Design of Experiments Catalyzes Optimization of Monoclonal Antibody Purification

Ever-increasing demand for monoclonal antibodies (mAbs) makes it imperative that their production be continually improved for cost, quality and yield. Design of experiments (DOE), by its multifactor testing methodology and statistical rigor, provides a sure path to mAb process optimization. This was demonstrated recently in a series of tests at a biotechnology company. By using the tools of DOE versus the traditional scientific method of one-factor-at-a-time (OFAT) experimentation, its mission was achieved in a matter of weeks rather than months with a far more comprehensive mapping of process conditions.

Purification processes for mAbs generally involve chromatography. The challenge of these experiments was to assess a new chromatographic resin (NCR) that removes process- and product-related contaminants (such as host cell proteins). Within only a matter of several months, the need was to establish with a high degree of confidence if this new single-use, disposable resin could deliver on its promise of streamlining the purification process while maintaining high selectivity.

Process Engineers at the corporation estimated that it would take in excess of 6 months to quantify the effects of four factors across two molecules if the OFAT method was employed. With the methods of DOE, the job was finished in a fraction of that time and with statistically valid results.

Fortuitously, just a few months before the NCR review project, the biotechnology company hosted a three-day *Modern DOE for Process Optimization* workshop taught by an expert from Stat-Ease, Inc., Minneapolis. Equipped with this statistical knowhow, and Stat-Ease's DOE-dedicated Design-Expert® software (DX), the researcher laid out a multifactor test plan to accomplish his mission. His 27-run experiment design, optimally customized by DX to detect main effects and two-factor interactions, explored four mAB-purification factors at 2 to 3 levels each:

A. Process Step: Pre vs Post Protein A (the binder for mAB)
   [Determine if the sample should be run through the NCR column before or after the Protein A column.]
B. Residence Time: 3 levels
   [Find the optimal time that the sample should be exposed to the NCR.]
C. Protein Loading (normalized): 3 levels
   [What is the best ratio of protein to column material.]
D. pH: 3 levels
   [Determine the optimal pH to run the column.]

A full factorial would have required 54 combinations (2x3x3x3), thus the custom design saved half the number (27) of costly runs. To broaden the applicability of the findings, DX was directed to optimally divide this experiment into two blocks by molecule.

To assess the purification-process performance, the following four responses were measured [goals shown in square brackets]:

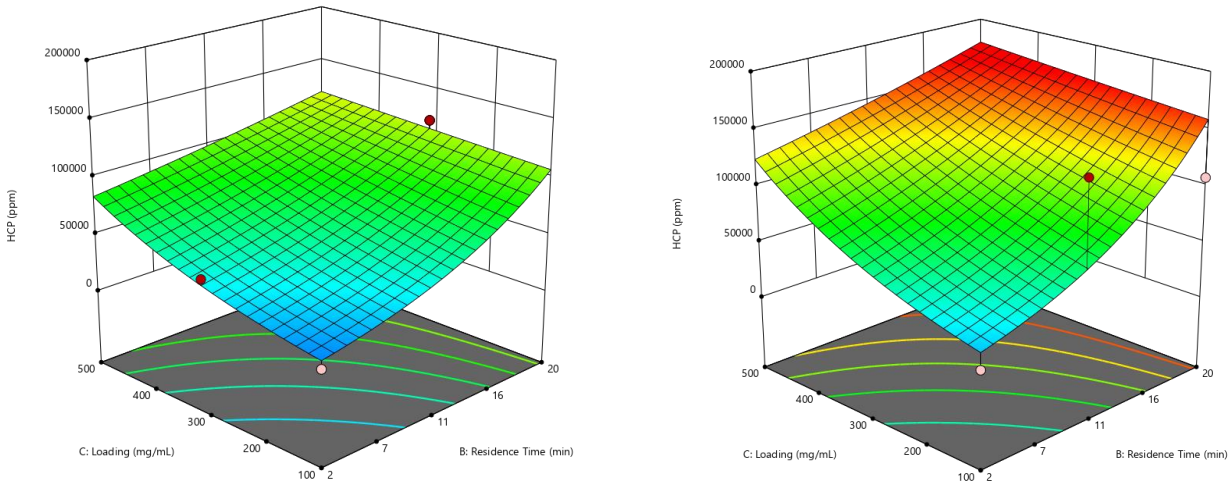
1. Size exclusion chromatography (SEC) aggregates, percent (%Agg)[<3]
2. Host cell proteins (HCP), parts per million (ppm)[< 100]
3. DNA, parts per billion (ppb)[<25]
4. Yield, percent (%)[>85]

The results of the experiment were all highly significant ($p < 0.001$). Here are details on the modeling:

- To facilitate comparison of terms on a relative basis, these predictive models operate on the factors coded to -1 at the lowest level of the numeric factors and +1 at the highest level (the categoric levels for factor A—the Process Step—are set to -1 for Pre and +1 for Post Protein A).
- Factors B and C, having three levels, provided enough data to fit squared terms, e.g., B^2 for predicting SEC aggregates. However, factor A was tested only at two levels, thus it only appears as a main effect or part of an interaction, e.g., AD in the model for SEC aggregates.
- Some responses, e.g., SEC aggregates, modeled significantly better when transformed to the Log scale.
- All models block out the variable of molecules, i.e., they work for either one, albeit with a shift up or down that turned out to be immaterial.

The software provided statistical guidance and explanatory notes for all these matters.

Before applying these models to a numerical solution for the most desirable combination of factors, various views of the response surfaces were looked at, such as those depicted in Figures 1a and 1b for HCP.



Figures 1a,b: HCPs at low and high pH, left to right

These data visualizations set the stage for the final optimization per the goals listed above in the square brackets for each of the four responses. The program recommended an optimal setup for the mAb purification process. At these optimal conditions, the fitted models predicted very compelling results, all of which exceeded the goals.

By putting the tools of DOE to good use, it was demonstrated with a high degree of confidence that NCR could, indeed, streamline the purification process while maintaining high selectivity. This statistically sound, multifactor testing method greatly accelerated the project versus traditional OFAT experimentation.