Improving Protein Purification: Application of Excipients in Downstream Processing

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Introduction and Objectives

The production of antibodies and Fc-fusion proteins involves several downstream processing unit operations. The widely used purification template with Protein A chromatography, virus inactivation at low pH, and subsequent ion exchange chromatography steps is mostly able to remove impurities like aggregates, host-cell proteins, and viruses, which could affect the safety and efficacy of the product.

The low pH elution during Protein A chromatography, as well as during virus inactivation may induce aggregation. Preventing protein aggregation during these unit operations instead of removing the multimeric forms during subsequent polishing steps would be a more efficient strategy. Excipients have shown that they can minimize aggregation levels in the final product formulation. For this reason, **we have investigated the benefits of adding excipients during downstream processing on protein stability, chromatographic performance and viral inactivation**.

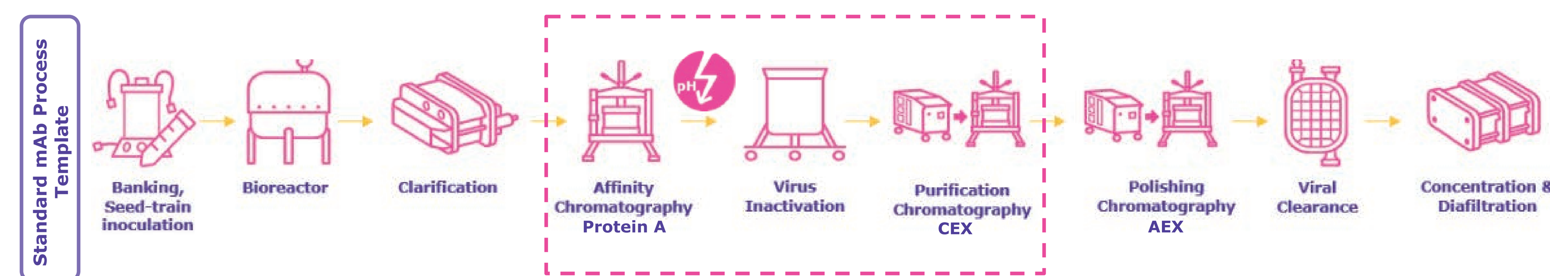

Figure 1: Outline of steps involved in a general monoclonal antibody (mAb) manufacturing process.

