Procedure

mAb polishing step development using Capto™ MMC ImpRes resin in bind/elute mode

MULTIMODAL CHROMATOGRAPHY RESINS

Introduction

Capto™ MMC ImpRes resin is a multimodal cation exchange chromatography resin (MMC) that has a high selectivity compared to traditional ion exchangers. This document provides you with recommendations to optimize conditions for removing impurities such as host cell protein (HCP), leached protein A, and aggregates. The approach described applies to polishing of mAbs, bispecific antibodies (bsAbs), and other large biomolecules using Capto MMC ImpRes multimodal chromatography resin in bind/elute mode.

Figure 1A shows two typical processes for purifying mAbs. In challenging separations such as for bsAbs, other chromatography purification techniques — such as those using multimodal (mixed-mode) cation exchange resins — may be required to reach the desired purity of your target protein (Fig 1B). As multimodal chromatography enables multiple modes of interaction between the ligand and the target molecule, you will need to screen pH and salt conditions to find the optimal conditions.

Bind/elute mode

In bind/elute mode, both loading, wash, and elution conditions should be screened to quickly determine the range for design of experiments (DoE) to be performed in columns. The procedure described below can identify the starting point for optimized binding and elution conditions if high-throughput process development (HTPD) tools are not available. The procedure involves four main steps:

1. Establishing load conditions
2. Determining dynamic binding capacity (DBC)
3. Gradient elution (low and high load)
4. Step elution

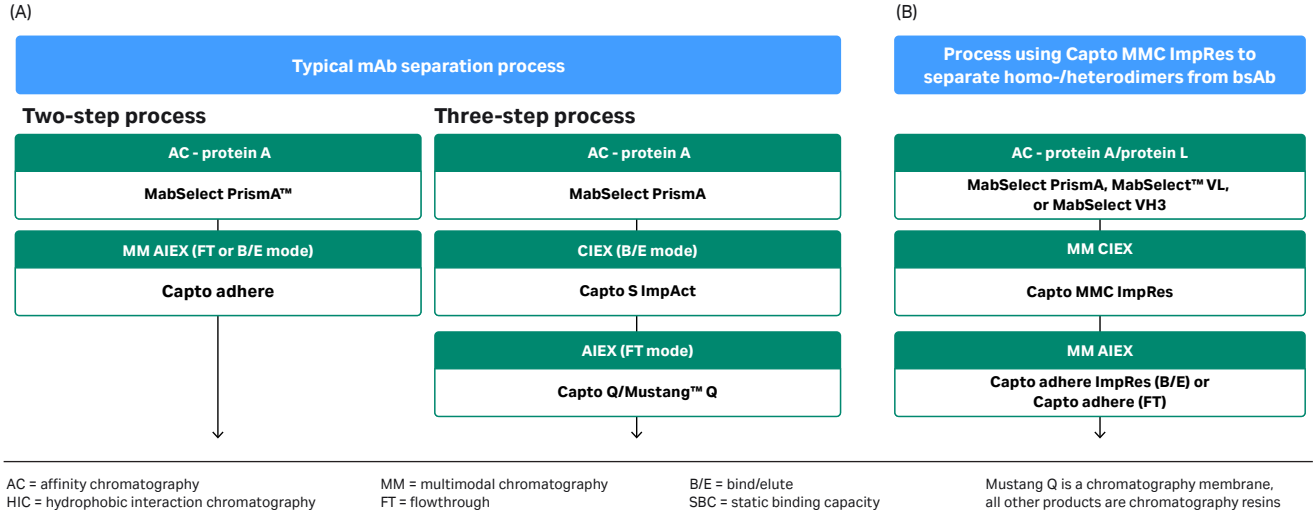

Fig 1. Multimodal Capto MMC ImpRes resin has novel selectivity and is well-established in challenging separations such as the removal of mispaired species from bsAbs. (A) In the Cytiva mAb toolbox, we recommend either a two- or three-step process for mAb purification. (B) Recommended purification process for separating homo-/heterodimers from bsAb using the novel properties of multimodal resins.

