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Scale-Down Models to Support Process Characterization

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Abstract

Process characterization using qualified scale-down models (SDM) offers time and resource-saving advantages to companies developing biotherapeutics. Current approaches with glass benchtop bioreactors as SDMs have demonstrated the ability to predict process performance and product quality, but are throughput-limited by infrastructure that requires significant operational input, as well as large volumes of media and reagents. In this article, the Sartorius Stedim Biotech ambr® 250 high-throughput, single-use mini bioreactor system will be discussed for its suitability as an SDM for process characterization.

Introduction

Process characterization is a pivotal part of the validation master plan filed with regulatory authorities, such as the FDA Biologics License Application (BLA). It is where all process aspects are evaluated to determine if the process is capable of reproducible commercial manufacturing to current Good Manufacturing Practices (cGMP). This evaluation includes identifying critical process parameters (CPP), demonstrating process robustness, and understanding sources and acceptable ranges of variation, as well as their impact on the process and how to control these variations to minimize risks to the process and product. Process characterization in cell culture evaluates the relationship between operating parameters, including temperature, pH, dissolved oxygen (DO), media components, and feed programs on critical quality attributes such as glycosylation, sialylation, charge variants, and aggregation.

Cell culture process characterization generally uses a design of experiments (DoE) approach and can take many months to test and evaluate processes and determine proven acceptable range data for the many parameters required to manufacture a biologic. As this approach can use anywhere from tens to hundreds of costly and time-consuming experimental bioreactor runs, while producing large data sets, there is a need for scale-down models (SDM) that represent commercial manufacturing and good statistical analysis tools such as univariate and multivariate data analysis.[1,2]

For process characterization, regulatory requirements state that a qualified SDM must be used which takes into consideration scale effects and represents the commercial-scale process.[3] Traditionally, SDMs used for process characterization are 2–5 L benchtop bioreactors. Due to the scale of these studies, using glass benchtop bioreactors as SDMs can be labor intensive and expensive to run, in terms of the large volumes of media used, as well as staff costs required to decontaminate, clean, build, and sterilize vessels before each run.

FIGURE 1. The ambr 250 single-use, mini bioreactor system.

Single-Use Mini Bioreactors

According to a recent survey[4], over 80% of the biopharma industry uses the Sartorius Stedim Biotech (SSB) ambr® single-use (SU) mini bioreactors. The ambr 250 system (**Figure 1**), which was originally developed in 2013[5] as an SDM for process development, consists of SU mini bioreactors (100–250 mL working volume), an automated cell culture workstation, and system control software. Key to the success of the SU ambr 250 bioreactors is that they are geometrically similar to larger stirred tank bioreactors, which helps develop process conditions for direct transfer to manufacturing-scale.

The workstation provides independent parallel control of 12 or 24 bioreactors, and independently controls stirring speed, gas supply, temperature, off-gas, foaming, and provides liquid handling for bioreactors via both a pipetting robot and reagent supply pumps. Each bioreactor incorporates sensors for real-time measurement and automated control, and settings for these can be bioreactor-specific. Controls can be customized as needed, and samples can be delivered into a range of integrated analysers or sample formats for analysis.

The system is supported by easy-to-use, intuitive software with a highly flexible control architecture for manipulating parameters, and a drag-and-drop interface for adding and linking process steps. It also has integrated BioPAT® MODDE software for DoE, powered by Umetrics. This allows implementation of DoE into the workflow for simpler process optimization and scale-up to larger SU BIOSTAT® STR pilot and manufacturing-scale bioreactors.

Currently, there have been approximately 250 ambr 250 systems installed world-wide, replacing many glass bioreactors for pre-clinical process development studies. The ambr 250 is also increasingly being qualified as an SDM used for late-stage process characterization studies. Therefore, it is sensible to review how the ambr 250 technology can be beneficial in process characterization, and the recent progress seen in this area.

Scalability

Comparisons with Benchtop Bioreactors

To qualify lab-scale bioreactors as SMDs, conditions must be determined that simulate the selected manufacturing-scale process performance as closely as possible. Once ideal operating conditions are determined, SDM performance is then compared with manufacturing process performance to determine and describe the capability of the SDM for process characterization studies. Most SMDs cannot totally replicate commercial manufacturing processes in every aspect, and determining when models are predictive of responses is critical. It is also important to characterize any differences that exist between the SDM and production-scale.

