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Optimization of the Elution Protocol for Recombinant Human Serum Albumin expressed in *Pichia pastoris* on Capto MMC using Design of Experiments

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Introduction

The multimodal resin, Capto[™] MMC, is a novel cation exchange type of chromatography resin that maintains its protein binding capacity at high conductivity. Due to the multimodal nature of the ligand (Figure 1), different types of interactions, such as ionic interactions, hydrogen bonding and hydrophobic interactions, are possible. Capto MMC therefore generally requires elution conditions that differ from those used with traditional ion exchangers. This poster describes how Design of Experiments (DoE, multivariate analysis) can be used to optimize the elution protocol to maximize the recovery and purification factor of recombinant human serum albumin expressed in *P. pastoris* on Capto MMC.

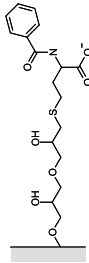


Figure 1. The Capto MMC ligand.

Chromatography

The sample was a clarified feed of recombinant human serum albumin (rHSA, Ip ~5.5, MW 67 kDa) expressed in *P. pastoris*. The experimental setup was as follows:

- Column: 1-ml HiTrap[™] packed with Capto MMC resin
- Equilibration: 10 ml 25 mM sodium acetate, pH 4.5
- Sample: 4.5 ml Clarified rHSA feed (conductivity 15 mS/cm)
- Sample concentration: 5 mg/ml
- Wash: 10 ml 25 mM sodium acetate, pH 4.5
- Elution: step-elution, 10 ml elution buffer (See Table 1)
- CIP: 15 ml 1 M NaOH
- Flow rate: 1.0 ml/min, except during CIP (0.5 ml/min)

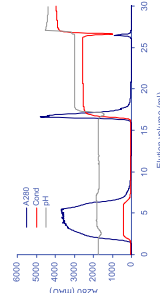


Figure 2. Clarified rHSA feed purified on Capto MMC.

The eluate was collected in 1-ml fractions and the first three eluate fractions (3 column volumes) from each run were pooled. The starting material and the pools were assayed for rHSA by use of a Superdex[™] 200 gel filtration column and recovery and purification factor were calculated for each run. An example of a chromatogram is shown in Figure 2. Purification was done on an AKTAexplorer[™] 100 system.

Design of Experiments

Setup

The experimental design was a full factorial design in three variables (pH, salt concentration (NH₄Cl) and buffer ionic strength (BIS) with additional experiments to resolve an observed curvature. The total number of experiments was 12. The responses were recovery and purification factor. Table 1 below shows which variables were used and how they were varied.

Table 1. Design parameters and their settings.

Parameters	Low setting	High setting
pH	5.75	7.75
BIS	0.026	0.300
Salt conc. (M)	0.25	1.75

Results

Two models were calculated, one for the recovery and one for the purification factor. The model for recovery fits the data quite well and explains ~95 % of the observed variation, and the purification factor model explains ~85 % of the observed variation. The coefficient plots (Figure 3) show that pH and BIS have significant effects on both responses. The salt concentration parameter was shown not to be significant within the specified limits. For the recovery, pH has a large positive effect and the effect from BIS is due to a curvature detected in the data, which has a negative effect, but also to an interaction effect with pH. The curvature means that if BIS is too low or too high the recovery will decrease. For the purification factor, pH has a positive effect and BIS has a negative effect. The curvature effect seen for the recovery is absent for the purification factor. The effect from both pH and BIS is also due to an interaction between the two.

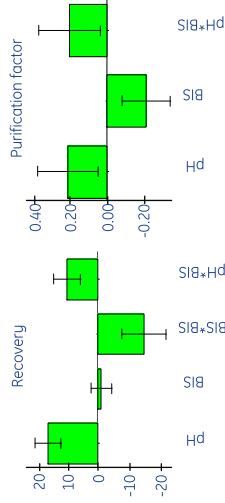


Figure 3. Coefficient plots for the recovery model.

The response surface plots (Figure 4) show that a compromise is needed between the two responses. The purification factor did not vary to any large extent under the tested conditions, so in this application the elution conditions would probably have been chosen to maximize recovery. A relatively high pH and high buffer ionic strength was needed to achieve good recovery.

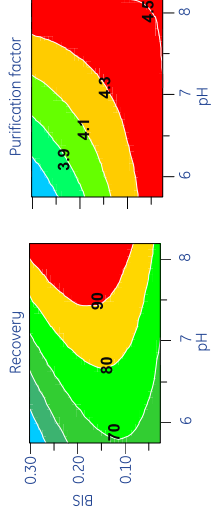


Figure 4. Response surface plots for the recovery and the purification factor. Buffer ionic strength is plotted versus pH. Labels correspond to recovery in percent and the purification factor, respectively.

Discussion

The use of DoE was an effective tool for optimizing the elution conditions on Capto MMC. As stated in the introduction a number of interactions, i.e. hydrophobic interactions and hydrogen bonding are possible with the Capto MMC ligand and they may contribute to the binding. To address these interactions in applications where elution is more challenging than here, the effect of salts of different lyophilicity and of hydrogen bonding disruptors (e.g. organic modifiers and urea) can be tested. Only two responses were included in this study, but for other applications, other responses, e.g. endotoxin clearance or level of certain impurities, may also be used as selection criteria for elution conditions and can be included in the DoE design.

Conclusions

- The use of DoE is a good way to scout for the best elution conditions on Capto MMC. The advantage of using DoE is that this can reveal interaction and quadratic effects that can easily be missed if multivariate analysis is not applied.
- DoE may be preferentially applied for optimisation of elution conditions were many factors and responses need to be considered.



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