High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates

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Introduction

Protein A affinity chromatography is the industry standard for capture of monoclonal antibodies in the downstream purification process. The Protein A step is a straightforward unit operation where the monoclonal antibody (MAb) is captured at near neutral pH and eluted using acidic pH. It is highly generic - most of the operating parameters can be predefined and identical for a number of different MAbs. However, some of the parameters, such as elution conditions, can benefit from further optimization for each individual MAb. Antibody aggregation during cell-culture and/or in the downstream processing, particularly during the low pH elution from the Protein A column and subsequent low pH virus inactivation, can be a challenge. Monoclonal antibodies susceptible to low pH and prone to aggregation will profit from optimizing the elution step for lowest possible aggregation and high monomer recovery. Additives, such as arginine, in the elution buffer, can increase the pH where elution of the MAb occurs, and thereby preventing aggregation (1).

In this study, PreDictor™ plates were used for screening of elution pH for five different MAbs on the Protein A derived chromatography medium MabSelect SuRe™. The use of PreDictor plates, 96-well filter plates with chromatography media, for screening of elution buffer conditions significantly reduces the development time required. The principle for PreDictor plates is batch uptake, corresponding to a separation where one single theoretical plate is involved, as compared to packed bed chromatography, where a cascade of theoretical plates refines the separation. Nevertheless, PreDictor plates are useful for prediction of chromatographic behavior. The focus of this study was to compare the elution pattern obtained from PreDictor plates with the elution pattern obtained using traditional packed bed chromatography.

Materials and methods

Plate experiment overview

PreDictor plates filled with 50 µl MabSelect SuRe per well were used. Removal of liquid between equilibration, sample load, wash, elution and acidic strip was performed by centrifugation. The monoclonal antibodies were loaded at a concentration of 1.3 mg/ml. Four of the MAbs (MAb #1, #3, #4 and #5) were diluted from purified MAb solution to the correct concentration in loading buffer. For MAb #2, unpurified clarified murine myeloma (NS0) cell supernatant at a concentration of 1.3 mg/ml was used as the start material. Equilibration, wash and acidic strip buffers were identical for all wells, whereas elution was performed with 20 mM sodium citrate between pH 5.8-3.0 in steps of 0.2 pH units according to the plate design in Figure 1. For convenience, elution buffers were distributed in a separate deep-well plate according to the plate design, prior to the plate experiment. This preparation enabled transfer of elution buffer with an eight-channel pipette from the deep-well plate to the filter plate when running the experiment. Analysis was made by measuring the absorbance at 280 nm in the unbound fractions (flowthrough), wash, eluate, and acidic strip fractions, collected in UV-readable plates. The MAb concentration in the elution fractions was determined and the recovery was calculated for the different elution pH values.

Plate procedure

1. Equilibration steps 1-3

After removal of storage solution, 200 µl of equilibration buffer was added to each well and then subsequently removed. This procedure was performed three times. Equilibration buffer was 20 mM sodium phosphate, 0.15 M sodium chloride, pH 7.4.

2. Loading step

Each well was loaded with 300 µl of 1.3 mg/ml MAb solution and incubated for 20 minutes on a shaker at approximately 1100 rpm before removal of the liquid by centrifugation. The unbound fraction was collected into a UV-readable plate for analysis.

3. Wash 1 and 2

Wells were washed with 200 µl loading buffer. The buffer was removed and the procedure was repeated.

4. Wash 3

Wash was performed with 200 µl of 20 mM sodium citrate, pH 6.0, and the wash solution was then removed. The wash fraction was collected into a UV-readable plate for analysis.

5. Elution 1, 2 and 3

Elution was carried out with 200 µl of 20 mM sodium citrate, with different pH in different wells according to the plate design (Figure 1). The chromatography medium was incubated with elution buffer 2 minutes before removal. This was performed three times. The three eluates were collected into three different UV-readable plates for analysis.

