High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process

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We have used MabSelect SuRe™ and Capto™ adhere chromatography media (resins) to significantly reduce the level of IgG antibody aggregates in a sample using an efficient two-step method that resulted in high yields and purity. In addition, we have developed a screening format employing the exceptional capabilities of PreDictor™ 96-well filter plates, HiScreen™ prepacked columns, and a Design of Experiments (DoE) approach for effective and rapid screening for optimal experimental conditions. Application of the optimized protocol led to a reduction in aggregate levels from 12.6% to < 0.5% in a single step with a monomer yield of 87%. Host cell protein (HCP) and ligand leakage were reduced to negligible amounts. In total, 192 conditions (flowthrough and selective elution experiments) were screened in approximately 4 h and analyzed in 48 h. The use of a highthroughput method in the process described here led to a speedy identification and subsequent optimization of the initial conditions. This application note describes the development of the Capto adhere step. For details on the MabSelect SuRe step, see Application note 28-9468-58.

Introduction

In the purification of monoclonal antibodies, Protein A affinity media is often used for the capture step because it produces high purity and yield after a single chromatography step. Subsequent downstream processing can be performed according to a variety of protocols including different combinations of chromotography media (e.g., ion exchange and hydrophobic interaction chromatography).

An efficient approach to monoclonal antibody purification involves a two-step process (Fig 1) whereby a multimodal chromatography medium capable of both hydrophobic and ion exchange interactions can be designed into a single product thus allowing for the selective removal of antibody aggregates from the monomeric forms. MabSelect SuRe is used for the Protein A-mediated capture step and Capto adhere is used for the selective removal of antibody aggregates.

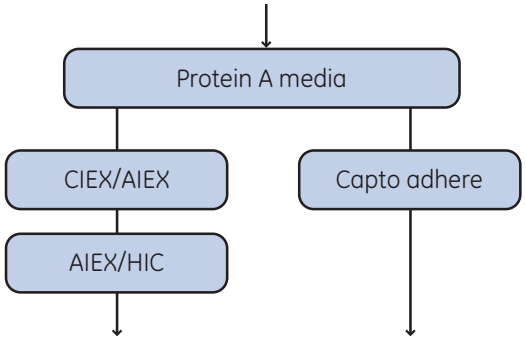

Fig 1. A classical three-step method (left) and an alternative two-step antibody process (right) based on MabSelect SuRe and Capto adhere. (CIEX = cation exchange chromatography; AIEX = anion exchange chromatography; HIC = hydrophobic interaction chromatography).

The complexity of multimodal media requires a more thorough process optimization study in order to take full advantage of the outstanding potential of this technology. This calls for the development of efficient and rapid screening methods for optimal process conditions.

This is the second of a set of four application notes focused on the development of operational excellence in MAb process development and manufacturing. The others are:

- High-throughput screening and optimization of a protein A capture step in a monoclonal antibody purification process (28-9468-58)
- Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom™ column formats (28-9403-49)
- A flexible antibody purification process based on ReadyToProcess™ products (28-9403-48)

This application note describes the development and optimization of a polishing step for the purification of a monoclonal antibody on Capto adhere. In the initial part of this study, we used PreDictor 96-well filter plates prefilled with Capto adhere to screen a large experimental space quickly. Promising results from the plate study were further optimized with HiScreen columns and a DoE approach to establish the final process conditions.

Materials and methods

Liquid handling

All the experiments were performed with PreDictor plates containing 6 µL of Capto adhere in each well. The buffers were prepared in an automated Tecan Freedom EVO™-2 200 Robotic System, but procedures such as sample addition were performed manually. Liquid removal during equilibration of the media was performed in a vacuum manifold and sample collection was performed by centrifugation (300 × g for 60 s).

Screening for initial conditions

The MabSelect SuRe elution pool was used as the sample after buffer exchange on a HiPrep™ Desalting column. The final IgG concentrations used were 0.53, 2.65 or 5.3 mg/mL depending on the experiment. The antibody solution contained approximately 14% of aggregates.

