GE Healthcare

Application Note 28-9078-93 AA Process-scale antibody purification

Selective removal of aggregates with Capto adhere

Abstract

This application note describes the selective removal of antibody dimers and aggregates from a two-step process based on MabSelect SuRe™ and Capto™ adhere.

Capto adhere is a strong anion exchanger with multimodal functionality designed for post-protein A MAb polishing. Removal of remaining contaminants is achieved in flowthrough mode under conditions that allow the antibodies to pass directly through the column while the contaminants are adsorbed.

This study presents results from optimization of the loading conditions with the help of Design of Experiments (DoE). The effects of buffer, pH, conductivity, and sample load were investigated. At optimal buffer conditions, the dimers and aggregates content were reduced 10-fold from 6% to 0.6% at a load of 120 mg MAb/mg medium. At higher load, 265 mg/ml, the dimers and aggregates reduction was 80% and the total yield was antibody was 94%.

Introduction

Over the last 20 years, the use of antibody titers in mammalian cell culture has increased dramatically. Recent industry reports demonstrate increase in antibody titers from 1 to 5 g/l. The associated increase of aggregates is a new challenge for manufacturers. Since aggregates are potential immunogens and important to keep at a low level, upgraded processes for aggregate removal are required.

Capto adhere is a strong ion exchanger with multimodal functionality (Fig 1), which offers a different selectivity compared to traditional ion exchangers.

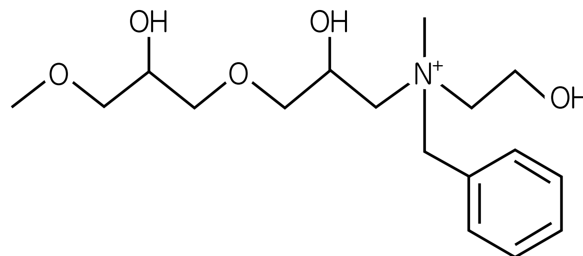


Fig 1. The Capto adhere ligand, N-benzyl-N-methyl ethanolamine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

Capto adhere is designed for intermediate purification and polishing of MAbs. Removal of protein A, aggregates, host cell proteins, nucleic acids, and viruses is performed in flowthrough mode.

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

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

As a member of the BioProcess™ media family, Capto adhere meets 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.

Capto

Design of Experiments (DoE) – basic principles

DoE is a systematic approach to study how variation in experimental factors affects the responses in a system. DoE is used to plan experiments so that the maximum amount of information can be extracted from a minimum of performed experiments.

The factors in a DoE study are varied so that they are independent of each other in a statistical sense. This makes it possible to evaluate the effect on the response of each factor separately (main effects). In addition, interaction effects between factors can be evaluated. For optimizing purposes, the use of DoE will almost always ensure that the real optimum for a response is reached.

A commonly used type of DoE is full factorial design where all main effects and interaction effects are independent of each other and therefore, their individual effect on the response can be resolved in the evaluation.

A replicated center point is usually included in the list of experiments and will give information on the variation in the responses. The center point also provides information on possible curvature in the data.

Material and methods

Clarified NS0 cell culture supernatant containing approximately 1.3 mg IgG_1/ml (supplied by BioInvent International AB) was purified on MabSelect SuRe and the elution pool was neutralized to pH ~ 6 with 1 M Tris pH 9. The pI of the MAb is 7.5 to 8.4. The elution pool was frozen and thawed several times to force the formation of dimers and aggregates. The pool contained approximately 6% soluble aggregates as determined by gel filtration chromatography on Superdex™ 200.

In the DoE, pH, conductivity, and load must be included. It is important to include conditions at the higher pH range (resulting in lower yield and higher purity) as well as conditions at lower pH range (resulting in higher yield and lower purity).

To find conditions suitable for the DoE, initial experiments were performed at pH 5.5 and 7.0, keeping sample load and conductivity constant.

