mAb polishing step development using Capto™ adhere in flow-through mode

CHROMATOGRAPHY RESINS

Introduction

The similar properties of different monoclonal antibodies (mAbs) allow a platform approach. Working from the wealth of information from other mAb applications means that processes often do not need to be developed from scratch. 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 resins and low pH hold step (for virus inactivation), the concentration of the mAb product is relatively high compared to the level of impurities, which are typically present at only a few percent or less. These impurities include host cell proteins (HCP), low level of leached Protein A ligand, product-related impurities such as modified antibody forms or aggregates, and trace levels of host cell DNA.

Capto™ adhere is a multimodal BioProcess™ resin specifically designed for post-Protein A purification of mAbs at process scale (1). The strong multimodal ligand gives a different selectivity compared to traditional ion exchangers. It is typically recommended to run the Capto™ adhere step in flow-through mode, meaning that the mAb goes in the flow-through fraction and the impurities bind to the ligand. However, Capto™ adhere can also be used in Bind/Elute mode, and due to the novel selectivity from the multimodal ligand, Capto™ adhere has also been found to be useful in non-mAb applications. If needed to reach the specified purity, Capto™ adhere can also be used in combination with anion or cation exchange chromatography for polishing. If the load material contains high levels of aggregates and/or the mAb tends to aggregate it is recommended to put the Capto™ adhere step as the last chromatographic step in the process (Fig 1).

The higher complexity of this multimodal ligand – compared to traditional ion exchangers – provides extensive benefits but also requires somewhat more process optimization in order to take full advantage of the high potential. For Capto™ adhere in flow-through mode, it is primarily the loading conditions that need to be screened. The purpose of this Procedure is to give a starting point for such optimization work.

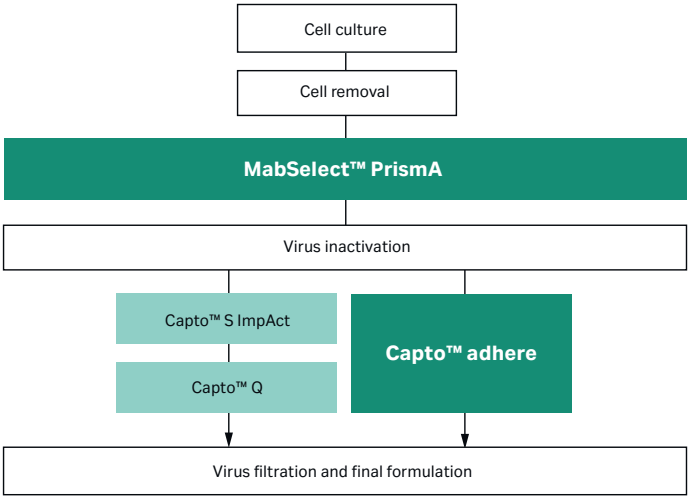

Fig 1. Platform process examples for mAbs based on two or three chromatographic steps.

Simplified process development

Optimization of loading conditions is preferably performed by using Design of Experiments (DoE). 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. By systematically varying important parameters (e.g., pH, conductivity, sample load) response surfaces can be obtained for yield and for clearance of key impurities.

Using a non-DoE approach is possible, but there is a risk that the number of experiments needed becomes excessive and that some interactions between parameters are not fully visible. The proposed approach in the DoE case is to perform a full factorial design with three variables. This will give 2^3 = 8 different conditions + 3 center points = 11 experiments. The center points are important because they give an indication of whether there is curvature in the data. Other designs, such as Central Composite Face (CCF) centered, may be used as they allow identification of 2nd degree curvature effects. A graphical view of how the full factorial design experiments are organized is shown in Figure 2.

For optimization of loading conditions on Capto™ adhere we have found that pH, conductivity, and load are the most important factors. Optimization of these factors will influence both yield and clearance of key impurities like dimer/aggregates, HCP, and leached Protein A. Before the optimization is run, an initial experiment (part 1 below) is performed to define a suitable pH range for the mAb of interest.

As an alternative to running small columns, high-throughput process development (HTPD) tools such as PreDictor™ 96-well plates may be used for process optimization. HTPD using PreDictor™ plates is explained fully elsewhere (2).

Part 1

Setting ranges for the experimental design

Initial screening of pH range

To define which pH range should be used in the design, an initial experiment should be performed in bind/elute mode. This is done by loading of analytical amounts of the mAb on a Capto™ adhere column at high pH, followed by elution in a linear pH gradient. Details are given in Table 1.

Start material: mAb initially purified on protein A resin.

Load preparation: The staring material must be buffer exchanged to loading buffer. This can be done using HiPrep™ 26/10 Desalting or HiTrap™ Desalting.

