# CaPure-HA™: DoE Optimization of Elution Conditions

Chromatography Resin
APPLICATION NOTE

Design of Experiments (DoE), also called experimental design, is a statistical approach to process development that has gained wide acceptance in the biopharmaceutical industry. DoE is used to reduce development costs by speeding up the design process and to optimize the parameters of a particular step in the manufacturing process. With its inherent ability to take into account multiple variables at once, designed experiments are also used to minimize process variation leading to a more robust manufacturing process.

## Introduction

CaPure-HA (hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$) is a form of calcium phosphate used in the chromatographic separation of biomolecules. Unlike other resins available from Tosoh Bioscience, CaPure-HA is both the ligand and the base bead. Hydroxyapatite has unique separation properties for biomolecules and CaPure-HA offers unparalleled selectivity and resolution for process scale operations. Its highly selective nature often separates proteins otherwise shown to be homogeneous by electrophoresis and other chromatographic techniques.

CaPure-HA resin is a spherical, macroporous form of the hexagonal crystalline structure of hydroxyapatite. It has been sintered at high temperatures for increased mechanical and chemical stability, allowing it to withstand the rigors of industrial-scale applications. *Table 1* lists the properties of CaPure-HA.

**Table 1.** Properties of CaPure-HA

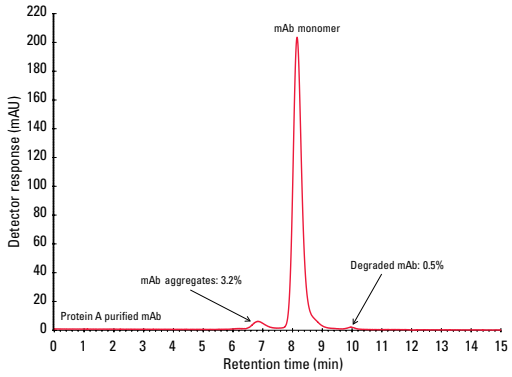
| | |
|---|---|
| Particle size (mean): | 39 µm |
| Pressure rating: | 10 MPa |
| Shipped as: | dry powder |
| pH stability: | 6.5 - 14 |
| Shelf life (estimated): | 10 years |

The data presented here demonstrate the optimization of the CaPure-HA elution using a DoE approach. The variables analyzed were mobile phase concentrations of NaCl and $NaPO_4$, with the objective of maximizing product yield while minimizing host cell proteins, host cell DNA, and mAb aggregate concentration in the final product.

## Experimental Conditions/Results

*Figure 1* is a size exclusion chromatography analysis of an aliquot of a protein A purified $IgG_1$ mAb on a TSKgel® G3000SWXL column. This sample is representative of the material that will be purified using a column packed with CaPure-HA. In this figure, the presence of monomer, dimer, and higher order aggregates can be seen. The presence of a peak at greater than ten (10) minutes retention time is due to residual citrate from the protein A elution step.

**Figure 1.** SEC Analysis of mAb Used for CaPure-HA DoE Experiments




| | |
|---|---|
| Column: | TSKgel G3000SWXL, 7.8 mm ID × 30 cm |
| Mobile phase: | 0.2 mol/L phosphate buffer, pH 6.8 |
| Gradient: | isocratic |
| Flow rate: | 1 mL/min |
| Detection: | UV @ 280 nm |
| Injection vol: | 20 µL |
| Sample: | protein A purified mAb $IgG_1$ |

Prior to executing the DoE experiment, the design space for column elution in NaCl *(Figure 2)*, and $NaPO_4$ *(Figure 3)* was determined. A 6.6 mm ID × 15 cm column was packed with CaPure-HA resin. The column was equilibrated with 20 mmol/L MES, 10 mmol/L $NaPO_4$, 100 mmol/L NaCl, 1 mmol/L $CaCl_2$, pH 6.5 (mobile phase A) for 5 CV.