DoE was performed and evaluated using Umetrics Modde™ 7.0 software (www.umetrics.com). A full factorial design was used including three variables (pH, conductivity, and load) and two center points. The experiments were performed in the pH interval 5.5 to 7.0. The conductivity was varied from 10 to 50 mS/cm and the load from 100 to 200 mg (Table 1).

Preload conditioning of samples was performed by buffer exchange on HiPrep™ 26/10 Desalting column[1].

Table 1. DoE setup, including two center points (blue and bold)

Loading buffer	pH	Cond (mS/cm)	Load (mg IgG/ml resin)
25 mM BIS-TRIS, 50 mM NaCl	5.5	10	100
25 mM BIS-TRIS, 50 mM NaCl	5.5	10	200
25 mM BIS-TRIS, 500 mM NaCl	5.5	50	100
25 mM BIS-TRIS, 500 mM NaCl	5.5	50	200
25 mM BIS-TRIS, 300 mM NaCl	**6.25**	**30**	**150**
25 mM BIS-TRIS, 300 mM NaCl	**6.25**	**30**	**150**
25 mM BIS-TRIS, 50 mM NaCl	7	10	100
25 mM BIS-TRIS, 50 mM NaCl	7	10	200
25 mM BIS-TRIS, 500 mM NaCl	7	50	100
25 mM BIS-TRIS, 500 mM NaCl	7	50	200

Results

Initial experiments

A comparison of chromatograms of the Capto adhere flowthrough at different pH is shown in Figure 2. Relatively steep breakthrough and wash curves are obtained at pH 5.5 (10 mS/cm). An increase in pH to 7.0 (i.e., closer to pI for the MAb) results in stronger electrostatic interaction between the MAb and the medium, giving a somewhat delayed breakthrough during sample load. In addition, the breakthrough and wash curves become more shallow. Significant amounts of MAbs are adsorbed to the column, resulting in a lower overall yield.

[1] For larger volumes of feed, sample conditioning is preferably performed by diafiltration or directly by adjustment of pH and conductivity. Desalting by buffer exchange or diafiltration may result in reduction of host cell protein levels and improved column performance.

Column:	Tricorn™ 5/50, bed height 3 cm
Sample:	MabSelect SuRe packed with Capto adhere elution pool, desalted
Sample load:	100 mg of MAb/ml medium
Loading buffer:	25 mM BIS-TRIS, 50 mM NaCl, pH 5.5 and 7.0 (conductivity 10 mS/cm)
Residence time:	2 min
System:	ÄKTAexplorer™100

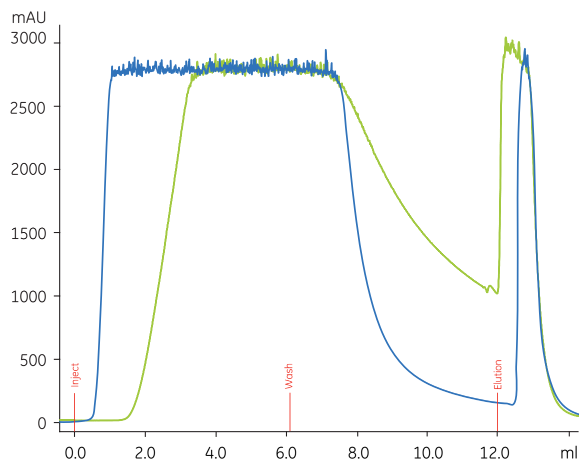


Fig 2. Comparison of chromatograms obtained at different pH. Loading buffer 25 mM BIS-TRIS, 50 mM NaCl pH 5.5 (blue), and pH 7.0 (green).

DoE

The experimental results from the DoE are summarized in Table 2. The model shows that yield is controlled by pH, but is independent of conductivity and load within the range 10-50 mS/cm and 100-200 mg/ml, respectively. Going from high to low pH, a non-linear increase in yield is obtained (Fig 3).

Dimers and aggregates clearance is influenced by pH, conductivity, and load (Fig 4). Higher pH, higher conductivity, and/or lower load results in higher aggregates clearance. An interaction effect is obtained between pH and conductivity. Higher pH and high conductivity gives the lowest dimers and aggregates response.