A 2× buffer stock solution was prepared for each experimental condition. The same volume of sample and buffer stock solution was then mixed and dispensed into each well of the PreDictor plate. The following parameters were tested in the initial screening phase: 50 mM sodium citrate pH 5.5 or 6.5; 50 or 450 mM NaCl; three different IgG concentrations (0.53, 2.65, and 5.3 mg/mL); and four different incubation times (2.5, 10, 30, and 60 min).

The final plate layout is shown in Figure 2. The following protocol was used:

1. The medium was equilibrated with 3 × 200 µL of buffer and excess liquid was removed by vacuum
2. The sample (200 µL) was added and incubated at four different incubation times (2.5, 10, 30, and 60 min) at room temperature on an orbital shaker at 1100 rpm
3. After the longest incubation time of 60 min, the flow through fraction was collected by centrifugation (300 × g for 60 s at room temperature) into 96-well plates

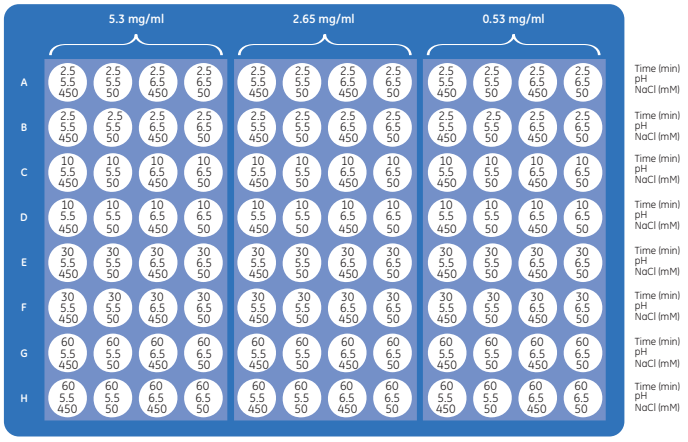

Fig 2. Plate layout of the initial screening experiments.

The starting material and flowthrough fractions were analyzed by size exclusion chromatography (SEC) with two Superdex™ 200 5/150 GL columns connected in series with a run time of 15 min/sample.

Flowthrough experiments

Analysis of the initial screening conditions enabled us to select appropriate conditions for the flowthrough experiments (Fig 3). The final IgG concentration was 5.3 mg/mL and the sample was incubated for 60 min. Sample and buffer handling were performed as described (see "Screening for initial conditions"). In these experiments, 96 different conditions were studied in one single plate as follows:

- 8 different pH levels with 50 mM sodium citrate (pH 4.0 to 6.0) or 50 mM sodium phosphate (pH 6.5 to 7.5)
- 12 different concentrations of NaCl (0 to 550 mM)

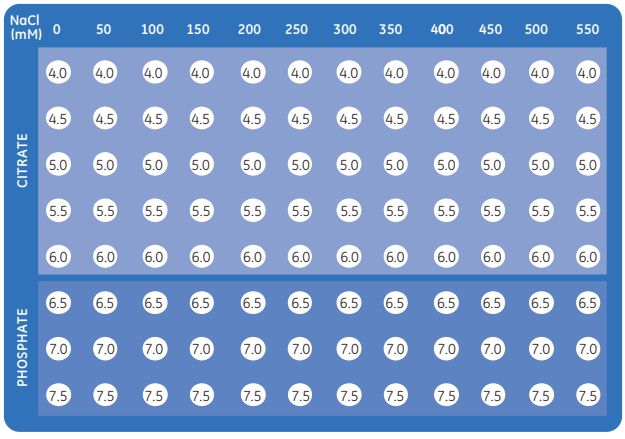

Fig 3. Plate layout of the flowthrough experiments.

Apart from an incubation time of 60 min, the protocol for the flowthrough experiments was the same as described (see "Screening for initial conditions").