6. Acidic strip 1, 2, and 3

Acidic strip was performed with 200 µl of 0.1M sodium citrate, pH 3.0 (the same condition in all wells). The chromatography medium was incubated with acidic strip buffer 2 minutes before removal. This was performed three times. The acidic strip fractions were collected into three different UV-readable plates for analysis.

7. Analysis

The absorbance at 280 nm was measured and the MAb concentration was determined in each fraction as well as total recovery for all three elutions.

Note that in the steps above, centrifugation was used to remove liquid from the PreDictor plates.

The concentration in the eluate fractions was calculated according to Lambert- Beer´s law and the recovery was determined by equation 1.

Equation 1:

$$\text{recovery (\%)} = (C_{eluate} \times V_{eluate}) / (C_{load} \times V_{load}) \times 100$$

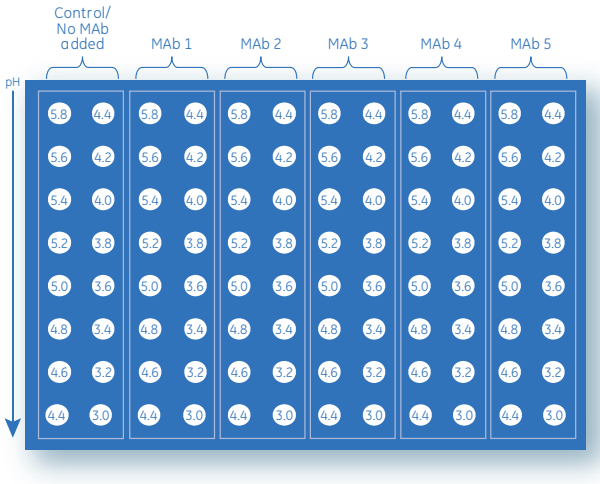

Fig 1. Plate design for the elution study on MabSelect SuRe. The chromatography medium in the plate was equilibrated with 20 mM sodium phosphate, 0.15 M sodium chloride, pH 7.4 before the antibodies at a concentration of 1.3 mg/ml were loaded onto the plate. The load was 7.8 mg MAb/ml chromatography medium. Elution was performed with 20 mM sodium citrate between pH 5.8-3.0 in steps of 0.2 pH units according to the scheme.

Column experiments

The column experiments were performed in a Tricorn™ 5/100, column volume (CV) 2 ml, packed with MabSelect SuRe. Sodium phosphate (20 mM) and sodium chloride (0.15 M) at pH 7.4 were used as equilibration/loading buffers and the sample concentration was 1.3 mg MAb/ml. The pure antibodies were diluted in equilibration buffer to the correct concentration before sample application. One ml of MAb solution and unpurified clarified NS0-cell supernatant, corresponding to a load of 0.7 mg/ml, was applied to the column in five different cycles. After sample loading, a short wash with loading buffer was performed, followed by a wash with 20 mM sodium citrate, pH 6.0. A decreasing linear pH gradient from pH 6-3 was then applied for 15 column volumes for elution of the monoclonal antibodies. UV absorbance at 280 nm was used for tracing of elution.

Results

Elution pattern

In Figure 2 the recovery in the elution fractions from the PreDictor plate is shown together with the corresponding chromatogram from the column experiment. Very good correlations between plates and columns were obtained for all five MAbs. Four of the MAbs showed generic elution at approximately pH 3.6, a behavior typical for MabSelect SuRe (2). MAb #5 showed different behavior with a 20% recovery at pH 5.8. The maximal recovery in the 96-well plate for MAb #5 (94%) was obtained at pH 4.6. In the column experiment, MAb #5 eluted as a broad peak, with the peak maximum at pH 4.8.

