Displacement chromatography for mAb charge separation

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Abstract

Monoclonal antibodies (mAbs) are large proteins susceptible to several post translational modifications (PTMs) that might cause charge heterogeneity. In the production of biosimilars these modifications play an important role, and similarity with the originator molecule must be shown. To accomplish charge distribution similarity, the cell culture must be carefully optimized, but still, chromatography polishing is often required. Separation of the different entities by displacement chromatography, a method invented by Tiselius in 1943 and further developed by Horváth's group, will be discussed. During loading a competition occurs between various entities in the sample because of different affinity to the sorbent. Hence, overloading the resin can replace and overcome limitation with gradient separations. Here load and pH were varied on a high capacity cation exchanger and the clearance of acid charge variants and recovery was studied.

Introduction

Differences in the charge distribution for a biosimilar compared to the originator drug may affect their biological activity and stability. From a safety and efficacy perspective, the charge distribution must be carefully monitored and controlled. Polishing the charge distribution in the downstream process often requires high resolution separation techniques that will significantly affect the recovery. It may be a better alternative to spend more time on the clone selection and the optimization of the cell culture condition in order to minimize the polishing requirement downstream. As there was no possibility to compare to an originator, a group separation to remove acidic charge (ACV) variants was performed. As the acidic charge variants elute before the main and basic variants on a cation exchanger, an experiment was designed where the Capto™ S ImpAct was overloaded to try to displace the ACV out of the column while still binding the other variants.

Material and methods

The sample was MabSelect SuRe™ LX purified mAb (IgG1) that was buffer exchanged to 25 mM sodium phosphate with the pH in the design of experiment (DoE). The buffer exchanged material was loaded onto the Capto S ImpAct column at 5 minutes residence time to a load determined by the design. After a wash with the loading buffer, the material was eluted with 25 mM sodium phosphate containing 250 mM NaCl at pH 7.5. The charge distribution was analyzed by an analytical cation exchanger using a pH gradient from pH 6.5 to 9.5. The design used was a Central Composite Orthogonal design, where pH and load were varied between pH 7.0 to 7.5 and 70 to 140 g mAb/L resin.

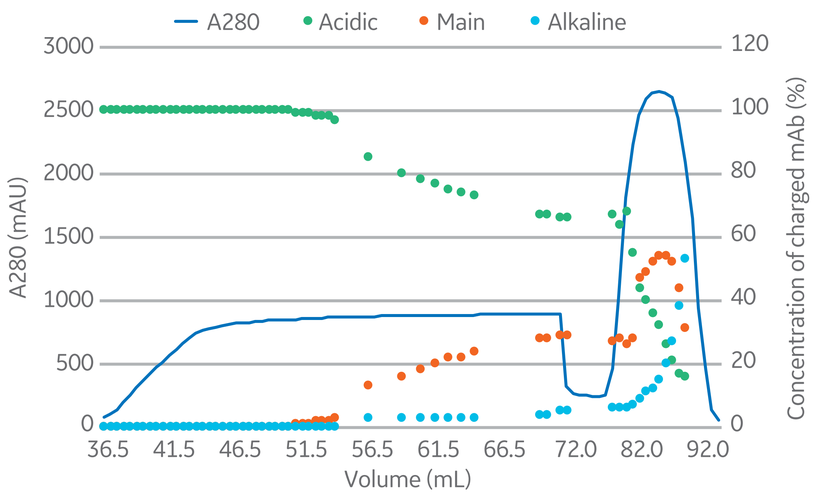


Fig. 1 A frontal analysis of a mAb on Capto S ImpAct, absorbance at (royal blue). The offline analysis of collected fractions on an analytical CIEX shows the breakthrough of acidic (green), main (orange) and basic (light blue) charge variants.

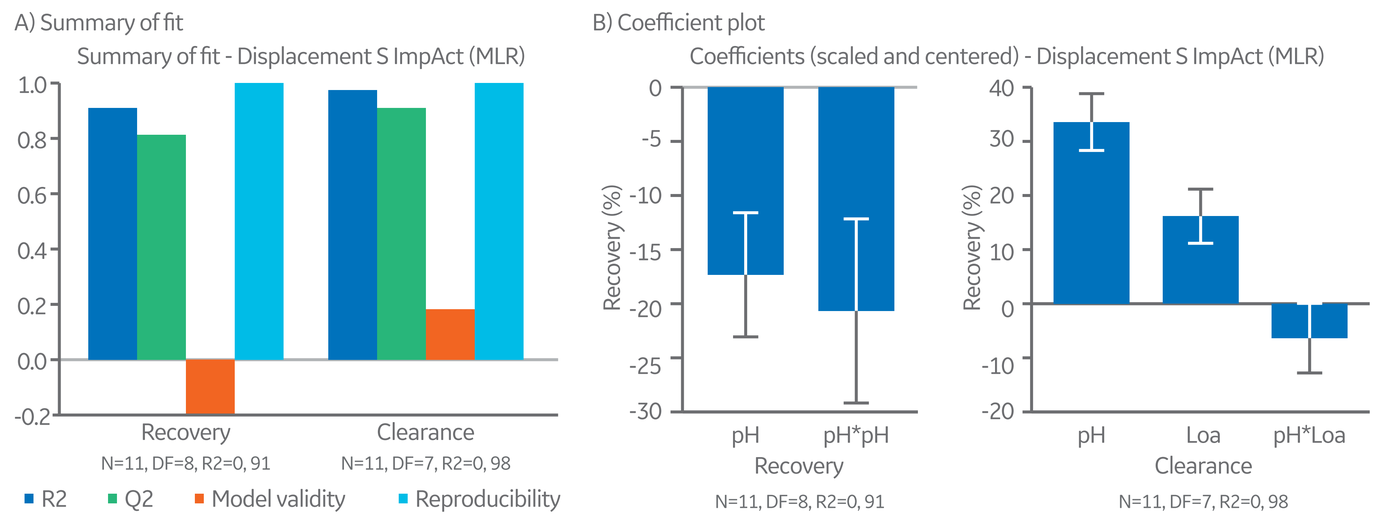


Fig 2. A good model for both recovery and clearance (A). The coefficient plot shows effect of pH for recovery. Both pH and load had effect on clearance (B).

