GE Healthcare
Life Sciences

Two-step purification of monoclonal IgG_1 from CHO cell culture supernatant

Abstract

The increasing demand for monoclonal antibodies (MAb) as biopharmaceuticals has promoted the development of cell cultures with increased expression levels. As a consequence, the demand for more efficient purification processes has increased. This application note describes a two-step process for MAb purification based on MabSelect SuRe™ and Capto™ adhere, a strong multimodal anion exchanger.

The two-step process is applied to the purification of monoclonal IgG_1 antibodies from CHO cell culture supernatant and the results are compared with data from a process based on a three-step process. If necessary, Capto adhere can be used as a second or third polishing step in any MAb purification platform.

Introduction

Over the last 20 years, the use of antibody titers in mammalian cell culture has increased dramatically. Recent industry information reports antibody titers from 1 to 5 g/L. The associated increase in contaminant levels, such as host cell proteins (HCP), viruses, aggregates, and protein A, leads to new challenges for MAb purification, and upgraded processes with efficient protein purification steps are required.

Large-scale purification of MAbs usually consists of three chromatographic steps. Initially the MAb is captured using protein A affinity chromatography, giving a product with high purity, typically 99%. The product is then further polished by cationic and anionic exchange chromatography.

The GE Healthcare chromatography media toolbox simplifies this process.

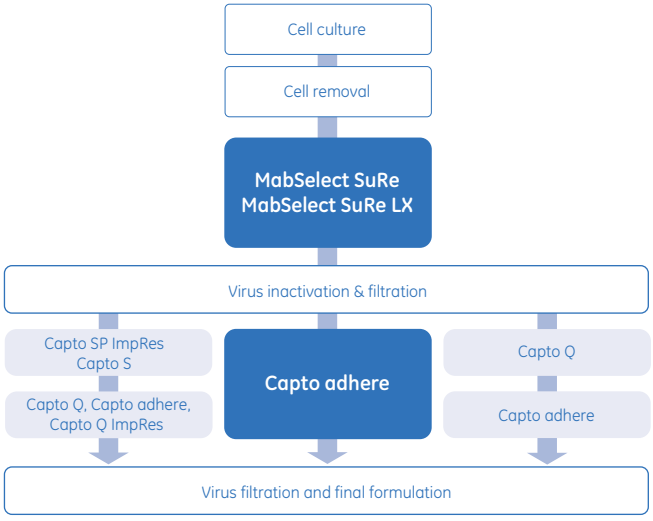

Fig 1. GE Healthcare chromatography media toolbox.

MabSelect SuRe, characterized by alkaline stability, enhanced protease resistance and a generic elution profile, is used for the initial protein A capture step. MabSelect SuRe LX has been further developed from MabSelect SuRe to give even higher binding capacity at longer residence time.

The medium of choice for polishing is Capto adhere. Capto adhere is a strong multimodal anion exchanger that offers a different selectivity compared to traditional ion exchangers, and is designed for intermediate purification and polishing of MAbs. Removal of protein A leakage, aggregates, host cell proteins, nucleic acids, and viruses is performed in flowthrough mode.

With Capto adhere it is possible to replace the two post-protein A steps with a single polishing step, and end up with a two-step process (Fig 1). If necessary, Capto adhere can be used in combination with other media in a three step process. An alternative intermediate step is represented by cation exchange chromatography using Capto S.

Capto adhere improves yield, productivity, and process economy by offering:

- High capacity in flowthrough mode
- Contaminant removal to formulation levels in one post-protein A step
- Wider operational window of pH and conductivity
- Potential savings in time and operating costs with a two-step chromatographic process

As members of the BioProcess™ media, Capto and MabSelect media families meet the demands of industrial biotechnology with validated manufacturing methods, security of supply, and comprehensive regulatory support to assist process development, validation, and submission to regulatory authorities.

Material and methods

Filtered CHO cell culture supernatant containing approximately 0.8 mg IgG_1/mL was supplied by Polymun Scientific (Austria). The pI of the antibody was 6.5 to 9.0. Due to very low HCP levels after MabSelect SuRe, the supernatant was spiked with 10% HCP (host cell protein) concentrate. All chromatographic steps were performed on an ÄKTAexplorer™ system with UNICORN™ software.

MabSelect SuRe

Clarified feedstock spiked with 10% concentrated HCP was applied to MabSelect SuRe. After washing with loading buffer and intermediate wash*, elution was carried out with 60 mM sodium citrate, pH 3.4, followed by low pH virus inactivation and filtration. Buffer exchange to 25 mM Tris, 175 mM NaCl, pH 7.5 was done on HiPrep™ 26/10 Desalting. For larger volumes of feed, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity.