Both scale-dependent and scale-independent parameters are important when scaling down. Scale-dependent parameters such as gas transfer rates and stirring speeds need consideration to ensure translatable model selection. Scaling strategies such as volumetric gas flow rate (vvm), power per unit volume (P/V) and mass transfer coefficient (k_La) are typically used in the development and qualification of SDMs for cell-culture processes.[2,6]

Currently, many process characterization studies are performed using benchtop bioreactors (such as SSB's Biostat® B-DCU) as a qualified SDM, based on historical success and institutional experience with these well-established systems. Therefore, for ambr® 250 technology to be generally applicable in this type of study, it should demonstrate broadly comparable performance to established SDMs, which means that ambr 250 should be reasonably representative of the chosen manufacturing-scale process performance with a clear understanding of the SDM boundaries for a given process.

In 2015, a DoE study with *Pichia pastoris* at Merck[7] demonstrated that ambr 250 bioreactors showed comparability of cell growth, titer, and product quality (<0% CV) to 1.5 L benchtop bioreactors. Additionally, using the automated SU mini bioreactors enabled a cell culture DoE to be completed in two weeks (up to four times faster than using 24 3 L bioreactors), and reduced total running costs by up to 50%.

A further study published by Bristol-Myers Squibb (BMS) in 2017[8] compared vvm, P/V, and k_La and determined that for the specific process, k_La was the optimum criteria to scale their process down into the ambr 250. Using k_La, 13 Chinese hamster ovary (CHO) clones were cultured in SU mini bioreactors and 5 L bioreactors. Eleven clones matched cell growth, viability, titer, and metabolite profiles of benchtop bioreactors very closely, and two clones showed slightly slower growth in ambr 250 vessels with minimal impact on titer (0–15% discrepancy). Peak viable cell density (VCD) reached 25–35 × 10^6 cells/mL and the differences in peak VCD, final viability, and titer between ambr 250 bioreactor duplicates were within 1–3%.

In a recent 2019 Sartorius Stedim Cellca (SSC) study[9], ten DG44 CHO clones were cultured in ambr 250 vessels and 5 L benchtop bioreactors (used as qualified SDMs in process characterization). In three representative clones (**Figure 2**), cell growth, product titers, and a variety of growth characteristics were found to be comparable in both bioreactor types, with most parameters in the range of ± 10% of the 5 L bioreactor, with the exception of pCO_2 which was slightly elevated in the SU mini bioreactor under

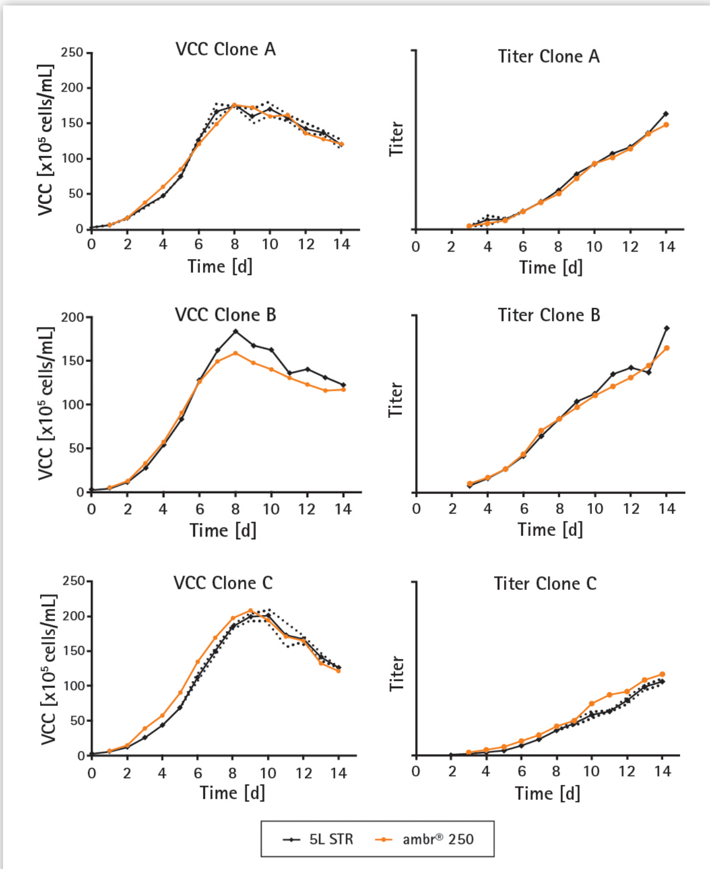

FIGURE 2. Comparison of viable cell concentration and titer of CHO cells cultured in ambr 250 and 5 L bioreactors.

the selected agitation and aeration conditions.

