WHITE PAPER

Making the most of your scale down model

G.E. McCreath and M.R. Shivhare
Fujifilm Diosynth Biotechnologies, Billingham, UK

Why are small scale process models required?

In 2002 the US Food and Drug Administration began its initiative to modernise the regulation of pharmaceutical manufacturing and product quality and initiated the concept of Quality by Design (QbD) for pharmaceuticals. QbD principles and advice for implementation are outlined in regulatory guidances such as ICH Q8, Q9 and Q10. One of the foundations of QbD, underpinning its operation is that products and processes are sufficiently understood so that those elements most critical for product quality can be identified, codified and controlled.

While process understanding can come from a variety of sources - including early process development activities, experience with similar products and manufacturing campaigns for clinical supply - scaled down process models of unit operations have particular utility. Advantages such as ease of set up compared with pilot or full scale activities, smaller requirement for materials and possible automation through the use of robotics allow for a depth and span of investigation that is impractical at larger scale. For example, in design space and control strategy development they allow a comprehensive understanding to be developed of the multivariate effect of operating conditions and material attributes on product quality. Once established they also allow for trouble shooting issues that can be encountered during commercial manufacturing, testing of pathogen and impurity removal and reduction strategies and can complement process performance qualification studies reducing the dependence on testing of commercial batches (1). Small scale process models (SSPM) also provide information that can be used in simulation studies to predict performance of the commercial process avoiding problems before they occur.

Considerations for SSPM development

There are a number of considerations that have to be taken into account in the development of a SSPM including:

- Is the final commercial scale process defined prior to SSPM development? SSPM's can be used prospectively as a development and investigational tool before the final commercial process has been defined. In this instance qualification, while ideally prospective, can be a retrospective activity. If data from the final scale is not yet available, the SSPM should be qualified against the largest scale for which data exists. The degree of variability in the large scale data set should be understood.
- Demonstration of equivalence across operating ranges. While most SSPM are qualified at set point operating conditions, there is value in including additional runs at off-centre conditions to test the relevance of the model under conditions that may be expected in manufacture.

- The equipment used in the SSPM should have equivalent design characteristics and process control capabilities; good engineering principles should always be applied.
- Identification of the scale independent parameters that will be used to establish the SSPM e.g. pH, dissolved oxygen, temperature, aeration, nutrient addition and the impact of scale dependant parameters e.g. working volumes.
- Limitations of the equipment utilised in the SSPM should be understood. While most unit operations can be scaled down effectively, even the best SSPM can have differences e.g. dead volumes, materials of construction, mixing patterns, process times etc.
- Identification of the key process outcomes that will be used to judge the significance of the model e.g. performance measures and product quality attributes.
- Raw materials used in the SSPM should be identical to those used at large scale. A good SSPM can be used to look for the impact of raw material variation on process performance.
- Assays utilised at both scales should be identical.

The usefulness of a SSPM is dependant on its ability to mimic and predict performance of full scale in a meaningful way and to demonstrate equivalency of key process outcomes (1). If equivalency cannot be demonstrated between scales, an understanding for the unequivalency and its impact on the relevance of the information should be obtained. Inadequate models can mislead process understanding and thus a critical step in the application of the SSPM lies in how verification and qualification of the model is undertaken.

Qualification of Small Scale Process Models

One approach to SSPM qualification, which remains the most common, is to compare the key quality and performance attributes from both the small and commercial scale operations performed at set point conditions (2). For fermentation operations, a model can be qualified both by overlaying continuous data such as the growth profiles, gas evolution rates and by comparing the data with a set acceptance criteria, typically mean $\pm$ three times sample standard deviation, which is derived using statistical analysis on historical batches. Additionally, univariate data analysis (UVDA) on discrete data e.g. the Students t-test and F-test are also applied to demonstrate equivalency by comparison of means and variance of key attributes with a certain confidence level (generally 95%). In some instances where the normal distribution of the data cannot be established, then non-parametric tests like Mann-Whitney can be used to demonstrate the equivalency between different scales (3). **Figure 1** *(over)* demonstrates the application of the classical approach using UVDA method.