Selective elution study

An elution study (Fig 4) was performed to improve the proportion of monomer yield. We investigated two different binding conditions (500 mM NaCl, 50 mM sodium citrate pH 4.5; and 50 mM NaCl, 50 mM sodium phosphate pH 7), for both the sample solution as well as the wash buffer. Each elution step was performed with the same buffer that was used in the binding step. The elution conditions were:

- pH 4.0 to 6.0 with 50 mM sodium citrate
- pH 6.0 to 7.0 with 50 mM sodium phosphate
- 0 to 550 mM NaCl

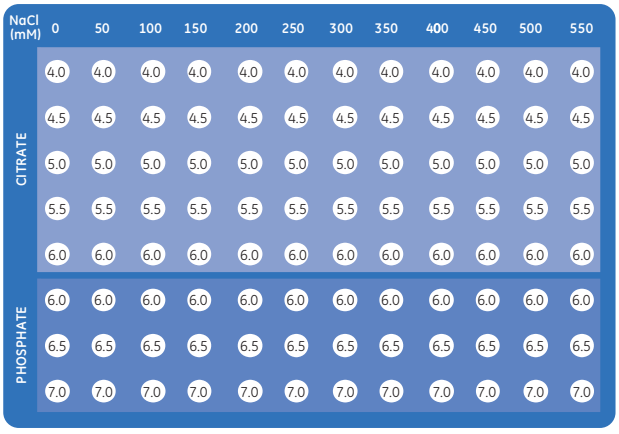

Fig 4. Plate layout of the selective elution study.

Briefly, the following protocol was used:

1. The medium was equilibrated with 3 × 200 µL of buffer and excess liquid was removed by vacuum filtration.
2. The sample (200 µL) was added to each well. The plate was incubated at room temperature on an orbital shaker at 1100 rpm for 60 min followed by centrifugation 300 × g for 60 s at room temperature) into an empty 96-well plate.
3. Each well was washed with 2 × 200 µL of equilibration buffer.
4. Elution was performed with 3 × 200 µL of elution buffer.

Column optimization with a factorial design

The MODDE™ software v8 was used to set up a Central Composite Face (CCF) design with a response surface modeling (RSM) objective. This resulted in 26 design runs plus replicated center points. The factors investigated are summarized in Table 1.

Table 1. Factors investigated in the optimization study

Aggregates	9% to 14%
Concentration	5 to 15 mg/mL
Load	60 to 100 mg/mL
Elution pH	6.1 to 6.5
NaCl for elution	150 to 450 mM

Loading was carried out according to the optimal conditions discovered in the screening phase. The pH and NaCl concentrations (Table 1) refer to the selective elution conditions from the column. The residence time was 5 min throughout the entire study. The starting IgG sample for this study consisted of two MabSelect SuRe elution pools containing 9% and 14% of aggregates, respectively. The center points were created by mixing equal amounts of the two samples to produce a final sample containing 11.5% of aggregates. A HiScreen Capto adhere column (4.7 mL) was used for the optimization study.

A freshly produced IgG sample containing 12.6% aggregates was used for the column verification experiment on a 1 mL HiTrap™ Capto adhere column.

Size exclusion chromatography (SEC) analysis

Aggregate content and concentration of Mab were analyzed by SEC using two interconnected Superdex 200 5/150 GL columns. An aliquot (10 µL) of each sample was applied to the column and run in phosphate buffered saline (PBS) at a flow rate of 0.35 mL/min for 15 min.

Yield and purity were calculated from the SEC results as follows:

$$Yield = \frac{Area_{monomer\ (eluted)}}{Area_{monomer\ (loaded)}} \qquad Equation\ 1$$

$$Purity = \frac{Area_{monomer}}{Area_{monomer\ +\ aggregates}} \qquad Equation\ 2$$

(in the elution or flowthrough)

Host cell protein (HCP) and ligand leakage analyses

HCP levels were measured using commercial anti-CHO HCP antibodies (Cygnus Technologies). Essentially, an ELISA methodology was adapted to a Gyrolab™ Workstation LIF using Gyrolab Bioaffy™ 200 HC microlaboratory discs.

Ligand leakage measurements were performed using a commercial ELISA kit (Repligen Corporation) with a slightly modified protocol compared to the one supplied by the manufacturer.

