

Applied Advanced Process Analytics in Biopharmaceutical Manufacturing: Challenges and Prospects in Real-time Monitoring and Control

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Abstract: Biopharmaceutical manufacturing processes are inherently complex due to their nonlinear bioprocess dynamics, variability in batch operations and manufacturing schedule, raw materials involved, and automatic process control. A typical processed lot generates large amounts of data that need to be analyzed and interpreted for process troubleshooting and continuous improvement purposes in addition to product release. Multivariate Batch Process Modeling, Monitoring and Control approaches in real-time are elaborated by providing industrial examples from the commercial manufacturing processes. Examples and opportunities in cell culture (e.g., bioreactor applications) and purification (e.g., large-scale chromatography) operations are summarized. Impact of Process Analytical Technologies (PAT), soft-sensor development, first principles modeling applications and commercial-scale examples are presented. Copyright © IFAC 2009.

Keywords: Process Analytical Technologies, Quality-by-Design, Biopharmaceutical Manufacturing, Real-Time Multivariate Process Monitoring, Soft-sensors.

1. INTRODUCTION

Biotechnology-based products have become increasingly important in recent years in treating chronic diseases such as cancer and arthritis. Although biopharmaceutical drugs constitute a small portion of (about 8% in 2004) of the pharmaceutical market, approximately 27% of new medicines in active development are now biotech products (Business Insights, 2005). Process development and commercial manufacturing of these products, such as therapeutic proteins; require a good understanding of chemistry, manufacturing and controls. Advanced process analytical technologies can be incorporated in process development as well as into commercial-scale manufacturing for advanced monitoring, control, continual process improvement, cost reduction and risk management.

Biopharmaceutical processes are typically comprised of a series of unit procedures operated in batch mode to produce therapeutic proteins, and have complex biological mechanisms that result in non-linear and time-variant process dynamics. This makes their modeling, monitoring and control challenging. In a typical commercial-scale biopharmaceutical manufacturing process there are multiple batch processing unit operations where off-line samples are taken to ensure in-process control and quality objectives are met and real-time measurements are made for open and closed-loop control and monitoring. A number of measurements are also made for

raw material release testing towards use in manufacturing. When this is looked at from a holistic perspective; there are many batches, variables and operational characteristics to analyze in a meaningful and proactive manner. While this goal can be achieved via post-mortem analysis, it is more desirable to monitor and control these multivariable multi-stage biopharmaceutical processes in real-time. Advanced process analytics in the form of real-time multivariate process monitoring and control provides an efficient means of identifying/reducing variation, managing process risks, relating process information to critical quality attributes (CQAs) and determining process improvement opportunities such as increasing yields and decreasing impurities. Due to long lead times and inflexibility in the regulatory approval process, applying process changes for improvement has not been very straightforward in the conventional regulatory paradigm. The United States Food and Drug Administration has published a series of new guidelines to address this issue and provide more flexibility for innovation while appropriately managing the risk around proposed process changes for continual improvement via process analytics and quality-by-design (QbD) principles (US FDA, 2002, 2004, 2006, Chirino and Mire-Slius, 2004). *Process Analytical Technology* (PAT) is defined as a system for designing, analyzing, and controlling manufacturing through real-time measurements of critical quality and performance attributes of the process as well as raw materials and other process inputs. QbD promotes improved process understanding

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during process and product development and building quality in the design instead of testing for quality. This can be achieved via correlative, causal, or mechanistic knowledge and at the highest level via first principle models (Cinar, et al., 2003, Rathore and Winkle, 2009).

Process analytical approaches including deploying multivariate techniques along with on-line/at-line/in-line sensors and analyzers and the use of chemometrics in biotechnology have received significant attention in recent years (Junker and Wang, 2006). Applications included monitoring and control of microbial fermentations (Albert and Kinley, 2001, Lopes, et al., 2004, Undey et al., 2004, Gnoth, et al., 2007), cell culture (Arnold, et al., 2003, Undey, et al, 2006) and purification processes (Larson, et al., 2003, Lee et al., 2006, Rathore, et al., 2008). Development of new measurement technologies for real-time bioprocess monitoring and control is also crucial (Becker, et al., 2007).