Capto adhere

The Capto adhere step was performed in flowthrough mode. After sample application, the column was washed with loading buffer until the MAb had passed through. The flowthrough and wash fractions were then pooled. Adsorbed material, mainly aggregates and other impurities, was eluted for further analysis.

Capto S

Following MabSelect SuRe chromatography, the eluate was virus-inactivated by low pH and then applied to Capto S without further conditioning. After washing, the MAb was eluted with 100 mM sodium phosphate pH 7.0.

Capto Q

Polishing was performed in flowthrough mode. Before loading onto Capto Q, the pH of the eluate was adjusted to 8.0 with 0.1 M sodium hydroxide. The conductivity was adjusted to 4.6 mS/cm by addition of MilliQ™ water. The pH was kept as high as possible to retain HCP, protein A, and dimers aggregates while allowing the monomeric MAbs to pass through the column without being adsorbed.

Determination of dimers and aggregates clearance

Analytical size exclusion was performed on Superdex™ 200 10/300 GL. The aggregate content was determined by integration of peaks at 280 nm.

Protein A ELISA

The protein A concentration in the start materials and flowthrough fractions was determined according to reference 1.

HCP ELISA

Samples for ELISA were pre-treated by addition of 10% 2.0 M TRIS, 1% BSA, 0.5% Tween™ 20, pH 8.0 (50 µL sample buffer to 450 µl sample). The samples were diluted in Sample Diluent Buffer and analyzed by a CHO HCP ELISA kit (Cygnus Technologies; CM015) using the High sensitivity protocol specified in the kit insert.

Results and discussion

The two-step purification process was performed by capture on MabSelect SuRe followed by polishing on Capto adhere. The results were compared to a three-step purification procedure based on capture on MabSelect SuRe followed by polishing with Capto S and Capto Q.

Two-step process

MabSelect SuRe

Clarified feedstock was purified on MabSelect SuRe and the chromatogram is shown in Figure 2. The HCP concentration was reduced from 95 000 ng/mL in the feedstock to 250 ng/mL. After this step, the protein A concentration was < 5 ng/mL and the aggregates content was < 0.7%.

Analytical size exclusion of the intermediate wash fractions showed that they contained mainly aggregates and HCP. Further purification of the MabSelect SuRe eluate was then performed either by one or two chromatographic steps.

* Intermediate wash is performed after loading of feed onto the column.

Column: XK 16/20 packed with 20 ml MabSelect SuRe
Bed height: 10 cm
Sample: Clarified CHO supernatant spiked with HCP
Sample load: 26 mg IgG_1/mL medium
Loading buffer: 0.02 M sodium phosphate, 0.15 M sodium chloride pH 7.4
Intermediate wash: 2 CV 0.025 M sodium phosphate, 5% isopropanol, 0.5 M sodium chloride pH 7.0
Flow rate: 250 cm/h (residence time 2.4 min)
Elution: 0.06 M sodium citrate, pH 3.4
System: ÄKTAexplorer 100

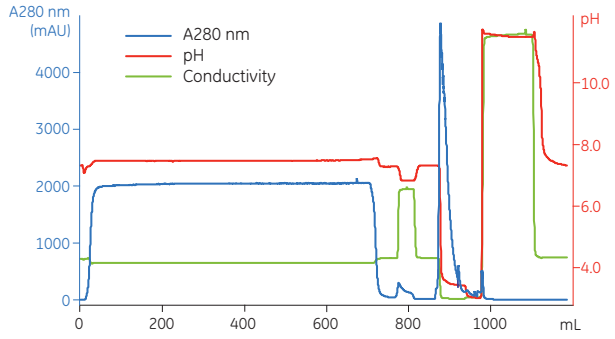

Fig. 2. Purification of IgG_1 on MabSelect SuRe.

Column: Tricorn™ 5/100 packed with 0.51 ml Capto adhere
Bed Height: 2.6 cm
Sample: MabSelect SuRe eluate after virus inactivation and filtration
Sample load: 200 mg MAb/mL medium
Loading buffer: 0.025 M TRIS, 0.175 M sodium chloride pH 7.5
Flow rate: 78 cm/h (residence time 2 min)
Elution: 0.1 M acetic acid pH 3.0
System: ÄKTAexplorer 100

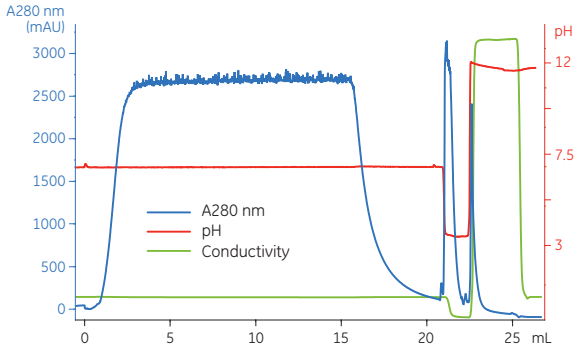

Fig. 3. MAb purification, two-step process, polishing on Capto adhere.

