EMA Expert Workshop on Validation of Manufacturing for Biological Medicinal Products

Tuesday 9th April 2013

Scale down models for Cell Culture

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Introduction

- “Small-scale models can be developed and used to support process development studies. The development of a model should account for scale effects and be representative of the proposed commercial process. A scientifically justified model can enable a prediction of quality, and can be used to support the extrapolation of operating conditions across multiple scales and equipment.”  
  ICH Q11 Step 4

- “It is important to understand the degree to which models represent the commercial process, including any differences that might exist, as this may have an impact on the relevance of information derived from the models.”  
  FDA Process Validation Guidance

- “Essentially, all models are wrong, but some are useful.”  
  George E. P. Box
Introduction

By definition, a scale-down model is an incomplete representation of a more complicated, expensive and/or physically larger system.

Scale down models must be used because of the limitations to conduct experimental studies with the at-scale equipment.
Key Elements of SDM Design

- Inputs: raw materials and components, feedstock/cell source, environmental conditions
- Design: selection of scaling principle(s), equipment limitations, on- and off-line analytical instruments
  - Use of sound scientific and engineering principles for scaling
- Outputs: performance and product quality metrics (CQAs), sample handling/storage, analytical methods.
  - Match full-scale as much as possible and feasible. Understand and/or control for differences between scale-down and full-scale (e.g., materials of construction, use of different assays)

These elements should be described and justified as part of the overall qualification of a scale-down model.
Key Elements of SDM Design

- Key Design Aspects for Cell Culture Processes:

  - Mixing
  - Heat Transfer
  - Mass Transfer

  - Gas Dispersion
  - Bubble Microorganism
Key Elements of SDM Design

- It is important to meet the same operating window for SDMs as for the at-scale process, if possible
- These window can be process and cell line specific
Many scale down criteria are used
- None is optimal, choice depends on project and cell line specific characteristics

<table>
<thead>
<tr>
<th>Unit Operation</th>
<th>Determining Criteria</th>
<th>Relation / Function</th>
<th>Expected Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspending</td>
<td>Tip Speed ( u )</td>
<td>( u = \pi \cdot n \cdot d \approx V^{1/9} )</td>
<td>inferior with increasing scale</td>
</tr>
<tr>
<td>Micro Mixing</td>
<td>Energy Dissipation Rate ( \varepsilon )</td>
<td>( \varepsilon = \frac{P}{V \cdot \rho} )</td>
<td>equal</td>
</tr>
<tr>
<td>Macro Mixing</td>
<td>Mixing Time ( \Theta )</td>
<td>( \Theta \approx \frac{D}{u} \approx \frac{1}{V^{2/9}} )</td>
<td>inferior with increasing scale</td>
</tr>
<tr>
<td>Mass Transfer</td>
<td>Mass Transfer Coefficient ( k_L a )</td>
<td>( k_L a = K \cdot \left( \frac{P}{V} \right)^{\alpha} \cdot (v_{SG})^{\beta} )</td>
<td>comparable</td>
</tr>
<tr>
<td>Shear Force</td>
<td>Turbulent Shear Force ( \tau )</td>
<td>( \tau = 0.0676 \cdot \rho \cdot d_p^2 \cdot \frac{\varepsilon}{V} )</td>
<td>comparable</td>
</tr>
</tbody>
</table>
Scale Down Model Justification

- Acceptance criteria: the performance of the scale down model should match the large scale product and process
- Process outputs of the manufacturing scale process and the SDM needs to be compared

Examples of product quality attributes
- Charge heterogeneity (Oxid., Deamid., Lysine-het., etc.)
- Glycosylation pattern (Galactose content, Mannose structures, non-fucose content, etc.)

Examples of key performance indicators (KPIs)
- Product titer
- Cell density and viability
- Concentration of substrates and byproducts (Gluc, Gln, NH$_4^+$, etc.)
Scale Down Model Justification

- Justification is documenting evidence a model is suitable for evaluating the effect of input material and parameter variation on process performance and product quality outputs.
  - The same change in inputs results in a substantially similar change in outputs.
- Through adequate description that the design provides the data it is intended to provide.
- Compare “at-target” performance
Justification by Qualitative Assessment

- Qualitative assessment of time-course trends and product quality attributes
  - Similar behavior between scales supports model suitability
  - Dissimilar behavior may indicate a problem, and can be valuable for troubleshooting and model improvement
Justification – Statistical Approach

E.g. Equivalence testing:
- Define an interval within which a difference is not scientifically meaningful, a “practically significant difference” (PSD)
- Compute the difference in means and associated statistic testing if difference is within the PSD (e.g., two-one-sided-t-test [TOST] and p-value)
- Null Hypotheses are $\delta > PSD$ or $\delta < -$ PSD. Achieving statistical significance (e.g. $p<0.05$) supports “equivalence” (both null hypotheses rejected)
- Outcome depends strongly on the definition of the PSD
  - The PSD should be based on a scientific/engineering considerations

Advantages
- Rewards greater data replication
- Similar to Bioequivalence calculations
- Supports a direct claim that model output is “not different”
Scale Down Model Justification

• An “Ideal Scenario”: Model is compared against full-scale at-target and off-target to verify the scale-down model is fully representative under various process parameter conditions

  Process Characterization
  Scale-down

  Full-scale PC/PV studies?