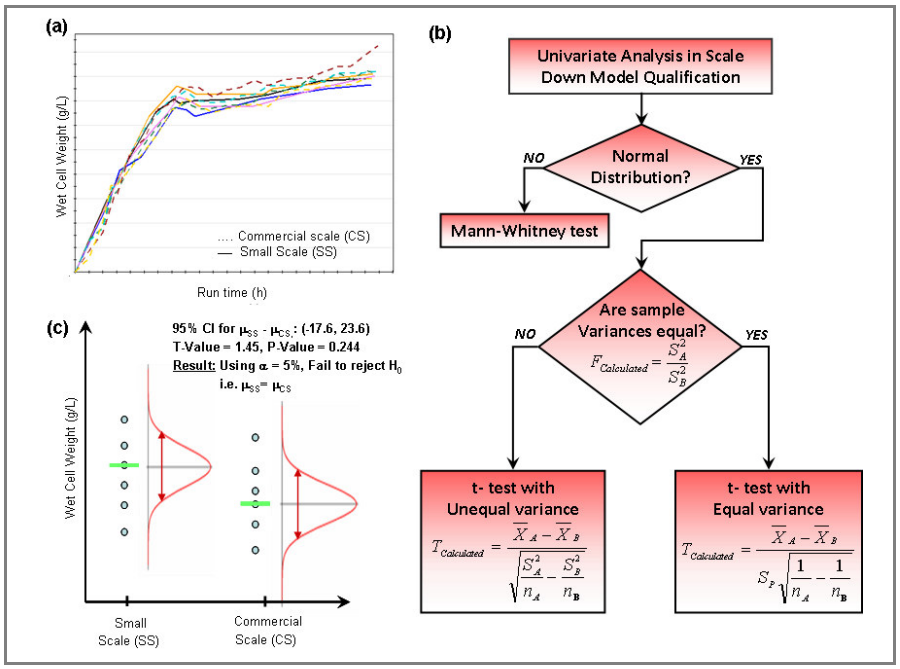

Figure 1: Univariate data analysis (UVDA) method in SSPM qualification.
(a) Overlaying plots from different scale, (b) Typical flow path for application of UVDA and (c) Output from students t-test along with a schematic visual interpretation. For detail statistical formula given in flow chart refer to References 3 and 4.

The classical approach of UVDA typically compares only the final end determination of the key attributes and hence it is straightforward and readily applied. However, to fully understand the multidimensional link between the various critical parameters and their effect on the product critical quality attribute throughout the whole batch profile (i.e. with respect to batch evolution) requires the application of multivariate data analysis (MVDA) techniques. MVDA techniques including Principal Component Analysis (PCA) and Partial Least Squares (PLS) not only take into account the multidimensionality of the data but also any missing values, variation due to different scale and experimental noise or error (5, 6). The resulting model derived from the application of MVDA is more sensitive than the one developed using UVDA methods because it can detect observations that don't fit the predicted response patterns while resulting in fewer false positive signals (7).

Application of MVDA in scale down model qualification

In this case study two data sets, one derived from commercial scale fermentation at 3000 L and one derived from laboratory scale fermentation at 15 L, were used to demonstrate the application of MVDA in SSPM qualification. These two data sets were screened for any univariate outliers or faulty measurements and then imported into SIMCA-P+ software (version 12.0.1.0 from Umetrics AB, Sweden). For qualification studies using MVDA only the scale independent variables are included in the analysis (7). Hence offline measurements from scale independent input and output variables such as Temperature (°C), pH, pO_2 (%), Methanol flow rate (vvm), Glycerol flow rate (vvm), Exit CO_2 (%), Dry Cell Weight (g/L), Wet Cell Weight (g/L) and OD_{600} (AU) were included as a predictor variables (X) in the analysis. In addition to this, the Run Time of fermenter representing the local batch time was also included as a response variable (Y) in the analysis.

The three dimensional data (Batch x Variables x Time) was unfolded by preserving variable direction (6). In order to give all the variables an equal chance of contributing to the multivariate model after unfolding, each variable was first centred with respect to their means and then scaled to unit variance. To compare the evolution of batches at different scales an Observation Level modelling approach was adopted for both the data sets (8). In this approach, two models - one for commercial scale and the other for laboratory scale - were built by projecting observations on the hyper-planes and translating them into latent variables. PLS modelling approach was used to relate the process data (X-variables) to the run time of production fermenter (Y-variable) (5,6,9). This provided an appropriate maturity index model that was used to explore the batch trajectory and progress at both scales with respect to run time. Next, an iterative model diagnostics step was performed and in this step, if required, any outlying multivariate observations were eliminated, and a new model was fitted to the remaining data. Once a suitable model for both commercial and laboratory scale was determined, the final step involved generating various plots from each model for comparison to aid in the qualification the SSPM. To highlight the advantage of MVDA in SSPM qualification only two resulting plots from the analysis are explained here in detail.

