Modes of controlling charge variant distribution in MAb production by upstream and downstream process parameters

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Modes of controlling charge variant distribution in MAb production by upstream and downstream process parameters

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Abstract

The characteristics of a biological drug, for example, charge variant profile, are determined to a large extent by the upstream expression system and conditions, but can also be influenced by the purification process. Charge variant distribution is a critical quality attribute for MAbs and is therefore of interest to monitor and control during the production process.

In this study, we investigated the effects of cell culture process parameters and medium components on charge variant distribution of a human IgG1 MAb. Furthermore, the MAb charge variant clearance in the downstream chromatography polishing step was evaluated.

Conclusion

Our results demonstrate that the distribution of acidic charge variants could be influenced during upstream cell culture by adjusting:

- Process time
- pH
- Cell culture components such as sugar and iron citrate concentration

During culture, pH as well as iron citrate and sugar concentrations contributed to increased amount of acidic charge variants, whereas higher MAb titers had a lowering effect.

Optimized clearance of acidic charge variants at good MAb recovery could be achieved in the downstream purification process by adjusting:

- Column bed height
- Sample load and pH
- Elution gradient type and slope

Large bed height and elution using a salt gradient resulted in improved clearance of acid charge variants at good MAb recovery.

The DoE study results indicate that low sample load and high pH as well as shallow elution salt gradient optimized clearance of acidic charge variants at total MAb recoveries of 70% or 80% MAb.

At a sample load of 40 g MAb/L medium, a more than 50% clearance of acidic charge variants could be achieved at a total MAb recovery of 70%.

In summary, both upstream MAb titer and downstream MAb recovery need to be balanced against MAb quality for optimized productivity (Fig 7). For a challenging MAb process, a balance between upstream production and downstream purification is also important.

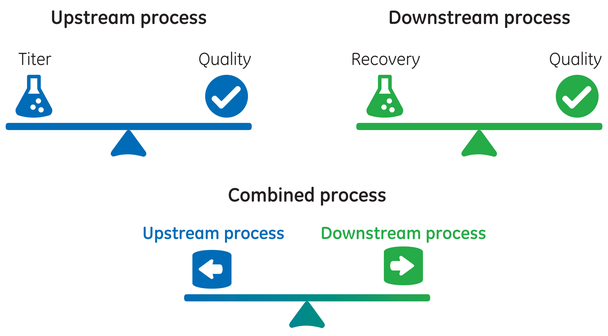

Fig 7. Both upstream MAb titer and downstream MAb recovery need to be balanced against MAb quality.

Results

Upstream process

A set of standard fed-batch cultures were evaluated in both stirred-tank bioreactor and WAVE Bioreactor™ systems in working volumes of 5, 10, 25, and 100 L. Average peak cell density was consistently around 20 × 10^6 viable cells/mL and final product concentration was above 4 g/L. Cell viability was high throughout the culture (Fig 1).

During cell culture time, a notable increase in acidic charge variants were observed (Fig 2). The reason for this was further investigated using a design of experiments (DoE) approach. In the design; pH, MAb titer, and concentrations of iron citrate and sugar were selected as factors partly from a process point of view, and partly because they are known to contributes to the generation of acidic charge variants through various biochemical pathways. Different MAb concentrations were incubated for 14 days in MES/HEPES buffer at different pH and in various iron citrate and sugar concentrations. After incubation, the charge variant distribution was analyzed using cation exchange chromatography (CIEX). The 4D contour plot shows that pH, iron citrate, and sugar contribute to increased amount of acidic charge variants and that higher MAb titers partly counteract this effects (Fig 3).

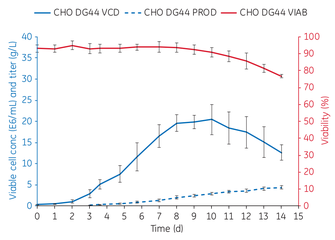

Fig 1. Cell growth, cell viability, and IgG titers over culture time.

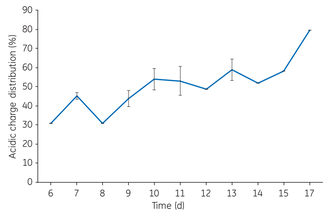

Fig 2. Average distribution of acidic charge variants over culture time.

