Quality by Design Approach Results in the Discovery of a Process Parameter that Increases Afucosylation

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ABSTRACT

Avid Bioservices, Inc. applied a Quality by Design (QbD) approach to characterize the effects of upstream process parameters on Critical Quality Attributes (CQAs). A risk assessment exercise using Failure Mode and Effects Analysis (FMEA) assigned Risk Priority Numbers (RPN) based on our prior knowledge, expertise in CHO cells, and extensive manufacturing experience with support from primary literature. Design of Experiments (DoE) using a fractional factorial design assessed process parameters with medium to high RPNs. One parameter in particular was found to significantly affect afucosylation levels on the antibody. We found that afucosylation levels can be increased by modifying the design space without introducing new variables to the process. Our ongoing QbD project will continue with a Response Surface Design DoE which will evaluate the effect of additional critical parameters on CQAs which will support increased antibody production levels for Phase 3 Clinical Trials and commercialization.

BACKGROUND

Initial upstream development identified a new chemically defined cell culture medium and animal component-free feeds which significantly increased the titer and productivity of a client's proprietary cell line.

To characterize process effect on product profile of the antibody, Critical Quality Attributes (CQAs) were identified and characterization work initiated. Twenty five bioreactor process parameters were evaluated using FMEA as a risk assessment tool to associate potential Critical Process Parameters (CPPs) with CQAs. Risk Priority Number (RPN) were calculated. Eleven parameters with medium and high RPN were identified as potential CPPs and were selected for further experimentation.

Following FMEA and RPN assignment, a bioreactor scale down model was developed using kLa and calculated power/volume ratio value. Oxygen mass transfer coefficient (kLa) was determined using static gassing out methods at 3L, 10L, and 1000L bioreactor scales.

DoE experiments were performed using fractional factorial and blocking designs at bioreactor scale. While executing our experiments, we discovered that afucosylation levels could be controlled by changing the design space alone. In addition, the increased afucoslation did not lead to increase in complex mannose glycoform such as Man5. Through research of primary literature and experimentation, we found that glycoform G0f, G1F and G2F can be controlled by bioreactor conditions. However, aside from this, we have not seen other cell lines that show that bioreactor parameters could control afucoslation without affecting other product quality attributes. Here we present effects of CPPs on afucosylation.

DISCUSSION

Fractional factorial matrix is useful in screening a large number of potential CPPs with fewer number of runs. Half Normal plot from fractional factorial DoE hinted at process parameters that may increase G0 (Figure 1).

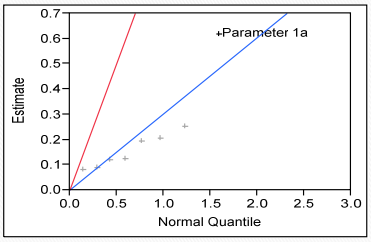


Figure 1
G0 Actual by Predicted Plot

Full DoE was performed to characterize CPPs effect on G0 profile. A model was presented as a regression function to predict the effects of changing variables. The P-value is statistically significant at 0.0024. The R-square value indicated that the model can explain 82% of the observed effects on G0.

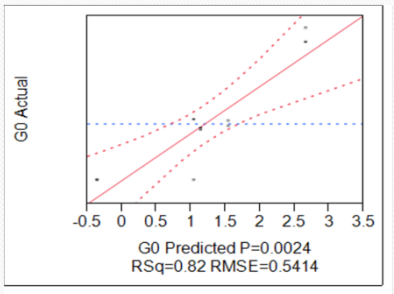


Figure 2:
G0 Response

Prediction profiler (Figure 3) shows the effect on G0 of each individual parameter investigated. The Contour Profiler shows the acceptable range to operate critical parameter without affecting CQA (Figure 4).

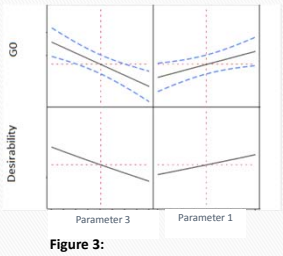


Figure 3:
Prediction Profiler

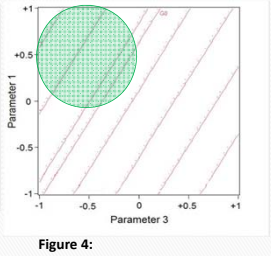
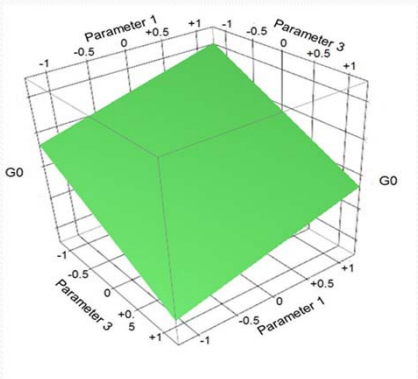


Figure 4:
Contour Profiler

Effect of parameter 1 and 3 on G0 profile. below in Figure 5.



As a result of these DoE studies, a set of optimized parameters was selected. Overall average afucosylation levels were increased.

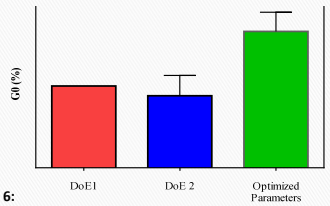


Figure 6:
G0 results indicating an increase in G0 was seen.

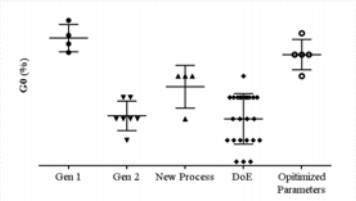


Figure 7:
G0 profile shown after optimized parameters.

CONCLUSIONS

- Identified Critical Process Parameters that affect CQAs.
- Showed through experimentation that G0 can be controlled by manipulating identified CPPs.
- Increasing G0 only increased bioassay activity but did not affect other CQAs.

ACKNOWLEDGEMENTS

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