# Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates

## Introduction

Finding the optimal conditions for a downstream purification process is of critical importance to gain high productivity and to achieve a robust biopharmaceutical manufacturing process at large scale. Screening for those conditions in columns is tedious and requires a large amount of sample. This is particularly true for ion exchange chromatography media, which can behave in a non-traditional manner (i.e., with optimal capacity at intermediate ionic strength) or for media with multimodal ligands where a different selectivity (relative to conventional ion exchange media) is expected. The ability to test many conditions in a parallel manner allows screening of a large experimental space. This improves the throughput in early stages of process development and also increases process knowledge, thereby allowing for identification of a robust design space (Fig 1).

Parallel screening in a miniaturized format allows higher throughput, lower sample consumption, and the design of more robust manufacturing processes. The launch of PreDictor™ 96-well filter plates, prefilled with GE Healthcare chromatography media, enables high-throughput screening while keeping sample consumption at a minimum.

This application note discusses some general considerations in the design of experiments using 96-well formats. In addition, a detailed description is presented of the experimental methods and the resulting data from the screening of loading conditions for two protiens, α-chymotrypsin and conalbumin, on the cation exchange chromatography medium Capto™ S.

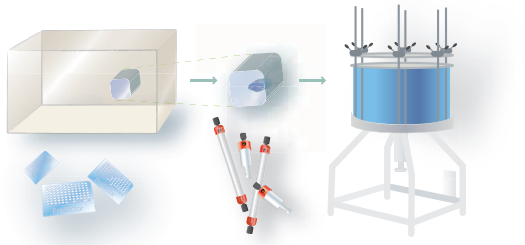

**Fig 1.** Conceptual visualization of a workflow for process development. Parallel screening using PreDictor plates makes it possible to explore a large experimental space (left). Once optimal conditions have been identified, fine-tuning and verification are carried out on columns using ÄKTAdesign™ systems (middle). The design space, shown in blue (middle), is identified and scaled up to a robust production scale process (right).

## Experimental approach

The binding capacity for two proteins, conalbumin, showing non-traditional behavior on Capto S, and α-chymotrypsin, behaving in a traditional manner, are investigated at various ionic strengths and pH values using Design of Experiments (DoE) as shown in Table 1. Corresponding results from column experiments (see reference 1) are shown to verify that data generated in plate format correspond well to data obtained in columns.

**Table 1.** The factors investigated, associated ranges, and response. Buffer was sodium acetate at approx. 20 mM.

| Factors | Range | Response |
|---|---|---|
| Ionic strength[1] | 20, 52, 85, 117, and 150 mM | Binding capacity, q, at 60 min incubation time |
| pH | 4.25, 4.75, and 5.25 | |

[1] The ionic strength of the loading buffer was adjusted by addition of sodium chloride after calculation of the contribution from the buffer substance (at the specific pH) using the Henderson-Hasselbach equation without correction for pKa shifts at different ionic strengths.

## *The theoretical basis for batch uptake*

Each well in the PreDictor plate can be considered as a batch system in which adsorption takes place. Batch adsorption is based on the interaction of sample molecules with a chromatography adsorbent suspended in solution. Under sufficient mixing conditions all chromatography particles are in contact with a solution of the same composition. As in the case of adsorption in a chromatography column, in a batch system a solute must diffuse from the bulk solution to the surface and the interior of an adsorbent particle, and be adsorbed onto an available active site. Thus, from the mass transfer perspective the steps are exactly the same as in the case of adsorption in a column. In a batch system mass transfer continues until a semi-equilibrium state is reached, where the overall adsorption and desorption rates are almost equal and no significant changes in solute concentration in the liquid phase takes place. Consequently overall rates of adsorption can be measured by monitoring changes in solute concentration in the liquid phase employing a mass balance principle. The batch uptake experiment occurring in the wells of PreDictor plates is schematically shown in Figure 2.

It should be emphasized that the binding capacity obtained in plates is not quantitatively comparable to dynamic binding capacity (DBC). The reason for the apparently long incubation time in plates (60 minutes as seen in Figs 5A and 6A) as compared to residence time in columns (2 minutes in Figs 5B and 6B) relates to the differences in the techniques. The incubation time corresponds better to the loading time in columns since this reflects the total time the media particles are in contact with the sample.