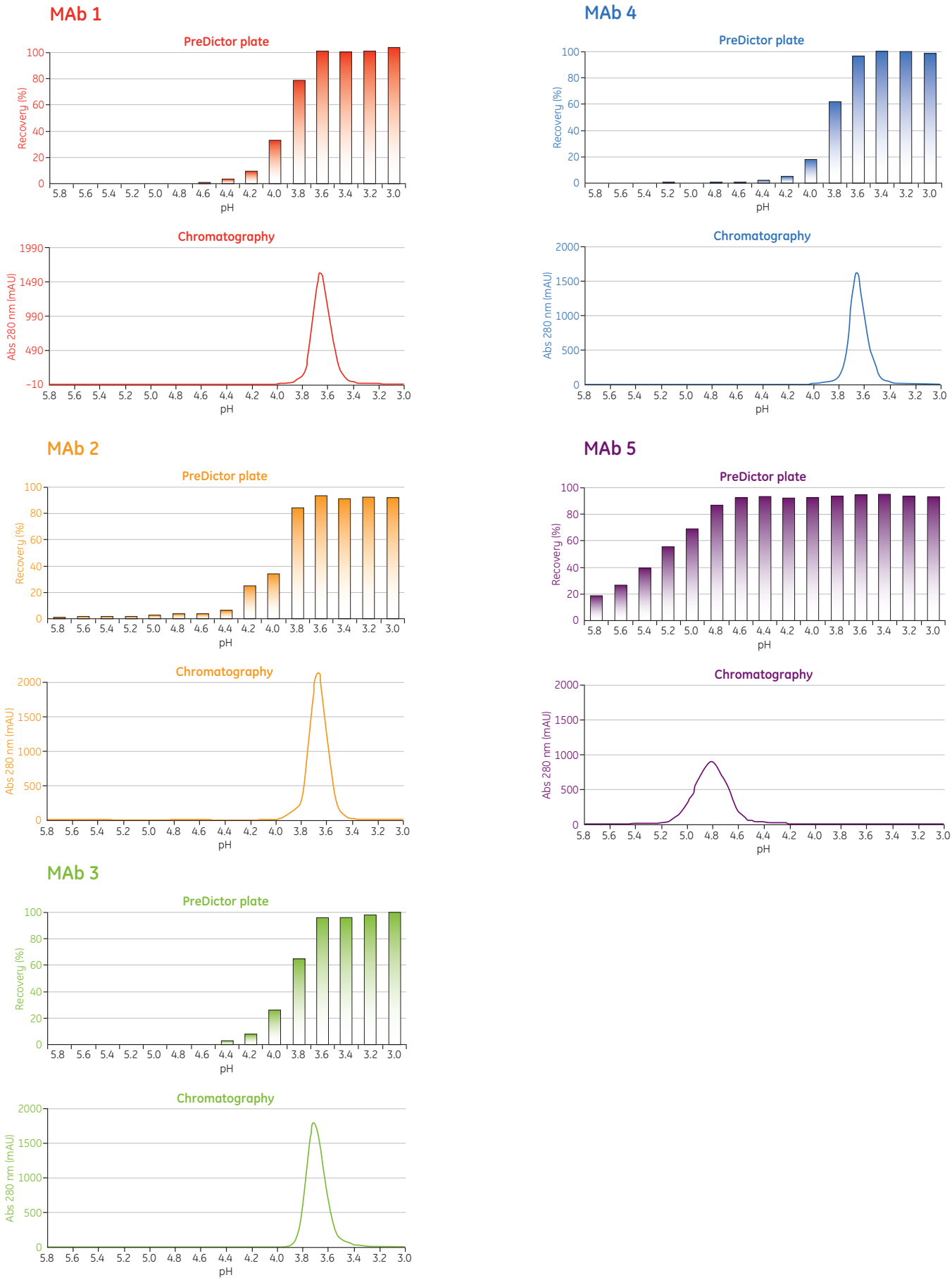

Fig 2. Comparison of elution patterns on MabSelect SuRe 96-well format relative to column chromatography. The bars in the histogram correspond to the cumulative relative amount of MAb recovered after elution at different pH values for each MAb. Below the histograms the corresponding chromatograms are shown.