The data from these studies described establishes the SU mini bioreactors as having comparability with benchtop bioreactor process performance, and indicates they could be used in their place as SDMs for process characterization studies.

Comparison with Pilot and Manufacturing-Scales

The next stage in qualifying an SDM is to determine how its process performance compares at pilot and manufacturing-scales. In the Merck study[7], ambr® 250 data from two CHO clones were compared with historical data from a 200 L bioreactor, using P/V as the SDM criteria. The clones had comparable cell growth/viability and titer, as well as comparable mAb quality, but did show different pCO_2 profiles across scales.

In a 2017 study[8], BMS showed that for two CHO clones (designated A and B), ambr 250 cell growth and titer performance with clone A matched 5 L and 250 L bioreactors, while with clone B, cell growth in the SU mini bioreactor was similar to that seen in 5 L, 250 L, and 1000 L bioreactors. The product titer from the ambr 250 varied by 5% from that generated at larger scale. Additionally, differences in peak VCD, viability, and titer between SU mini bioreactor duplicates were within 1–3%.

More recently, in the 2019 SSC[9] study, cell growth, product titer, and metabolite levels in the ambr 250 were compared to fed-batch processes at 5 L, 200 L, and 2000 L scales. VCD, titers, glucose, and lactate levels were comparable at all scales with peak VCDs of $2–2.5 \times 10^6$ cells/mL observed. Key performance measures and process parameters for all clones confirmed that most were in the range of ± 10%, with the exception of pCO_2 which was slightly elevated in the ambr 250.

To date, these published studies have focused on comparing the SU mini bioreactor with 200–2000 L bioreactors and demonstrate ambr 250 has good comparability with process performance of VCD and product titer, as well as metabolite profiles. However, at this scale it should be noted that pCO_2 levels are not always comparable, and this can affect process performance in both large and small scales.[7, 9] Where process knowledge, risk analysis, and process data highlight pCO_2 as a CPP, special attention must be made to gassing and agitation conditions to ensure that pCO_2 profiles are matched well to the larger reference-scale process. The sum of the ambr 250 control architecture, sparger/headspace gassing control schemes, and integrated CO_2 analysis options provides a highly flexible and capable platform, enabling a range of approaches to pCO_2 matching.

A recently published paper[10] describes a very thorough approach to pCO_2 matching in an ambr 250 SDM, characterizing both k_La (O_2) and k_La (CO_2), and applying a surface transfer-compensated model for gassing in ambr 250. Comparable pCO_2 profiles, culture performance, and product quality attributes were achieved between scales from 0.25–2000 L and for multiple mAbs.

Comparisons with Commercial-Scale Bioreactors

A range of biopharmaceutical companies and contract development and manufacturing organizations are assessing the applicability of SU automated bioreactors as qualified SDMs for process characterization studies. A significant number are already using ambr® 250 for projects at this stage of development. In their review of SU mini bioreactor technology[1], FUJIFILM Diosynth Biotechnologies has proposed that ambr 250 bioreactors could be qualified as SDMs for use in process characterization studies. More recently in 2019[6], Merck went one step further and published a study establishing the ambr 250 as a qualified SDM for two commercial mAb production processes. They compared two monoclonal antibodies (mAb1 and mAb2) expressed in CHO cells with ambr 250 vessels and commercial-scale bioreactors over 10,000 L scale.

For mAb1, a scale-down criterion was used to maintain the same constant P/V from ambr 250 to an 18,000 L bioreactor where matching the pCO_2 profile was not a concern. The study found that gassing strategies and vvm were consistent between scales for mAb1.

To scale down the mAb2 process, where pCO_2 affected process performance, vvm of sparge gas was limited to match the commercial process' pCO_2 profile. In order to maintain oxygen transfer, the speed in the SU mini bioreactor was increased to 500 rpm (the direct scale-down of P/V from commercial-scale is 300 rpm). This gassing strategy resulted in a more stable pH profile, making the process more representative of the larger scale bioreactor.