In this paper, a generic approach for developing further process understanding, modelling, monitoring and control is summarized. Specific applications and case studies are provided from commercial manufacturing experience in the use of process analytics. The potential of soft-sensors and first principles modeling in biopharmaceutical manufacturing is discussed with industrial examples. Use of process analytics and real-time multivariate monitoring technology in operational success is also demonstrated. Challenges and prospects of adaptive process control are discussed.

2. PROCESS UNDERSTANDING VIA MODELING AND ADVANCED MONITORING

Aforementioned QbD approach demands a high-level of process understanding for ensuring control of CQAs. Levels of process understanding and knowledge can be categorized in increasing order (lowest to highest) as descriptive, correlative, causal, mechanistic and first principles-based. While mechanistic and first principles models provide the highest level of understanding and predictability, their development and adaptation may not be very straightforward in biopharmaceutical process development and manufacturing that use design of experiments (DOE)-based approaches along with other heuristic knowledge about the process and product. During commercialization of a product an essential level of process understanding is demonstrated to ensure process consistency, product safety, efficacy and purity. However, in order to expedite time-to-market (while the drug is meeting the efficacy, purity and safety requirements) and make the product available to the patients, its manufacturing process may not be sufficiently optimized early in the product lifecycle. Additionally, there may be scale-up effects, raw material lot-to-lot variability (i.e., as an unmeasured load disturbance to the process) and other operational aspects such as maintenance schedules and human factors collectively driving the overall process variability. Real-time multivariate statistical process monitoring (RT-MSPM) provides a means to proactively monitor this overall process variability and build the necessary foundation towards predictive monitoring and multivariable control. In the generic methodology proposed

in this paper, a multivariate model for each unit operation is developed for advanced monitoring and prediction purposes where applicable. Based on the frequency of data availability, models are used in real-time and/or via post-mortem batch analytical purposes. Making the data available and establishing required databases, connections to source systems, data pre-treatment and reconciliation are practical considerations that need to be addressed in industrial setting prior to enabling RT-MSPM (Undey, 2008).

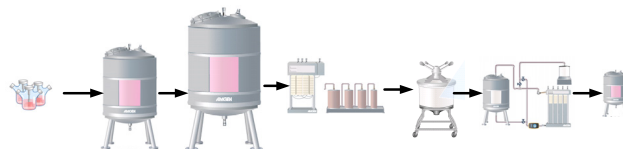


Fig. 1. Simplified process flow of a biologics manufacturing process (inoculation, scale-up bioreactors, clarification and purification train).

As shown in Fig. 1 typical biopharmaceutical manufacturing processes involve multiple unit operations including bioreactors for scale-up, cell growth/protein production, clarification ultrafiltration and chromatography columns and skids. There may be more steps based on each product's requirement and several parallel trains for plant throughput maximization.

3. MULTIVARIATE BATCH MODELING AND MONITORING

Multivariate (MV) modeling techniques such as Principal Components Analysis (PCA) and Partial Least Squares (PLS) are used to handle batch process data issues such as large number of variables, colinearity and missing data while summarizing the overall variability in the principal component and latent variable space. Historical in-control batches are used for MV model development. Control limits for MV statistics are calculated and MSPM charts are used for efficient monitoring (Nomikos and MacGregor, 1995).

In this study batch process data are analyzed and monitored in two hierarchical levels. The first level is called the observation level and used to monitor the batch evolution with respect to a maturity variable in real-time. The second level is the batch level which is used for monitoring the batch fingerprint and can be used in predicting a final performance variable (Wold et al., 1998). Since early in the progress of a batch the confidence in the prediction is typically low, care needs to be taken when to start using batch level MV charts for real-time monitoring after a certain amount of data is available. All the real-time batch level cases presented in this paper starts computing the batch level MV statistics when 50% of the batch is completed and it has provided a good predictive performance.

Umetrics' Simca-P+ and SBOL (Simca-Batch On-Line) modules are used for the MSPM cases presented in this paper. Simca-P+ is the tool used for offline MV model development. SBOL is the tool used for real-time MSPM and uses the MV models developed by Simca-P+.