## *General Experimental Considerations*

For the experimental set-up, start by determining relevant screening parameters. Consider suitable conditions for subsequent column chromatography such as buffer species, concentration of target protein, etc. Since the plate format provides the opportunity to run a large number of parallel experiments, it is advisable to screen a broader range of conditions per parameter than traditionally done in columns. With buffers or samples containing certain detergents special precautions during sample loading and/or incubation may be needed as described more thoroughly in the PreDictor plate Instructions (2).

When determining the layout to be used in plates, consider the following:

- Number of parameters for investigation
- Manual or automated handling
- Order of pipetting

Analytical methods suitable for high-throughput should be chosen for analysis in PreDictor plates. As a rule of thumb, minimize the number of analyses and/or use simple analyses in early stage screening. More thorough analysis can be performed in later stages when the experimental space and the number of samples have decreased. Finally, after evaluating data, the experimental space identified as most relevant can be further optimized in small columns, or in a modified plate experiment for more thorough screening.

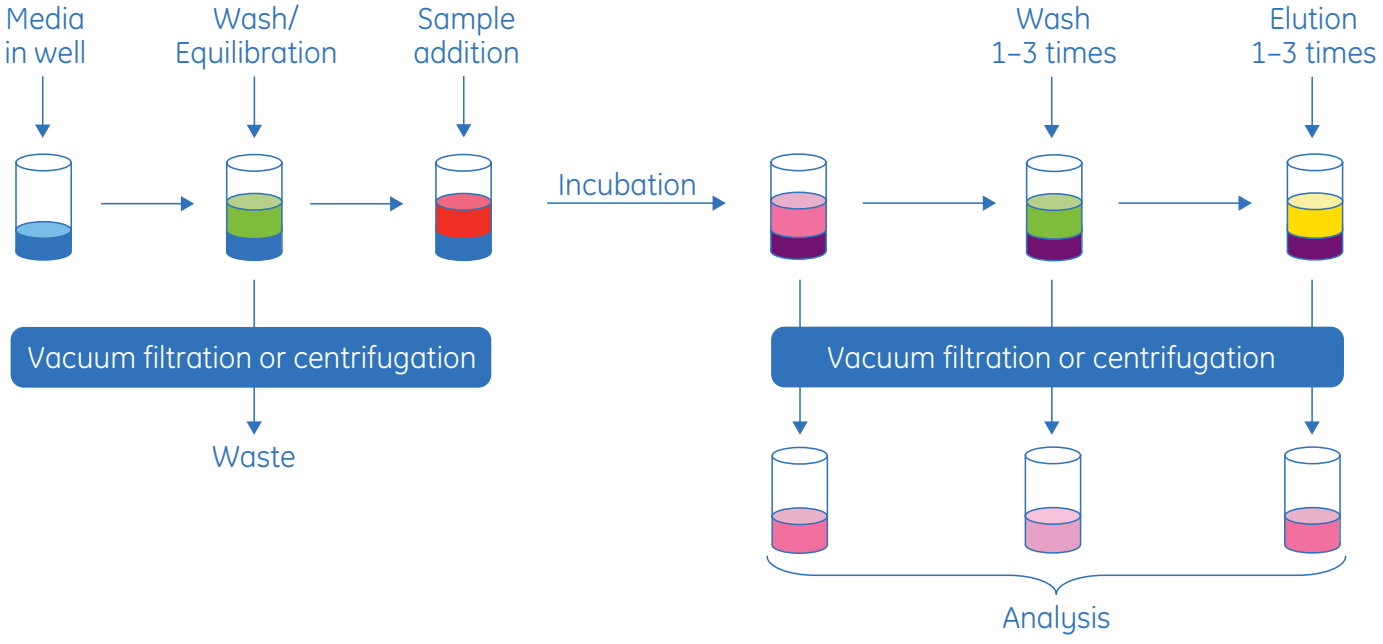


**Fig 2.** Schematic drawing of the batch uptake experiment occurring in the wells of the PreDictor plates. See text for details