Recovery in unbound fraction (flowthrough), wash and strip

The unbound material from the plate experiment, corresponding to the flowthrough when running a column, was also analyzed for UV absorbance at 280 nm. Less than 1% of MAbs #1, #3, and #4, and 1% of MAb #5 was found in the unbound fraction. For antibody #2, purified from cell culture supernatant, it was not possible to determine the amount of antibody in the unbound fraction merely by UV detection. This was due to the intrinsic UV absorbance of the feed caused by the high amount of host cell proteins and other UV absorbing components. However, the high recovery in the elution fractions at pH 3.0-3.6 indicated that there was no significant loss of MAb #2 in the flowthrough. To be able to determine the antibody concentration in a complex mixture, an affinity method such as a Biacore™-based assay (real time SPR), ELISA, or an analytical Protein A method would be needed.

The maximum recovery during elution obtained for MAb #5 was 94%, which was somewhat lower than for the other MAbs tested. The lower recovery can be explained by loss of MAb #5 during the pH 6.0 wash. Analysis of the third wash fraction (pH 6.0) showed that 3%-4% of MAb #5 was found in this fraction, whereas the other antibodies showed no loss in recovery due to the pH 6.0 wash. Enlarging the chromatogram close to the baseline shows that MAb #5 starts to leak off the column at pH 6.0, and is then continuously leaking off the column at pH < 6 (Figure 3).

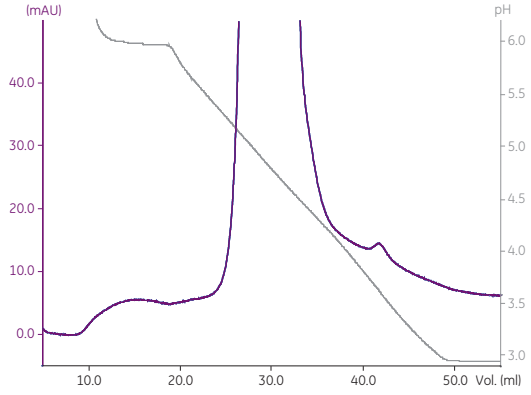

Fig 3. Enlargement of the MAb #5 chromatogram. Using the PreDictor format it was found that 3%-4% of MAb #5 was lost during wash with the pH 6.0 buffer. This was confirmed with traditional chromatography. Magnification of the area close to the baseline in the chromatogram confirms that MAb #5 leaks off the column at pH 6.0, and is then continuously leaking off at pH < 6, resulting in a broad elution peak with its maximum at pH 4.8.

For removal of the MAb still bound to the chromatography media after elution, and to be able to calculate the mass balance, all wells were stripped with 0.1M sodium citrate, pH 3.0. As expected, the histograms showing the MAb eluted during this acidic strip are mirror images of the histograms showing the recovery in the elution fractions (Fig 4). The wells where an elution pH gave a poor recovery subsequently gave a high recovery during the acidic strip (and vice versa).

Conclusions

The results obtained on PreDictor plates show good correlation with results obtained using traditional packed bed chromatography. PreDictor plates are a time-saving and accurate tool for screening of chromatographic conditions. As shown here, many different targets can be screened for suitable elution conditions with very little time and sample spent. None of the five antibodies contained significant amounts of soluble aggregates and dimers. If aggregation had been an issue, this type of methodology would have been useful for screening of elution conditions aiming for low aggregate concentration and high monomer recovery in the product pool. Screening for additional parameters such as buffer species, conductivities, and buffer additives (preventing aggregation during elution) could easily be included in this type of study.