The study demonstrated that the SU mini bioreactors achieved comparable cell growth to commercial-scale with similar peak VCDs, ranging from $4.5–5.2 \times 10^6$ cells/mL, and percentage viability profiles matching well up to day 12. Antibody titers were consistent across scales, but a difference was observed later in culture, and at day 13, final titer was 13% higher in ambr 250 vessels than commercial bioreactors. Similar percentage VCD profiles and cell growth with mAb1 were seen between both ambr 250 systems with no evidence of a bioreactor (workstation deck) position effect. The harvest viabilities for both mAb1 and mAb2 processes were also comparable between scales.

For product quality attributes, the SU mini bioreactor was representative of an 18,000 L bioreactor with mAb1 for acidic and basic variants, mannose and agalactosylated species, with a difference of fucosylated species across a scale of less than 2%, a difference that is not significant. The mAb2 process in ambr 250 was also representative of the commercial-scale process for acidic and basic variants, and fucosylated species.

Multivariate and univariate statistical analysis of performance and product quality data demonstrated that ambr 250 data were clustered with the majority of commercial-scale data at the 95% confidence level, establishing the ambr 250 as a qualified SDM for two commercial-scale cell culture processes for use in process characterization studies.

Sampling, Analytics, and Data Analysis

With the ambr® 250 system, process parameter values such as culture volumes and DO/pH are logged continuously. Cell culture performance and metabolite values can be obtained offline or by at-line sampling with automated cell culture analyzers including the BioProfile® FLEX2 (Nova Biomedical) and ambr pH analysis module. While for many benchtop bioreactors (3–5 L size), typical sample volumes can be neglected, in the case of ambr mini bioreactors, sample volumes should be accounted for (*e.g.*, calculating daily feed volumes according to current culture volume) rather than approximating to the nominal working culture volume.

In order to ensure the best possible match between an SDM and the reference process, all sources of variation, including analytical variation, should be minimized. Therefore, a helpful complimentary approach is to apply exactly the same analytical techniques (such as cell count, metabolites, product quality) in both manufacturing reference-scale and ambr 250 SDM. An optimal method for ambr 250 SDM development would likely include both of these approaches, namely appropriate accounting of sample and addition volumes, plus implementing a comprehensive and precise set of analytics with low sample volume requirements alongside both manufacturing reference-scale and ambr 250 SDM.

Process characterization studies feed into a process control strategy and validation master plan, as part of a BLA regulatory filing. Therefore, data management and integrity are an important consideration. The ambr 250 systems include a variety of data export tools such as periodic comma-separated values output, an online open platform communications connection, and also the recently offered batch application programing interface. These tools provide users with a range of efficient and reliable interfaces to automate data flows and meet the needs of both established data infrastructure and record keeping compliance policies.

Conclusions

In this article, evidence has been discussed which demonstrates that, with appropriate scaling approaches in place, ambr® 250 mini bioreactors match well with bioreactors ranging in scale from 1.5–18,000 L for process performance, in terms of cell densities, metabolite levels, product titer, and product quality attributes. The precise scaling strategy for each project may vary according to specific process sensitivities. For example, pCO_2 is a CPP for many cell culture processes, and focused efforts may be required to match large-scale pCO_2 profiles in the small-scale. It is advantageous that the ambr 250 system has flexible capabilities that can be applied to successfully match pCO_2 profiles to much larger scales.[10]

The two key factors supporting application of ambr 250 as a qualified SDM are: (A) achieving the best practicable match to the large-scale reference process; and (B) understanding and describing comparable performance (and differences) of the ambr 250 SDM. The essential tools and capabilities are already available today to develop and qualify ambr 250 SDMs able to mimic key performance and quality attributes up to commercial-scale cell culture. These tools include established scaling practices adapted for this smaller volume, appropriate analytical techniques across scales, and both univariate and multivariate data analysis methods, Application of this SU mini bioreactor technology in place of glass benchtop bioreactors in some, or all, process characterization studies could minimize downtime between experiments, use less media, and reduce system hands-on time by around 50%. The results are significant reductions in development timelines and cost of goods for biologics manufacturing.

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