Column prediction

The data obtained from the PreDictor plate experiments was used to predict the column conditions as follows: assuming that monomer plate capacities equaled dynamic binding capacities (most likely valid for longer residence times), then purity and yield can be calculated based on the following equations:

$$Q_m = (C_{ini,m} - C_{FT,m}) \frac{V_{sample}}{V_{medium}} \qquad \textit{Equation 3}$$

$$Yield = \frac{V_{load} \times C_{ini,m} - CV \times Q_m}{V_{load} \times C_{ini,m}} \qquad \textit{Equation 4}$$

$$Purity = \frac{V_{load} \times C_{ini,m} - CV \times Q_m}{V_{load} \times (C_{ini,m} + C_{ini,a}) - CV \times (Q_m + Q_a)} \qquad \textit{Equation 5}$$

where V_{load} is volume loaded, C_m is monomer concentration, C_a is aggregate concentration, ini represents initial, FT represents flowthrough, CV is column volume and Q_m or Q_a are the binding capacities for monomer and aggregates, respectively.

Results and discussion

The two-step process was based on MabSelect SuRe as the capture step, followed by Capto adhere in flowthrough mode. Screening and optimization of the process conditions were performed with the goal of decreasing aggregate content from approximately 14% in the feed, to less than 1% aggregates (~ 99% monomer purity)in the final sample with acceptable yields (> 85%). A secondary goal was to explore new formats such as PreDictor plates and HiScreen columns—in combination with a DoE approach—to produce rapid screening and reduce the number of experiments required to establish optimal process conditions.

Screening for initial conditions with PreDictor plates

One of the goals of the initial screening phase was to determine the incubation time required for all the components to reach a state of equilibrium so that the binding properties of both monomers and aggregates can be estimated (Fig 5). Adsorption was completed after approximately 10 min and 30 min for the monomer and aggregate species, respectively. The aggregates produced slower kinetics so an incubation time of 60 min was chosen for the remaining experiments.

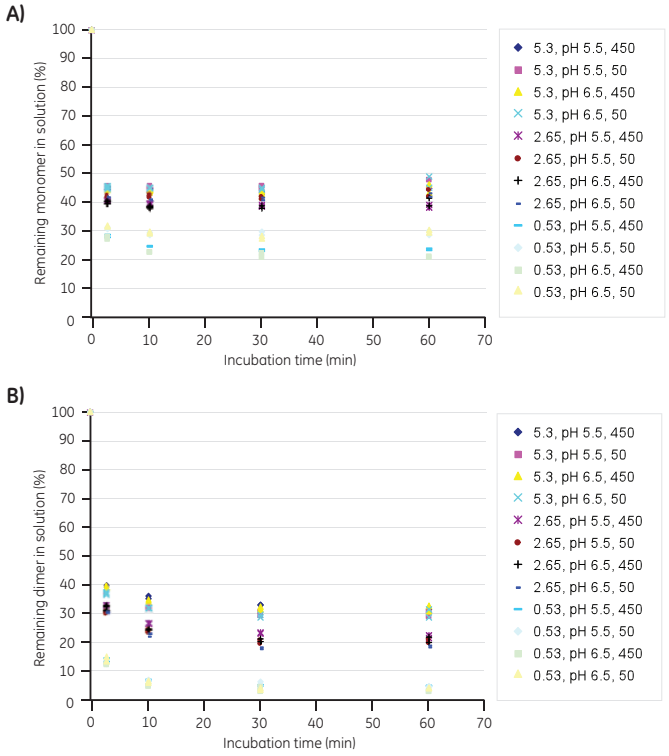

Fig 5. Adsorption curves of (A) monomer and (B) aggregates. This shows the remaining monomer and aggregate concentrations in the flowthrough fractions under the investigated conditions of antibody amounts, NaCl concentrations, and pH).

Flowthrough experiments with PreDictor plates

An IgG sample containing 14% of aggregates was used. After applying the sample, the flowthrough fractions were subjected to SEC analysis. The capacities for monomer and aggregate IgG (Fig 6) were calculated (Equation 3). The capacity for IgG monomers exceeded that of aggregates under all the conditions tested, which implied that the removal of aggregates would result in the inevitable loss of some monomer IgG.