References

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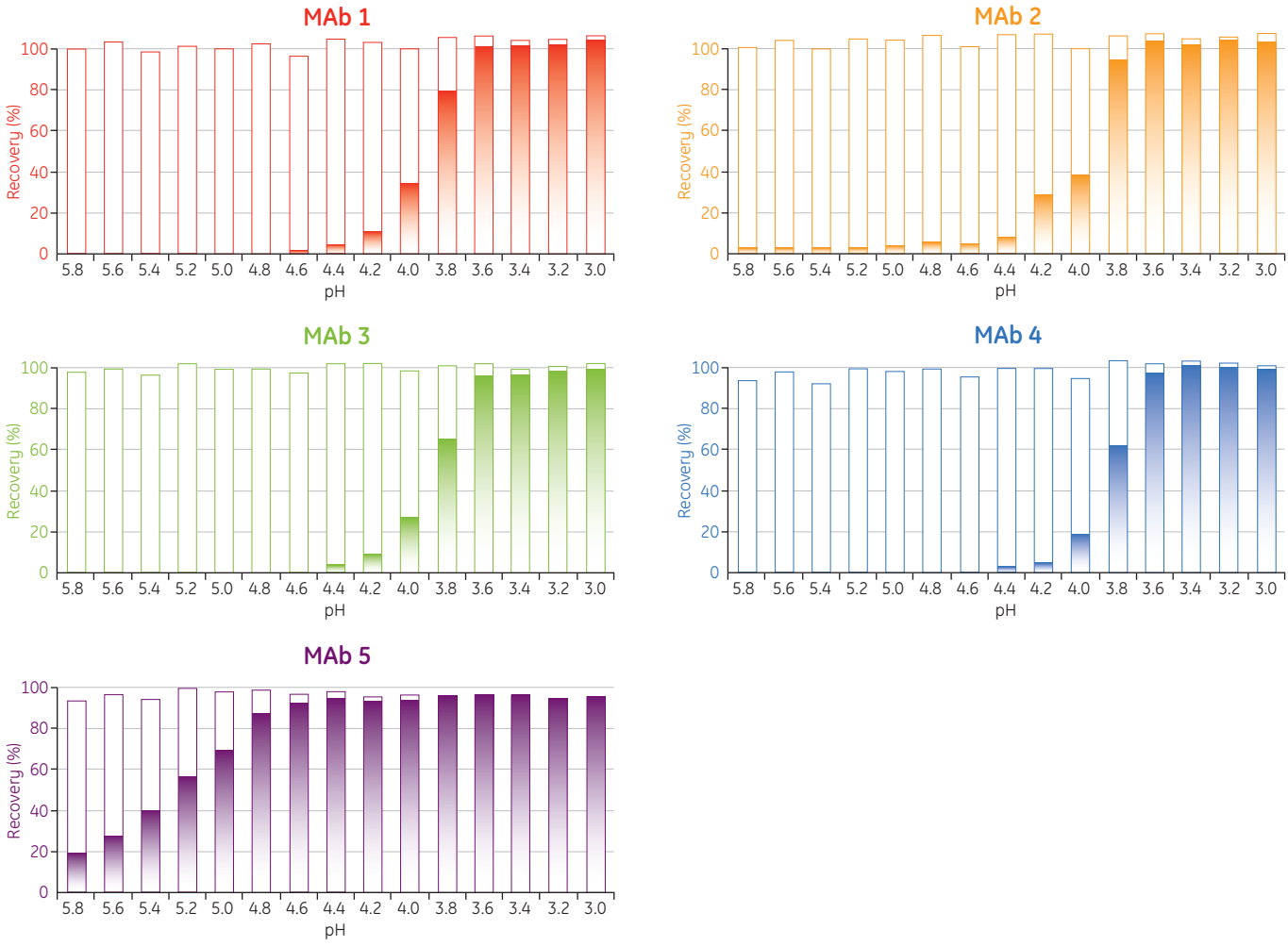

Fig 4. The recovery in the eluates and the acidic strip steps. Acidic strip of MAb still bound to the chromatography media after elution using various pH was done with 0.1M sodium citrate pH 3.0. The recovery in the acidic strip is depicted as unfilled bars whereas the recovery in the elution fractions is depicted as filled bars. As expected, the histograms showing the MAb fraction recovered during acidic strip are mirror images of the histograms showing the recovery during elution. A poor recovery during elution resulted in a high recovery during strip, and vice versa.

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