Application of Definitive Screening Design (DSD) to the icIEF Assay Development of Antibodies and Therapeutic Proteins

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ABSTRACT

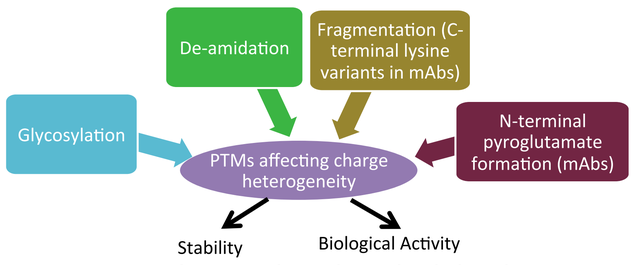
Post-translational modifications (PTMs) of therapeutic proteins and antibodies have a great impact on their biological function and stability. Charge heterogeneity arising due to PTMs is an important quality attribute in therapeutic proteins and antibodies. Therefore, understanding charge heterogeneity and its impact on the stability of biologics is of paramount importance. Imaged-capillary Iso-Electric Focusing (icIEF using iCE280) is a pI-based high-resolution technique that can be used to monitor charge heterogeneity of antibodies and proteins caused by PTMs. Here we describe a Design of Experiment (DoE) approach using a Definitive Screening Design (DSD) model to optimize the icIEF development of a glycosylated protein transferrin and an IgG1 monoclonal antibody. Initial scouting experiments were performed to identify critical factors. By applying a DSD model, we were able to efficiently optimize the icIEF assay development for transferrin and IgG1 with five factors in a small number of experiments. Overall, the use of DSD to icIEF method development has minimized the high cost associated to multiple icIEF runs when using One-factor-at-a-time (OFAT) approach, reduced development time, and most importantly, improved robustness of the assay.

INTRODUCTION

Quality by Design (QbD) defined as a "systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control" by ICH [1], is applied in a variety of processes within the biopharmaceutical industry. However, QbD applications have limited focus on analytical methods [2] and a traditional or One Factor at a time (OFAT) approach is more commonly used for analytical method development. The Analytical Development laboratory at Cytovance Biologics aims to gain a better understanding of QbD applications to reduce method development time and decrease costs by using Design of Experiments (DoE) approach. The application of a DoE approach to develop an imaged-capillary Isoelectric focusing method (icIEF) for monitoring Post-translational modifications (PTMs) in therapeutic proteins is presented in this poster.

Post-translational modifications (PTMs) are important components of cell signaling and can occur at every stage of bioprocessing. Therapeutic proteins display a wide range of PTMs, the most common ones are glycosylation, amidation, phosphorylation, sulfation etc. PTMs can alter charge heterogeneity thereby profoundly affecting the biological activity and stability of the proteins (Figure 1). The charge variants are required to be monitored since most therapeutic proteins carry one or more forms of PTMs that can alter the charge heterogeneity[3]. At Cytovance Biologics, charge heterogeneity of antibodies and therapeutic proteins is monitored by icIEF method using iCE280 by Protein Simpl.

Figure 1: Post-translational modifications affecting charge heterogeneity



An OFAT approach to analytical method development involves testing one factor at a time instead of all factors simultaneously; therefore it is difficult to identify the impact of two factor interaction on the response. An OFAT approach can also miss the optimized settings of factors and requires more resources. A DoE approach allows testing several factors simultaneously with less resources and is more efficient at determining impact of two factor interaction on the response.

In this poster, the case study of the icIEF method development of transferrin, a glycosylated protein is presented using a simple four-step procedure (Figure 2) applying QbD principles.

Figure 2: Design of Experiment (DoE) – A problem solving tool to icIEF method development

- Identify key factors and responses
- Relationship between factors and responses
- Best setting of factors for optimal response
- Perform confirmation runs. Are results comparable to predicted?

METHOD

Step 1 - Screening: Based on prior knowledge of icIEF method development with other glycosylated proteins, an initial screening was performed to identify the pI range for transferrin (Figure 3). Based on the initial results, a scouting experiment was performed and key factors (Table 1)and responses (Table 2) were identified (Figure 4).