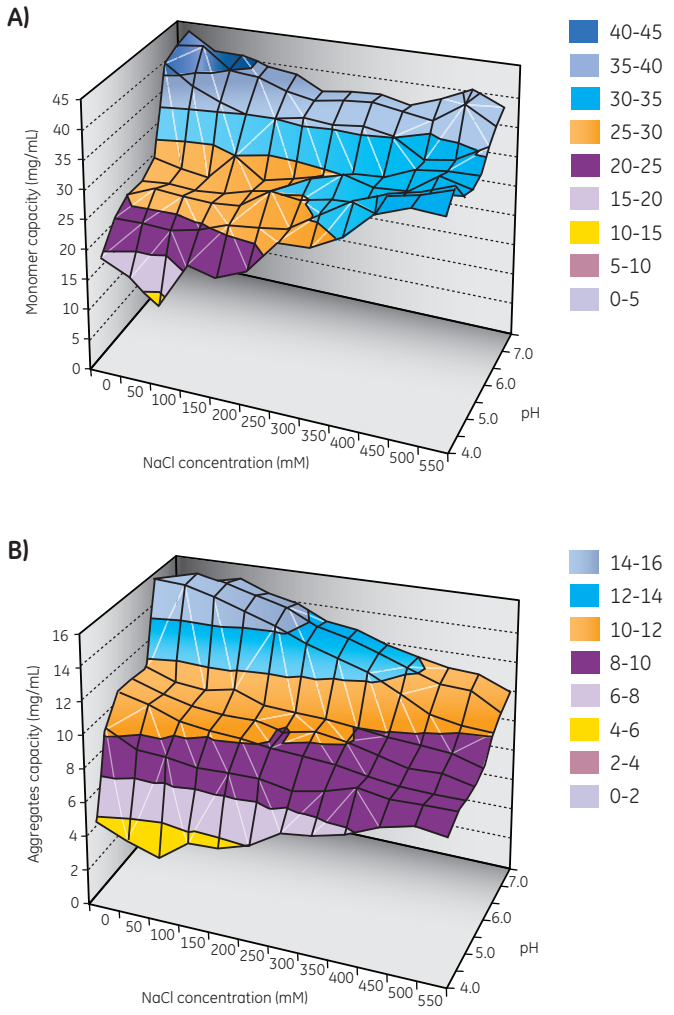

Fig 6. (A) Monomer and (B) aggregate capacities determined from the PreDictor plate experiments.

Column prediction

Data from the flowthrough experiments and the application of equations 3, 4, and 5 were used to predict column performance. In the example shown in Figure 7, a prediction based on a column volume of 10 mL and a sample load of 130 mg/mL produced > 98% monomer and a yield of 60% to 65%. A yield as low as that is not acceptable for a large-scale process so we opted for a selective elution study.

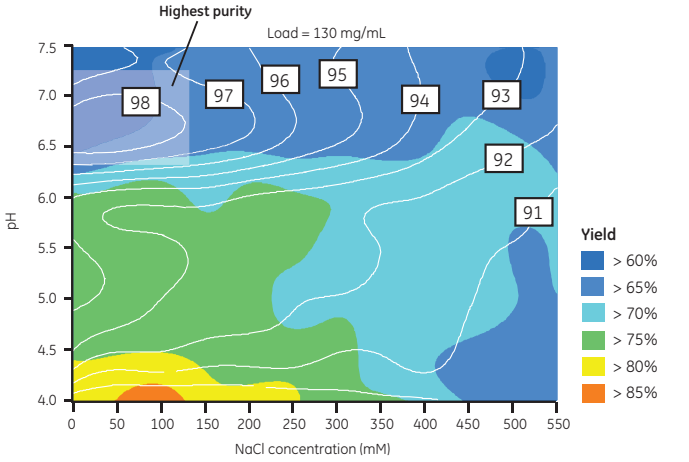

Fig 7. Column prediction of purity (iso-lines) and yield (color map) at a sample load of 130 mg/mL.

