Model-based Assessment of Process Sensitivity and Robustness in Biopharmaceutical Manufacturing

Karin Westerberga, Ernst Broberg Hansenb, Thomas Budde Hansenb, Niklas Borga, Bernt Nilssonb

aDepartment of Chemical Engineering, Lund University, 221 00 Lund, Sweden
bNovo Nordisk A/S, Hagedornsvej 1, 2820 Gentofte, Denmark

Abstract
A methodology for model-based assessment of both the sensitivity of a process to variations in the operating conditions and of the resulting process robustness is described. It is exemplified with a case study of preparative anion chromatography where eight process parameters are studied and ranked according to their effect on the product quality. Interactions between parameters are also studied, and a quantitative estimate of the probability of a failed batch is given. The analysis can also be used to find a control strategy for the process. The proposed approach is different from the current process development paradigm which relies on experiments and statistical analysis only.

Keywords: Preparative chromatography, process modeling, process verification, robustness

1. Introduction
In the development of a manufacturing process, it is necessary to verify the process’ capability to deliver a consistent high quality product. In the biopharmaceutical sector regulatory guidelines now stress the importance of adapting a risk-based approach where a scientific assessment of risks and their consequences, as well as a strategy for mitigating these risks, are required [1, 2]. A common approach is to perform a risk analysis such as FMEA followed by a Design of Experiments study of the selected parameters. The number of experiments that can be performed is however limited and the study is often restricted to linear effects and a selection of parameters has to be made based on prior knowledge or assumptions about the process.

Mechanistic modeling has become an interesting complement to the purely experimental studies. These models are based on a theoretical understanding of the physical and chemical mechanisms involved in the process step; some model parameters can be determined from correlations whereas others are fitted to calibration experiments in order to make the model predictions fit the experimental data. The calibrated model can then be used for analysis, optimization and design of the modeled process.

A model-based methodology is shown here for analysis of the sensitivity and robustness of biopharmaceutical manufacturing processes. The purpose of the study is to verify the process design by demonstrating process robustness and identifying the critical process parameters and their acceptable ranges. An example is given for a preparative anion chromatography step. The study starts with model development, followed by risk analysis identifying the process parameters which need to be included in the study. The model-based analysis then consists of a screening simulation of single parameter variations followed by full-factor simulation of significant process parameters. This will
identify the parameters which affect the process output the most, and also which parameters interact to give a strong combined effect. Finally, a Monte Carlo simulation sampling variations in all process parameters gives quantitative information on the overall process robustness.

2. Case Study
An anion exchange chromatography step was chosen as an example process for the model-based approach. In the step a target therapeutic protein is separated from two product-related impurities. One weakly adsorbing impurity elutes before and under the main peak and one strongly adsorbing impurity elutes after the main peak. The objective of the step is to meet two critical quality attributes (CQA:s): the target product purity should be above 95% and the amount of weakly adsorbing impurity below 3%. The separation is analyzed with respect to both of these CQA:s.

3. Model
3.1. Column model
The column was modeled with the kinetic-dispersive model which includes axial dispersion and adsorption kinetics. A set of differential equations describe the transient concentration profile in the column while concentration gradients in the radial direction as well as inside the stationary phase particles are neglected. The adsorption of proteins to the stationary phase was described by the self-association isotherm [3]. As in the steric mass action model, adsorption of a protein to the surface is balanced by displacement of counter-ions [4] but proteins can also adsorb by dimerization to other proteins of the same species already adsorbed on the surface. The number of displaced counter-ions depends on the protein charge which is pH dependent. The model calibration and final parameter set is described in detail in [5]. Figure 1 shows the model fit to experimental data at the studied operating point. Although the asymmetry of the main peak was not captured completely, the model fit was considered good enough for the purpose of this study.

Figure 1. The model fit at the operating point. The weak impurity co-elutes with the product and the strong impurity elutes after the product peak. Lines with dots show the experimental data.
3.2. Simulations

The model was implemented in a chromatography simulation platform developed at Lund University [6]. The column was discretized with 200 gridpoints using finite differences, and solved with the *ode15s* solver in MATLAB. Four components were modeled of which three adsorbed, giving seven states to be calculated at each gridpoint. Simulation times were brought down to approximately 5 seconds by extensive use of sparse matrix operations. A small computer cluster of 40 CPUs was used to perform the large number of simulations required for the robustness analysis.

