Computer-aided Assay Development for Charge Heterogeneity Analysis by iCE

Scott Mack, Susan Darling, Bob Gavin, and Annegret Boge,
ProteinSimple, Santa Clara, CA

Introduction

Analytical techniques like isoelectric focusing have become indispensable tools in evaluating biologic preparations. The resulting surge in charge isoform analysis has led to major advances in instrumentation, such as Imaged Capillary Electrophoresis iCE™. To maximize the benefits from improved instrumentation requires development of robust assays. Initially implemented in biopharmaceutical manufacturing, Quality by Design (QbD) has the potential to transform assay development. Key to QbD is comprehensively gauging the effects of process inputs on critical to quality (CTQ) attributes of the output. To this end, the Design of Experiments (DOE) methodology has proven itself to be a highly efficient tool in modeling the relationship between input and output.

The contents of this poster demonstrate the successful implementation of DOE tools in the iCE3 assay development process. Utilizing a Central Composite Design (CCD) strategy, a response surface of the relationship between ampholyte composition and focusing time on peak resolution was generated. Experimental validation of this model at optimal operational settings indicated a high level of accuracy with error between the predicted and experimentally derived values ranging between -0.68 and 8.06 percent.

iCE3 Separations

Separations were performed on an iCE3 system equipped with either a PrinCE Next MicroInjector or an Alcott 720 NV Autosampler. Samples were injected into the HT cIEF cartridge for a duration determined by the transfer time measurement step performed during cartridge installation. After injection the sample was pre-focused for 1 minute at 1500 V followed by a focusing period of y minutes at 3000 V. The focusing time (y) ranged between 9 and 11 minutes.

Results and Discussion

Human IgG1 Kappa Charge Profile

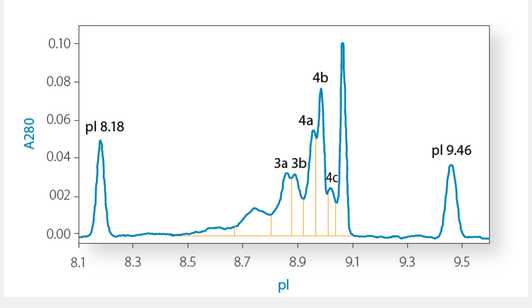

Figure 1 A human IgG1 Kappa screening run trace generated using 2% Pharmalyte 3 to 10, 2% Pharmalyte 8 to 10.5 gradient with 4M urea, focused for 1 min at 1500 V and 10 min at 3000V. Peaks 3a through 4c were identified as resolution indicating and assigned as critical to quality attributes.

Attribute Screening

The high resolution charge profile in Figure 1 shows the presence of five separate peaks clusters with maximums spaced by 0.03 to 0.05 pH units apart in isoelectric point (pI). Consistent resolution of these peaks will have a great effect on downstream peak integration and quantitation of charge profile's Peak Cluster Percent Composition (PCPC), so their specific resolution was selected as the CTQ's to evaluate assay performance.

Central Composite Experimental Design

In DOE, combinations of mulitple factor settings form a design space. Comprised of a balanced distribution of experimental points surrounding a center point, regions of the design space are defined by discrete values called levels. In factorial designs these outer points, called cubepoints, test conditions above (High Level: 1) and below (Low Level: -1) the center point (0). The three levels in this design space are sufficent to model a factor's first order (Linear) effects, combined effects with other factors (Interactions), and test for the presence of second order (Curved) effects on output. Central Composite Designs (CCD), through the utilization of a second set of axial (α, -α) experimental levels, is capable of modeling first order, second order (Quadratic), and combined effects.

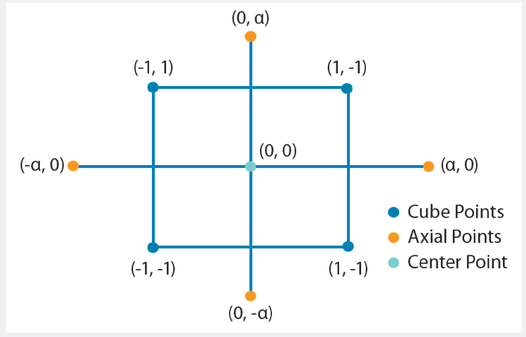

Figure 2: A plot of a two factor Circumscribed Central Composite design.

Response Surface Analysis

The various points of the CCD were run on the iCE3 system in a random order determined by Minitab 16 during construction. These runs were calibrated, converted and imported into ChromPerfect to be integrated. Specific peak resolutions were calculated for peaks 3b, 4b, and 4c. Once populated with the specific resolution values, the CCD worksheet was analyzed by Minitab 16 and response surface models built.

Contour Overlay Plot

A contour plot combines individual response surfaces into a single graphical representation in order to investigate overlapping conditions that allow the assay to achieve a desired peak resolution performance. In Minitab 16 the contour plot is an active graphic allowing for predicted assay performance to be calculated at any point in the design space.