The raw data was also plotted as a function of purity times yield for all the elution conditions with the aim of finding a compromize for the two responses yield and purity (Fig 8). The optimum spot in such a plot is expected to produce the highest purity and yield at the same time. The peak values were found at an approximate pH of 6 and 250 mM NaCl.

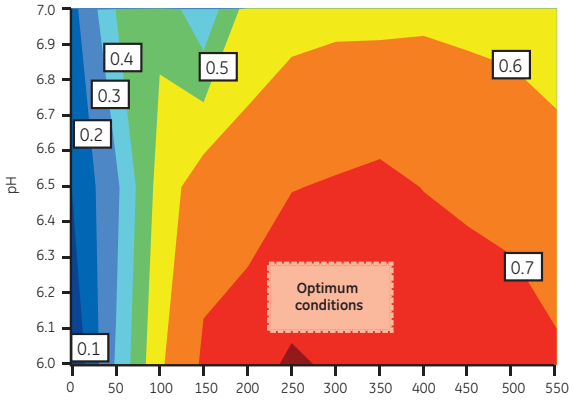

Fig 8. Effect of NaCl concentration and buffer pH on a normalized objective function purity × yield.

Optimization study with HiScreen columns (DoE)

We investigated the following factors:

- Protein concentration
- Aggregate content of the start sample
- Aggregate content of the load sample
- Elution pH
- Elution NaCl concentration

The experiments were performed to find the best conditions for monomer purity (> 99% in the final sample) and acceptable monomer yield (> 85%).

The purity of the monomer IgG (Fig 9) was adversely affected by an:

- Increase in start aggregate level (Aggr)
- Increase in start protein concentration (Conc)
- Increase in load (Load)
- Increase in NaCl concentration (NaCl)

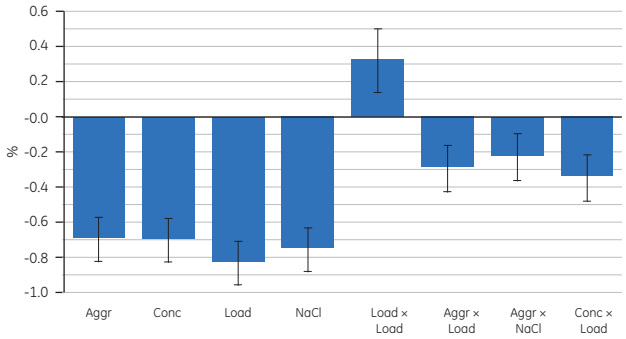

Fig 9. Coefficients plot for monomer purity.

We found that the yield of monomer IgG was adversely affected by an increase in the amount of aggregate IgG in the starting sample and also, by an increase in the pH of the elution buffer. On the other hand, the yield of monomer IgG was enhanced by an increase in the sample load and also, by an increase in the amount of NaCl in the elution buffer. Although the effect of the sample concentration was not significant, it was left in Figure 10 because one of the interactions contained this factor. For both models, quadratic terms and other interactions were present.

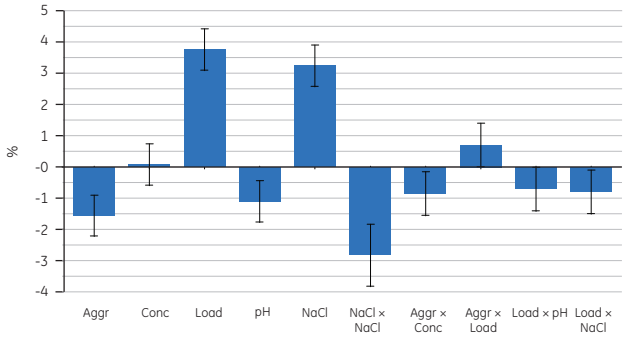

Fig 10. Coefficients plot for monomer yield.

The models for purity and yield can be combined to produce a sweet spot for a particular set of user-defined criteria (Fig 11). In this case, the set criteria were: > 85% monomer yield and > 99% monomer purity (which is equivalent to less than 1% of aggregated IgG). The load was set to 60 mg/mL and the NaCl concentration for elution was 300 mM.

