an alternative for commercial production (4).

The ligand density of this new adsorbent has been increased and optimized, with reported dynamic binding capacities (with polyclonal and monoclonal human IgGs) of ≥60 g/L (5). One potential limitation is that longer residence times during column loading (>6 minutes) are required. This time is to permit interaction with protein A coupled to the inner surfaces of the beads. Linear flow rates could be increased for other steps of the chromatographic process (such as equilibration, washing, regeneration, etc). Therefore the slower flow rates during product loading are unlikely to have a significant impact on process capability at production scale.

Reported data indicate that the adsorbent’s binding capacity is independent of MAb feed concentration, with comparable resin binding capacity at feed concentration of 1–10 g/L IgG expressed in a CHO cell supernatant. The concentration independent performance is of interest, particularly if higher-titer MAbs are expressed for commercial processes and smaller-diameter columns are used in downstream processing steps. Pressure flow characteristics and alkaline stability of the protein A is also reportedly maintained, with an extended lifetime for MabSelect SuRe LX media (80% of the dynamic binding capacity reported after 150 cycles with cleaning buffer consisting of 0.5M NaOH) (6). With an increased density of protein A ligand, analysts might anticipate an increase in leached ligand, particularly during the first few affinity cycles. Low levels of leached protein A (10–15 ppm) have been reported. These levels are higher than those...
Increasing protein A

At least 30 mg human IgG/mL

6 min residence time

Recombinant protein A

50V

3–12 (long term), 2–14 (clean-in-place)

MabSelect SuRe LX (MabSelect media, technical information, GE Healthcare).

<table>
<thead>
<tr>
<th>Adsorbent Property</th>
<th>MabSelect SuRe</th>
<th>MabSelect SuRe LX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand*</td>
<td>Recombinant protein A</td>
<td>Recombinant protein A</td>
</tr>
<tr>
<td>Average particle size $d_{avg}$</td>
<td>85 μm</td>
<td>85 μm</td>
</tr>
<tr>
<td>Ligand coupling method</td>
<td>Epoxy activation</td>
<td>Epoxy activation</td>
</tr>
<tr>
<td>Matrix</td>
<td>Highly cross-linked agarose</td>
<td>Highly cross-linked agarose</td>
</tr>
<tr>
<td>Dynamic binding capacity$^c$</td>
<td>At least 30 mg human IgG/mL</td>
<td>~60 mg human IgG/mL</td>
</tr>
<tr>
<td>Residence time</td>
<td>2.4 min residence time</td>
<td>6 min residence time</td>
</tr>
</tbody>
</table>

* Details of agarose cross-linking, protein A ligand density and coupling orientation are proprietary and have not disclosed by the manufacturer. $^b$ $d_{avg}$ is the median particle size of the cumulative volume distribution. $^c$ Value is determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a column with a bed height of 20 cm for MabSelect SuRe and at 100 cm/h in a column with a bed height of 10 cm for MabSelect SuRe LX (MabSelect media, Technical Information, GE Healthcare).

Figure 2: Ribbon and space-filling model of the structure of the protein A-Fc complex show the interaction between fragment B of protein A and the CH3-CH2 domains of IgG1 (8).
HCIC adsorbents may be more difficult to apply as a MAb platform process.

Researchers have developed synthetic protein A ligands based on the hydrophobic IgG binding domain of protein A using techniques such as protein engineering, phage display, and synthetic chemistry (11). Initial studies showed that capture of antibodies using a protein A synthetic resin could yield comparable product quality and throughput (13). More comprehensive studies, however, showed that synthetic ligands generally reduce selectivity, affinity, and a degree of nonspecific adsorption compared with biological ligands such as protein A (11, 14, 15). Some studies demonstrated differences in selectivity between two antibodies (15), resulting in the need for operational and/or process modifications for similar MAb products.

Synthetic ligand adsorbents are potential alternatives to protein A, but they may be challenging to implement as part of a platform process for commercial antibody purification. Although the capital expenditure required for MabSelect resin may not be insignificant and primary capture may require the highest level of capital investment for a typical MAb platform process, it may represent only ~3% of total manufacturing costs (10).

Furthermore, if the new generation of protein A resins are considered (e.g., MabSelect SuRe and MabSelect LX brands) with greater ligand density and stability permitting ≥150 reuse cycles (6), then the associated contribution of the resin to the cost of goods per cost per batch is reduced. Such cost then may be comparable with those of single-use virus removal filters (depending on column/batch size) often used in MAb manufacturing. Replacing protein A with a less expensive ‘alternative’ could reduce consumable costs, but it may not have a significant impact on cost of goods because of resulting productivity losses (10).

**Options for the Next Generation**
The potential decline of the dependence on affinity chromatography for the primary capture of MAbs may have been quelled — for now. But synthetic-ligand, mixed-mode, or protein A affinity adsorbents may need to evolve further still to meet ever increasing demands. Some options for a next-generation adsorbent development could include higher ligand density, alternative ligand orientation and accessibility, changes in bead and pore size, or modified support matrices that demonstrate increased size and potential mass transfer (15). Although protein A affinity adsorbents may be here to stay, investment in subsequent adsorbent evolution for the primary capture of antibodies may be driven by throughput requirements, process economics, and the number of monoclonals progressing to commercial manufacture.

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**References**

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