Table 2. Experimental results from the DoE

pH	Cond (mS/cm)	Load (mg IgG/ml)	D/A (% in flowthrough)	Yield (%)
5.5	10	100	0.77	94
5.5	10	200	0.98	100
5.5	50	100	0.3	94
5.5	50	200	0.52	99
6.25	**30**	**150**	**0.29**	**93**
6.25	**30**	**150**	**0.25**	**95**
7	10	100	0.13	47
7	10	200	0.29	76
7	50	100	0.24	74
7	50	200	0.35	68

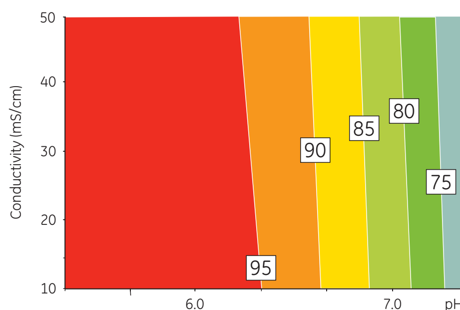
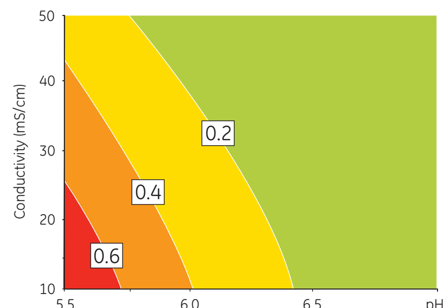
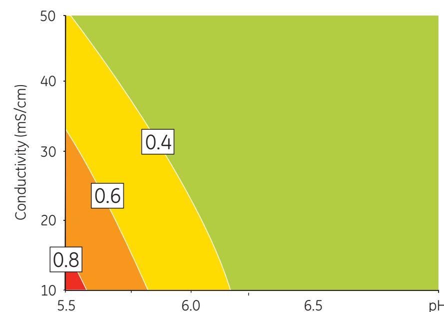


Fig 3. Response surface plot demonstrating the effect of pH on the yield. Neither the load nor the conductivity did signficantly affect the yield. Low pH facilitates high yield. Yield expressed in percent (labels).





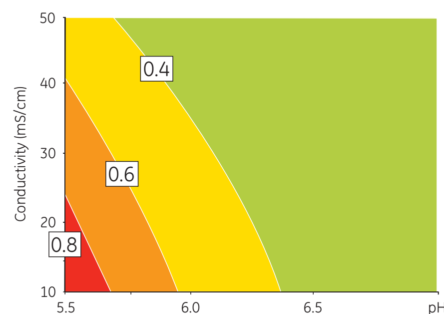


Fig 4. Response surface plots demonstrating the effect of pH, conductivity, and load on the clearance of aggregates. High pH, high conductivity, and low load gives the best reduction of aggregates. Aggregate concentration in the flowthrough pool is expressed in percent (labels).

Selective removal of aggregates

Starting from the results above, loading conditions were chosen to favor dimers and aggregates removal (i.e., pH 6.5 and conductivity 30 mS/cm).

A chromatogram from the Capto adhere step is shown in Figure 5. A summary of how load affects the dimers and aggregates clearance is shown in Table 3 and Figure 6.

Column:	Tricorn 5/50, bed height 3 cm
Sample:	MabSelect SuRe packed with Capto adhere elution pool
Sample load:	265 mg of MAb/ml medium
Loading buffer:	20 mM citrate, 300 mM NaCl, pH 6.5 (conductivity 30 mS/cm)
Elution buffer:	0.1 M acetic acid, pH 3.0
Residence time:	2 min
System:	ÄKTAexplorer 100

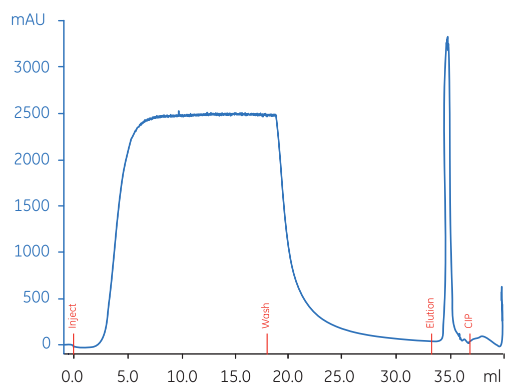


Fig 5. Polishing on Capto adhere.