We found a broad zone within the investigated pH interval where both criteria were fulfilled. The broadest operational area was discovered at the most acidic elution pH of 6.1.

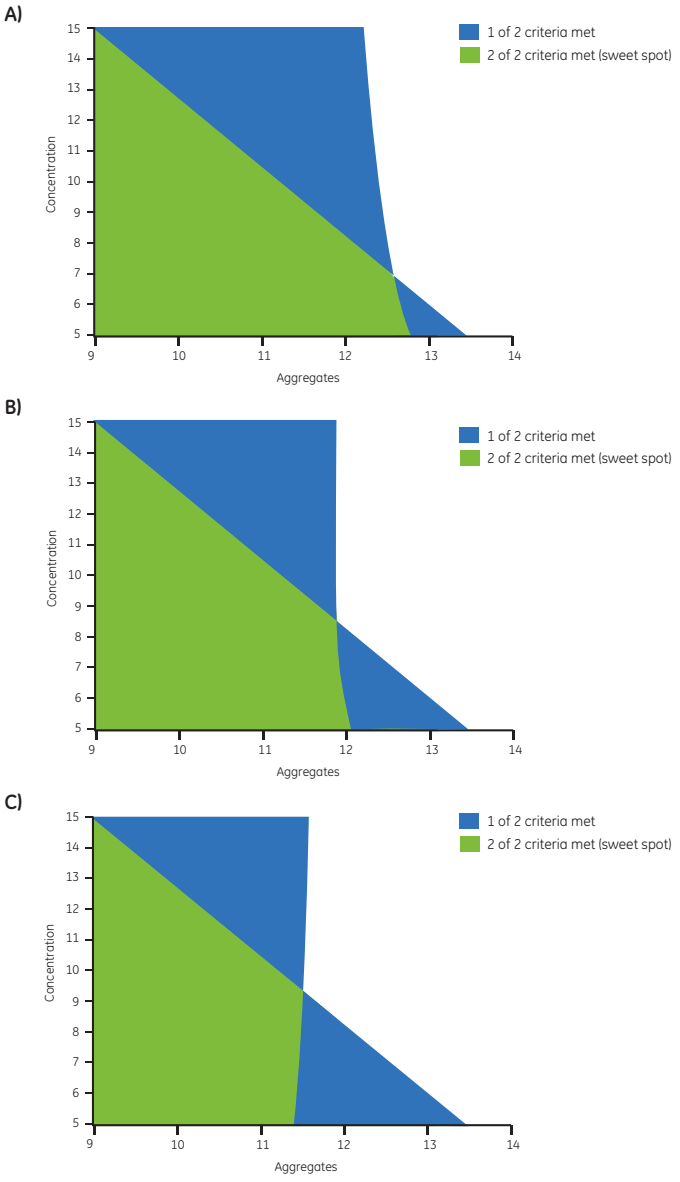

Fig 11. A sweet spot plot for IgG monomer yield and purity. The conditions for these plots were a sample load of 60 mg/mL media and elution with 300 mM of NaCl at the three different pH levels of: (A) 6.1; (B) 6.3; and (C) 6.5.

Column verification

Since there was a good correlation between the data from the optimization study and that from the Predictor plate experiments, we set up a column verification study with a 1 mL HiTrap Capto adhere column using similar run conditions to those from the sweet spot analysis:

- The sample load was 60 mg/mL
- The concentration of IgG aggregates in the starting sample was 12.6%
- The starting concentration of the IgG sample was adjusted to 5 mg/mL
- The elution buffer had a pH of 6.1 and a NaCl concentration of 250 mM

The column verification study (Fig 12) produced an eluted IgG monomer yield of 87%, which was a significant improvement on the 60% to 65% yield obtained from the PreDictor plate experiments in which only the flowthrough was included in the process step. The purity level (99.5%) of the eluted IgG monomer met the sweet spot analysis criteria of > 99.0% (Fig 13). In addition, the HCP content of the eluted IgG monomer was reduced from 131 ng/mL (26 ppm) to under the limit of quantification < 5 ng/mL. MabSelect SuRe ligand leakage was also reduced from 10 ng/mL (2 ppm) to under the limit of quantification < 3 ng/mL.

