Strategies for optimized cell culture media: effectively using design space to influence product quality

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CY15145-12Jul20-PT

Strategies for optimized cell culture media: effectively using design space to influence product quality

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Abstract

Effective biopharmaceutical production is achieved in part through target-specific, quality-optimized cell culture media. Balancing the many individual media components required to achieve high titers and support critical protein quality attributes can be challenging. Here we highlight a statistical design strategy to optimize cell culture media to influence the charge variant profile of a mAb produced in CHO DG44 cells.

Media optimization requires incorporation of progressive statistical strategies. First, a screening model is used to identify the key components that influence critical culture attributes (e.g., titer, protein quality). Multivariate analysis can be used to identify factors correlating with the various attributes. Further rounds target optimization of factor levels. The following case study presents a two-part screening campaign that was used to target charge variant factors. Results indicate that several conditions improved titer and maximized main variant expression. A subsequent round focused on finding optimal levels for key drivers of higher titer and main variant expression.

Introduction

Well designed experiments coupled with progressive statistical analysis strategies provide the opportunity to reduce the number of experimental rounds required to achieve desired project goals. The following case study demonstrates the use of these strategies to increase main fraction charge variants to ≥ 50% through feed component optimization and to maintain peak titer values of > 1 g/L. In Phase I, historical data from development of the baseline media, feed, and culture process was analyzed. Throughout several rounds of past experimentation a charge profile made up of mostly basic variants was consistently observed.

In our first round of experimentation (Phase II) a two-part screening design was employed. Part 1 screened six feed prototypes at two concentrations each (35 g/L and 50 g/L). Prototypes with varying concentrations of key components were selected in order to provide meaningful data for Multivariate analysis. Part 2 was a D-optimal design varying 4 factor groups in 2 base feed prototypes. The factor groups were components targeted to modify charge profile. Phase III utilized a Definitive Screening Design to vary individual components from factor groups significantly affecting the response variables, as well as additional components identified from Phase II Partial Least Squares and Multivariate correlation analyses.

Results

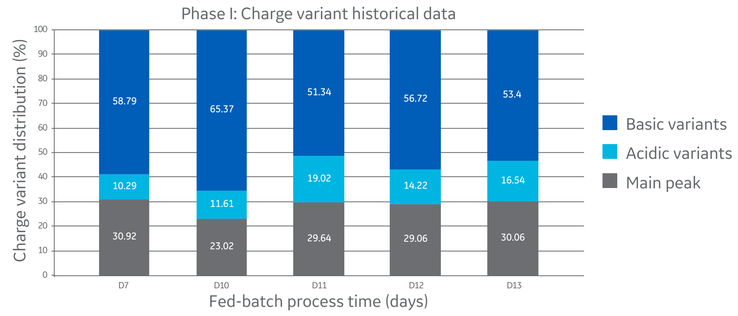

Fig 1. Phase I charge variant historical data. Results represent the typical percent charge distribution from the baseline media and feed system. Data show a large basic variant fraction with the typical range between 50–65%.

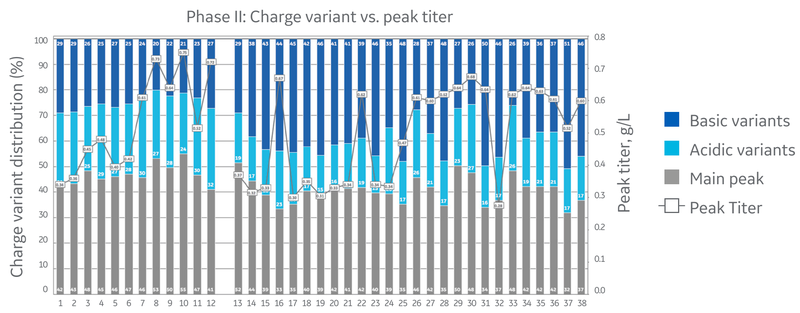

Fig 2. Average (n=2) charge variant distribution and peak titer results across Phase II feed conditions. Condition numbers 1–12 are part 1 results and 13–38 are from part 2 of the experiment. Several conditions provided a substantial increase in percent main fraction, yet peak titer did not reach the goal of >1 g/L.

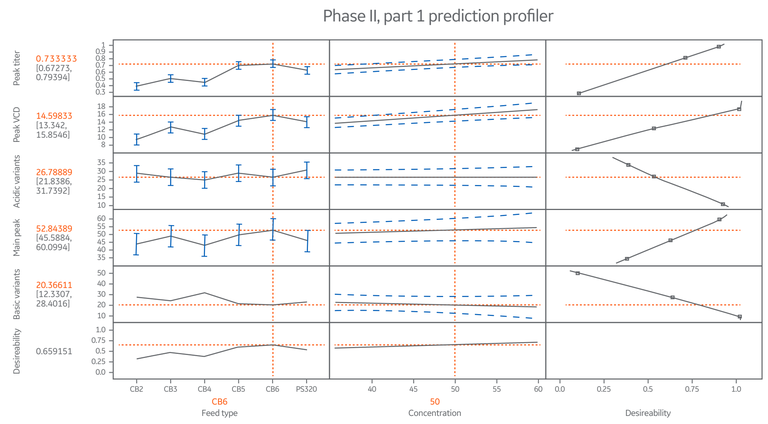

Fig 3. Prediction profiler produced from Least Squares Regression analysis on Phase II, part 1 data. The profiler is set to maximize peak titer and percent main charge variant peak (main peak), displaying the predicted best feed type and hydration concentration. Feed type CB6 at the highest tested hydration concentration provided the best results. The trace lines for the concentration factor predict that a concentration higher than what was tested could be beneficial.