Good reduction of dimers and aggregates is obtained, even at loads up to 265 mg MAb/ml medium. The levels are reduced from 6% to 0.6% (10 fold reduction) with a sample load up to 120 mg/ml. A high load (outside the design; Table 1) results in high yield at the expense of reduced aggregates clearance, as predicted by the model (Fig 4).

Bound material eluted at pH 3 contains approximately 60% dimers and aggregates, confirming that they are adsorbed to Capto adhere during sample load while most of the monomers pass through the column. The total yield of monomer after sample application of 265 mg/ml is 94% and the dimers and aggregates content is reduced from 6% to 1.3% (4.8 times reduction). Chromatograms from the gel filtration on Superdex 200 are shown in Figure 7.

Table 3. Dimers and aggregates (D/A) content in starting material, fractions, and eluate during sample loading

Load (mg IgG/ml)	D/A (%)	Reduction
Starting material	6	ND
60	0.7	8.8
120	0.6	10.3
150	0.9	6.4
180	1.2	4.9
265	2.2	2.7
Pooled fractions	1.3	4.8
Eluate	~ 60	ND

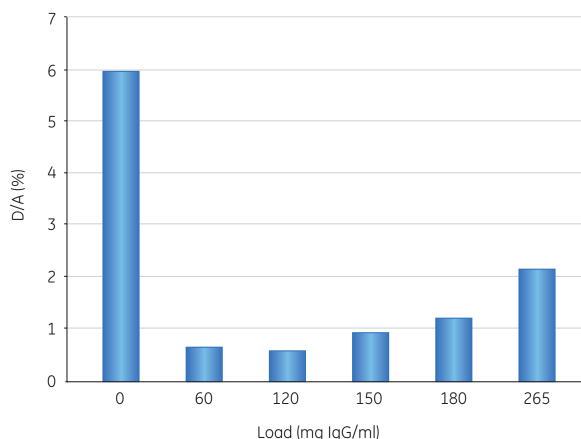
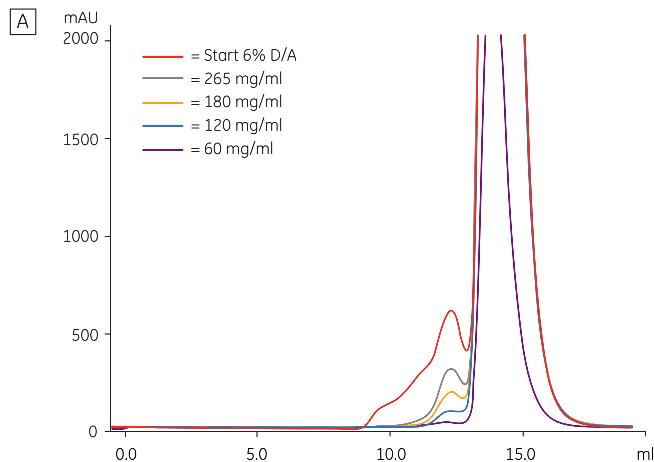


Fig 6. Dimers and aggregates content in starting material and fractions collected during sample loading.

