mAb polishing step development using Capto™ adhere ImpRes in bind-elute mode

MULTIMODAL CHROMATOGRAPHY RESINS

Introduction

With tighter and tighter timelines in the development of downstream processes for biopharmaceuticals, developing a platform approach has become increasingly important. The similar properties of monoclonal antibodies (mAbs) allow for such a platform approach to processing, based on the wealth of information available from a wide variety of mAb applications.

The industrial standard and the most standardized step is the first, the capture/purification step, based on protein A chromatography. The greatest variability between different mAb purification platform processes usually occurs in the intermediate and polishing purification step(s). Following the capture purification on protein A resin and low pH hold step (for virus inactivation), the concentration of the mAb product is relatively high compared with the level of impurities, which are typically present at only a few percent or less. These impurities include host cell proteins (HCP), low levels of leached protein A ligand, product-related impurities such as modified antibody forms or aggregates, and trace levels of host cell DNA.

Capto™ adhere ImpRes is a BioProcess™ chromatography resin for high-resolution polishing of mAbs as well as other biomolecules. The strong anion exchange multimodal ligand displays high selectivity (compared with traditional ion exchangers) allowing efficient separation of the main contaminants in mAb processes such as DNA, HCP, leached protein A, aggregates, and viruses.

These features allow for a two-step purification process of mAbs, with Capto™ adhere ImpRes in the polishing step (Fig 1). If needed to reach the specified purity, Capto™ adhere ImpRes can also be used in combination with anion or cation exchangers or other multimodal chromatography resins.

Utilizing the full power of multimodal ligands is sometimes perceived as requiring rigorous optimization procedures. However, by adaptation of straightforward principles based on years of experience of mAb polishing using the adhere ligand, rapid process designs can be achieved. As a result, it is possible to generate data in early development studies that can be used for further optimization in later stages of development.

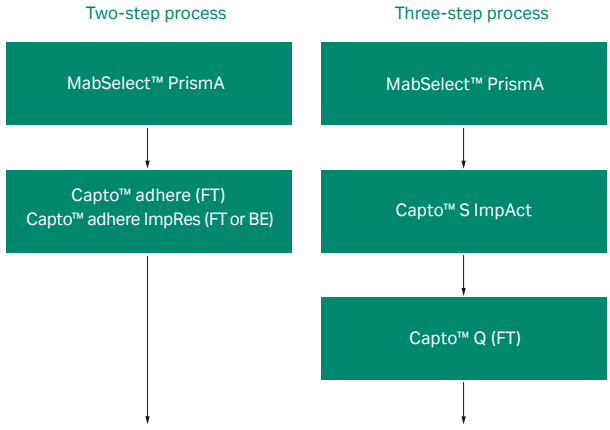


Fig 1. Platform process alternatives for mAbs based on two or three chromatographic steps. Capto™ adhere ImpRes can be operated both in flow-through (FT) and bind-elute (BE) mode.

Bind-elute mode

In bind-elute mode both loading and elution conditions should be optimized. The purpose of this procedure is to provide a starting point for such optimization work and to offer a quick approach to designing a chromatographic purification protocol in bind-elute mode.

Screening of loading and elution conditions is recommended using design of experiments (DoE) and high-throughput process development (HTPD) tools, such as PreDictor™ 96-well filter plates or PreDictor™ RoboColumn™ units. DoE is a systematic approach

to study how variation in experimental factors affects the responses of a system, and is used to plan experiments so that the maximum amount of information can be obtained from a minimum number of experiments. An example of such method development using Capto™ adhere ImpRes in bind-elute mode is given in reference 1.

The simplified method development described in this procedure is less extensive but should be applicable for a majority of mAbs. The method involves four main steps: 1) establishing loading conditions, 2) determining dynamic binding capacity, 3) gradient elution, and 4) step elution.

Establishing loading conditions

To find suitable loading conditions, an initial experiment is performed using a pH gradient for elution. Capto™ adhere ImpRes is packed in a Tricorn™ 5/50 column to a bed height of 5 cm.

