High-throughput screening for elution conditions on Capto MMC using PreDictor plates

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY13156-04May20-AN

High-throughput screening for elution conditions on Capto MMC using PreDictor plates

Carina Engstrand, Tryggve Bergander and Karol Lacki
GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden

Introduction

The increasing demands on the Biopharma industry with shorter time lines and cost constraints have challenged the use of conventional column chromatography for early process development. High-throughput screening of chromatographic conditions can substantially decrease the time and amount of sample needed during this process. The work presented here describes the use of PreDictor™ plates, 96-well filter plates filled with chromatography media, for parallel screening of elution conditions on the multimodal cation exchange medium Capto™ MMC using Design of Experiments (DoE). The results are compared with data generated in chromatography columns. Comparisons of time and sample required to perform the study in the two different formats are made.

Experimental approach

In this elution study, buffer ionic strength (BIS), pH, and concentration of two eluting salts were varied using DoE (MODDE™, version 8.0) within a range of conditions listed in Table 1.

Theoretically, the batch adsorption/desorption occurring in the wells of PreDictor plates involves the same mass transfer mechanisms as in column chromatography (1). To verify that data derived from PreDictor plates give the same qualitative information as data generated by column chromatography, a corresponding study was performed in a Tricorn™ 5/100 column (column volume (CV) 2 ml).

Table 1. The factors, associated ranges, and responses measured in this study

Factors	Range	Responses
Salt concentration	0.25, 1.00, 1.75 M	Recovery in 1st elution (for plates) or in 3 CV (for column)
Salt type	NaCl or NH_4Cl	
Buffer Ionic Strength (BIS)[1]	0.026, 0.163, 0.300 M	
pH[2]	5.75, 6.25, 6.75	

[1] Calculated from the Henderson-Hasselbach equation without correction for pKa shifts, and at different ionic strengths. Obtained by addition of sodium hydroxide.

[2] The buffers used were sodium malonate (pH 5.75 and 6.25) and sodium phosphate (pH 6.75).

Materials and Methods

Prototype PreDictor Capto MMC plates filled with 6 µl chromatography medium per well were used to study elution conditions for bovine serum albumin (BSA). Equilibration buffer was 50 mM sodium acetate, 250 mM NaCl, pH 4.75. Removal of liquid from the plates was done using a vacuum manifold.

Design of Experiments

For the experimental design the factors and responses listed in Table 1 were chosen. The experimental layout in the PreDictor plate was done according to the RED-MUP design in MODDE. The design was a full factorial design in multiple levels in order to resolve main, interactive, and curvature effects. The software used for the design and evaluation was MODDE version 7.0 for column experiments and MODDE version 8.0 for plate experiments (2).

Prestudy: Binding step

To identify experimental settings (e.g., incubation time and protein concentration) for the elution study, a pre-study of the binding step was performed. Batch uptake curves at four different protein concentrations were monitored by adding sample at different times, followed by simultaneous removal and measurement of unbound protein. The dynamic binding capacity at 10% breakthrough (QB10%) for BSA had previously been determined in columns to be approximately 35 mg/ml chromatography medium at 1 minute residence time. A suitable load is often considered 70% of QB10%, in this case 25 mg/ml. Thus, for the experimental procedure, conditions giving a binding capacity of 25 mg/ml should be chosen.

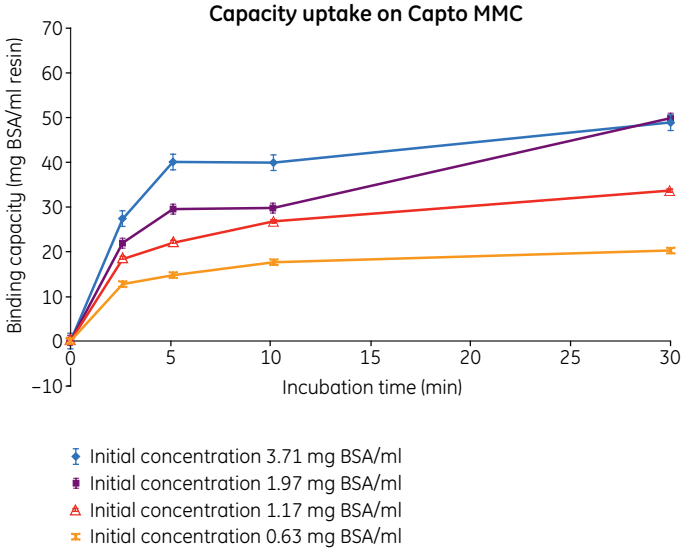

Fig 1. Batch uptake curves for BSA at four different concentrations at various incubation times.

Elution study

Chromatography in 96-well format

After equilibration of the chromatography media in the wells, 200 µl of 1 mg BSA/ml equilibration buffer was loaded onto each well in the PreDictor plate and incubated on an orbital shaker for 10 minutes. The wells were washed three times with equilibration buffer, whereafter elution was done using the different conditions described in the experimental set-up. The elution step was repeated three times. Eluted material was collected in a UV-readable microplate (Costar).