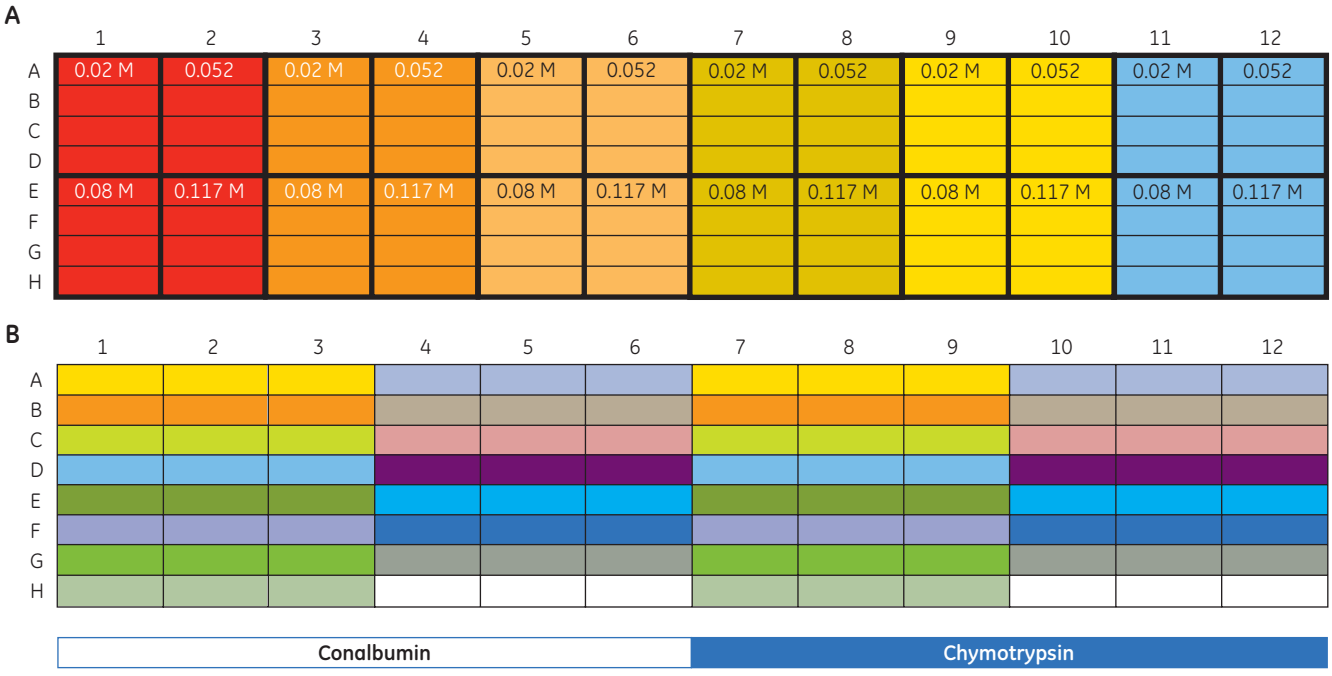


**Fig 3. A**: Experimental design and plate layout for the contact time batch uptake study to identify settings for the subsequent recovery study. The incubation at six contact times are shown in different colors. Each color is divided into four regions corresponding to the four different experimental conditions, resulting in one plate for each protein. **B**: Experimental design and plate layout for the screening of loading conditions. The 15 different conditions shown in different colors were distributed as indicated. Six wells (in white) were not used. After 60 minutes incubation was ended by vacuum filtration of the plate. Buffer conditions were as outlined in Table 1.

# Materials and Methods

## *Plate experiment*

PreDictor Capto S plates filled with 2 µl of chromatography medium per well were used. The plates were kept on a collection plate throughout the experiment as described in the PreDictor plate Instructions (2). Removal of liquid was done using a vacuum manifold, following the general recommendations in the PreDictor plate Instructions (2). Prior to the experiments, buffers were prepared in a separate deep-well plate to facilitate liquid transfer in a manual workflow.