The column was loaded with TBL-mAb-01 to a concentration of 1.0 g/L and the column was washed with mobile phase A for 3 CV. The column was then eluted with a 10 CV linear gradient to either 1.0 mol/L NaCl or 500 mmol/L $NaPO_4$, both in mobile phase A.

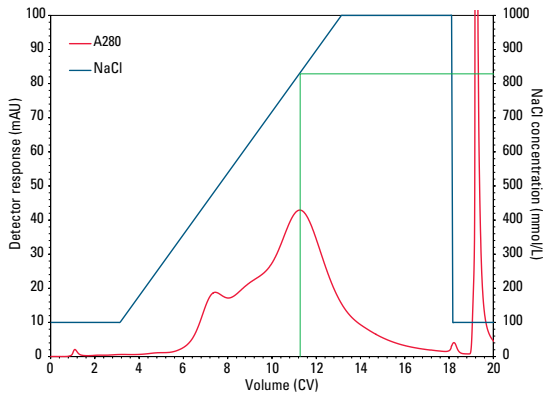
The dynamic binging capacity of the CaPure-HA resin for TBL-mAb-01 was next determined using an 8.34 g/L solution of the mAb in mobile phase A. At 10% breakthrough, the measured DBC for TBL-mAb-01 was 47.0 g/L.

For the DoE experiments, a 3.0 mm ID × 15 cm column was packed with CaPure-HA and equilibrated with mobile phase A. A 9.85 g/L solution of TBL-mAb-01 in mobile phase A was loaded to approximately 80% of DBC. The column was washed with two additional column volumes of mobile phase A.

The column was then eluted with 5 CV of mobile phase A containing 10, 130, or 250 mmol/L $NaPO_4$ and 100, 500, or 900 mmol/L NaCl. The eluted mAb was then evaluated for purity, HCP, DNA, and yield. Results of these experiments can be seen in *Table 2*.

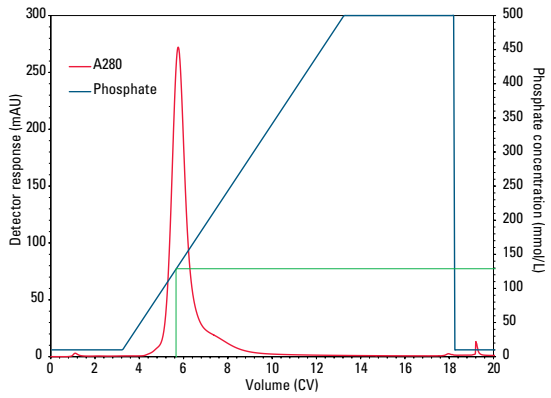
A contour plot of the DoE experiments can be seen in *Table 3*, highlighting the optimum elution conditions for this particular monoclonal antibody on CaPure-HA.

**Figure 2.** Determination of Elution Design Space, NaCl Elution




| | |
|---|---|
| Resin: | CaPure-HA |
| Column size: | 6.6 mm ID × 15 cm (5.1 mL) |
| Mobile phase: | A: 20 mmol/L MES, 10 mmol/L $NaPO_4$, 100 mmol/L NaCl, 1 mmol/L $CaCl_2$, pH 6.5 |
| | B: mobile phase A + 1.0 mol/L NaCl |
| Flow rate: | 300 cm/hr (1.71 mL/min) |
| Detection: | UV @ 280 nm |
| Temperature: | ambient |
| Gradient: | 0 - 100 % B (10 CV) |
| Sample: | TBL-mAb-01 (1.0 g/L-resin) |
| Instrument: | ÄKTA avant 150 |