4. Results

4.1. Sensitivity analysis

Eight process parameters were identified as potentially critical process parameters in the risk analysis: the amount of buffer, acid and salt in the elution buffer, the protein concentration and the salt concentration in the feed, the relative amounts of the weak and strong impurities in the feed and the measurement of the feed protein concentration. The load volume and the pooling of the product were both dependent on the measured protein concentration, which justified its inclusion in the study. The parameters with relative standard deviations are listed in table 1 below. The process model was used to simulate variations in the eight parameters plus/minus one standard deviation and the resulting purities were used to calculate the sensitivity as the change in each CQA relative to the difference between the CQA requirement and the CQA obtained at the process setpoint:

\[
\text{Sensitivity} = \frac{CQA_{\text{setpoint}} - CQA_{\text{set}}} {CQA_{\text{setpoint}} - CQA_{\text{requirement}}} \cdot 100
\]

*\(CQA_{\text{set}}\) denotes the value of the critical quality attribute which was obtained after varying the process parameter one standard deviation in the direction which gave a worse value of the CQA (i.e. lower purity or higher amount of weak impurity). The sensitivities are included in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setpoint</th>
<th>rsd (%)</th>
<th>Sensitivity weak impurity</th>
<th>Sensitivity product purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (mol/L)</td>
<td>0.020</td>
<td>5</td>
<td>0.1</td>
<td>34</td>
</tr>
<tr>
<td>Acid (mol/L)</td>
<td>0.011</td>
<td>5</td>
<td>0.4</td>
<td>33</td>
</tr>
<tr>
<td>Salt (mol/L)</td>
<td>0.25</td>
<td>5</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Salt feed (mol/L)</td>
<td>0.010</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Protein feed (g/L)</td>
<td>3.5</td>
<td>15</td>
<td>0.3</td>
<td>25</td>
</tr>
<tr>
<td>Weak impurity (g/g)</td>
<td>0.027</td>
<td>25</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>Strong impurity (g/g)</td>
<td>0.079</td>
<td>25</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>Protein analysis (g/g)</td>
<td>1.0</td>
<td>10</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1. The potential critical process parameters included in the study and the sensitivities calculated in the nominal range analysis.
Parameters with a sensitivity score above 10 were considered critical and analyzed for interaction effects in a full-factor design of three levels of the five critical process parameters: buffer, acid, protein feed, weak impurity, and strong impurity. The full-factor simulations showed interaction effects between the amount of buffer, acid, the amount of protein, and the amount of strong impurity in the feed. Variations in more than one of these parameters led to a lower purity than a purely additive effect would give. The underlying effect was that the separation of the strong impurity was sensitive to the pH.

4.2. Uncertainty analysis
After identifying the critical process parameters and the significant interaction effects, the question still to be answered was that of the probability of a failed batch, i.e., a batch where the specifications on either the product purity or the amount of weak impurity in the product were not met. A Monte Carlo simulation was made using Latin Hypercube sampling [7] on the process parameters within the distributions specified in Table 1. 800 samples were simulated giving a representative probability distribution of the process output represented by the CQA:s, Figure 2.

The requirement on the amount of weak impurity was fulfilled in all samples while the product purity was too low in 12 of the 800 samples. This meant that the process was not robust enough.

![Figure 2](image-url)

Figure 2. The result from the Monte Carlo simulation. The amount of weak impurity in the product never exceeds the limit which is represented by the dashed line in the histogram, whereas the product purity was below the requirement of 95% in some simulations.

4.3. Control strategies
The number of failed batches can be reduced in two ways: either the variations are minimized, so that the product purity does not vary outside the specification or else the operation at the setpoint is changed so that the purity of all batches is increased, moving the low-purity batches inside the specification. The second option will normally lead to a reduced yield as a tradeoff, it is therefore of interest to reduce the variability.

From the sensitivity analysis, study of parameter interactions and the Latin Hypercube simulations it was decided that the most important parameter to control in order to reduce the purity variations was pH. pH was strongly correlated to the product purity and depended on the amounts of buffer and acid in the elution buffer. The purity was
also strongly correlated to the amount of impurities in the feed. However, from the perspective of the productivity of the entire downstream process, it is better to design a chromatography step which is able to handle larger amounts of impurities than to put up stricter requirements for the steps upstream as this might increase the number of rejected batches.

5. Conclusions

The model-assisted approach could be used successfully to screen the process parameters thought to have an effect on the purity obtained in the chromatography step. Out of the eight original parameters five were classified as critical and interaction effects were demonstrated between four of these. In addition to ranking the process parameters based on their effect on the process, the uncertainty of the process output could be assessed through Monte Carlo simulations.

The model-assisted approach suggested here can be compared to the traditional experimental approach, where Design of Experiments is commonly used to select a number of experiments on a reduced parameter set selected by risk analysis and experience. In the model-assisted approach parameters need not be excluded a-priori but can be analyzed by simulations, and the relevant experiments can be selected based on the output. Parameter variations which are difficult to control in experiments, such as the amount of impurities in the presented example, can be studied easily with the model.

Naturally, the model cannot predict anything which is not already known and included in the model equations. But by allowing parameters to be tested without restriction and quick analysis of the results it is a valuable tool and contributes to new understanding of the overall process. It also has the potential to continue being useful for documenting process knowledge and making continuous improvements as it can be updated and made more precise as process data becomes available. Modeling and model-based analysis can therefore be made to fit well into the regulatory framework for biopharmaceuticals.

References

5. K. Westerberg et al. 2011, Model-Based Process Challenge of an Industrial Ion-Exchange Chromatography Step, Chemical Engineering and Technology, accepted for publication