Evaluation of data

The absorbance at 280 nm was used to calculate the concentrations of the elution pools according to Lambert-Beer´s law. The recovery of protein was then calculated using Equation 1. The recovery in the first elution was used as the response in MODDE.

Equation 1: $m = C \times V$, recovery (%) = $100 \times m_{recovered} / m_{loaded}$

Column chromatography

Tricorn 5/100 columns with a CV of 2 ml were used for the column experiments (3). Equilibration buffer was 50 mM sodium acetate, 250 mM NaCl, pH 4.75. The sample was BSA (4 mg/ml) in equilibration buffer. The dynamic binding capacity at 10% breakthrough (QB10%), 1 minute residence time, had previously been determined using frontal analysis. For the recovery runs, 70% of QB10% was loaded to the column.

Results

From the batch uptake in Figure 1, it was apparent that an initial concentration of 1 mg/ml and an incubation time of 10 min resulted in a robust response with a capacity of approximately 25 mg/ml. Thus, this was used as the sample concentration and the incubation time in the elution study in PreDictor plates.

Evaluation of the recoveries in MODDE showed that the results in PreDictor plates were similar to those obtained in column chromatography (Fig 2).

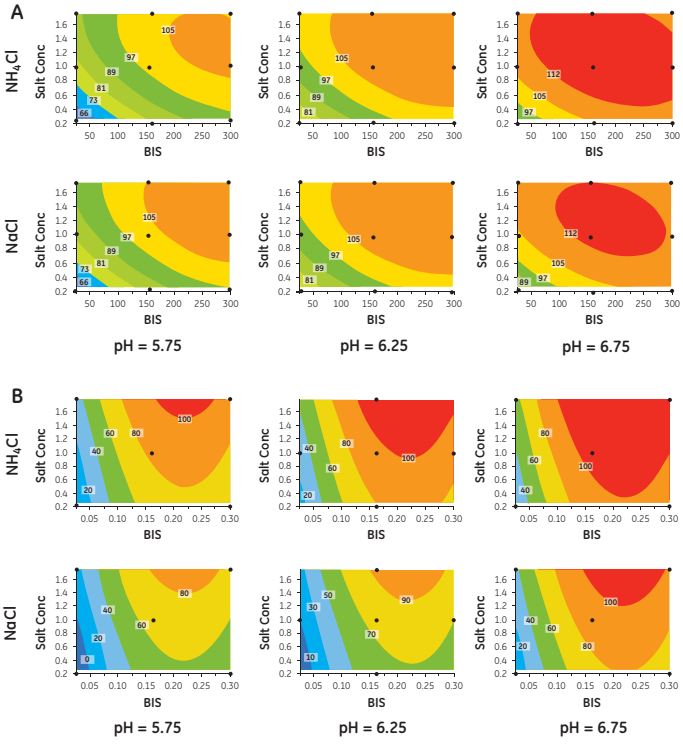

Fig 2 A. Contour plots for the recovery of BSA in A) PreDictor plates and B) Tricorn column (data from reference 3). Recovery is plotted as a function of salt concentration and buffer ionic strength (BIS) at three different pH values for the two salt types NaCl and NH_4Cl. Experimental data points are shown as black dots.

Both studies define the best conditions for high recoveries at pH 6.75 at buffer ionic strength around 0.23 M and a concentration of NH_4Cl above 0.5 M. When NaCl is the preferred eluting salt, higher salt concentrations are needed (i.e., above 1 M). The difference in the two models

could be due to a significantly larger elution volume relative the amount of chromatography media in the plate compared to the column experiment.

A comparison of the experimental time and sample consumption for this experiment performed in plates and in columns is shown in Figure 3.

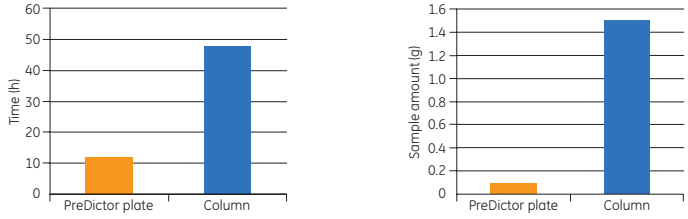

Fig 3. Estimate of experimental time and sample consumption for this screening using PreDictor plates and conventional column chromatography (Tricorn 5/100, CV 2 ml).

Conclusions

The optimal conditions for high recoveries of BSA on Capto MMC obtained on PreDictor plates show good correlation with conditions identified using traditional packed bed chromatography. At the same time PreDictor plates allow for shorter experimental time and lower sample consumption, making them a time-saving and accurate tool for screening of chromatographic conditions during process development.

References

1. Application Note. Screening of loading conditions on Capto S using a new highthroughput format, PreDictor plates. 28-9258w-40 AA.
2. MODDE software vesion 7.0 and version 8.0, Umetrics AB, www.umetrics.com.
3. Application Note. Optimizing elution conditions on Capto MMC using Design of Experiments. 11-0035-48 AA.

For contact information for your local office, please 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 Bio-Sciences AB,
Björkgatan 30,
751 84 Uppsala, Sweden

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

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

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

28-9277-90 AA 05/2008