4. DESIGN SPACE MONITORING AND POSTMORTEM ANALYSES OF BATCH PERFORMANCE

During initial bioprocess design and characterization the full variability due to raw material lots, scale-up parameterization and large-scale operations cannot be estimated and therefore the process is monitored to ensure consistency. Process design space is constructed to understand the ranges on key and critical operating variables (process inputs) and their impact on the key/critical performance variables (process outputs) at bench and pilot-scale manufacturing and later scaled-up to commercial operation. Multivariate models can be helpful in also comparing the bench and/or pilot-scale experience against large-scale manufacturing to study similarities and identify any differences in performance. It can also be used to improve bench/pilot-scale model representation against large-scale so that process development can improve on the scale up parameterization. Representative scaled-down process models (i.e., actual scaled-down equipment) are crucial for troubleshooting and process improvement experimentation. In the following example (Fig. 2), PCA-based multivariate models were applied to process performance variables from batches performed at both bench and large-scale manufacturing.

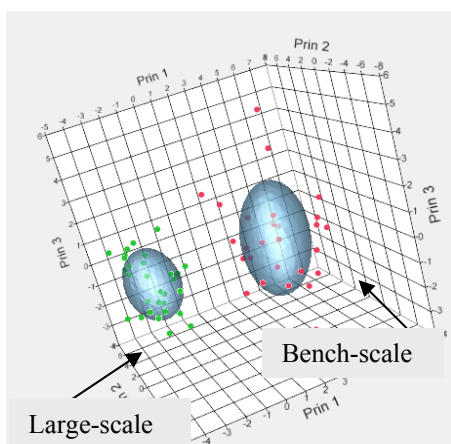


Fig. 2. Multivariate comparison and monitoring of different manufacturing scales against design space

With this approach, variability in process data from more than twenty performance variables is summarized with only three principal components. Ellipsoids represent 95% confidence volume. In this case, large-scale batches seem to be more tightly controlled compared with bench-scale batches (partly due to a wider range of input space explored in bench-scale and scale-up differences). Multivariate monitoring of both scales provide a means of comparison as well as identifying improvement areas where changes can be made to move the two spaces closer to each other in terms of expected correlations, variability and means.

As a postmortem case study, multivariate modeling is performed retrospectively on commercial manufacturing batches to support technical investigations, identify process and/or operational improvement opportunities, the sources of process variation to increase process understanding. Data

from historical batches is used to develop PLS-based models for key process performance variables. Information from process characterization studies can also be leveraged to further refine the PLS models. As an example, a low product titer trend was observed in manufacturing of a commercial biologic. A PLS-model was developed for product titer using multiple process inputs from 36 historical batches. Based on a review of exceptional batches in the MV charts (fingerprint in Fig. 3 and variable contribution chart in Fig. 4), it was determined that the cell specific productivity was significantly lower in the decreased product titer batches. This information guided process analysts to focus on operational parameters likely to adversely impact cell specific productivity. This analysis revealed that a shift in induction timing potentially contributed to the low product titer trend. After the induction timing was adjusted back to target, higher product titers were obtained.

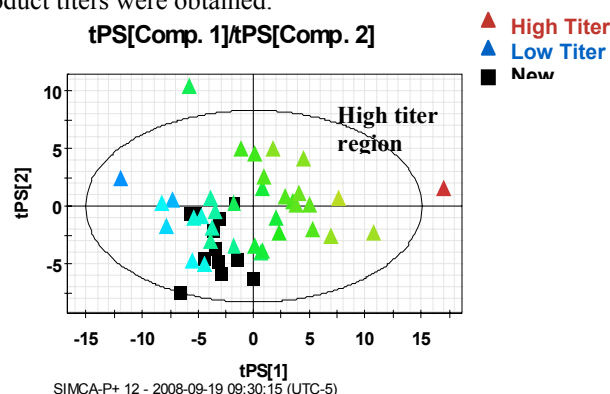


Fig. 3. A score scatter plot showing new manufacturing batches relative to historical ones.