Protein A Chromatography

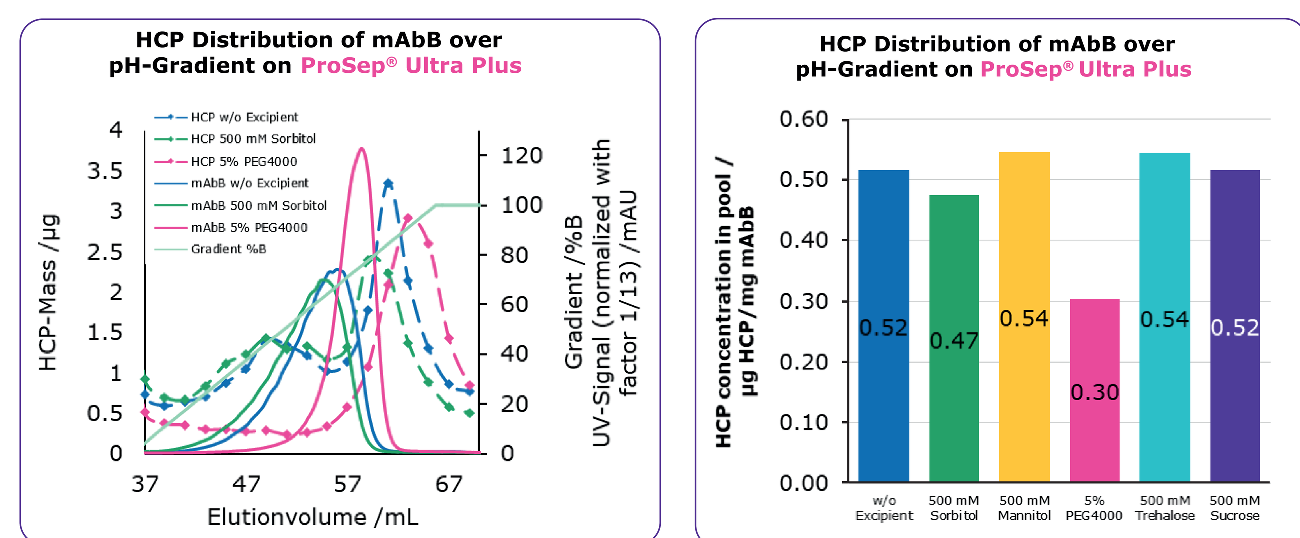

Figure 2: Influence of excipients on HCP removal during gradient elution of Protein A chromatography using ProSep® Ultra Plus; Purity of elution pool in aspect of HCP content of elution pool from collected fractions based on UV280 collection criterion of >30 mAU, with total mAb content during pH gradient.

Addition of 5% PEG4000 lead to sharper peak and provides benefit not only in the reduction of pooling volumes but also HCP content of elution pool.

- Potential excipients (Sorbitol, Mannitol, Sucrose, Trehalose and PEG4000) have been selected by results of low pH (pH 2.8) screening assay (Fig. 6) to be investigated further in real life DSP conditions.
- Selected excipients were added to the buffer system during chromatographic run and their effect on chromatographic performance have been observed.
- 5% PEG4000 causes a shift of sharper elution peak to lower pH during pH gradient elution, while elution without excipient or with the use of disaccharides and polyols show broader elution peak.
- Addition of PEG4000 led also to a comparable or lower elution pool volume (Results not shown in this poster).
- Elution behavior of the HCP's in the presence of PEG4000 differs significantly from control and other selected excipient conditions (Fig. 2, left).
- Purer elution pool profile with lowest HCP concentration down to 0.3 µg HCP/mg mAbB was achieved during chromatography run with addition of 5% PEG4000 (Fig. 2, right).
- Addition of all selected excipients have no negative effect on chromatographic performance.

Virus Inactivation and Viral Clearance Study

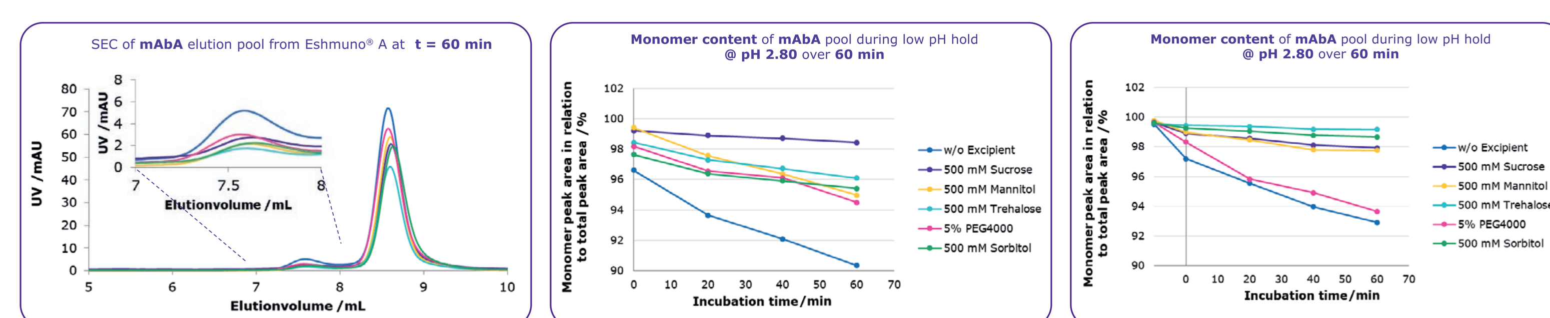

Figure 3: Results overview of low pH viral inactivation; Size exclusion analysis of elution pool of different excipient conditions (left), mAb purity (monomer in %) of elution pool of mAb A (middle) and mAb B (right) after 60 min low pH virus inactivation step at pH 2.8.