Sample: mAb pool, initially purified on protein A resin, is buffer exchanged on HiTrap™ Desalting column to 20 mM phosphate, 20 mM citrate pH 7.8

Loading buffer: 20 mM phosphate, 20 mM citrate pH 7.8

Elution buffer: 20 mM phosphate, 20 mM citrate pH 4.0

1. Equilibrate the column with loading buffer.
2. Sample load: 1 mg mAb/mL resin.
3. Elute in a linear gradient for 20 column volumes (CV) from loading buffer to elution buffer.
4. Re-equilibrate with loading buffer for 5 CV.

The elution position for the mAb (i.e., the pH at peak maximum), defines the "elution pH". The elution pH is used for defining loading conditions for binding capacity studies.

Determination of dynamic binding capacity

Dynamic binding capacity (DBC) is determined by frontal analysis, according to a standard procedure.

Sample: mAb pool, initially purified on protein A resin, and buffer exchanged on HiPrep™ 26/10 Desalting column to loading buffer

Loading buffer: 20 mM phosphate, pH of the loading buffer should be elution pH + 2 pH units (2)

Elution buffer: 0.1 M acetate pH 3.0

System and column recommendation: ÄKTA™ avant 25 system with prepacked HiScreen™ Capto™ adhere ImpRes column, or Tricorn™ 5/50 column packed with Capto™ adhere ImpRes (bed height ≥ 5 cm). The UV absorbance at 280 nm (A_{280}) is used for determination of breakthrough. Before frontal analysis, the mAb solution is injected (bypassing the column) to obtain a maximum absorbance value (3).

1. Equilibrate the column with loading buffer.
2. mAb is loaded to the column using a residence time of 4 min (0.25 mL/min for a 1 mL column) until > 10% breakthrough.
3. Wash out of unbound material with 3 CV loading buffer.
4. Strip with 0.1 M acetate pH 3.0, 5 CV.
5. Re-equilibrate with loading buffer, 5 CV.

The DBC at 10% breakthrough ($DBC_{10\%}$) is calculated according to:

$$DBC_{10\%} = (V_{10\%} - V_0) \times C_0/V_c$$

where $V_{10\%}$ = load volume (mL) at 10% breakthrough, V_0 = void volume (mL), C_0 = mAb concentration in the sample (mg/mL), and V_c = volumetric bed volume (mL).

Notes:

1. To avoid deamidation of the mAb, pH should normally be maintained below 8.
2. If the mAb is unstable or aggregates at high pH, a lower pH should be used.
3. If the absorbance at 280 nm in bypass is > 1000 mAU, change wavelength to 254 nm.

Gradient elution

Gradient elution experiments are typically the initial step used to determine elution conditions.

Sample: mAb pool, initially purified on protein A resin, is buffer exchanged on HiPrep™ 26/10 Desalting column to 20 mM sodium phosphate at loading pH (as defined above)

Loading buffer: 20 mM phosphate at loading pH

Elution buffer: 20 mM phosphate, 20 mM citrate pH 4.0

Strip buffer: 0.1 M acetate pH 3.0

1. Equilibrate the column with loading buffer.
2. Load 70% of $DBC_{10\%}$ using a residence time of 4 min (0.25 mL/min in a 1 mL column).
3. Wash out unbound material with 2 CV of loading buffer.
4. Elute in a linear gradient for 20 CV from loading buffer to elution buffer.

   Collect fractions and measure aggregate, HCP, and protein A concentration. Pool according to desired recovery and purity. If purity level is not achieved with 70% load, use lower load and/or a shallower gradient.

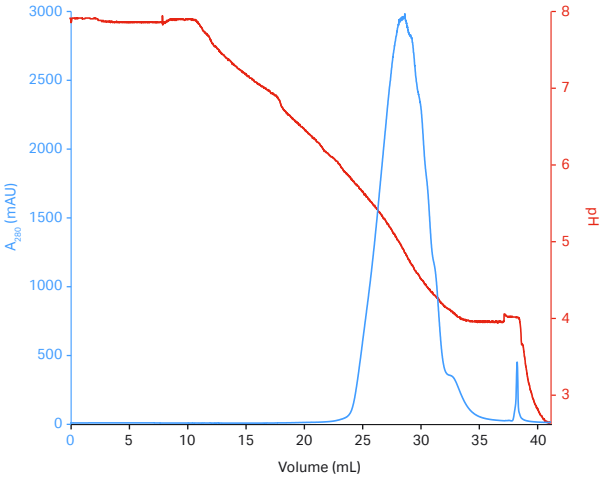


Fig 2. Chromatogram using gradient elution.