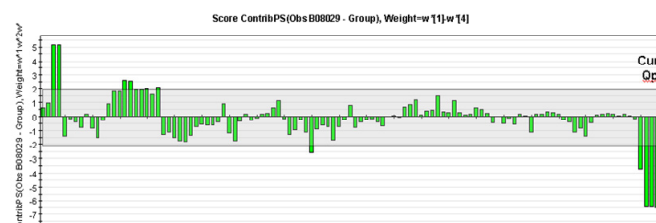


Fig. 4. Variable contribution plot to Hotelling's T^2 showing low cell specific productivity as a significant contributor to low titer (horizontal band indicates +/- 2 standard deviation about the mean batch).

With introduction of new on-line technologies such as cell density probes, pCO_2 probes and Glucose/Lactate sensors comes the possibility of applying multivariate process modeling to predict and control biologic manufacturing processes towards achieving target productivity and quality end points. Several opportunities exist to improve the process models using new and available characterization data and increase model sensitivity and predictability. While it is very informative to use MSPM for postmortem exploratory analysis of process upsets, it is more desirable to monitor the process in real-time to detect and diagnose those upsets and take preventive actions where possible. In addition to monitoring, prediction of key end points while the process is in progress is also possible (a.k.a. soft-sensors) and provides

various opportunities towards more efficient process operations and advanced process control and optimization.

5. REAL-TIME MULTIVARIATE BIOPROCESS MONITORING CASE STUDIES

5.1. CASE STUDY-1: REAL-TIME LARGE-SCALE BIOREACTOR CELL CULTURE MONITORING

The first example is from a bioreactor monitoring and shows how real-time monitoring technology is used towards operational excellence, hence identifying equipment and mechanical related issues as well. A transient decline (~3%) is observed in Final Viabilities (measured offline) in a Perfusion Bioreactor across batches. Deviations in real-time MV charts are detected for those low viability batches (*Hotelling's T²* chart for one of the low viability batches is shown in upper Fig. 5). Variable contribution plots identified that the low Final Viability batches that are run on particular bioreactor and its skid had higher Perfusion Feed and Retentate Temperature compared to the historical batches. Further investigation revealed that the wrong size gaskets were installed in the pump seal flush line for these batches. This made the control of temperature and pressure on the seal flush line very difficult. The correct size gaskets were replaced prior to the next batch and the temperature profile and final viability were within their normal ranges.

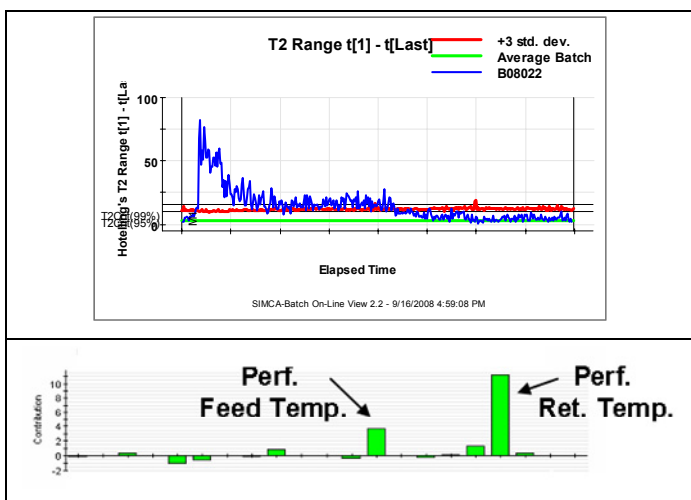


Fig. 5. Hotelling's T^2 chart (top) for one of the low final viability batches and contributions to the deviation (bottom)

5.2. CASE STUDY-2: REAL-TIME MONITORING OF LARGE-SCALE CHROMATOGRAPHY PROCESS

PCA models were developed for each phase of the Protein A affinity chromatography unit operation (e.g., pre-equilibration, elution) from historical process data. In general, only two or three principal components were needed for a given phase to summarize the batch process. Future batches could then be projected onto the model to allow rapid detection of deviations from the normal operating space and corrective actions would be taken where possible. Model has detected failure of a pre-column pH probe during purification

of a batch through Protein A chromatography. The online pH probes are commonly used to verify proper equilibration of a column or end-point of a titration for a viral inactivation step prior to taking a confirmatory offline sample. In this example, the *Hotelling's T²* plot identifies the out of trend batch (on the left at Fig. 6) in real-time and the variable contribution plot (on the right hand side) identifies the pH probe having a significant effect. This information was used to replace the probe prior to further processing and avoided compromising any offline verification samples.