Establishing loading conditions

To find suitable loading conditions, an initial experiment is performed using a pH gradient for elution. Capto MMC ImpRes resin is packed in a Tricorn™ 5/100 chromatography column to a bed height of 10 cm. At this stage, residence time (and flow rate used) is less important. You may perform a relatively fast run at this low bed height. The chromatography running conditions used in this example were:

Column:	Tricorn 5/100 column self-packed with Capto MMC ImpRes resin, 10 cm bed height, or prepacked HiScreen™ Capto MMC ImpRes column
Sample:	mAb pool, initially purified on protein A resin, is buffer exchanged on HiTrap™ Desalting column to loading/wash buffer.
Sample load:	1 mg mAb/mL resin
Loading/wash buffer:	Use buffer with 2 pH units below pI of target molecule; 50 mM acetate at pH 5.0 to 6.0 or 25 mM phosphate at pH 6.0 to 8.0
Elution buffer:	Loading/wash buffer + 500 mM NaCl.
Strip buffer:	25 mM phosphate, pH 8.0 + 1000 mM NaCl
Cleaning-in-place (CIP) agent:	500 mM NaOH
Gradient:	Elution buffer, 0% to 100%
System:	ÄKTA avant™ or ÄKTA pure™ 25 chromatography systems

1. Equilibrate the column with loading/wash buffer.
2. Sample load: 1 mg of mAb/mL resin.
3. Use a flow rate that gives 4 to 6 min of residence time.
4. Wash: Wash the column with 3 CV of loading/wash buffer.
5. Elute in a linear gradient from 0% to 100% of elution buffer for 20 column volumes (CV) and keep it at 100% for 3 CV.
6. Strip the column with 5 CV of strip buffer, followed by 5 CV of 500 mM NaOH CIP agent.
7. Re-equilibrate with loading buffer for 5 CV.

Use absorbance at 280 nm (A_{280}) to track the antibody. The elution position for the antibody (i.e., the pH at A_{280} peak maximum) should be in the middle of the gradient. If elution is in the first third of the gradient (or not binding), decrease the pH by 0.5 to 1 pH unit. If elution is in the last third of the gradient (or not eluting at all), increase the pH by between 0.5 and 1 pH units. The pH where your peak elutes in the middle of the gradient should be used as the loading buffer in the following experiments.

Determination of dynamic binding capacity

DBC is determined by frontal analysis according to a standard procedure using our recommended running conditions:

Column:	Tricorn 5/100 column packed with Capto MMC resin (bed height 10 cm) or HiScreen Capto MMC ImRes prepacked column
Sample:	mAb pool, initially purified on protein A resin, and buffer exchanged on HiPrep™ 26/10 Desalting column to loading buffer
Loading buffer:	As defined in the section above, *Establishing loading conditions*
Elution buffer:	Loading buffer + 500 NaCl
Strip buffer:	25 mM phosphate, pH 8.0 + 1000 mM NaCl
CIP:	500 mM NaOH
System:	ÄKTA avant or ÄKTA pure 25 chromatography systems

Suggested steps to determine DBC

1. Pump the sample in bypass of columns until A_{280} is stable. If your UV signal is > 1200 mAU, dilute the sample with loading buffer until the signal is < 1200 mAU or change the absorbance wavelength to 254 nm. The absorbance at which your signal is stable is defined as UV_{max}. Remove the sample from your chromatography system by rinsing with loading buffer.
2. Equilibrate the column with loading buffer.
3. mAb is loaded to the column using a residence time of 6 min at A_{280} until you reach 10% of UV_{max} (10% breakthrough).
4. Wash out unbound mAb with 3 CV of loading buffer.
5. Elute in a step with elution buffer, 5 CV.
6. Strip the column with 5 CV of strip buffer, followed by 5 CV of CIP agent.
7. Re-equilibrate with loading buffer for 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).

If the determined DBC is lower than expected, try to decrease pH by 0.5 pH units to see if your DBC improves.

Gradient elution

Gradient elution experiments at a relevant load are typically the initial step you should use to determine elution conditions. Use the following running conditions:

Column:	Tricorn 5/100 column packed with Capto MMC ImpRes resin, 10 cm bed height, or prepacked HiScreen Capto MMC ImpRes column
Sample:	mAb pool, initially purified on protein A resin, and buffer exchanged on HiPrep 26/10 Desalting column to loading/wash buffer
Loading/wash buffer:	As defined in *Establishing loading conditions* above
Elution buffer:	Loading/wash buffer + 500 NaCl
Strip buffer:	25 mM phosphate, pH 8.0 + 1000 mM NaCl
CIP:	500 mM NaOH
System:	ÄKTA avant or ÄKTA pure 25 chromatography systems

1. Equilibrate the column with loading buffer.
2. Load sample to 70% of the $DBC_{10\%}$ as determined in the section above, *Determination of dynamic binding capacity*.
3. Wash out unbound material with 3 CV of wash buffer.
4. Elute in a linear gradient for 20 CV from 0% to 100% of elution buffer, and keep it at 100% for 3 CV.
5. Collect fractions and measure aggregate, HCP, and mAb concentration. Pool according to desired recovery and purity.
7. Strip the column with 5 CV of strip buffer followed by 5 CV of CIP agent.
8. Re-equilibrate with loading/wash buffer for 5 CV.