**Figure 3.** Determination of Elution Design Space, $NaPO_4$ Elution



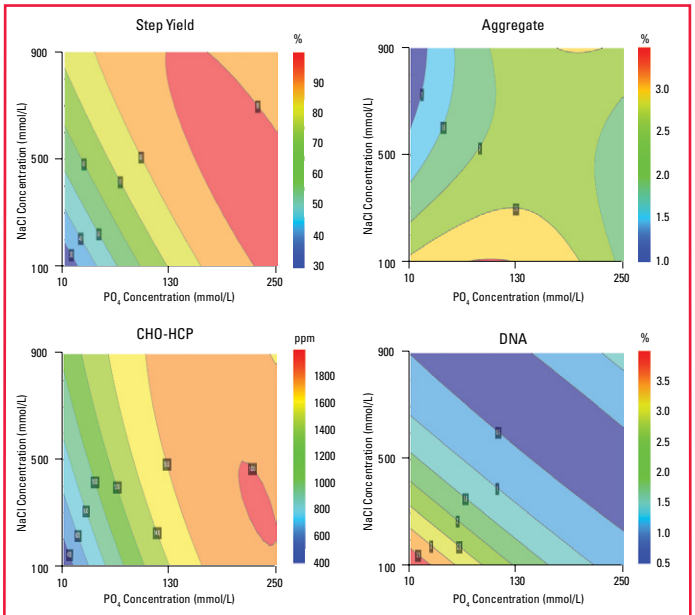

| | |
|---|---|
| Resin: | CaPure-HA |
| Column size: | 6.6 mm ID × 15 cm (5.1 mL) |
| Mobile phase: | A: 20 mmol/L MES, 10 mmol/L $NaPO_4$, 100 mmol/L NaCl, 1 mmol/L $CaCl_2$, pH 6.5 |
| | B: mobile phase A + 500 mmol/L $NaPO_4$ |
| Flow rate: | 300 cm/hr (1.71 mL/min) |
| Detection: | UV @ 280 nm |
| Temperature: | ambient |
| Gradient: | 0 - 100 % B (10 CV) |
| Sample: | TBL-mAb-01 (1.0 g/L-resin) |
| Instrument: | ÄKTA avant 150 |

**Table 2.** Individual DoE Experiment Results

| Experiment | NaCl (mmol/L) | $NaPO_4$ (mmol/L) | Yield (%) | Aggregate (%) | CHO-HCP (ppm) | DNA (%) |
|---|---|---|---|---|---|---|
| 1 | 100 | 10 | 12 | 3.1 | 360 | 4.7 |
| 2 | 900 | 10 | 74 | 0.9 | 1129 | 0.7 |
| 3 | 100 | 250 | 88 | 1.9 | 1764 | 0.8 |
| 4 | 900 | 250 | 87 | 2.1 | 1690 | 0.7 |
| 5 | 100 | 130 | 86 | 2.5 | 1492 | 1.1 |
| 6 | 900 | 130 | 82 | 2.3 | 1472 | 0.7 |
| 7 | 500 | 10 | 62 | 0.5 | 696 | 0.7 |
| 8 | 500 | 250 | 89 | 2.0 | 1672 | 0.8 |
| 9* | 500 | 130 | 84 | 2.3 | 1531 | 0.8 |
| 10* | 500 | 130 | 84 | 2.4 | 1780 | 0.8 |
| 11* | 500 | 130 | 84 | 2.5 | 1685 | 0.7 |
| **Load Material** | | | | 3.6 | 4422 | 1.2 |

* Center points

**Table 3.** DoE Evaluation of mAb Elution Conditions, Contour Plots




## Conclusions

CaPure-HA is an effective resin for the removal of degradation products, DNA, dimer, and higher order aggregates from mAb monomer in a protein A purified antibody. The highly selective and robust nature of CaPure-HA gives chromatographers the flexibility to use this resin for the removal of process impurities at almost any stage in the manufacturing process.

As shown by the response plots in *Table 3*, CaPure-HA elution conditions can be modified to fit your process. Low phosphate, high salt elution may be used to selectively remove aggregates and HCP, while a moderate phosphate, moderate salt elution provides greater DNA removal with the tested mAb.



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