

Bioprocess Scale Up

Product and Process Development Interactions

Bioprocess scale up poses specific challenges with long, complex development timelines where process development and clinical development interact together. Delays in developing scalable processes will be further compounded by the manufacturing scale required not being finalized until late in development when dosage and efficacy data become available. The article reviews approaches to scaling bioprocess unit operations, as well as their interaction with the less recognized areas of technology transfer of processes and manufacturing scheduling.



Biopharmaceutical Process Scales

Since its inception 30 years ago the biopharmaceutical industry has launched more than 100 molecules and is anticipated to maintain a growth rate of 15–30% annually [1].

Commercial production of biopharmaceuticals covers a wide range of scales depending on factors including clinical indication, dose, and length of treatment. Typically efficacy and dosages for a molecule are clarified during PII studies, which then allows good estimates of process scale to be made.

For commercial processes, 0.5–50 kg/y would encompass the majority of non antibody proteins products, although outliers, like insulin, have an annual production of ca. 5,000 kg/y. Antibodies typically have high dosages, so typical scales are 10–500 kg/y with some estimated at ca 750 kg/y [2].

With modern expression systems productivity can be high. Antibody titres of 7–10 g/L are reported while with microbial systems, state of the art microbial expression system such as Avecia's pAVEway system, can achieve titres > 13 g/L [3].

Scaling Down to Scale Up

Small scale studies are not just limited to initial process development studies but

also to small scale experimental studies designed to characterize key process scale up variables, process characterization (determination of proven acceptable ranges (PAR)), reuse studies (chromatography media, membranes) and storage stability studies (chromatography media). There is a need for confidence in the scale up and scale down approaches.

The development of ultra small scale experimental methods is starting to allow the collection of scale up relevant data while minimizing material requirements. This development has facilitated the use of multi parallel experimentation using robotic systems. The parallel development of modeling approaches allows data extrapolation from small scale data sets. Analytical methods matching these smaller scale experiments are essential [4, 5].

Scaling from Laboratory Data

Scale up/down is achieved through understanding of the underlying science and selecting key variables to be maintained constant with scale. This in practice is illustrated with the following examples:



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1. Fermentation

Laboratory studies must define the biological requirements for the system and also provide a process compatible with scale up [6].

A recommended scale up check list for development studies would include:

Organism:

- Effect of multiple seed steps
- Inoculum transfer criteria
- Strain stability with greater number of generations

Medium:

- Raw material grades consistent across scales
- Complex media variability and sterilization strategy
- Sterilization strategy differences across scales

Process control:

- Tightness of control, e.g. pH, temperature
- Hydrostatic head differences across scales
- Different control instrumentation, i.e. spectrophotometer differences for OD600 measurement

Hydrodynamics:

- Voidage and foaming propensity
- Vessel aspect ratio differences
- Aeration rate
- Hold up
- Heat and mass transfer limits on process intensity (metabolic heat load proportional to the oxygen uptake rate (OUR)).
- Maximum demand in terms of time and fermenter volume
- Chilled water capacity

2. Chromatography

Scale up is based on increasing column diameter to achieve a greater column volume (CV) = $\pi r^2 \times \text{height}$ (fig. 1). Everything normalized to CV means that scale up is linear (fig. 2). The CV of load, wash, step elution volume, gradient elution volume remains constant. Superficial velocity or linear flow rate (LFR, cm/h) should remain constant between the scales [7].

The removal of wall effects with increasing bed diameter means identical linear flow rates result in increasing bed compression. If the process scale flow rates are developed based on small column diameters without consideration of potential flow rate limitations of large diameter columns, bed instabilities may occur that are not evident during small scale development stud-



Fig. 1: A 1.2m diameter chromatography column used in large scale purification of a biopharmaceutical protein illustrating the approach to scale up of increasing column diameter to achieve larger column volumes.

ies. The relationship between superficial velocity and column pressure drop is a function of column height, column width, resin bead diameter and resin bead rigidity.

Complications can arise due to effects not related to the chromatographic separation but due to large scale specific factors such as column packing method (large scale may use different approaches to packing from small scale), extra column volumes (volume of system and pipework relative to the chromatography column) and column distributor design (differing as columns scale up).

Constant Factors:

- Chromatography bead chemistry and size
- Load pH

- Load conductivity
- Load volume loaded (CV)
- Target protein concentration (mg/ml)
- Target protein loaded (mg/ml bed)
- Total protein loaded (mg/ml)
- Linear flow rate (cm/h)
- Wash / elution buffer volume (CV)
- Gradient volume / CV

The scaled up chromatography run protocol should follow the laboratory scale protocol including any pre-column sanitizations, use of high ionic strength pre-equilibration buffers and the number of CV of equilibration buffer used. This is simplified by the use of equipment across scale using similar control software and valving etc.

