Pilot-scale capture of monoclonal antibody IgG_4 on MabSelect Xtra with high purity and yield

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CY14311-09Jun20-AN

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Summary

MabSelect Xtra™ is a recombinant protein A-based affinity medium for capturing monoclonal antibodies. Its dynamic binding capacity for polyclonal human IgG is about 30% higher than conventional high-capacity protein A media, which makes it especially attractive for capturing antibodies from high expression feedstocks.

The application of MabSelect Xtra described here is the pilot-scale capture of monoclonal antibody IgG_4 on ÄKTApilot™ chromatography system with the medium packed in a FineLINE™ Pilot 35 column (Fig 1).

The goal of achieving an IgG_4 product of high purity and yield was clearly met. The monomer content in the eluate from three test runs was in excess of 99% and the average yield was 94%. Furthermore, the capture/CIP process appears very robust and should be suitable for further scale-up.

Fig 1. ÄKTApilot chromatography system with FineLINE Pilot 35 column. The column was packed with MabSelect Xtra to a bed height of 20 cm.

Introduction

Protein A-based affinity media have become the industry standard for capturing monoclonal antibodies. Thanks to its high selectivity, protein A gives very high purity and recovery at this first processing step. This both reduces the demands on the following purification steps, and has a positive effect on the overall process economy.

MabSelect Xtra is a member of the MabSelect™ family of recombinant protein A-based affinity media. This Application Note describes the capture of monoclonal antibody IgG_4 on an ÄKTApilot chromatography system with MabSelect Xtra packed in a FineLINE Pilot 35 column. The goal was to achieve an IgG_4 product of high purity and yield.

Product characteristics

MabSelect Xtra

Like MabSelect, MabSelect Xtra is based on a high-flow agarose base matrix, but with larger pores, a smaller mean particle size, and a higher ligand density. This results in higher capacity; MabSelect Xtra has 30% higher dynamic binding capacity for polyclonal human IgG than conventional high-capacity protein A media. In a packed bed, the maximum recommended operating velocity for MabSelect Xtra at large scale is 300 cm/h at 20 cm bed height.

MabSelect Xtra shares the same recombinant Protein A ligand as MabSelect. Coupling conditions, designed to favor single-point attachment of the ligand, are also the same.

ÄKTApilot system

ÄKTApilot chromatography system is a bench-top process development and production system based on the ÄKTAdesign™ platform. The system purifies milligrams to tens of grams of product, which enables easy scale-up and scale-down. Hygienic design, a high level of automation, accuracy, reproducibility, and reliable operation make ÄKTApilot perfect for scale-up, process optimization, and production.

Materials and methods

General details

The NS0 cell line feedstock containing the IgG_4 monoclonal antibody was supplied by Lonza Biologics, UK (see Acknowledgement). The pI of the antibody was 6.5–7.5.

All chromatographic steps were performed on ÄKTApilot system with UNICORN™ 4.11 software. Figure 1 shows the ÄKTApilot chromatography system and the FineLINE Pilot 35 column. The column was packed to a bed height of 20 cm using a two-step procedure. HETP and asymmetry factor testing before and after the runs showed that the efficiency of the bed was good.

The general disposition of the study was to perform a series of eight runs as follows:

1. 'Pre-feed CIP' (manual)*
2. Blank run no. 1†
3. Feed + CIP (capture run no. 1)
4. Blank run no. 2
5. Feed + CIP (capture run no. 2)
6. Blank run no. 3
7. Feed + CIP (capture run no. 3)
8. Blank run no. 4

* The 'pre-feed CIP' with a CIP solution of 50 mM NaOH + 500 mM Na_2SO_4 was performed to avoid high amounts of protein A in the eluted material. See Results.

† Blank runs were made to measure possible carryover. No sample was applied and no CIP was performed. Equilibration buffer was used instead.

Column:	FineLINE Pilot 35 (bed height 20.1 cm, 1 column volume = 193 ml) packed with MabSelect Xtra
Sample:	IgG_4 clarified NSO supernatant,
Sample load	5.16 l (0.55 g/l)
Buffer A:	10 mM sodium phosphate, 150 mM NaCl, pH 7.2
Buffer B:	100 mM glycine-HCl, pH 3.5
Flow rate:	300 cm/h (48 ml/min)
Gradient:	0–100 % B in 0 CV
CIP:	50 mM NaOH, 500 mM Na_2SO_4, contact time 10 min (46 ml/min)
System:	ÄKTApilot™

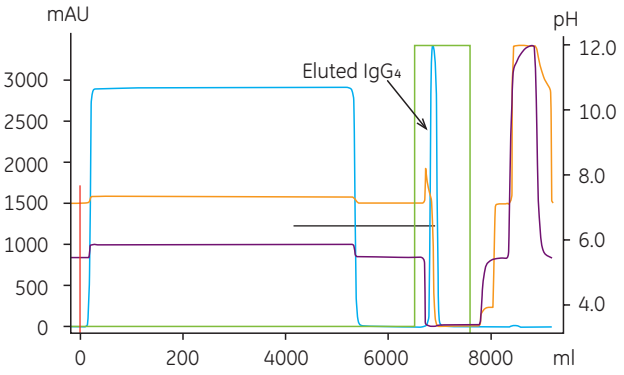

Fig 2. Capture of monoclonal antibody IgG_4 on MabSelect Xtra from the first capture/CIP run. The same procedure was repeated twice with blank runs in between, and the same chromatographic separation result was obtained each time. The average yield of IgG_4 was 94%. No significant carryover was detected in the blank runs.