System and column recommendation: ÄKTA™ avant 25 system with prepacked HiScreen™ Capto™ adhere column, or Tricorn™ 5/100 column packed with Capto™ adhere, 10 cm bed height.

Based on the results from this experiment, the pH limits for the design can be defined. The lower limit is set to the elution pH (i.e., pH at elution peak maximum, see Fig 3). The upper pH limit should typically be about 2 pH units higher. It is important to include both non-binding and partially binding conditions in the design.

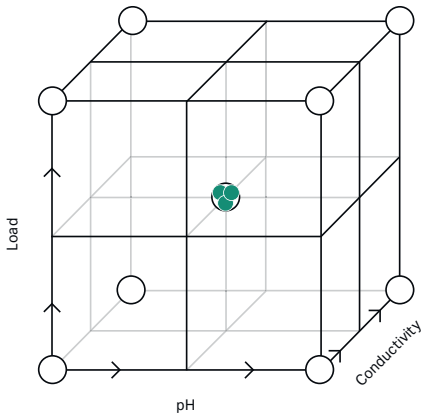

Fig 2. A graphical representation of the DoE approach (full factorial design) to optimization of loading conditions.

Ranges for conductivity and sample load

Conductivity: A conductivity range of 2-30 mS/cm is appropriate for most mAbs. If it proves difficult to get down to 2 mS/cm (e.g., due to precipitation of the mAb) levels slightly above 2 mS/cm should be acceptable.

Sample load: Appropriate settings for the load depend on the requirements for the specific process but in many cases 50-250 mg/mL work well.

Recommended buffer systems

Choice of buffer system for the experiments will be dependent on the pH range in the experimental design. Suitable buffer systems for different pH ranges are given in Table 2.

Table 2. Recommended buffer systems

Resin	pH range*	Buffer composition†
Capto™ adhere	4-6	25 mM citrate
	6-7.8	25 mM sodium phosphate + 50 mM sodium citrate
	6.5-8.5	25 mM sodium phosphate + 25 mM Tris

* pH above 8.5 should be avoided due to risk of de-amidation.

† Adjust buffer conductivity by addition of NaCl.

Table 1. Chromatography method for initial screening of pH range.

Step	Volume (CV)	Buffer composition	Linear flow rate (cm/h)
Equilibration	2	20 mM sodium citrate, 20 mM sodium phosphate, pH 7.8 (Buffer A)	300
Loading @ 1 mg/mL	0.5	20 mM sodium citrate, 20 mM sodium phosphate, pH 7.8	300 (2 min residence time)
Wash	3	20 mM sodium citrate, 20 mM sodium phosphate, pH 7.8	300
Elution	10	Gradient 0%-100% B, 20 mM sodium citrate, 20 mM sodium phosphate, pH 4.0 (Buffer B)	300
	7	100% B, 20 mM sodium citrate, 20 mM sodium phosphate, pH 4.0	300
CIP	3	1 M NaOH	100
Re-equilibration	5	20 mM sodium citrate, 20 mM sodium phosphate, pH 4.0	300
	7	20 mM sodium citrate, 20 mM sodium phosphate, pH 7.8	300

Part 2
Running the experimental design

When the ranges for the three factors have been set, the Design of Experiments can be performed according to the procedure described below. The experiments should be performed in a randomized order. Table 3 gives an example of an experimental set-up. Using ÄKTA™ avant and UNICORN™ 6, a suggested experimental order will automatically be generated.

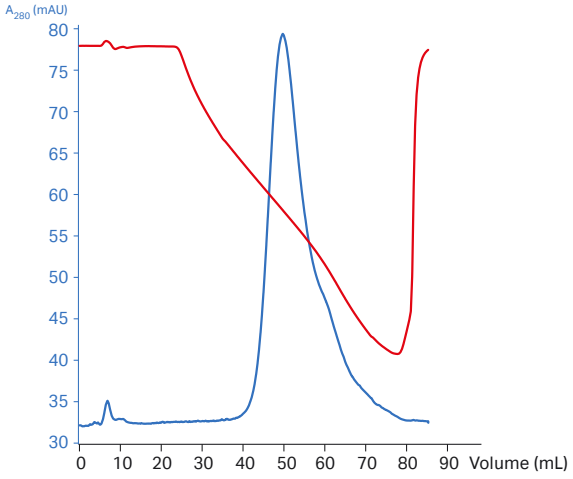

Fig 3. Determination of binding and elution conditions on HiScreen™ Capto™ adhere. The red curve represents pH.

Simplified chromatographic set-up

Choice of system and column: The choice of system and column depends on the amount of starting material that is available. Larger columns give more material for analysis but require significantly more starting material. Table 4 lists different options.

Start material: mAb initially purified on protein A resin.

Load preparation: The samples must be conditioned to the respective loading buffers, according to the experimental design. This can either be done using HiTrap™ Desalting, HiPrep™ 26/10 Desalting, or by direct adjustment of pH and conductivity.