Figure 3 shows the plate layouts for determining suitable contact times for each of the proteins (Fig 3A), and for screening of the 15 combinations of pH and ionic strength (Fig 3B) shown in Table 1. Modde 8.0 software (3) for DoE was used to help optimize the plate layouts within the experimental design.

Samples were collected in UV-readable collection plates (Costar) and the concentration of protein was determined spectrophotometricaly using Lambert-Beer's law. The binding capacity (q) was calculated according to equation 1.

Equation 1: $q = (C_{initial} - C_{flowthrough}) * V_{sample} / V_{medium}$

### 1. Equilibration steps 1-3

After vacuum removal of the storage solution, 200 µl of equilibration buffer was added to each well and the wells were drained. This procedure was performed three times. Equilibration buffer was 20 mM sodium acetate as outlined in Table 1.

### 2. Loading step

A. Contact time study (Fig 3A)

Using the layout shown in Figure 3A, 200 µl of sample at a concentration of 3.5 mg/ml protein solution was added and incubated for 60 minutes. Samples with the longest incubation time were added first (columns 1 and 2 in Figure 3A). Subsequently, two columns were filled with sample at the following intervals: 30, 45, 50, 55, and 57.5 minutes after the first sample addition. This resulted in incubation times of 60, 30, 15, 10, 5, and 2.5 minutes. For all samples, mixing was performed on an orbital shaker at 1100 rpm during sample incubation. Two and a half minutes after the last sample addition, the plate was placed on a vacuum manifold and all liquid from each well was collected into wells in a UV-readable microtiter plate. Four replicates were used in the contact time study. The capacity at zero incubation time was assumed to be zero.

B. Screening study (Fig 3B)

Using the layout shown in Figure 3B, 200 µl of sample at a concentration of 3.5 mg/ml protein solution was added to each well and the plate was then placed on an orbital shaker and mixed for 60 minutes at 1100 rpm. The plate was then placed on a vacuum manifold and all liquid from each well was collected into wells in a UV-readable microtiter plate. The 15 conditions in this study were run in three replicates to allow for statistical evaluation.

### 3. Analysis

The absorbance at 254, 280, 295, and 310 nm was measured on the collected samples. The use of four different

wavelengths allowed the absorbance to be measured within the linear region for at least one of the wavelengths.

### Column experiments

The dynamic binding capacity at 10% breakthrough ($Q_{B10\%}$) at 2 minutes residence time (= approximately 90 minutes loading time) was determined using frontal analysis. Columns used were Tricorn™ 5/100, column volume (CV) 2 ml. Buffers were identical to those used in the plate-based experiments. Sample concentration was approximately 4 mg/ml (for details, see reference 1).

## Results

### Contact time studies

Results obtained from the experiment focusing on the effect of contact time on binding capacity are shown in Figure 4. For all conditions tested, the chromatography medium was saturated with proteins after 45 minutes of incubation. Based on these results, a contact time of 60 minutes was chosen for the screening study (Figs 5 and 6).