• Is this practical?
  - Short answer: No
    ▪ Multiple additional runs, may also require sufficient replication at off-target points for statistical confidence.
    ▪ Full scale runs are prohibitively expensive
  - Long answer: part-way…, sometimes…, it depends…
    ▪ Some parameters are tested: cell age, run duration, hold times
    ▪ Testing at pilot scale instead of full-scale?
Scale Down Model Justification

- The evidence for predictability of small scale models can be gathered throughout development:
  - Satellite experiments in the small scale models with feed streams directly from the large scale systems during clinical grade manufacturing, and by using the same lots of raw materials and consumables as in the manufacturing lots are an ideal option.
  - Deviations during manufacturing can be reproduced in the satellite model as they occur (with a small time offset) and their impact on process performance/product quality can be assessed in large and small scale in parallel.

- The above approach has limitations:
  - Not all development units have large and small scale readily available.
  - It is also possible to have clinical manufacturing with few or no significant deviations and hence no chance to gather data measuring the predictability/reliability of small scale models.
Scale Down Model Justification

- Some outputs are more important than others
  - Product quality attributes
  - Key performance indicators (e.g., titer)
  - Other characteristics (e.g. metabolic measures)

- A model can be “equivalent” for some outputs, but not all, and still be a representative model – and even still be representative of those outputs that are not statistically equivalent!
Dealing with offsets

- Evaluating the acceptability of an observed offset
  - Is the mechanism understood and/or specific source known (e.g., light exposure, hold time differences, sample handling)?
  - Is the magnitude of the offset, and absolute value of the output near a "natural limit" (e.g., % Monomer near 100%)?

- A question of confidence…
  - Unlikely to have sufficient replication of on- and off-target conditions at full-scale for a statistically robust comparison of factor effect sizes between scales.
  - Scientific understanding, offset stability and off-target full-scale testing add incrementally to the totality of evidence that an offset is acceptable.
Traditional Applications of SDM

- What scale down models have been used for from a traditional point of view:
  - Cell line selection
  - Process and media development
  - Investigation of Raw Material Variability
  - Characterization/Validation of cell age effects
  - Characterization/Validation of process parameter excursions
  - Determination of PARs for process parameters
  - Supporting Consistency claim when few at-scale batches are available

Validation / MAA relevant data
The Future? - Upstream Ultra SDMs in Validation

• Current –
  - bench top scale down reactors
  - Mainly 2–15 L systems used

• Soon/now… Ultra-scale-down reactors
  - 15-100 millilitres
  - Individually controlled
  - multiparallel reactors e.g. (ambr, 24 or 48-parallel rig)
  - Validate to model benchtop – generate large design space data sets
  - But will need the a similar degree of justification as the 2-15L bioreactor systems
The Future? - Upstream Ultra SDMs in Validation

- Erlenmeyer flask data – relate to benchtop reactor data
  Approximation to bioreactors for process characterisation
  30-50ml- litres volumes - individual flasks,
  Simulation of pH, D.O. control, stirrer speed, fed-batch

- Shaking multi-well plates
  - 1-2 ml cultures, 24+ plates, 1500 wells/incubator
  - Approximation to Erlenmeyer flask control, engineering / mixing design and characterisation
  - Automation of feeding and sampling
  - Generate larger design space data sets
Summary

- Scale-down models are a tool for developing and characterizing “the process”
  - Enables evaluation of input material and parameter variability on a process to an extent that is simply not feasible at manufacturing scale
  - By definition of a “model”, even the best is an incomplete representation, but can still provide useful and accurate information.

- Scale-down models should be designed and demonstrated as appropriate representations of the manufacturing process.
  - Industry must demonstrate a model is appropriate and applicable
  - Regulators must recognize models cannot be absolutely perfect, but understand their value and permit industry to utilize them for the information they can appropriately provide.
The Upstream Team

- Arie van Oorschot  Uniqure
- Kristopher A Barnthouse  Janssen Pharmaceuticals
- Vijay Chiruvolu  Amgen
- Ranjit Deshmukh  Medimmune
- Ray Field  Medimmune
- Jason Gale  Pfizer
- Christian Hakemeyer  Roche
- David Kirke  UCB
- Li Malmberg  Abbvie
- Karin Sewerin  Consultant for Medimmune
- Juergen Wieland  Ratiopharm

Thank you!
Back-up
Dealing with offsets

- The statistical evaluation of at-target performance is really an evaluation of risk, where offsets suggest higher risk.
- The risk: an offset may indicate the model will have a different response to the same change in process conditions.

At-target (Input=0) comparison

more likely

assumed
Dealing with offsets

When is an offset acceptable, when not, and what to do

• Constant offset - account for offset in data interpretation, need sufficient data supporting magnitude of offset used.

• Magnified response in model
  - Factor effect directionality and ranking still valid, direct prediction difficult
  - Robust interpretation possible by comparison to scale-down controls.

• Attenuated response in model
  - Same as magnified response, but higher risk since effect sizes may be falsely interpreted as not significant.
The Future? - High Content Validation Tools

- Transcriptome Sequencing of Production Cell Lots?

- Use of o’omics profiling:
  - High content cell physiology / Characterisation / Multi-gene arrays / RNA-Seq
  - Map the metabolism in many pathways between different Process conditions / Map and model the metabolic design space
  - Currently used for process development