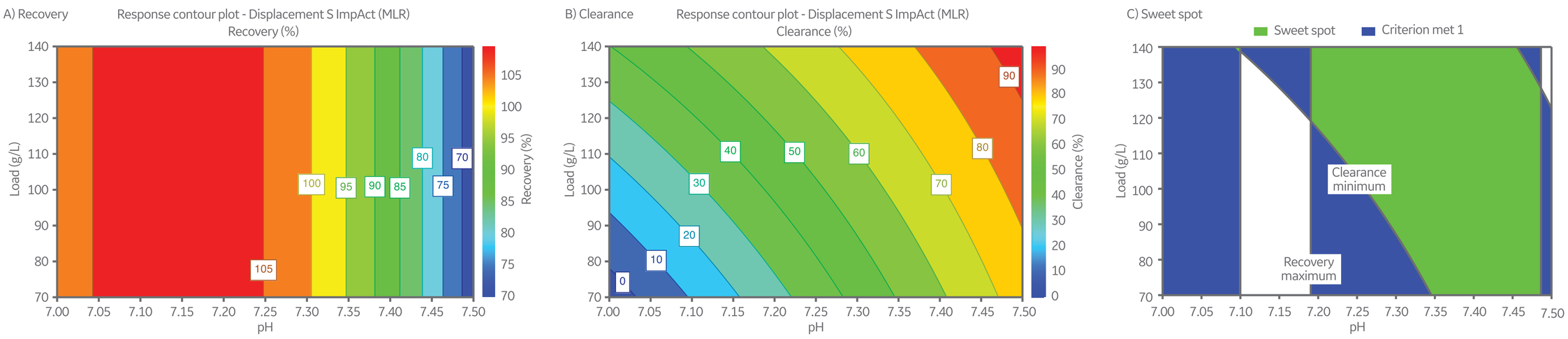
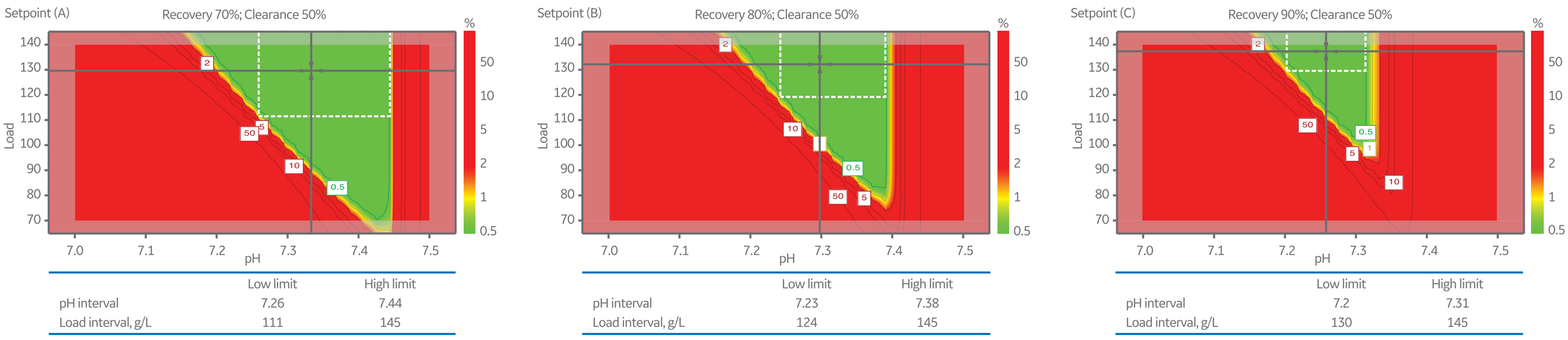


Fig. 3 Illustrates the effect of pH and load on mAb recovery (A) and clearance (B) of acidic charge variants. The sweet-spot analysis (C) shows the clearance of at least 50% of acidic charge variants, keeping the mAb recovery above 70%.



	Low limit	High limit
pH interval	7.26	7.44
Load interval, g/L	111	145

	Low limit	High limit
pH interval	7.23	7.38
Load interval, g/L	124	145

	Low limit	High limit
pH interval	7.2	7.31
Load interval, g/L	130	145

Fig. 4 As the sweet-spot analysis had a 50% risk of failure at the edges of the green area, a Monte-Carlo simulation was done. Here the failure risk at the green borders was 1%. The Monte-Carlo simulation indicates that a robust recovery and clearance can be achieved within pH and load levels indicated below the plots for the different set-points.

Conclusions

- The DoE shows the possibility to displace acidic charge variants by overloading the column.
- This results in capacities of up to 140 g/L and maybe more.
- It facilitates a load at a single pH and step elution with salt.

References

1. Tiselius A. Ark. Kemi. Mineral. Geol. 1943;16A:1.
2. Horváth Cs., Nahum A, Frenz J. J Chromatogr. 1981;218:365.

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