Optimizing elution conditions on Capto MMC using Design of Experiments

Abstract

The multimodal ligand of Capto™ MMC generally requires elution conditions that differ from those used with traditional ion exchangers. This application note describes how Design of Experiments (DoE) can be used to optimize elution conditions and thus recovery of a target protein. The results are summarized as follows:

- The effect of quantitative design factors , such as concentration of eluting salt, pH and ionic strength of the buffer, correlated positively with the recovery of the target protein. The qualitative factor, type of salt, also affected recovery.
- The use of DoE proved to be an efficient way of investigating the effect of several parameters on target protein recovery. Furthermore, DoE revealed a curvature effect that could have been overlooked if multivariate analysis had not been applied.

Introduction

Capto MMC has a ligand with multimodal functionality (Figure 1). This means that different types of interactions, such as ionic interactions, hydrogen bonding and hydrophobic interactions, are possible. The design of the ligand enables binding of proteins at high conductivity, however, it does limit elution using only increasing salt concentration. Thus a different elution protocol has to be used compared to traditional ion exchangers.

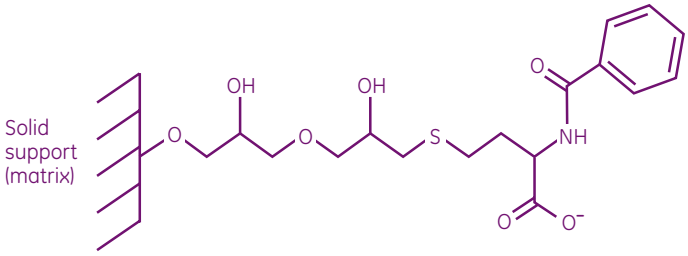

Fig 1. The multimodal ligand of Capto MMC.

Generally, Capto MMC requires an increase in both pH and salt concentration for effective elution of bound protein. Additionally, the ionic strength of the buffer and the type of salt will also affect elution. Elution conditions for Capto MMC were investigated by measuring the recovery of bovine serum albumin (BSA) in an elution volume corresponding to 3 column volumes as a function of four different parameters: salt concentration, type of salt, buffer ionic strength and pH.

Capto™

Material and methods

Frontal analysis

Chromatography system:	ÄKTAexplorer™ 10
Column:	Tricorn™ 5/100
Protein:	BSA
Loading buffer:	50 mM sodium acetate, 250 mM NaCl, pH 4.75 (30 mS/cm)
Loading concentration:	4 mg/ml
Amount loaded:	40 mg BSA
Elution buffer:	see DoE below
Flow velocity:	600 cm/h (1 min residence time)

Design of Experiments

The DoE setup was as follows:

Factors:		**Responses:**
Salt concentration	0.25, 1.00, 1.75 M	Recovery in elution volumes corresponding to 3 column volumes (CVs)
Salt type	NaCl or NH_4Cl	
Buffer ionic strength (BIS)*	0.026 – 0.300 M	
pH†	5.75, 6.25, 6.75	

* Calculated from the Henderson-Hasselbalch equation without correction for pKa shifts at different ionic strengths.

† The buffers used were sodium malonate (pH 5.75 and pH 6.25) and sodium phosphate (pH 6.75),

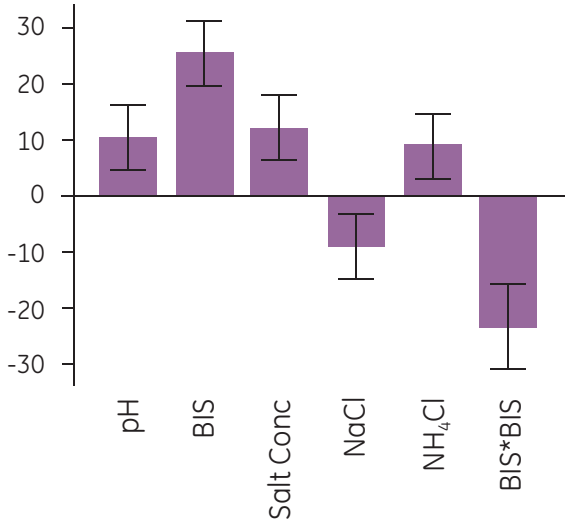

Fig 2. Coefficient plot for the model of recovery.

The design was a full factorial design in multiple levels in order to resolve main, interaction and an observed curvature effect [1]. The software used for the design and evaluation was MODDE 7.0 (Umetrics AB).

Results and discussion

All of the tested factors affected recovery of BSA as shown by the coefficient plot (Figure 2).

All of the main factors correlated positively with the recovery of BSA, indicating that an increase in any or all of the factors - pH, salt concentration or buffer ionic strength (BIS) - improves recovery. Although no interaction effects were identified a curvature effect was observed, suggesting that recovery will decrease if the ionic strength of the buffer is above 0.23 M.

In the DoE, the effect of salt type was also investigated. NaCl and NH_4Cl were studied, but the Hofmeister series can be consulted for choice of eluting salt [2]. The response surface plots for the recovery of BSA illustrate the results (Figure 3). Full recovery was obtained under different conditions. The highest pH (6.75) allowed for the greatest variation in salt concentration and buffer ionic strength. Under these conditions NH_4Cl gave better recovery than NaCl.