Chromatographic run:

Table 5 provides a summary of the chromatographic runs to be performed in the experimental design.

1. Equilibrate the column using the same buffer composition as for the loading step.
2. Load the columns with the correct amount of mAb, according the the design. This will be different for different runs according to the experimental design. See examples in Table 3. Collect the flow-through fractions.
3. After sample load, continue to wash out unbound mAb with loading buffer until the UV curve starts to level off. The wash fractions can normally be pooled together with the flow-through fractions.
4. During optimization, cleaning-in-place (CIP) is recommended after every cycle. After scale up, CIP is recommended every 1-5 cycles, depending on the nature and the condition of the feedstock.

Step durations: All step durations mentioned here are only indicative – the actual step durations may be shorter or longer if the chromatograms and fraction data so indicate.

Table 3. Example of an experimental set-up for Capto™ adhere

Run order	Target load (mg/mL resin)	Target load conductivity (mS/cm)	Target load pH
1	250	2	Lower limit
2	150	16	Center point
3	250	2	Upper limit
4	150	16	Center point
5	150	16	Center point
6	50	2	Upper limit
7	250	30	Lower limit
8	50	2	Lower limit
9	250	30	Upper limit
10	50	30	Lower limit
11	50	30	Upper limit

Note: pH values should be set according to the screening results described in Part 1. Target values are given, but measured values should be used in the data analysis.

Table 4. System and column recommendations for optimization of loading conditions

System*	System control	Resin	Column	Bed height (cm)	CV (mL)	Amount (g) of mAb required for 11 experiments
ÄKTA™ avant 25	UNICORN™ 6	Capto™ adhere	HiScreen™	10	4.7	11
ÄKTA™ avant 25	UNICORN™ 6	Capto™ adhere	Tricorn™ 5/100	10	2	5
ÄKTApurifier™	UNICORN™ 5	Capto™ adhere	Tricorn™ 5/20	2	0.5	1

* If sample availability is very limited, the ÄKTApurifier™ alternative with 2 cm bed height columns is recommended. However using 10 cm bed height is preferential.

Table 5. Chromatography methods for the experimental design

Step	Volume or eq.	Buffer composition	Residence time (min) and/or linear flow rate (cm/h)
1. Equilibration	5 CV	Identical to loading buffer	300 cm/h
2. Loading	X mL (corresponding to 50–250 mg mAb/mL resin	According to experimental design (see Table 3)	2 min 300 cm/h @ 10 cm bed height)
3. Wash out unbound material	Until UV has reached base line	Identical to loading buffer	75 cm/h @ 2 cm bed height
4. Strip	5 CV	0.1 M acetic acid pH 3	300 cm/h
5. CIP		1 M NaOH	15 min contact time 100 cm/h
6. Re-equilibrations	7 CV	Loading buffer	300 cm/h

Responses

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

For details on the analytical methods, see (5).

- Total yield is calculated based on size exclusion chromatography (SEC) or by A_{280} measurements as amount eluted/amount loaded.

$$\text{Yield} = \frac{(\text{Mon} + \text{Agg})_{\text{Eluted}}}{(\text{Mon} + \text{Agg})_{\text{Loaded}}} \times 100$$

- Aggregate content: flowthrough fractions are analyzed by SEC using two interconnected Superdex™ 200 5/150 GL columns connected to ÄKTApurifier™ 10 (Plus). Amount of aggregate is obtained from UNICORN™ as "Percent of total peak area" after integration of the chromatogram.
- HCP and protein A levels are 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.)

Data analysis

DoE data should be evaluated using the DoE module in UNICORN™ 6. If experiments are performed on ÄKTApurifier™, commercial DoE software (e.g., Modde™; www.umetrics.com) may be used. If data analysis is not performed using DoE software, additional experiments are recommended with a reduced experimental set-up in order to find optimal conditions.

Conclusions

The information provided here is based on previous experience and may be used as a starting point for step development. Considerations of the known properties of the specific mAb should always be taken. The recommended path for process development is to adopt DoE and HTPD. For further assistance with step or process development, please contact your local

Cytiva sales representative or Cytiva's Fast Trak™ organization.

References

1. Data file: Capto™ adhere, Cytiva, CY11848.
2. Handbook: High-throughput process development with PreDictor™ plates, Cytiva, CY16051.
3. Ljunglöf, A., K. Eriksson, and T. Frigård. Rapid process development for purification of a mAb. BioProcess™ International **9**, 62-68 (2011).
4. Application note: Rapid process development for purification of a mAb using ÄKTA™ avant 25, Cytiva, CY14504.
5. Application note: Optimization of loading conditions on Capto™ adhere using design of Experiments, Cytiva, CY13251.

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