Table 1: Key Factors
Methyl cellulose
Narrow range pharmalyte (5-8)
Protein concentration
Urea
Focusing time

Table 2: Responses - Resolution
Peak 2
Peak 3a
Peak 3b
Peak 4

Figure 3: Electropherogram (E-gram)of Transferrin (initial screening)

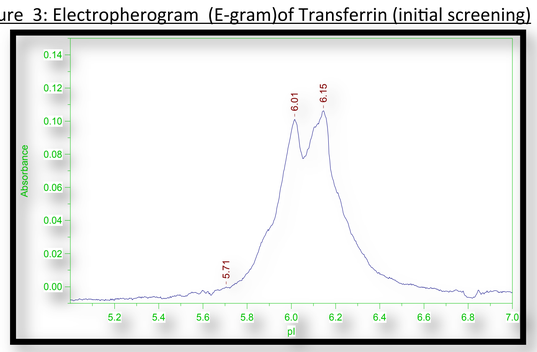
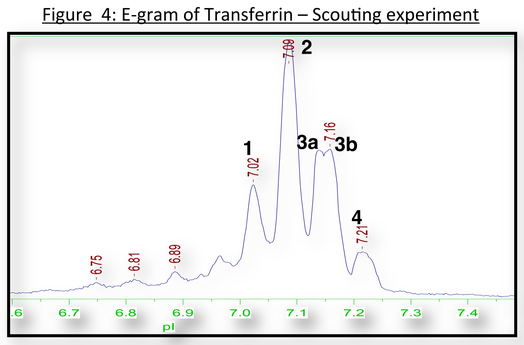


Figure 4: E-gram of Transferrin – Scouting experiment



Results indicate that for transferrin, Peak 2, 3a, 3b and 4 are resolution indicating. Since peak 3 was not well resolved, separation of peak 3a and peak 3b is critical.

Step 2: Characterize: A definitive Screening Design (DSD) model was constructed using JMP software to characterize the relationship between factors and responses. A DSD model offers practical advantages over other designs because it involves smaller number of runs (cost-effective), can identify important factors causing a non-linear effect on the response and allows continuous factors to be tested at three levels[4] [5]. A five factor DSD was designed with a low, center point and a high point (Table 3). A total of 16 runs were performed with three injections of each run (Table 4). Each E-gram was calibrated with pI markers, converted into ANDI files and imported into Chrome Perfect software. Peak integration and peak-specific resolution was calculated. Resolution values for peak 2, 3a, 3b and 4 were input into JMP worksheet. Predictive models were generated using JMP software as described in Figure 5.

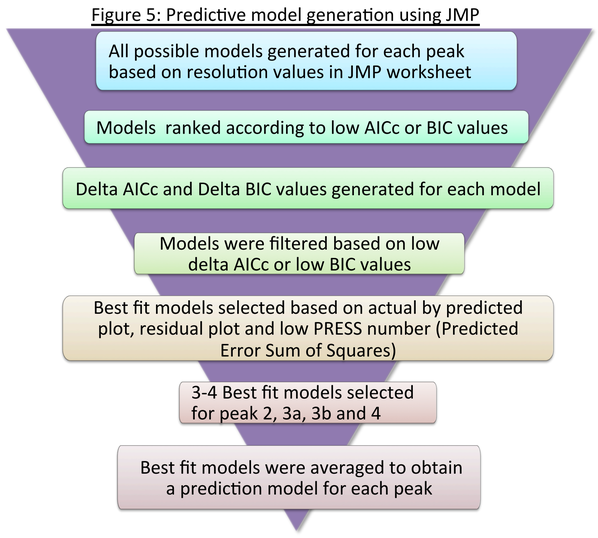
Table 3: A 5 factor DSD Design

Factors	Low (-1)	Centerpoint (0)	High (+1)
Urea concentration	4M	6M	8M
Narrow range pharmalyte (5-8)	1.5%	3%	4.5%
%Methyl cellulose concentration	0.25%	0.35%	0.45%
Protein concentration (final)	0.2 mg/mL	0.4 mg/mL	0.6 mg/mL
Focusing time	8 min	10 min	12 min

Table 4: DSD design construction using JMP software

Factors/runs	Urea	Narrow range pharmalyte	% MC	Protein concentration	Focusing time
1	1	1	-1	0	-1
2	0	0	0	0	0
3	0	-1	-1	-1	-1
4	-1	-1	1	0	1
5	1	0	-1	1	1
6	1	-1	1	1	-1
7	-1	1	0	1	-1
8	1	-1	0	-1	1
9	-1	0	1	-1	-1
10	-1	1	-1	-1	1
11	0	1	1	1	1
12	1	1	1	-1	0
13	-1	-1	-1	1	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0

Figure 5: Predictive model generation using JMP



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5.http://www.slideshare.net/jmpsoftware/jmp-definitive-screeningdesigns

Step 3 – Optimize: Two factor interaction for each response was predicted using the *Prediction profiler* in *JMP* software. For peak 3a (Figure 5a), several two-factor interactions were identified. % narrow range pharmalyte was predicted to interact with urea concentration, methyl cellulose concentration and protein concentration. Similarly, for peak 3b, peak 2 and peak 4, two-factor interactions were identified (Figure 5b, 5c and 5d). A combination of individual responses (Figure 6) demonstrate that there are quadratic effects to peak 3a and peak 4 resolution in response to changes in several factors.