Addition of all selected excipients led to an increased **monomer content**.

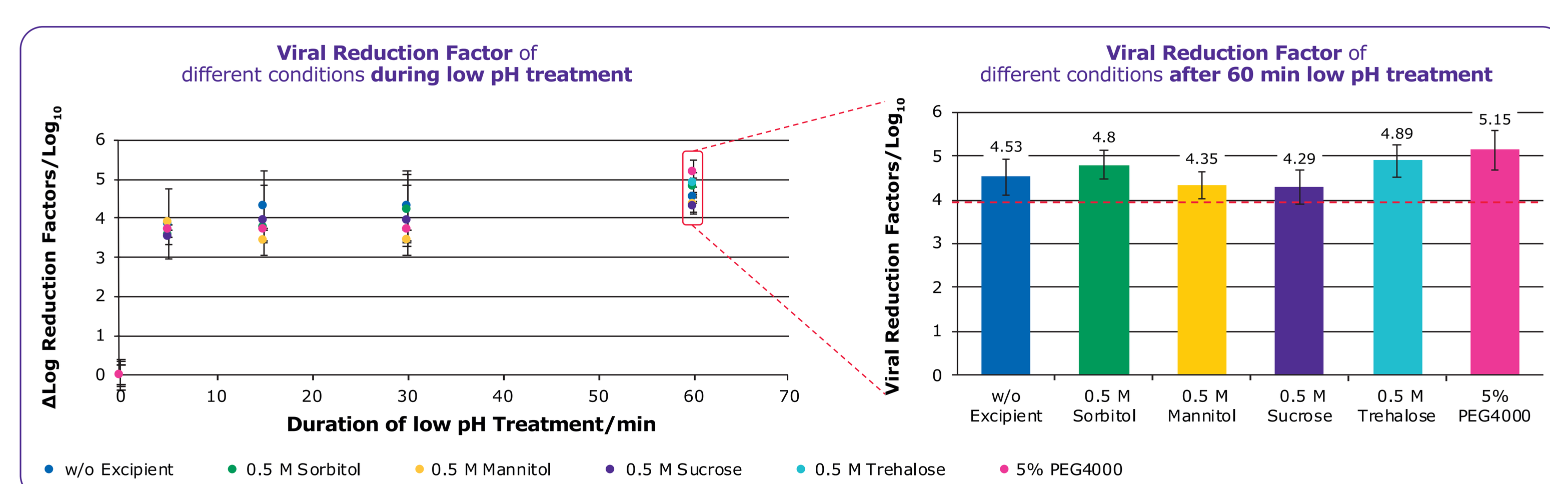

Figure 4: Overview of viral reduction factor of different conditions during low pH treatment in viral clearance study (left) and summarized results of 60 min low pH treatment (right).

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Reduction Factor	Effectiveness*
≤1 $\log_{10}$	Not significant
1–2 $\log_{10}$	Indicative/contributable
2–4 $\log_{10}$	Moderate
>4 $\log_{10}$	High

* Examples of effective virus reduction combined with reduction factor based on EMA guideline in consideration of ICH Q5A guideline.

Addition of selected excipients have no negative effect on viral inactivation. Slight improvement by addition of Sorbitol, Trehalose and PEG4000.

- Decreased aggregate content (dimers) could be observed in all excipients conditions (Fig. 3, left).
- Addition of all selected excipients led to an increased monomer content up to 6.3% (Fig. 3, middle and right). The results are well in line with excipient screening results.
- Based on EMA guideline in concideration of ICH Q5A guideline, Viral reduction of all tested conditions have been classified as highly effective (viral reduction factor > $4\log_{10}$, Fig. 4).
- Slight improvement by addition of Sorbitol, Trehalose and PEG4000 could be observed in viral clearance study (Fig. 4).
- Addition of selected excipients have no negative effect on viral reduction.