Capto adhere

The Capto adhere step was performed in flowthrough mode under conditions where most contaminants were adsorbed, while the antibodies passed through the column (Fig 3). The HCP concentration was reduced from 250 to 20 ng/mL (7.5 ppm). The aggregate content was initially low in the MabSelect SuRe eluate (< 0.7%), and after the Capto adhere step, the content was below the detection limit. The adsorbed material was eluted from the column and analyzed with size exclusion. The eluate contained approximately 5% aggregates, confirming that aggregates remaining after the capture step were efficiently adsorbed (Fig 4). The level of protein A was below the detection limit (Table 1).

Acceptable levels for key impurities

With the exception of DNA values, there are no absolutely defined acceptable levels of impurities in MAb formulations (2). Target acceptable levels are based on a risk assessment and depend on dosage, patient population, and indication.

Typical target values are < 5 ppm for protein A, < 10 ppm for HCP, and < 1% of dimers and aggregates. These levels are reached in the example above. To further lower the contaminant levels, in particular the HCP contents, a third step can be added to the two-step procedure. Increased purity will be at the expense of slightly lower yield.

A Capto Q step was added to the two-step model (data not shown). The yield in this step was almost quantitative (99.7%) and the HCP content was reduced by 50%.

Column: Superdex 200 10/100 GL
Sample: Flowthrough fraction (red) and eluate (blue) from the Capto adhere step
Sample load: 50 µL each
Loading buffer: 0.01 M sodium phosphate, 2.7 mM potassium phosphate, 137 mM sodium chloride, pH 7.4
Flow rate: 0.5 mL/min
System: ÄKTAexplorer

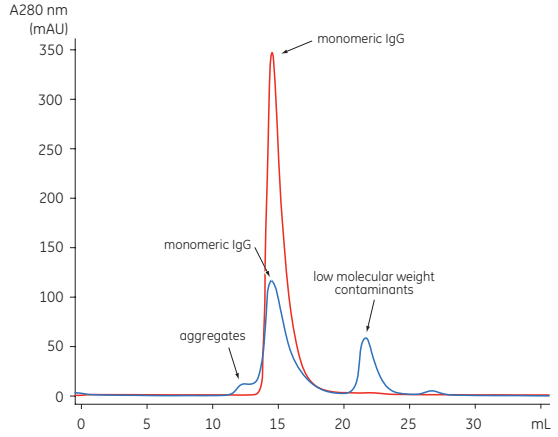

Fig. 4. Size exclusion analysis of flowthrough (red) and elution pools (blue) from Capto adhere.

Table 1. Two step process: summary of results

Two step process	Accumulated yield (%)	Dimers and aggregates (%)	Protein A (ng/mL)	Protein A (ppm)	HCP (ng/mL)	HCP (ppm)
Start material spiked with HCP	100	—	—	—	95 000	128 300
MabSelect SuRe[1]	95	< 0.7	< 5	< 1	250	55
Capto adhere[2]	90	< 0.1	< 5	< 1	20	7.5

[1] After virus inactivation and filtration.
[2] Preconditioning by buffer exchange gave 100% yield and slight reduction in HCP.

Three-step process

The results from the three-step process are presented in Figure 6 and 7 and Table 2.

MabSelect SuRe

Capture step on MabSelect SuRe was the same as for the two-step procedure.

Capto S

The yield of monomeric IgG_1 was close to 99%. About 65% of the HCP was removed, while the Capto S step was not effective in removing leaked protein A or aggregates (Table 2).

Capto Q

The polishing step was performed in flowthrough mode (Fig 7). The yield of monomer IgG_1 in the flowthrough fraction was 96%. The HCP level was 20 ng/ml (3 ppm), a more than 6-fold reduction. The protein A concentration was below the quantification limit and the aggregates level was less than 0.1%.

Fig. 5. As with all BioProcess media, MabSelect SuRe and Capto adhere are well-suited for use in AxiChrom™ process scale columns.