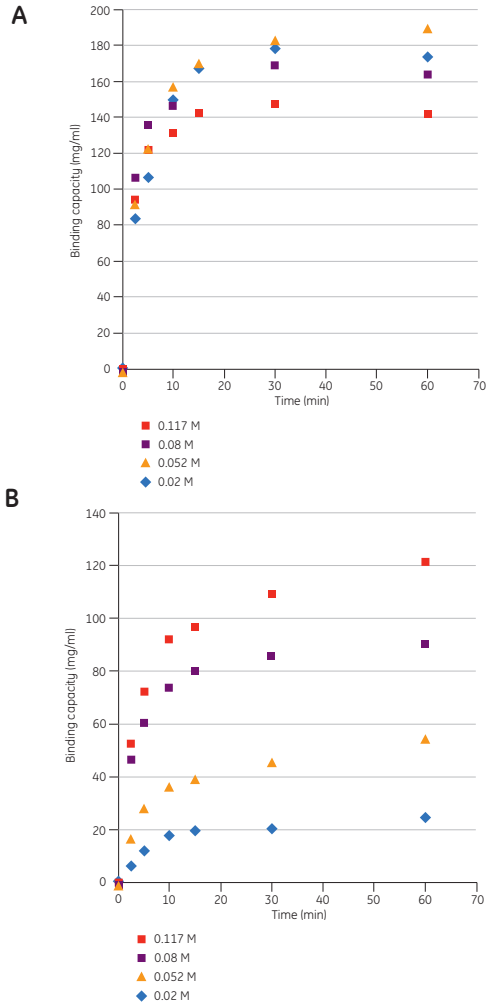


**Fig 4.** Effect of contact time and ionic strength on binding capacity for α-chymotrypsin (**A**) and conalbumin (**B**) measured in PreDictor Capto S plates filled with 2 µl medium per well. The capacity at zero incubation time was assumed to be zero. Legends to each figure describe the ionic strengths used (as outlined in Table 1).

### Screening studies

The plate experiments with α-chymotrypsin showed that the highest capacity was obtained at pH 4.75 at low conductivity as shown in Figure 5A. At pH 5.25 a somewhat non-traditional behavior is seen. At pH 4.25 the decrease in capacity with increasing ionic strength is less pronounced, in accordance with column experiments shown in Figure 5B. The results from the column chromatography experiments (Fig 5B, see also ref 1), at 2 minutes residence time, show good correlation with the results obtained in the plate at 60 minutes incubation time. The experimental space for optimum capacities coincides in both formats. As mentioned previously, the incubation time corresponds better to the loading time in columns, which in the example shown here is 90 minutes to reach $Q_{B10\%}$.

For conalbumin in PreDictor plates, a non-traditional behavior is seen at all three pH values (Fig 6A). The capacity optimum at the three different pH values shifts towards higher conductivities as pH decreases. This correlation, related to the protein net charge (4), is also observed in column chromatography as shown in Figure 6B.

### Parallel runs save time and sample

A comparison of time and sample spent using the two different experimental approaches is shown in Figure 7. The screening in PreDictor plates was performed in less than a tenth of the time required for screening in columns, the savings in amount of sample being even larger.

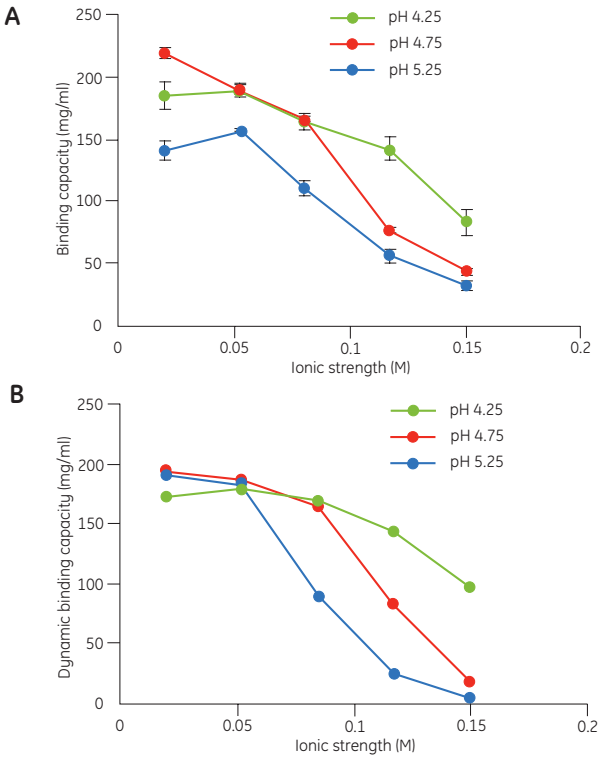


**Fig 5.** Determination of loading conditions for α-chymotrypsin on Capto S. **A**: binding capacities for 60 minutes incubation time in PreDictor Capto S 2 µl plates. Error bars indicate 1 standard deviation, based on triplicates. **B**: dynamic binding capacities (DBC) at 10% breakthrough for α-chymotrypsin on Capto S. Residence time 2 minutes, column Tricorn 5/100 (CV 2 ml).