Dynamic Binding Capacity of CEX

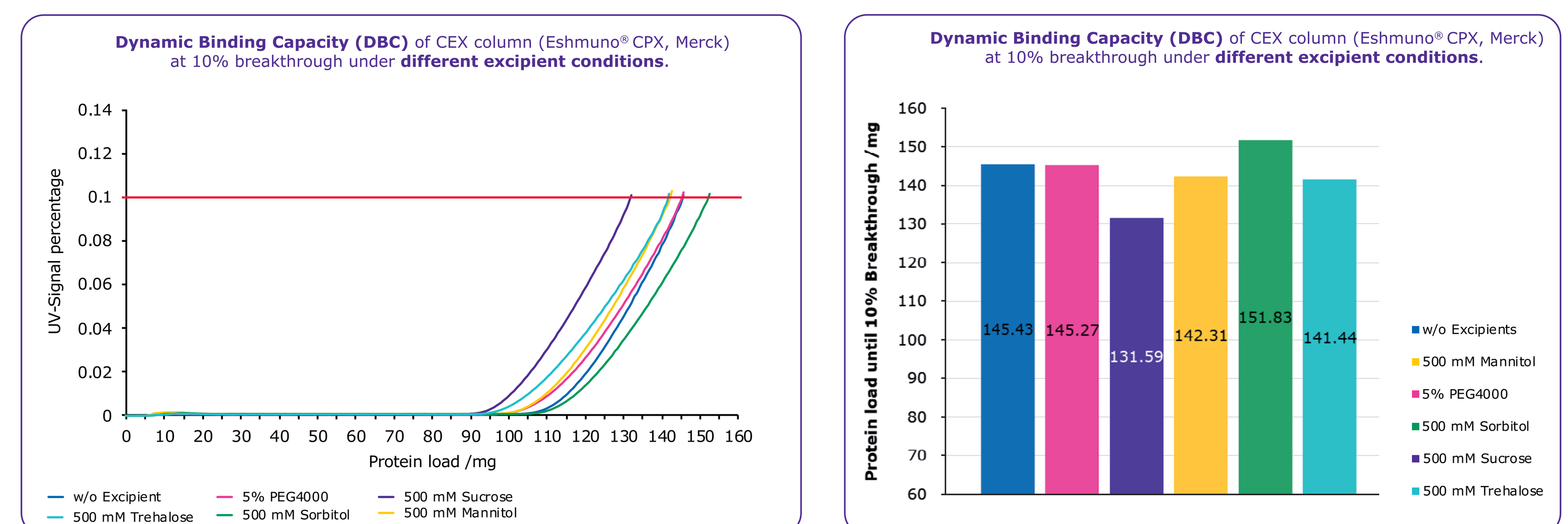

Figure 5: Dynamic Binding Capacity (DBC) of CEX column (Eshmuno® CPX, Merck) at 10% breakthrough under different additive conditions.

The addition of excipients don't affect the dynamic binding capacity in cation exchange chromatography. Slight improvement of DBC with addition of **500 mM Sorbitol**.

- The capacity was unaffected with addition of PEG4000, Trehalose and Mannitol (DBC 10% ca. 140–145 mg/mL CV).
- Slight reduction on binding capacity was observed in additive condition of 500 mM Sucrose.
- Nevertheless, the presence of all additives don't negatively affect the process condition, because safety factor is generally used on CEX process (e.g. 80% of the 10% breakthrough).