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

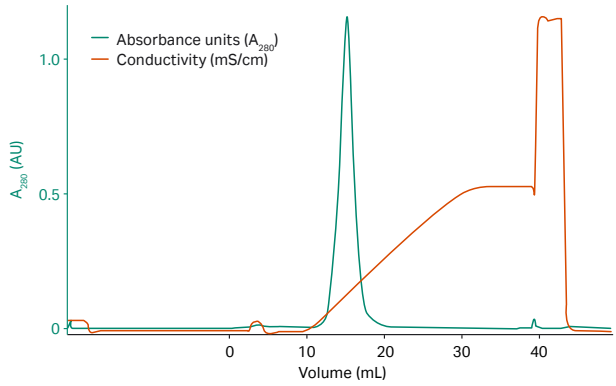

Fig 2. Chromatogram using salt gradient elution. Absorbance at 280 nm is shown on the left axis and conductivity is shown on the right axis. A reduction of pH with 0.5 to 1.0 pH units could be considered in this example to achieve elution of the peak in the middle of the gradient.

Step elution

Using the gradient elution analysis as a guide, the step elution conductivity should be between the start of the elution and the conductivity at A_{280} peak max. of the elution peak from the gradient run. Any delay of system hold-up volumes in the chromatography system must be considered.

Column:	Tricorn 5/100 column packed with Capto MMC ImpRes resin, 10 cm bed height, or HiScreen Capto MMC ImpRes column.
Sample:	mAb pool, initially purified on protein A resin, and buffer exchanged on HiPrep™ 26/10 Desalting column to loading buffer.
System:	ÄKTA avant or ÄKTA pure 25 chromatography systems

We recommended the buffers described in *Gradient elution*.

1. Equilibrate the column with the loading/wash buffer as defined in *Gradient elution*.
2. Load sample to 70% of the $DBC_{10\%}$ as determined in the section *Determination of dynamic binding capacity*.
3. Wash out unbound material with 3 to 5 CV of loading buffer.
4. Elute with 5 CV of elution buffer. Collect fractions and measure, for example, aggregate, HCP, and leached protein A concentrations, as well as monomer yield. Pool the fractions according to your desired purity. If the level of desired purity is not achieved, use a lower elution pH (or NaCl concentration). If the yield is too low or the elution peak is too broad, use a higher elution pH (or NaCl concentration).
5. Strip the column with 5 CV of strip buffer, followed by 5 CV of CIP agent.
6. Re-equilibrate with loading/wash buffer, 5 CV.

Important considerations

- To avoid deamidation of your mAb, you should maintain a pH below 8.0.
- If the mAb is unstable or aggregates are formed at high pH, use a lower pH.
- If mAb elutes during the wash step, use a buffer with lower conductivity and/or avoid buffer mixtures. Loading buffers using buffer mixtures leading to high conductivities (> 4 mS/cm) could result in high binding capacities, but mAb might leak from the column during washes and/or early in the gradient elution.
- Binding capacity of Capto MMC ImpRes resin is influenced by ionic strength. Consequently, the effects of buffers at a given pH should be measured at equivalent ionic strength.
- Addition of salt (e.g., 100 mM NaCl) to the elution buffer can result in sharper peaks.
- A reduced pH often gives a higher DBC. To increase throughput, load the sample at a lower pH and adjust both pH and salt during elution.

Conclusions

When developing a biopharmaceutical production process, we recommend that you adopt HTPD and DoE to identify optimal process conditions. However, you can use the general steps described here as a starting point for customized step development when HTPD tools are not used. The known properties of the specific mAb should always be considered. For further assistance, please contact your local Cytiva sales representative or our Fast Trak™ organization.

cytiva.com

CY38591-11Jan24-PD