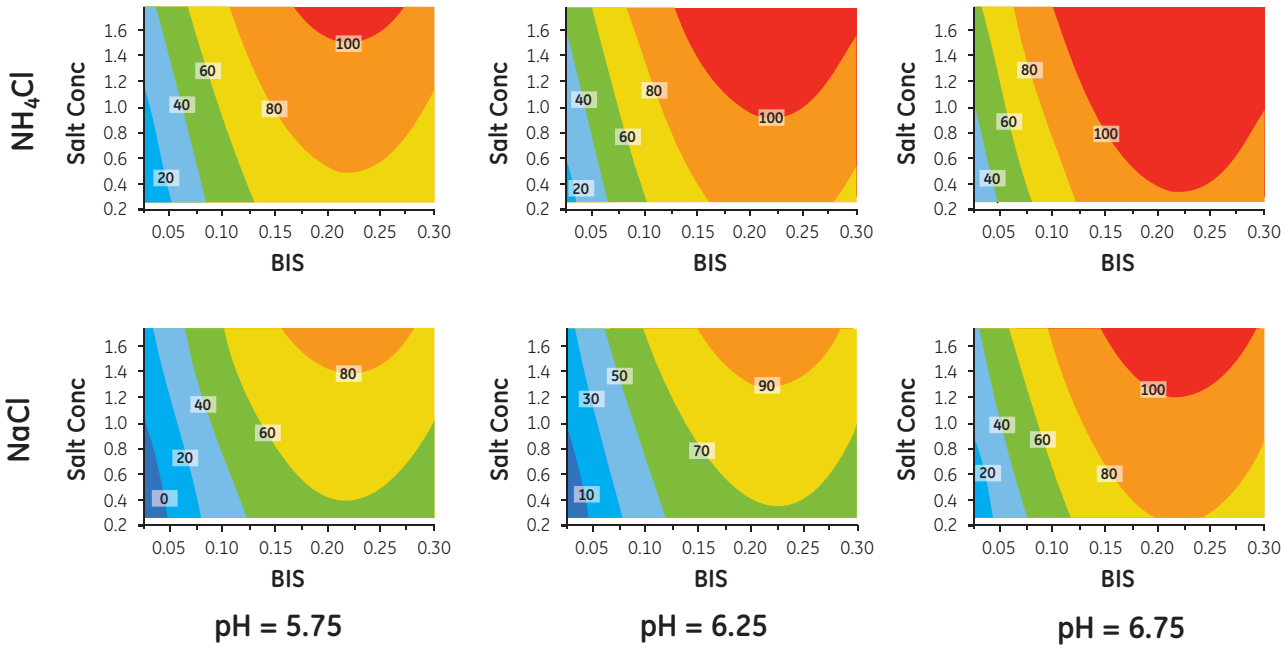

Fig 3. Response surface plots for the recovery of BSA. Recovery is plotted as a function of salt concentration and buffer ionic strength (BIS) for two different salts. Labels correspond to recovery in percent.

Figure 3 illustrates the optimum values for the selected parameters in the DoE required to achieve full recovery. Buffer ionic strength should be about 0.23 M at pH 6.75, which was the highest pH tested in this study. Salt concentration is dependent on the type of salt used; in this DoE, NH_4Cl gave a better recovery than NaCl at lower salt concentrations. For NH_4Cl, full protein recovery was achieved when the salt concentration ranged from about 0.5 M and upwards and the buffer ionic strength was 0.23 M at pH 6.75. It is possible to obtain full recovery at a lower pH with NH_4Cl, but it should be noted that the salt concentration range narrows with decreasing pH.

The response surface plots also show that full protein recovery is possible when elution is performed with NaCl, but only at the highest pH tested (pH 6.75). However, the salt concentration range is narrower for NaCl than for NH_4Cl. For NaCl, full protein recovery is achievable at a salt concentration of 1.3 M and above when buffer ionic strength is 0.23 M at pH 6.75.

In this DoE, the effects of selected parameters on the recovery of a pure protein were investigated. In a real feed application, other factors such as purity and removal of certain contaminants will need to be considered. As proteins/contaminants have different elution characteristics under different conditions, DoE may be used to optimize the purity of the target protein or removal of certain contaminants.

Conclusions

Both a change in pH and an increase in salt concentration were needed to maximize recovery of the target protein. The lyotropic [2] character of the eluting salt influenced recovery – elution with NH_4Cl gave a higher recovery than elution with NaCl. A high buffer ionic strength also facilitated recovery through a more rapid change of pH during elution and possibly through the lyotropic effect of the buffer salt.

In this study we have shown that DoE is a useful tool for scouting the best elution conditions on Capto MMC. DoE is beneficial in that it can reveal interactions and quadratic effects* that may otherwise go unnoticed. DoE can be preferentially applied for optimizing elution conditions in real feed applications where more responses, e. g., purity and concentration of major impurities are relevant for the application.

The DoE experiments were performed on Tricorn 5/100 columns. Scouting is most conveniently performed on prepacked HiTrap™ columns as they save both sample and time.

* The quadratic effect/term is used to quantify deviations from linearity in the data.

References

1. Box, G. E. P., Hunter, W. G., and Hunter, S. *Statistics for Experimenters,* John Wiley & Sons, Inc., New York (1978).
2. Melander, W., and Horváth, C. Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: an interpretation of the lyotropic series. *Arch. Biochem. Biophys.* **183** 200-215 (1977).

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