Screening Assay

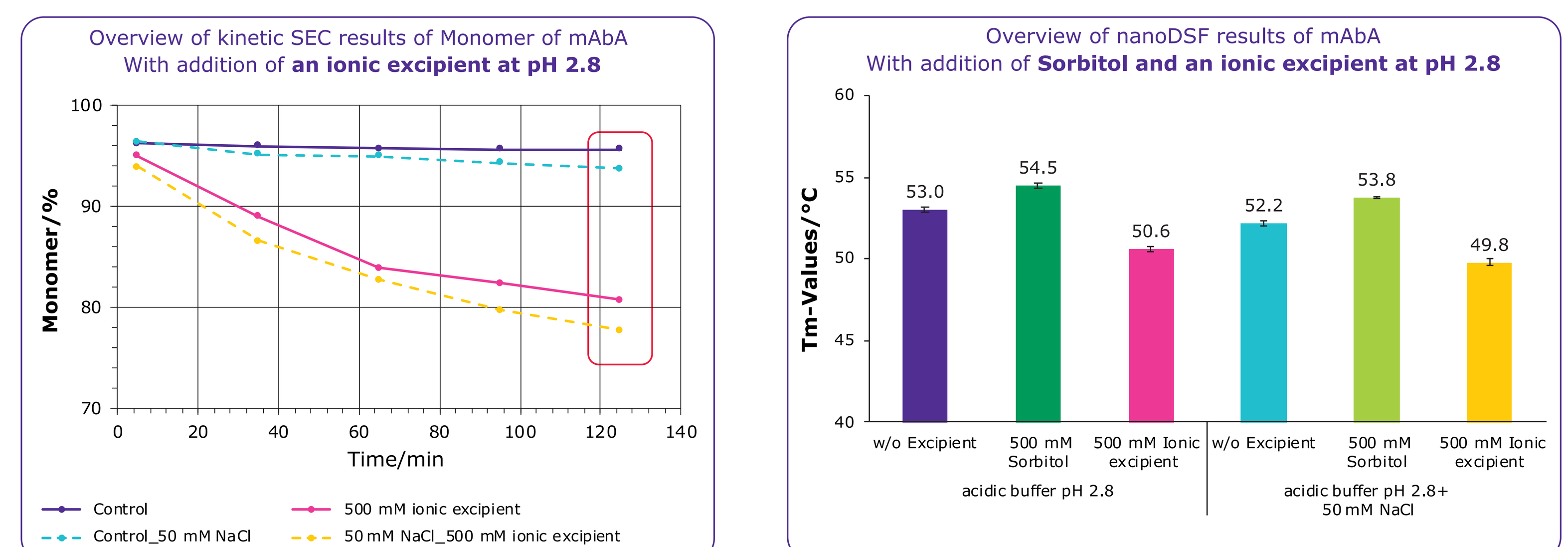

Figure 6: Exemplary results of screening in kinetic SEC diagram (left) and melting temperature (Tm) diagram by nanoDSF (right) showing stabilizing effect of certain excipients (Sorbitol and an ionic excipient as negative control) on mAbA during low pH treatment.

Addition of suitable excipients during aggregation kinetic assay prevents formation of aggregats **→ stabilization of mAbA during low pH stress**.

- To determine the influence of excipients in Protein A chromatography and virus inactivation, a low pH screening assay was established.
- Potential Excipients **(Sorbitol, Mannitol, Sucrose, Trehalose and PEG4000)** during screening in acidic conditions were investigated further in real life condition in Protein A chromatography and viral clearance study.
- Addition of suitable excipient (e.g. Sorbitol) prevents formation of aggregates (Fig. 6, left) and increase Tm up to 1.5 °C (Fig. 6, right) which indicates stabilization of mAbA during low pH stress condition.
- Polyols (e.g Mannitol, Sorbitol, Innositol) and Sugars (e.g. Sucrose and Trehalose) and PEG4000 were most effective excipients in low pH screening condition with and without the absence of sodium chloride.

Conclusion

In this study, we were able to confirm the addition of excipients can have a beneficial effect for the purification during Protein A chromatography and virus inactivation by stabilizing proteins, seeing that the level of monomer can be increased up to 6.3%, reduction of volume and HCP content of elution pool, without harming the subsequent chromatographic steps.

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