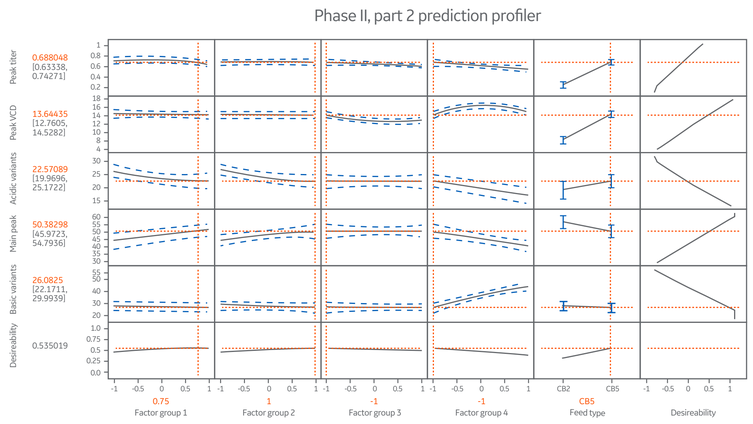

Fig 4. Prediction profiler produced from Least Squares Regression analysis on Phase II, part 2 data. All significant main and interaction effects are evaluated and explorable in the live version of the profiler. The profiler shown is set to maximize peak titer and percent main peak, displaying the predicted best levels of each factor based on the set weightings. Feed type CB5 had the largest positive effect on peak titer. With feed type set to CB5, the +1 level of factor group 1 and 2 significantly increased percent main peak as did the -1 level of factor group 4.

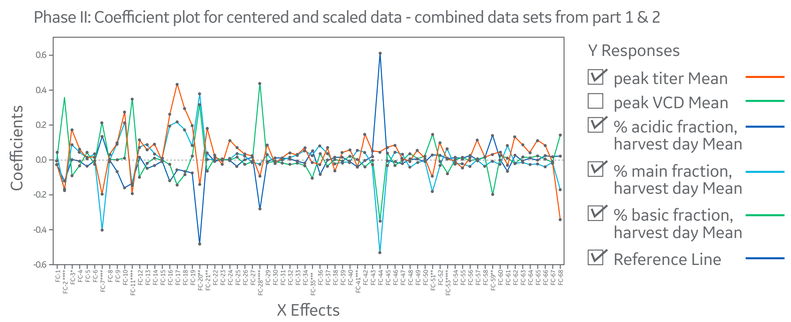

Fig 5. Partial Least Squares coefficient plot from both parts of the Phase II experiment. Feed components are shown on the X-axis and labeled FC1–FC68. Correlation coefficients for response variables peak titer, acidic variants, main peak, and basic variants are plotted and highlight differing relationships between individual components and each response variable. Combining the data sets helps elucidate individual effects of grouped components that are masked in the part 2 data alone. The coefficient plot also helps detect additional components that could be tested to improve peak titer and percent main peak.

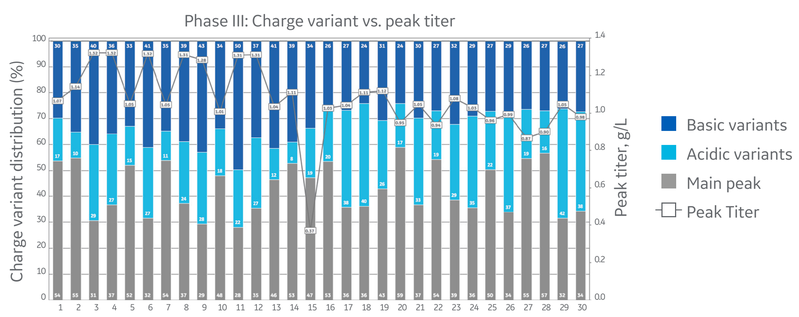

Fig 6. Charge variant distribution and peak titer results across Phase III feed prototype conditions. Inclusion of components significantly correlating with increased peak titer and percent main peak from Phase II screening produced several conditions with higher peak titer (achieving the goal of >1 g/L) as well as further increases in percent main peak.

Discussion and conclusions

In only two rounds of feed development experiments the percent main charge variant fraction more than doubled, from an average of 28.5% up to a maximum of 59%. Basic and acidic fractions reached minimums of 24% and 8% respectively. Peak titer reached a maximum of 1.32 g/L, satisfying goal requirements.

The two-part design from Phase II screening enabled testing of a wide range in concentration of feed components and led to the identification of factor component groups with significant effects on the response variables. Effects from individual components in each of the factor groups were detected through combined part 1 and part 2 Multivariate analyses. These analyses also identified additional components correlating with each of the response variables. Testing each of the identified components in a balanced manner according to their correlative relationships during the Phase III experiment led to additional increases in peak titer and percent main fraction. Quadratic effects and second degree interactions were able to be detected and revealed predicted levels to further increase titer while maintaining the desired charge profile (data not shown).

The earliest phase of media or feed optimization ideally leads to the identification of all components with potential to significantly impact culture responses of interest. Investigating complex correlation structures between culture responses and each formulation component is possible through screening formulations with sufficient concentration ranges. Partial Least Squares analysis can also produce estimates for desirable concentration levels for testing. The large number of components identified can be too high for traditional screening designs to produce a reasonable number of treatments. Definitive screening designs have the advantage of not only producing fewer treatments with a large number of factors but also allowing for curvature and interactions to be detected, resulting in fewer rounds of experimentation needed to find optimal levels. The use of efficient statistical methods in a logical progression led to substantial progress toward project goals in only two cell culture experiments. A final round could be done to optimize component levels from the Phase III predictions in order to further increase peak titer.

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