In **Figure 2** (*over)***,** we have shown the Variable Importance for Projection (VIP) plot a key plot obtained from PLS analysis. VIP plots indicate which variables included in the analysis have the greatest contribution to a batch evolution and thus this plot is helpful in scale-up comparison and in identifying influential variables (10). From both the VIP plots in **Figure 2** it can be seen that the fermentation process has a strong impact on OD_{600}, Dry cell weight (DCW) and Wet cell weight (WCW). Among the input parameters considered in the model, Methanol flow rate was the most influential parameter (VIP index >1). Comparatively the Temperature, pH and pO_2 have less influence on the fermentation process as these process parameters are tightly controlled during the entire batch evolution. Comparison of VIP plot across different scales showed that the partial pressure of oxygen (pO_2) increased in terms of its relative importance for projection in laboratory scale. As a consequence of better mass transfer, efficient gas mixing and distribution, the small scale fermenter rarely needs additional oxygen supply; therefore, oxygen demand in this fermenter can be sufficiently sustained by the airflow and agitation. In contrast, the manufacturing scale fermentation process requires additional supplementation with pure oxygen in addition to the oxygen supplied from air. This explains the rational behind the change in relative importance of pO_2 across different scales and provides additional information important when considering scale up. Overall the VIP plot across the two scales agreed well with each other and thus provides an additional quantitative assessment of the successful scaling of the production fermenter.

Figure 2

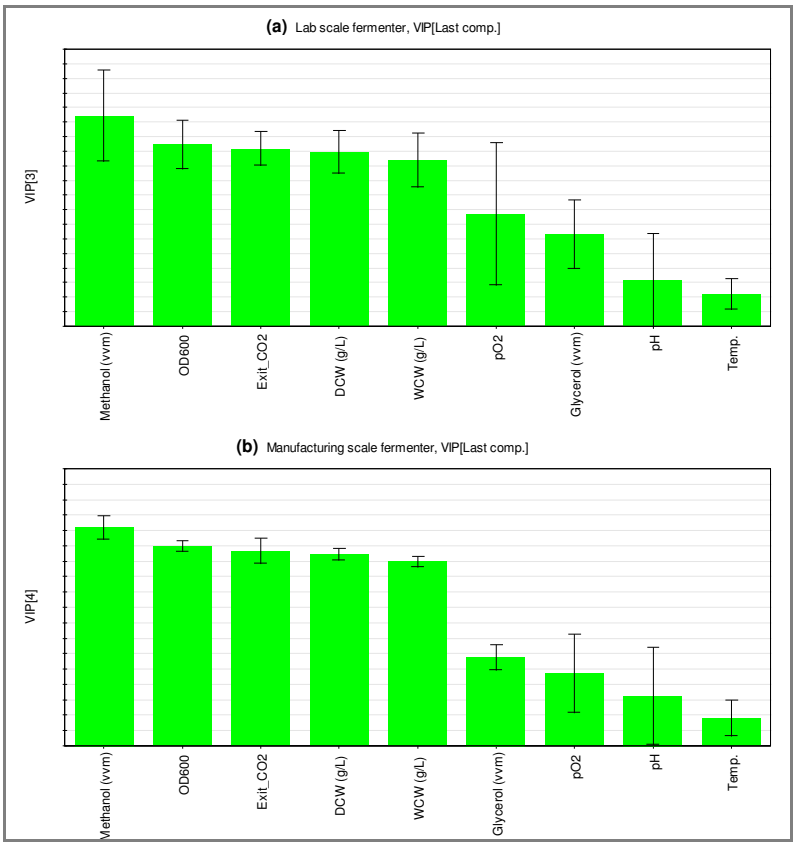

Figure 2: Variable Importance for the Projection plot for (a) small scale laboratory and (b) commercial scale fermenter

Further information towards the qualification of the SSPM was obtained from a 3-dimensional scatter plot obtained from PLS analysis and is shown in **Figure 3** *(over)*. This plot was created from the commercial scale data using the first three predictive score with a 99% confidence ellipsoid (7). Data from the laboratory runs was then used to predict the spatial coordinates of these batches in the multivariate space formed with the commercial scale batches. As shown in **Figure 3**, due to good prediction ability of the combined first three predictive score, the laboratory scale batches (blue triangles) reside well within the multivariate confidence ellipsoid of the commercial scale batches (black triangles) indicating that both data sets have comparable trends and possess similar correlation structures. This plot provides additional confidence that the 15 L laboratory scale is an acceptable SSPM for the 3000 L commercial scale fermenter.

