Characterization of Bioreactor Conditions Effect on Afucosylation

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ABSTRACT #P-226-W

Avid Bioservices, Inc. applied a Quality by Design (QbD) approach to characterize the effects of upstream process parameters on Critical Quality Attributes (CQAs). A preliminary 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. Interestingly, two parameters were 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. Since we have never seen this previously, a study was conducted to understand this phenomenon. The conventional one factor at a time approach revealed that the concentration of one component in cell culture medium allowed two process parameters to control afucosylation.

QbD APPROACH and 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) was 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. A model was generated from the DoE and was confirmed with a 1000L cGMP bioreactor.
- DoE experiments were performed using fractional factorial and blocking designs at bioreactor scale. While executing our experiments, we discovered that glycoform G0 could be controlled by changing the design space alone. In addition, the increased G0 did not lead to increase in complex mannose glycoforms such as Man5. Through research of primary literature and experimentation, we found that glycoforms G0F, G1F, and G2F can be controlled by bioreactor conditions. However, aside from this, we have not seen other cell lines that show bioreactor parameters could control G0 without affecting other product quality attributes. It is possible that another variable allowed this to occur.
- To further explore this, the process conditions were set based on a DoE model that predicted increased G0 through varying the cell culture media components. Using our previous experience on creating custom feeds for bioreactor processes, we tested five cell culture media components. The data show one cell culture media component allowed two process parameters to control the level of G0 glycoform.

Background on glycoform and ADCC activity

Based on primary literature, non-fucosylated glycoforms have been shown to increase ADCC activity, while fucosylated glycoforms have been shown to decrease ADCC activity. However, the previous studies utilized engineered cell lines or artificially stimulating samples to generate or enrich the desired glycoforms. In this study we apply multivariate regression analysis of glycoform to our proprietary ADCC surrogate bioassay on thirty-five bioreactor from the DoE study. This is more representative of the variations in glycoforms from normal cGMP bioreactor processes because the DoE study is based on the characterization ranges. A correlation between quality attributes and ADCC activity based on the characterization ranges will be presented.

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).

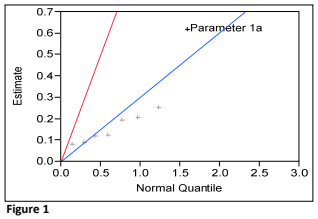

Figure 1
G0 Actual by Predicted Plot

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.

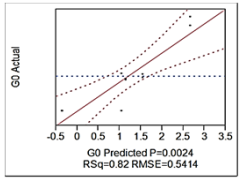

Figure 2:
G0 Response

OPTIMIZATION of PARAMETERS

As a result of these DoE studies (Figure 3), a set of optimized parameters were selected (Figure 4). Overall average afucosylation levels were increased.

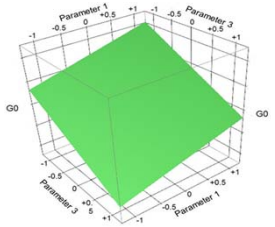

Figure 3:
Effect of parameters 1 and 3 on G0 profile

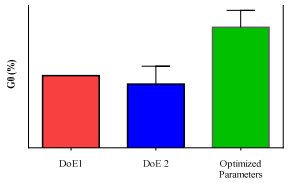

Figure 4:
G0 results indicating an increase in G0 was seen

RESULTS and DISCUSSION

Further analysis of media component 5 revealed that levels of G0 increased in a linear relationship to the concentration of media component 5 (Figure 5). Overall average afucosylation levels were increased with these optimized parameters (Figure 6).

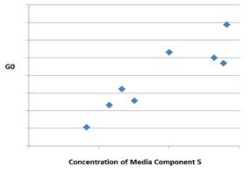

Figure 5:
At optimum process parameters, the concentration of one media component controls G0 glycoform.

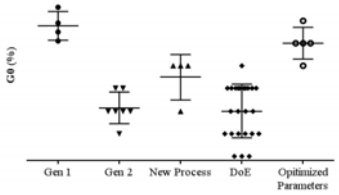

Figure 6 :
G0 profile shown after optimized parameters.

Predicted values from the DOE model were compared to the results from a 1000 L cGMP bioreactor run (Figure 7).

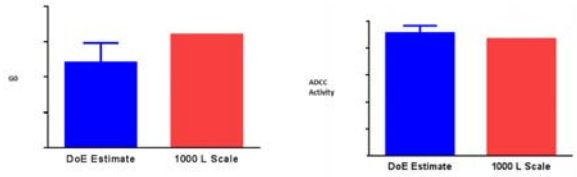

Figure 7:
DoE model prediction comparison to cGMP 1000 L Bioreactor run results.

RESULTS and DISCUSSION (continued)

When analyzing each quality attribute individually, G0, Man5 and basic peak (IEX) have a statistically significant correlation with ADCC activity. However, G0 is highly predictive of ADCC with a $R^2 = 88.19\%$, while basic peak is the less predictive (Figure 8).

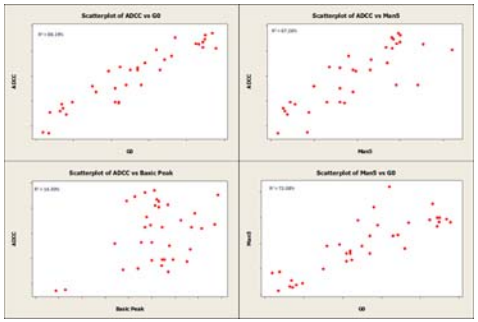

Figure 8: Scatterplots showing the relationship of ADCC activity, G0, Man5, and Basic Peak (IEX)

ADCC Activity = 5.077 + 19.367 G0 + 2.628 Man5 + 1.765 Basic		
Model R-Sq = 90.63% P-value = 0.000		
	Coef	P
Constant	5.077	0.428
G0	19.367	0.000
Man5	2.628	0.427
Basic	1.765	0.010

Table 1: Multivariate regression analysis

With a full model from multivariate regression analysis (Table 1), Man5 is no longer statistically significant because it is highly correlated with G0. Increasing G0 by 1% will result in an increase of ADCC activity by 19.4%.

CONCLUSIONS

- Two out of the twenty-five potential process parameters were identified as Critical Process Parameters (CPPs) affecting the Critical Quality Attributes (CQAs).
- At the optimal CPPs, one component in the cell culture media showed a linear correlation with G0. This component allowed the two process parameters to control G0.
- We showed through experimentation that G0 could be controlled by manipulating these two identified CPPs.
- Increasing G0 increased the ADCC activity as measured through a proprietary ADCC surrogate bioassay.
- A model generated from the DOE studies estimated results for a scaled down 3 L run.
- The DOE model for G0 glycoform and ADCC activity predicted, and thus confirmed, the actual 1000 L cGMP bioreactor run.
- Multivariate analysis of the DOE data showed that G0 is mainly responsible for the bioassay activity within the characterization range.

ACKNOWLEDGEMENTS

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