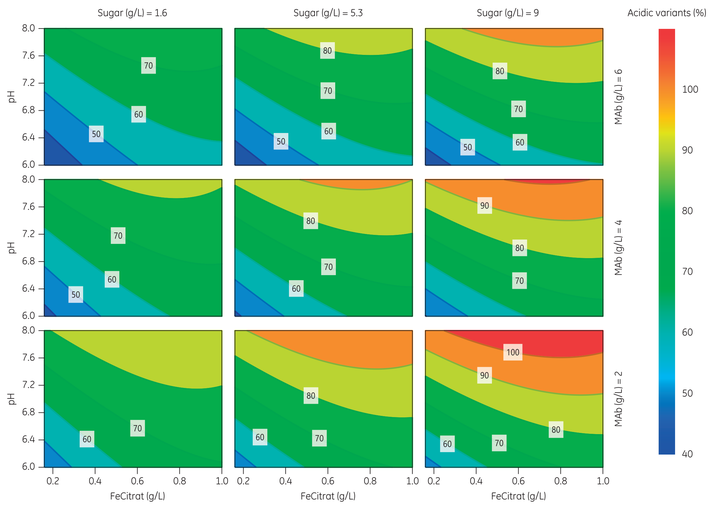

Fig 3. 4D contour plots for pH and concentrations of sugar, iron citrate, and IgG.

Downstream process

As start material, MAb purified on MabSelect SuRe™ LX chromatography medium from clarified cell culture harvest was used. The MAb sample contained 18% acidic charge variants. For the polishing step, Capto™ SP ImpRes was selected. First, the effect of bed height and gradient elution using pH or salt on acidic charge variant clearance was investigated. The results show that a larger bed height and elution using a salt gradient was favorable for clearance of acidic charge variants (Fig 4). Secondly, a DoE study was performed for optimizing the process, including Capto SP ImpRes at a 20 cm bed height and elution using a salt gradient. As factors, sample load and pH as well as slope of elution gradient were selected (Fig 5). As responses, remaining acidic charge variants at a total MAb recovery of 70% and 80% was evaluated (Fig 6). The results show that all factors tested affected clearance of acidic charge variants, where pH seams to contribute most. At a MAb recovery of 70%, an approx. 60% clearance of acidic charge variants was achieved.

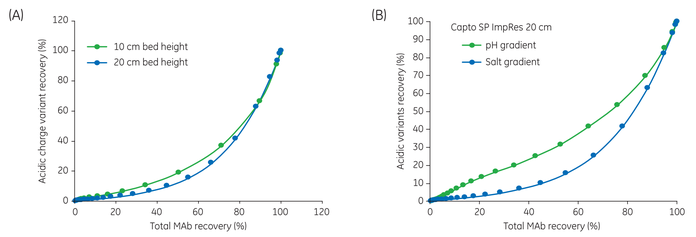

Fig 4. Recovery curves for total MAb versus acidic charge variants, where (A) bed height and (B) gradient type were compared.

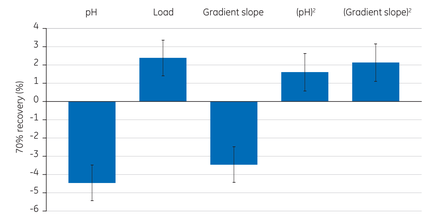

Fig 5. Effects of sample load and pH as well as slope of elution gradient on clearance of acidic charge variants at 70% total MAb recovery.

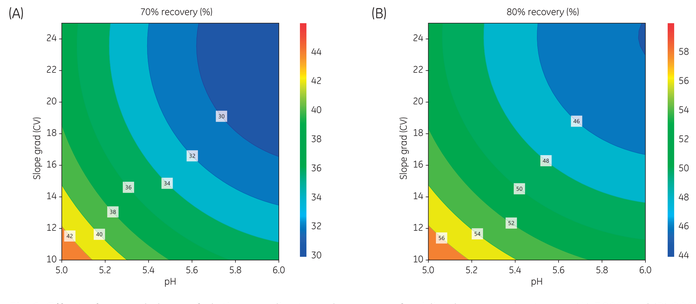

Fig 6. Effect of pH and slope of elution gradient on clearance of acidic charge variants at w(A) 70% and (B) 80% total MAb recovery and a load of 40 g MAb/L chromatography medium. White boxes = remaining acidic charge variants (%).

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