Column:	Tricorn™ 10/300 (bed height 30 cm, CV=23.56 ml)
Medium:	Superdex 200 HR
Sample:	IgG_4 purified on MabSelect Xtra from clarified NSO supernatant, Lonza Biologics plc, Slough, U.K.
Sample volume:	50 µl
Buffer A:	PBS, pH 7.4
Flow rate:	39 cm/h (0.5 ml/min)
System:	ÄKTAexplorer™ 100

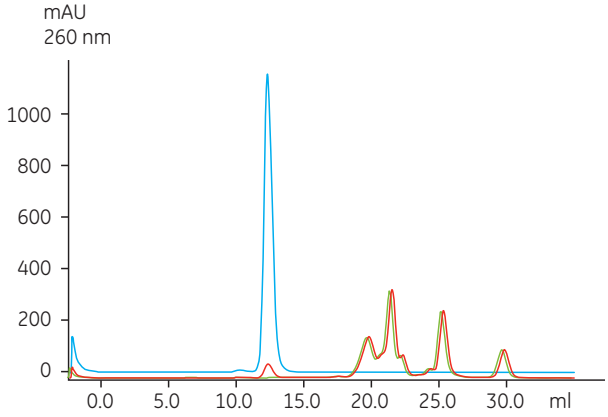

Fig 3. Dimer/aggregate analysis results of the start material (clarified IgG_4 supernatant, [red]), flowthrough (green) and eluate (blue) after capture on MabSelect Xtra. The monomer content of the eluate was approximately 99%. No monomer was found in the flowthrough.

Experimental conditions

IgG_4 capture comprised three repeated runs, including CIP, made on the FineLINE Pilot 35 column.

Clarified NS0 supernatant (5159 ml) was applied to the column at a nominal fluid velocity of 300 cm/h (48 ml/min, residence time [RT] = 4 min). This is equivalent to a sample loading of 14 mg IgG_4/ml bed volume.

IgG_4 was desorbed by decreasing the pH to 3.5 by eluting with 100 mM glycine-HCl using a step gradient (0–100% B) for 5 column volumes (CV). Neutralization buffer (1 M Tris-HCl, pH 9.0) was added to the eluted IgG_4 material to minimize exposure of the antibody to low pH.

Each capture step included CIP with 50 mM NaOH, 500 mM Na_2SO_4 (46.0 ml/min for 2.4 CV). Capture was repeated three times with in-between blank runs (no sample loaded) to verify the robustness of the capture and to see if the degree of protein A leakage differed after repeated CIP. IgG_4 content in the eluate was then calculated from the absorbance at 280 nm, from which the yield was determined.

In addition to calculating the purity and yield of the IgG_4 and measuring possible carryover, the following components were also analyzed; dimers/aggregates, host cell proteins, DNA, leaked protein A, and endotoxins.

Results

IgG_4 capture

Figure 2 shows the chromatogram of the first IgG_4 capture/CIP run on MabSelect Xtra.

Dimer/aggregate content

Figure 3 shows the result of measuring the dimer/aggregate content in the start, flowthrough, and eluted material by analytical gel filtration on Superdex™ 200 HR. The dimer/aggregate content in the eluate was approximately 1%. No monomer IgG_4 was found in the flowthrough. Note that the chromatogram shows the superimposed results of three analytical runs. Table 1 summarizes data from these runs.

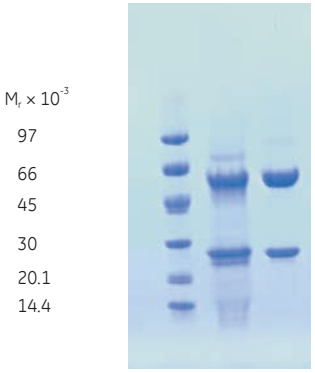

Fig 4. SDS-PAGE analysis on ExcelGel™ SDS Gradient 8–8. The light chain of the IgG_4 was visualized at M_r 25 000 and the heavy chain at approximately M_r 50 000. This result confirmed other analytical data by showing that there was a clear reduction of proteins in the eluted material.

Electrophoresis

SDS-PAGE was run to visually analyze the purity of the pooled start and eluted material. Samples were run under reducing conditions together with a low molecular weight standard. The gel was Coomassie™ stained. Figure 4 shows the results, which confirm the high purity seen with analytical gel filtration in Figure 2.