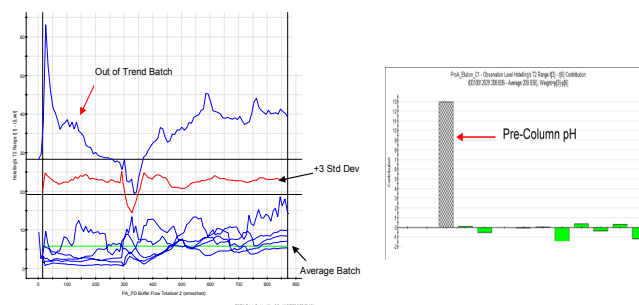


Fig. 6. Hotelling's T^2 chart (on the left) indicating an out-of-control batch and the contribution plot diagnosing the faulty pH probe (on the right)

6. REAL-TIME SOFT (VIRTUAL)-SENSOR APPLICATIONS IN BIOPHARMACEUTICAL MANUFACTURING

There are many on-line/at-line/in-line probes and analyzers available for measuring bioprocess variables and quality/performance indicators. In a typical setting, for instance a bioreactor has temperature, level, pH, pressure, agitator speed, aeration rate, and dissolved oxygen measurement systems and data acquisition. Additional measurement systems such as cell density, dissolved carbon dioxide, mass spectroscopy in the off-gas, on-line HPLC, and fluorometric sensors are among the available and desirable technology. Research has shown that there are mathematical means via empirical and first principles modeling to generate predictions on the performance end-points in real-time (Cheruy, 1997, Undey, et al., 2006). A generic framework is depicted in Fig. 7, where more frequently measured and readily available process variables are used for developing a soft-sensor to generate an on-line quality estimate. Industrial examples are given for a cell culture production bioreactor case where product final titer is predicted in real-time and a first-principles model for monitoring a chromatography column performance in real-time.

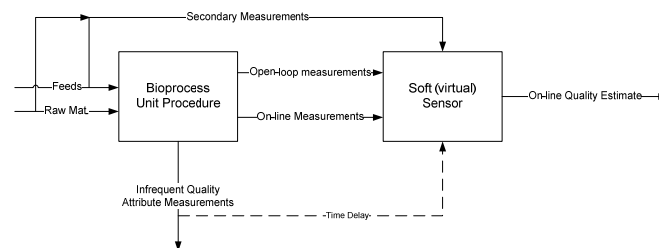


Fig. 7. Soft (virtual)-sensors for bioprocesses.

6.1. CASE STUDY-3: REAL-TIME SOFT-SENSOR FOR BIOREACTOR FINAL TITER PREDICTION

The production bioreactor is a critical step for biopharmaceutical processes since the target protein is expressed during this step. It is very important to closely monitor this unit operation in real-time. Any deviations from the normal operation during this step may lead to low productivity and out-of-spec product.

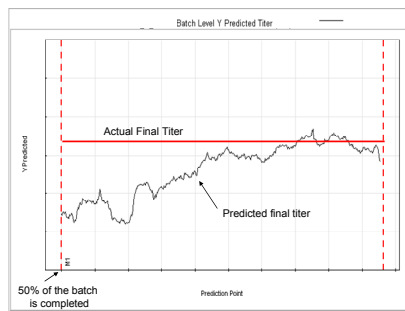


Fig. 8. Real-time Final Titer prediction starts when 50% of the batch is completed.

Final Titer is used as the performance parameter that is predicted in the batch level while monitoring the process in real-time. Accurately predicting Final Titer several days in advance of harvesting provides many PAT opportunities such as offline assay elimination (titer is typically determined via an HPLC method offline), titer optimization, schedule optimization and real-time control. Fig. 8 illustrates real-time final titer prediction for a batch by only using the continuously measured inputs and outputs such as pH, O₂ flow and bioreactor volume as predictors. Final titer is predicted within 0.5 % of the actual offline testing result.