Column:	Superdex 200 10/300 GL
Sample:	Start material and fractions collected after sample load of 60, 120, 180, and 265 mg MAb/ml medium (A); Bound material eluted with 0.1 M acetic acid (B).
Buffer:	PBS buffer pH 7.4
Flow rate :	0.5 ml/min
System:	ÄKTAexplorer



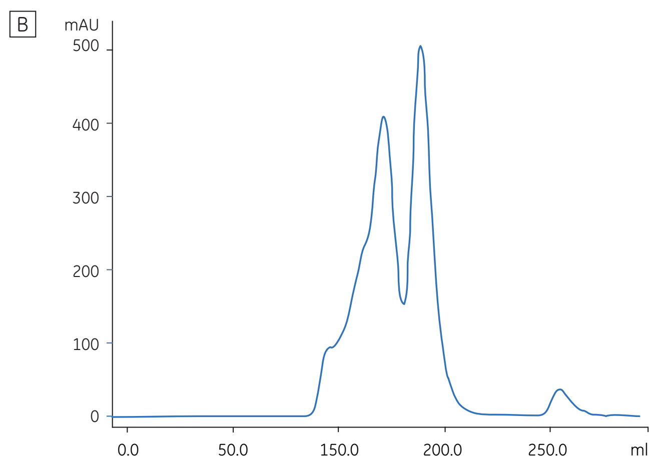


Fig 7. Gel filtration chromatography on Superdex 200 10/300 GL. **(A)** Start material and fractions collected after sample load of 60, 120, 180, and 265 mg MAb/ml medium. **(B)** Bound material eluted with 0.1 M acetic acid pH 3.

Conclusions

This study describes the optimization of the loading conditions using DoE, and the application of optimal conditions for the selective removal of dimers and aggregates from monoclonal antibodies purified by capture on MabSelect SuRe. At a sample load of 120 mg/ml, the dimers and aggregates content is reduced from 6% to 0.6%, giving a 10-fold reduction. Approximately 80% of the aggregates are adsorbed to the medium at a sample load of 265 mg IgG_1. The total yield of monomer is 94%. The results show that Capto adhere has a high potential to selectively remove dimers and aggregates from MAb preparations.

Acknowledgements

Filtered NS0 cell line feedstock was supplied by BioInvent International AB, Lund, Sweden.

www.gehealthcare.com/bioprocess

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

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Separating viral particles with Capto Q chromatography media may require a license under United States Patent 6,537,793 B2 and foreign equivalents owned by Centelion SAS.

The Tricorn column and components are protected by US design patents USD500856, USD506261, USD500555, USD495060 and their equivalents in other countries.

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First published Jan. 2007

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

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

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

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

Asia Pacific T +85 65 62751830 F +85 65 62751829 • Australasia T +61 2 8820 8299 F +61 2 8820 8200 • Austria T 01 /57606 1613 F 01 /57606 1614 • Belgium T 0800 73 890 F 02 416 8206 • Canada T 1 800 463 5800 F 1 800 567 1008 • Central & East Europe T +43 1 972 720 F +43 1 972 722 750 • Denmark T +45 70 25 24 50 F +45 45 16 2424 • Eire T 1 800 709992 F +44 1494 542010 • Finland & Baltics T +358 9 512 3940 F +358 9 512 39439 • France T 01 69 35 67 00 F 01 69 41 98 77 Germany T 0800 9080 711 F 0800 9080 712 • Greater China T +852 2100 6300 F +852 2100 6338 • Italy T 02 26001 320 F 02 26001 399 • Japan T 81 3 5331 9336 F 81 3 5331 9370 • Korea T 82 2 6201 3700 F 82 2 6201 3803 • Latin America T +55 11 3933 7300 F +55 11 3933 7304 • Middle East & Africa T +30 210 96 00 687 F +30 210 96 00 693 • Netherlands T 0800-82 82 82 1 F 0800-82 82 82 4 • Norway T +47 815 65 777 F +47 815 65 666 • Portugal T 21 417 7035 F 21 417 3184 • Russia, CIS & NIS T +7 495 956 5177 F +7 495 956 5176 • Spain T 902 11 72 65 F 935 94 49 65 • Sweden T 018 612 1900 F 018 612 1910 • Switzerland T 0848 8028 10 F 0848 8028 11 • UK T 0800 515 313 F 0800 616 927 • USA T +1 800 526 3593 F +1 877 295 8102

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