5. Strip with 0.1 M acetate pH 3.0, 5 CV.
6. Re-equilibrate with loading buffer, 5 CV.

Figure 2 shows the chromatogram resulting from the methods described above.

Step elution

By analyzing impurity levels in collected fractions from the gradient elution experiment, a suitable elution pH can be identified. Elution pH should be slightly higher (+ 0.2 pH units) than the breakthrough of critical contaminants. Any delay of pH in the chromatography system has to be considered.

Sample: mAb pool, initially purified on protein A resin, is buffer exchanged on HiPrep™ 26/10 Desalting column to 20 mM sodium phosphate at "loading pH" as defined above

1. Equilibrate the column with loading buffer as defined above.
2. Load 50% to 70% of $DBC_{10\%}$ using a residence time of 4 min (0.25 mL/min in a 1 mL column).
3. Wash out unbound material with 2 CV of loading buffer.
4. Elute with 5 CV of appropriate elution buffer.

   Collect fractions and measure, for example, aggregate level, HCP, protein A, and monomer yield. Pool the fractions according to desired purity. If purity level is not achieved, use a higher elution pH. If yield is too low or the elution peak is too broad, use a lower elution pH.
5. Strip with 0.1 M acetate pH 3.0, 5 CV.
6. Re-equilibrate with loading buffer, 5 CV.

Analyses and responses

Typical analyses or responses are recovery (%), aggregate content (%), HCP levels (ppm), and protein A levels (ppm), but this might vary depending on the requirements for the specific process.

For details on the analytical methods, see reference 4.

Total recovery: Calculated after the gel filtration ([GF] also referred to as size-exclusion chromatography [SEC]) step, by A_{280} measurements as amount eluted/amount loaded

Aggregate content: Flowthrough is analyzed by GF using two interconnected Superdex™ 200 5/150 GL columns connected to an ÄKTA™ system. Amount of aggregate is obtained from UNICORN™ control software as "percent of total peak area" after integration of the chromatogram

HCP and protein A levels: Determined using commercial ELISA assays, such as anti-CHO HCP antibodies (Cygnus Technologies Inc.) for HCP and MabSelect SuRe™ protein A ligand leakage kit (Repligen Corp.)

Conclusions

When developing a platform approach to biopharmaceutical production processes, the recommended path is to adopt DoE and HTPD to identify optimal process conditions. The general steps described here may be used as a starting point for customized step development. Consideration of the known properties of the specific mAb should always be taken into account. For further assistance with step or process development, please contact your local Cytiva sales representative or Cytiva's Fast Trak™ organization.

Tips and tricks

- If mAb elutes during the wash step, use buffer with lower conductivity and/or avoid buffer mixtures including Tris. Loading buffers using buffer mixtures resulting in high conductivities (> 4 mS/cm) can result in high binding capacities but mAbs might leak from the column during washes and/or early in the gradient
- Sample load using high salt concentration (500 to 1000 mM) can improve binding capacity and could also result in improved HCP clearance
- Binding capacity of Capto™ adhere ImpRes is influenced by ionic strength. As a consequence, effects of buffers at a given pH should be measured at equivalent ionic strength
- Tris buffer might give lower binding capacity than phosphate buffer with equivalent pH and ionic strength. Tris buffer can also give multiple peaks during gradient elution and might in some cases improve separation of charged isoforms
- Addition of salt (e.g., 100 mM NaCl) to the elution buffer can result in sharper and bolder peaks

References

1. Application note: Polishing of monoclonal antibodies using Capto™ adhere ImpRes in bind and elute mode, Cytiva, CY13429.
2. Handbook: High-throughput process development with PreDictor plates, Cytiva, CY16051.
3. Ljunglöf, A. *et al*. Rapid process development for purification of a mAb. *BioProcess International* **9**, 62–68 (2011).
4. Application note: Optimization of loading conditions on Capto™ adhere using design of experiments, Cytiva, CY13251.

Related literature

Data file: Capto™ adhere, Cytiva, CY11848.

Procedure: mAb polishing step development using Capto™ adhere in flow-through mode, Cytiva, CY20505.

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