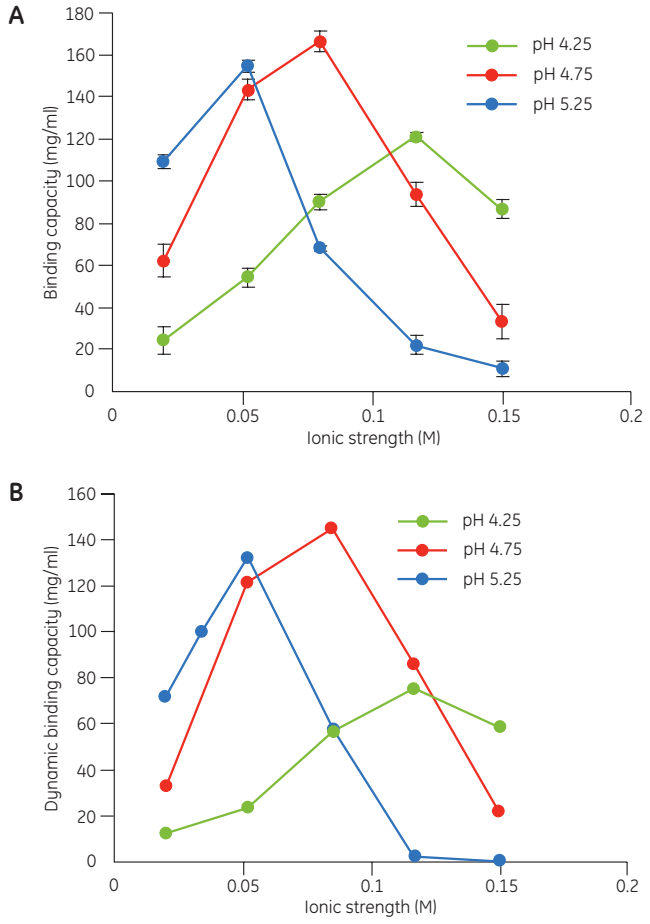


**Fig 6.** Determination of loading conditions for conalbumin on Capto S.
**A**: binding capacities at 60 minutes in PreDictor Capto S 2 µl plates. Error bars represent 1 standard deviation, based on triplicates.
**B**: dynamic binding capacities (DBC) at 10% breakthrough for Conalbumin on Capto S. Residence time 2 minutes, column Tricorn 5/100 (CV 2 ml).

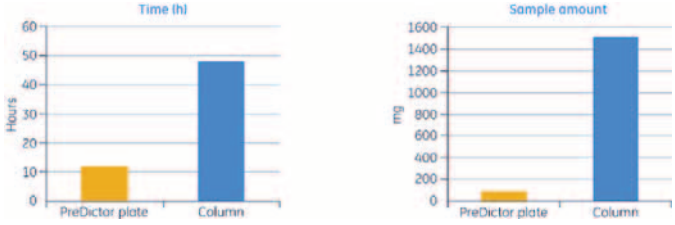


**Fig 7.** Estimate of time and sample consumption and time spent for this application on PreDictor Capto S 2 µl plates (yellow bars) and conventional column chromatography using Tricorn 5/100 with a column volume of 2 ml (blue bars).

## Conclusions

PreDictor plates provide a rapid method to distinguish between traditional and non-traditional behavior on Capto S. The optimal conditions for high binding capacity on Capto S found in PreDictor plates coincide with the best conditions found in column chromatography. The use of PreDictor plates also results in significant savings in both time and sample consumption. PreDictor plates, therefore, are an efficient tool for rapid screening in early stage process development.

**References**

1 Application Note. Screening and optimization of the loading conditions on Capto S. 28-4078-16 AA.
2. Instruction, PreDictor plates, 28-9258-34 AB.
3. Modde 8.0, Umetrics AB, www.umetrics.com
4. Harinarayan, C. *et al.* An exclusion mechanism in ion exchange chromatography. *Biotechnology and Bioengineering* **95 (5),** 775-787 (2006).

Elanders i Uppsala 2008

For contact information for your local office, please visit, www.gelifesciences.com/contact

www.gelifesciences.com/

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

imagination at work



GE Healthcare Bio-Sciences Corp., 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA
GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA UK
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

28-9258-40 AA 02/2008