Figure 5 – Predicted two factor interactions for (a) peak 3a (b) peak 3b (c) peak 2 (d) peak 4

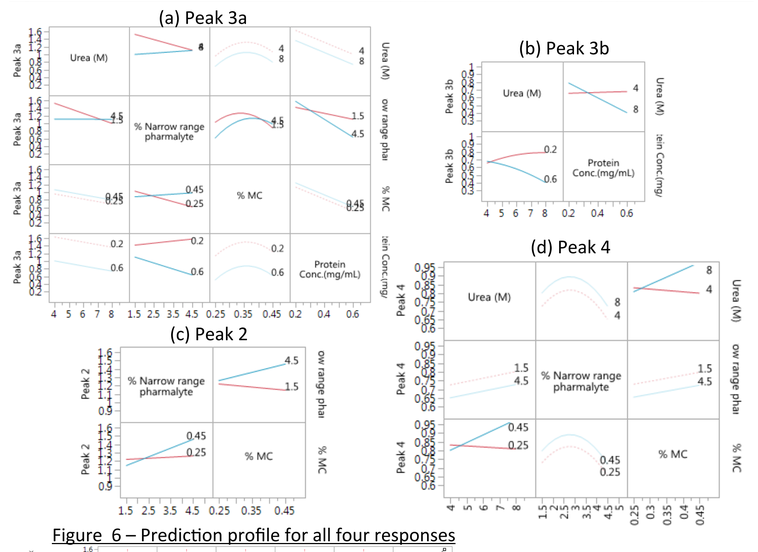

Figure 6 – Prediction profile for all four responses

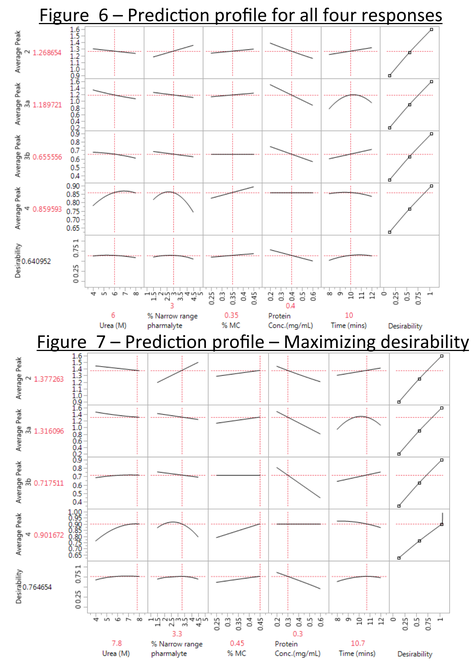

Figure 7 – Prediction profile – Maximizing desirability

Since separation of peak 3a and 3b is critical, the goal was to obtain a prediction profile that would maximize the resolution of these peaks. By maximizing desirability, the profile obtained is shown in Figure 7.
Based on prediction profiler, the conditions for the best setting for resolution of peak 3a and peak 3b were obtained (Table 5a and b).

Table 5 – (a) Optimized settings for verification (b) Predicted resolution values for all peaks

Factors	Conditions
Urea	7.8 M
% Narrow range Pharmalyte	3.3 %
% methyl cellulose	0.45 %
Protein Concentration	0.3 mg/mL
Focusing time	10.7 min

Peak #	Predicted Resolution
Peak 2	1.38
Peak 3a	1.32
Peak 3b	0.72
Peak 4	0.90

Step 4 – Verify: Confirmation runs were performed with five preparations of the optimized conditions. Resolution values from an average of the five preparations indicate good resolution was obtained for all the peaks with the optimized conditions using the DoE approach. The actual resolution values were comparable to the predicted values (Table 6). Peaks 3a and 3b were well resolved (Figure 8).

Figure 8 – E-gram of Transferrin – Validation run

Table 6 – Actual vs Predicted values

Peak #	Predicted Resolution from DoE Approach	Average Resolution from five preparations of TF
Peak 2	1.38	1.33
Peak 3a	1.32	1.28
Peak 3b	0.72	0.81
Peak 4	0.90	0.76

CONCLUSION

icIEF method development using a DoE approach