Column:	HiTrap Capto adhere 1 mL
Sample:	Diafiltered elution pool from MabSelect SuRe, 5 mg/mL
Load:	60 mg/mL
Binding buffer:	50 mM sodium phosphate, 50 mM NaCl, pH 7.0
Elution buffer:	50 mM sodium phosphate, 250 mM NaCl, pH 6.1
Flow rate:	0.2 mL/min
System:	ÄKTAexplorer™ 100

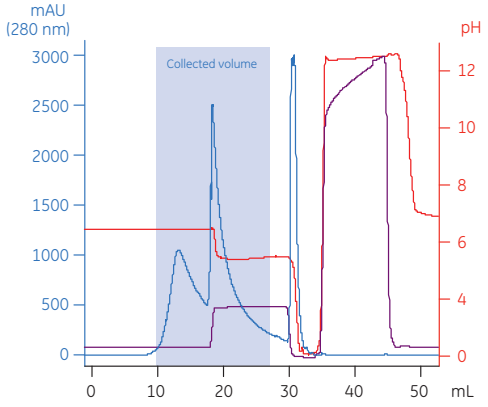

Fig 12. Chromatogram from the column verification study.

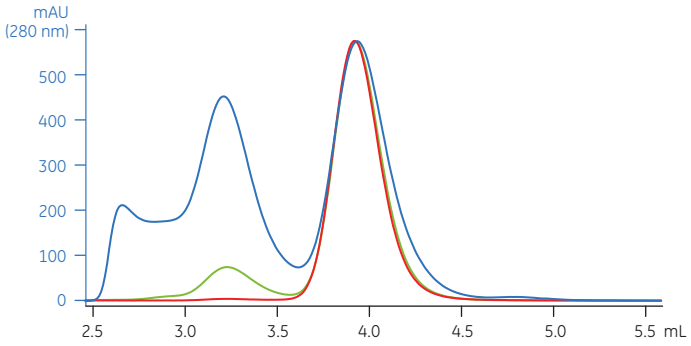

Fig 13. SEC analysis of the start material (green), flowthrough/elution fraction (red), and strip fraction (blue). All the curves were normalized against the flowthrough/elution fraction.

Conclusions

We have used Capto adhere (as the polishing step) with MabSelect SuRe (capture step) to reduce high levels of IgG antibody aggregates in an efficient two-step method that produced high yields and purity. In addition, we demonstrated an application of new screening formats employing the exceptional capabilities of PreDictor 96-well plates, HiScreen prepacked columns, and a DoE approach for effective and rapid screening for optimal conditions. The plate format is suitable for initial screening whereas the more refined screening, based on the findings from the plate results, should be performed with the column formats for optimal results. The optimized process was able to reduce aggregates levels from 12.6% to < 0.5% in a single step with a monomer yield of 87%. Furthermore, HCP and ligand leakage were reduced to negligible values. In total, 192 conditions (flowthrough and selective elution experiments) were screened in approximately 4 h and analyzed in 48 h. The high-throughput workflow produced a high-level knowledge of the process and allowed for a rapid identification of the conditions for optimization.

Ordering information

Product	Quantity	Code no.
PreDictor MabSelect SuRe, 6 µL	4 × 96-well filter plates	28-9258-23
PreDictor MabSelect SuRe, 20 µL	4 × 96-well filter plates	28-9258-24
PreDictor Capto adhere, 6 µL	4 × 96-well filter plates	28-9258-17
PreDictor Capto adhere, 20 µL	4 × 96-well filter plates	28-9258-18
HiTrap MabSelect SuRe	5 × 1 mL	11-0034-93
HiTrap Capto adhere	5 × 1 mL	28-4058-44
HiScreen MabSelect SuRe	1 × 4.7 mL	28-9269-77
HiScreen Capto adhere	1 × 4.7 mL	28-9269-81

Related literature

Data file: MabSelect SuRe	11-0011-65
Data file: Capto adhere	28-9078-88

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