Table 1. Analysis results of dimers/aggregates in the eluted material from three different capture runs. The monomer form of IgG_4 was found at approximately 12.3 ml retention volume. Only 0.9% of dimers/aggregates was found in the eluate

Sample	Retention (ml) area	Area/peak (volume [%])
Eluted material run no. 1	7.8	0.1
	10.3	0.8
	12.3	99.1
Eluted material run no. 2	7.8	0.1
	10.3	0.7
	12.3	99.2
Eluted material run no. 3	7.7	0.1
	10.3	0.7
	12.3	99.2

Host cell proteins and DNA

Host cell proteins (HCP) and DNA were analyzed by Lonza Biologics. Figure 5 shows the results from a Western blot assay (silver-stained gel) of samples from the capture step. The blue/gray bands are HCP and the red are antibody-derived components. A large amount of HCP found in the loaded material passed out in the flowthrough, thus giving a substantial reduction of HCP in the eluate.

Leakage of protein A

Analysis results for leaked protein A (SPA) are shown in Figure 6. Leakage declined after the manual pre-feed CIP run. Levels found after the three following CIP runs were relatively low. In the eluted IgG_4, levels of leaked protein A were low (6 ppm).

This result suggests that it is beneficial to perform a blank run, preferably including CIP, prior to loading sample and commencing the regular series of capture/CIP processing cycles.

Endotoxins

Endotoxins were detected using the limulus amoebocyte lysate (LAL) assay for lipopolysaccharides. Table 2 shows the results.

Table 2. The majority of the endotoxins were removed during capture on MabSelect Xtra, resulting in material of high purity after only one step. The final pooled eluate contained as little as 0.08 E.U./mg IgG_4

Sample	E.U./ml (analysis)	Volume (ml) result)	Total E.U.	Amount IgG_4 (mg)	E.U./mg IgG_4
Start material (clarified NS0 supernatant)	7.0	15477	108341	7950	13.6
Eluted IgG_4	0.83	704	584	7600	0.08

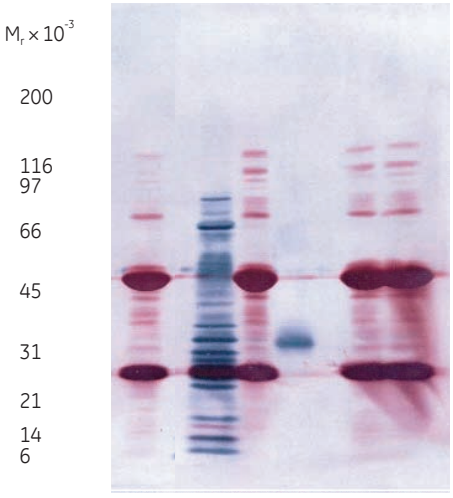

Fig 5. Western blot analysis of the HCP content of samples from the capture step on MabSelect Xtra. Much of the HCP passed out in the flowthrough, thus reducing HCP in the eluate.

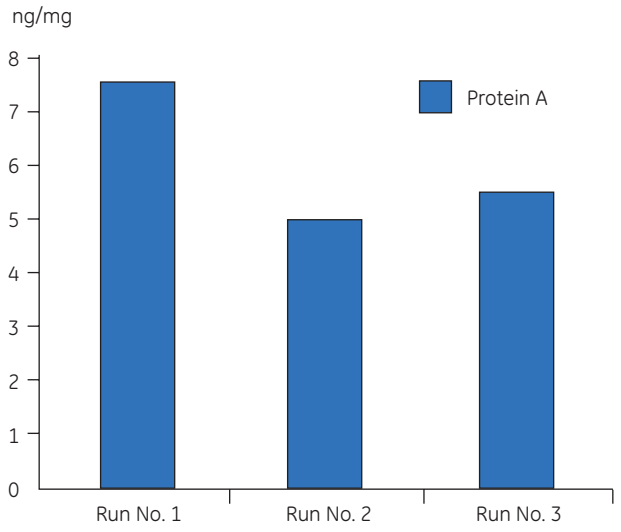

Fig 6. Levels of leaked protein A in the eluted IgG_4 fraction are low (6 ppm). Protein A leakage was measured according to reference 1.

Conclusion

This pilot-scale capture step of monoclonal antibody IgG_4 on ÄKTApilot chromatography system with MabSelect Xtra packed in a FineLINE Pilot 35 column achieved its set goal of giving a final product of high purity and yield. The monomer content in the eluate from the three runs was in excess of 99% and the average yield was 94%. In addition, the study also demonstrated the robustness of the process.

Table 3. Summary of purification and analytical results

Parameter	Result (IgG_4 fraction)
Purity	More than 99% monomer
Yield	94%
Host cell protein	Substantial reduction
DNA	Below detection limit
Protein A	6.0 ppm
Endotoxins	0.08 E.U./mg

The process described should thus be amenable to validation and scale-up to production. Well-proven methods for packing MabSelect Xtra in production-scale columns such as Chromaflow™, BPG™, and BioProcess™ are available to facilitate this transition.

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. *Journal of Immunological Methods* **235,** 61–69 (2000).

Acknowledgement

IgG_4-clarified supernatant was provided by Lonza Biologics, 224 Bath Road, Slough, SL1 4DX, UK. Lonza manufacture therapeutic grade monoclonal antibodies using mammalian cell culture processes. They also performed the HCP and DNA analyses.

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First published Dec. 2004

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11-0011-58 AB 11/2007

Elanders Östervåla 2007