Column: Tricorn 5/200 packed with 4 mL Capto S to a bed height of 20 cm
Sample: MabSelect SuRe eluate after virus inactivation and filtration
Sample load: 100 mg MAb/mL medium
Loading buffer: 0.02 M sodium citrate, 0.012 M sodium chloride pH 5.3
Intermediate wash: 6 CV of 40 mM sodium phosphate pH 6.5
Flow rate: 500 cm/h (residence time of 2.4 min)
Elution: 0.1 M sodium phosphate, pH 7.0
System: ÄKTAexplorer 100

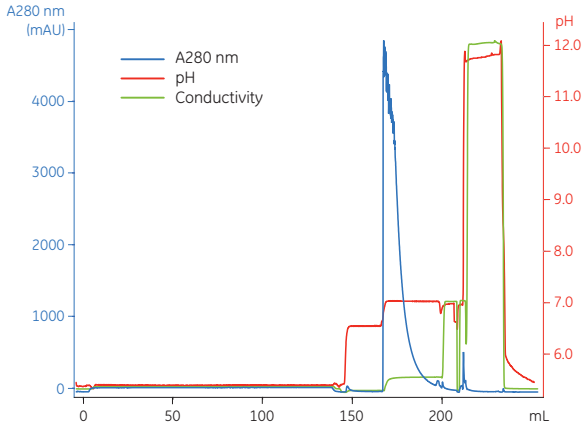

Fig. 6. MAb purification, three-step process, intermediate purification on Capto S.

Column: Tricorn 5/100, Capto Q bed volume: 2 mL
Sample: Eluate after Capto S, pH adjusted to 8.0, and conductivity to 4.6 mS/cm
Sample load: 130 mg MAb/ml medium
Loading buffer and wash: 0.025 M sodium phosphate pH 8.0
Flow rate: 500 cm/h (residence time of 1.2 min)
System: ÄKTAexplorer 100

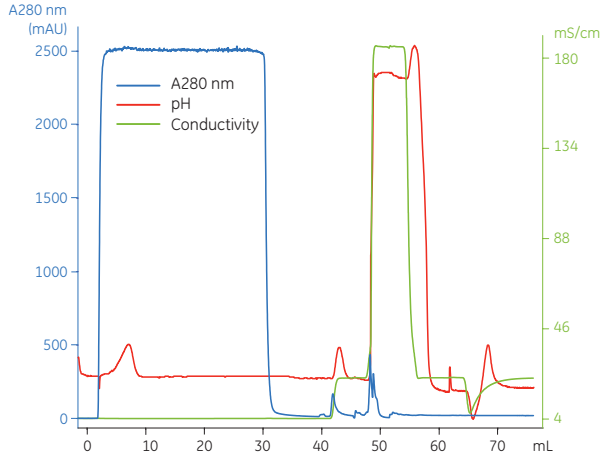

Fig. 7. MAb purification, three-step process, polishing on Capto Q.

Table 2. Summary of results from the three-step process

Three step process	Accumulated yield (%)	Dimers and aggregates (%)	Protein A (ng/ml)[1]	Protein A (ppm)	HCP (ng/ml)[1]	HCP (ppm)
Start material spiked with HCP	100	–	–	–	95 000	128 300
MabSelect SuRe[2]	95	< 0.7	< 5	< 1	250	55
Capto S	94	< 0.7	9.3	0.4	445	20
Capto Q	90	< 0.1	< 5	< 1	20	3

[1] Determined with ELISA.
[2] After virus inactivation, filtration, and pH adjustment.

Conclusions

This study demonstrates that it is possible to design a two-step procedure based on MabSelect SuRe and Capto adhere for the purification of MAbs.

In this study, both the two- and three-step processes successfully purified the MAb to impurity levels acceptable for formulation (i.e., HCP content < 10 ppm, protein A below detection limit, and dimers and aggregates concentration less than 0.1%; Table 3).

Table 3. Comparison of final results obtained with the two- and three-step processes

Final result	Three-step process	Two-step process
Total yield (%)	90	90
Dimers and aggregates (%)	< 0.1	< 0.1
Protein A (ng/mL)	< 5	< 5
Protein A (ppm)	< 1	< 1
HCP (ng/mL)	20	20
HCP (ppm)	3.0	7.5

Reference

1. Steindl., F. *et al.* A simple method to quantify staphylococcal protein A in the presence of human or animal IgG in various samples. *J. Immunol. Methods* **235**, 61–69 (2000).
2. WHO Technical Report 878, *Annex 1* (1998).

Acknowledgements

Filtered CHO cell line supernatant was supplied by Polymun Scientific Immunbiologische Forschung GmbH, Nassdorfer Lindell, 1190 Vienna, Austria.

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/bioprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

28-9078-92 AD 03/2011