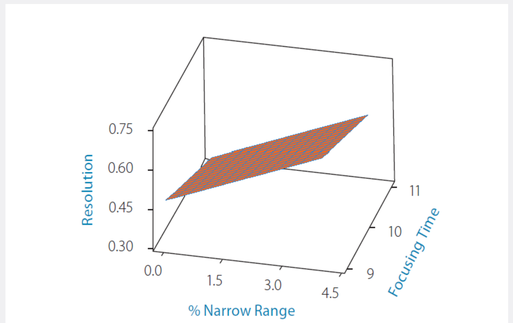

Figure 3. Surface plot for the regression model of peak 3b specific resolution. The plot shows a linear improvement in peak 3b resolution as % Narrow Range is increased and a linear decline in resolution as focusing time is increased.

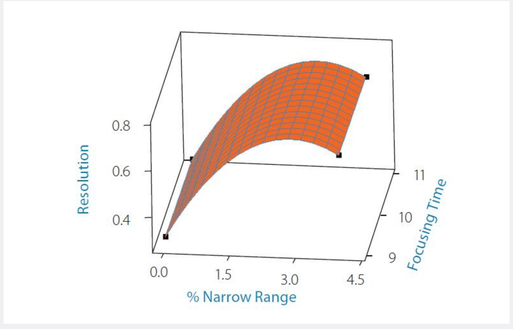

Figure 4. Surface plot for the regression model of peak 4b specific resolution. The plot shows quadratic effects in peak 4b resolution in response to changes in % Narrow Range. There appears to be a maximum setting for resolution near 3.0% Narrow Range.

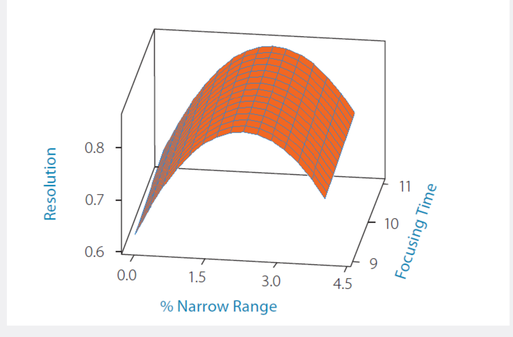

Figure 5. Surface plot for the regression model of peak 4c specific resolution. The plot shows quadratic effects in peak 4c resolution in response to changes in % Narrow Range. There appears to be a maximum setting for resolution near 2.5 % Narrow Range.

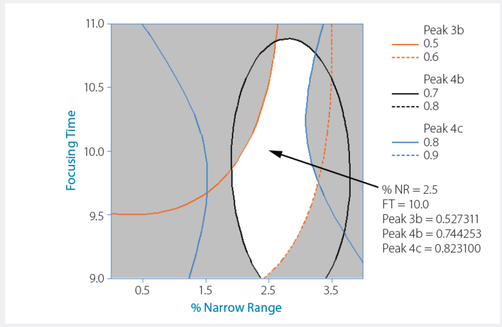

Figure 6. An overlay contour plots for peaks 3b, 4b, and 4c was generated. Operational parameters for the assay were set far away from the failure boundaries at factor values at 2.5 % Narrow Range and 10 minutes of focusing. The contour model predicted the resolution performance for peak 3b to be 0.527, peak 4b at 0.744 and peak 4c at 0.823.

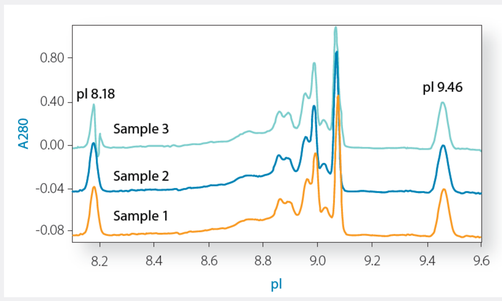

Figure 7. Three iCE 3 Human IgG1 Kappa validation runs. Samples were individually prepared and run using the Narrow Range concentration and focusing time duration identified in Figure 6

	Peak 3b	Δ	% Dev.	Peak 4b	Δ	% Dev.	Peak 4c	Δ	% Dev.
Predicted	0.527			0.744			0.823		
Exp. Run 1	0.561	0.034	6.06%	0.805	0.061	7.58%	0.833	0.01	1.20%
Exp. Run 2	0.597	0.07	11.73%	0.724	-0.02	-2.76%	0.822	-0.001	-0.12%
Exp. Run 3	0.563	0.036	6.39%	0.774	0.03	3.88%	0.798	-0.025	-3.13%
Exp. Average	0.574	0.047	8.06%	0.768	0.024	2.90%	0.818	-0.005	-0.68%

Table 1. Contains both predicted (contour overlay) and experimentally obtained (validation runs) resolution values for each of the three peaks used optimize assay performance.

Conclusion

Computer-aided method development techniques like DOE clearly provide for simultaneous multidimensional characterization of assay performance. The results obtained demonstrate that a properly executed central composite experimental design can provide accurate modeling of both linear and nonlinear effects on peak resolution. Using these experimentally validated models, robust conditions were identified for the Human IgG1 Kappa iCE assay.