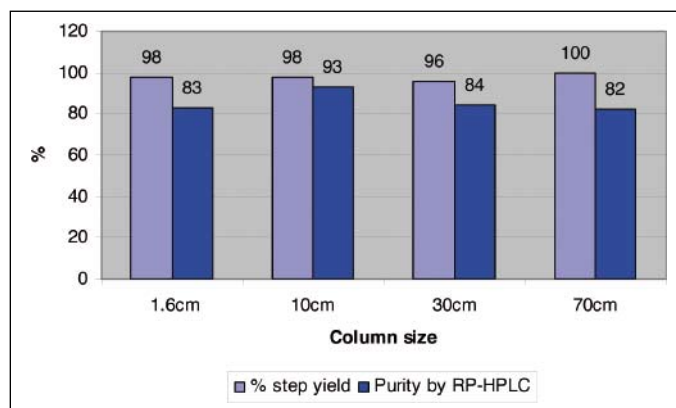


Fig. 2: Comparison of yield and purity data of a cation exchange chromatography step showing consistency of performance in scaling from 1.6 cm to 70 cm diameter columns.

3. Centrifugation

Batch centrifugation will be employed for laboratory development and small scale processes. As the process is scaled up, transition to continuous centrifugation may be necessary (fig. 3). Continuous centrifuges are limited in the extent that they can be scaled down and hence potentially significant amounts of material are required for scale up studies [8].

Development Approach:

The centrifugation characteristics of a particular suspension should be assessed empirically by carrying out trials in a centrifuge of similar design to that proposed for full scale use. Prior to this selected properties of the material to be processed should be assessed in laboratory scale experiments to provide an estimation centrifuge performance. Complete dependence on laboratory characterization is unwise because of the complexity of the effects involved in continuous centrifugation.

Centrifuge Scale Up:

The clarification capability of a disc centrifuge is quantified by the Sigma factor (Σ) which is a characteristic of the centrifuge.

Using small scale batch experiments the settling rates of suspensions and the compacted solids volume are characterized. With knowledge of the characteristics of the centrifuge to be used, Sigma factor and solids holding volume, an estimate can be made of the flowrate and rate of accumulation of solids. This then sets the frequency with which the centrifuge will need to be discharged to prevent solids being lost into the centrate as the solids holding space fills.

For clarification the feed rate for a centrifuge is given by the relation:

$$L \text{ (m}^3\text{/s)} = \text{settling rate (m/s)} \cdot \Sigma \text{ (m}^2\text{)}$$

Performance of different centrifuges is given by the ratio of the Sigma factors

$$\frac{\text{Throughput on Machine A}}{\text{Throughput on Machine B}} = \frac{\Sigma A}{\Sigma B}$$

The ratio of the solids holding spaces between the two centrifuges allows the discharge interval to be adjusted given the different solids accumulation rates of the two centrifuges.

4. Ultrafiltration

In cross-flow membrane processes parameters to be maintained constant are [9]:

- Volume of retentate per membrane area (V_r/A), equivalent to specifying mass of protein per membrane area
- Retentate flow rate per membrane area (Q_r/A) (this keeps the sweeping action across the membrane constant)
- Trans-membrane pressure to ensure that permeate rates are consistent

Membrane inlet and outlet pressures do not necessarily have to be kept constant to ensure performance upon scale-up. As process-scale UF is invariably conducted using a cross-flow configuration, all meaningful process development must seek to mimic this arrangement.

Process Scale and Technology Transfer

Technology transfer of the process from development aims to determine the logistics of process operation at the required manufacturing scale.



Fig. 3: A large scale intermittent discharge continuous centrifuge as used in cGMP manufacture.

Potential constraints that can limit the scaling of processes include:

- Refolding volumes
- Chromatography column diameter
- Fermenter heat transfer capability
- Number of separate chromatography columns of a given size operable within a purification suite
- WFI capacity
- Buffer volumes

These need to be considered as part of the technology transfer strategy and may feed back to additional laboratory work. Interacting with technology transfer and scale up is the manufacturing operation itself and how this is organized to achieve the most efficient operation. Development of plant models/ process schedulers allow the complexity of simultaneous operation of a number of multi step process to be assessed rapidly [10].

Using such tools to explore alternative manufacturing scenarios make it possible to achieve major savings in plant

capacity through relatively simple changes in manufacturing approaches.

Conclusions

Bioprocess scale up is a complex multidimensional problem involving not only consideration of the process but interactions with the facility in which it will be operated. Scale up is dependant in thorough characterization of the scaling variables. Technology transfer to the manufacturing plant depends not only on understanding of the process variables but also process constraints imposed by facility equipment performance. Detailed plant models can allow more efficient operation of manufacturing facilities by allowing better utilization of equipment and services and a better understanding of where bottlenecks are present.

Developments in modern expression systems gives the prospect of high yielding upstream processes which will reduce fermentation scale up necessary to manufacture a

given amount of product. The challenge to downstream development scientists will be to provide matching improvements in downstream processes.

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