6.2. CASE STUDY-4: REAL-TIME SOFT-SENSORS FOR CHROMATOGRAPHY OPERATION

In this case study for biologics purification has been real-time determination of unit operation step yields. These are typically calculated by measuring offline samples of the load and product pools, and results are often not available until well after batch completion. UV detection unit is typically available by the production unit, Beer's Law ($A=\epsilon BC$, i.e., absorbance is proportional to concentration of a solution, where ϵ is molar absorptivity, B, path length of the sample and C, concentration of the solution) can be used as a basis to correlate the absorbance to Protein Concentration UV in real time (Fig. 9). Estimated concentration can then be used to determine unit operation step yield.

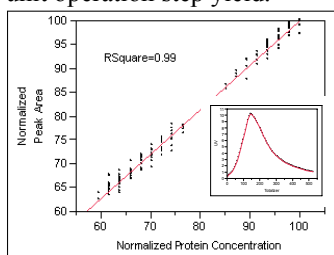


Fig. 9. Correlation of chromatography peak area (shown as inset) to protein concentration.

For process chromatography operating in bind and elute mode, the eluate peak can be integrated numerically. The same can be done for phases of the chromatography operation, such as regeneration, to complete the mass balance. Incorporating these mass balance terms into the PLS model significantly improves the predictive power.

Another area of exploration for advanced monitoring has been the use of first principles models to predict chromatography resin binding capacity. The resin capacity is typically determined during characterization studies by measuring the percentage breakthrough of protein during the loading phase. Due to the large volume requirements, this is typically not measured routinely once the process is implemented at manufacturing scale. This capacity measurement is useful to understand the changes in resin characteristics during the operational lifetime of the column and make decisions about when to change out the resin to ensure high performance.

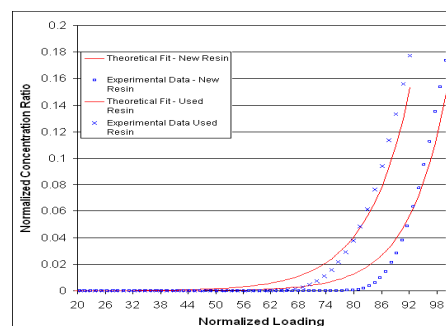


Fig. 10. Capacity changes of resin used in 90 batches of biopharmaceutical purification process.

Using the analytical solution to the differential mass-balance equation developed by Ghose et al. (2004), actual resin capacity at different points in the column lifetime was modeled. The model inputs were determined from literature and via process knowledge. This capacity information can then be compared against the expected column loading for a given batch to determine if any losses would be expected (Fig. 10). It can also be incorporated as an input variable to the soft-sensor (multivariate model in this case) to provide a more accurate prediction of step yield.

7. CONCLUSIONS

Process analytical technology (PAT) within the Quality-by-design framework provided by regulatory agencies define guidelines towards demonstrating good process understanding in product development and therefore requires improved monitoring and control for pharmaceutical manufacturing processes. Real-time multivariable monitoring framework for bioprocesses as shown in the examples not only provides proactive process supervision but also serves as an operational success management tool, hence, it has the potential to reduce production costs since it is also used to monitor and detect the equipment-related issues.

Soft (virtual)-sensors were also studied in this paper and promising examples were provided from cell culture and

purification areas to show how existing process and its data can be utilized for prediction of key performance end points. While advanced monitoring capabilities are demonstrated, it is important to understand how process control (e.g., feedback and/or feed forward) can be achieved to ensure consistent process outputs based on biological performance end points. This paper summarized the necessary preliminary steps of process understanding and advanced monitoring applied in biopharmaceutical manufacturing environment. New measurement technologies will be necessary to enable closed-loop control of key performance variables and CQAs to meet biopharmaceutical manufacturing needs. It is necessary that these on-line quality measurement systems that are available or being developed to have faster turn around times than the process time (i.e., the on-line test results can be available before the relevant biological phase is completed) so that a control action can be taken in a timely manner. Advanced multivariable monitoring with robust, accurate and improved on-line/at-line/in-line probes and analyzers with fully-automated data management and multivariable control capabilities hold great possibilities for 21st century PAT-enabled biopharmaceutical manufacturing.

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