Figure 3

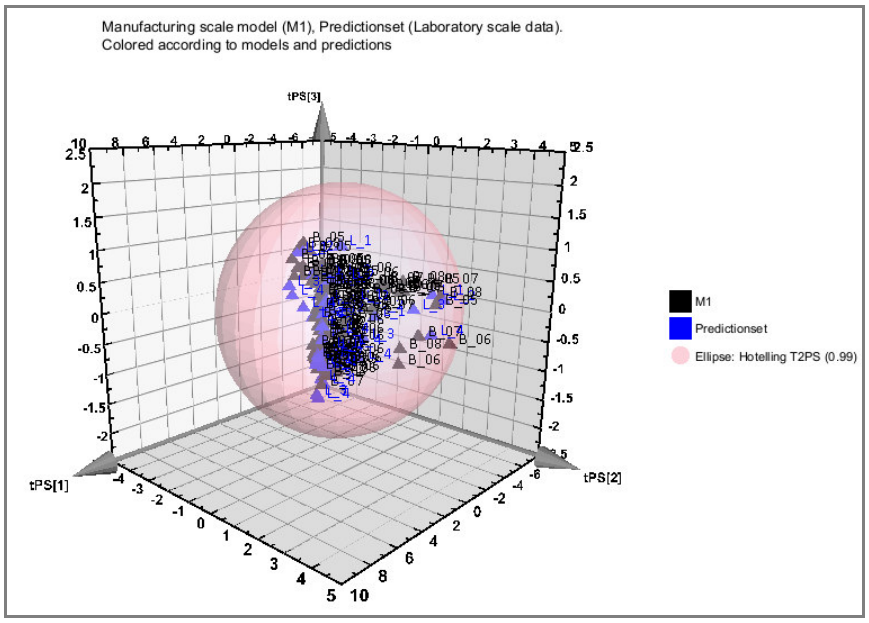

Figure 3: Scale comparison for commercial and small scale laboratory fermentation runs using PLS model. The sphere is a Hotelling's 99% confidence ellipsoid built from the manufacturing scale data (▲). The plot shows how all the laboratory scale runs (▲) fall within the ellipsoid thus showing that scales produce comparable process performance.

Summary

Successful manufacturing of biopharmaceuticals relies on good process design, scale up and control strategies. SSPM's have an important part to play in process design allowing for the generation of process understanding and definition of control strategies for commercial manufacture. However to be useful, SSPM's have to be shown to represent the commercial scale process as closely as possible.

MVDA have advantages over traditional UVDA techniques in that they provide a fingerprint of the process and hence the outcome can be used to compare future batch profile, fault detection and real time process monitoring. Variation introduced by uncontrollable factors and any missing values are accounted in the model and the analysis does not rely on the assumption of normal distribution of data. The resulting MVDA plots including the Score plot, Loading plot, VIP plot and Batch control chart illustrates batch evolution trends, determine clusters and outliers, and helps in identifying influential variables dictating the batch trajectory and improves process understanding.

This case study demonstrates that MVDA is a very useful technique for extracting process understanding from multidimensional data sets and using that information, together with traditional one dimensional and UVDA, to gain confidence in SSPM development.

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AUTHORS' BIOGRAPHY

Graham McCreath *is Head of Process Design at Fujifilm Diosynth Biotechnologies where he is responsible for late stage process characterisation, process validation and Quality by Design. He has 16 years experience in industrial bioprocess development gained at PPL Therapeutics, Avecia, MSD and Fujifilm in addition to 4 years' post-doctoral experience in purification process development. He has developed and supported a number of products derived from transgenic animals, mammalian and microbial sources throughout all stages of pharmaceutical development from Phase I to launched commercial product. He holds a PhD in Chemical Engineering from University of Cambridge.*
Email:Graham.McCreath@fujifilmdb.com

Mahesh Shivhare *is a Senior Process Statistician in the Process Design group at Fujifilm Diosynth Biotechnologies. He oversees and provides statistical support to all phases of biopharmaceutical drug development from early phase process development to process and product characterisation, validation as well as commercial manufacturing. He has experience in providing statistical support for both the small molecules and biologics pharmaceutical industries and has held similar positions previously with Avecia, MSD and Pfizer. He holds a PhD in Chemical Engineering from Newcastle University. He is also a certified Six Sigma Green Belt professional. Email:Mahesh.Shivhare@fujifilmdb.com*