sartorius stedim biotech

Advances in Single-Use Platforms for Commercial Manufacturing

In association with

BioProcess™ INTERNATIONAL

Welcome and Contents

Welcome to "Advances in Single-Use Platforms for Commercial Manufacturing." This special report explores recent advances in single-use process platforms for commercial production of biopharmaceuticals. Enormous developments have been made in single-use systems, allowing biomanufacturers to adopt the technology in critical applications within their commercial-scale facilities. The authors illustrate how companies can work with teams of experts from supplier organizations to implement end-to-end single-use bioprocesses at scales up to 2,000 L by carefully considering the approach from the earliest stages in process development. Finally, the report examines how cutting-edge developments in sensors and data analysis methods give biomanufacturers significantly improved control of their bioprocesses than they have ever experienced before. Each expert contributor presented on his or her respective topic to audiences at the Biotech Week Boston conference in 2017. We hope you'll find this report enjoyable and that it will be an invaluable resource for considering your own approach to implementing single-use process platforms.

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senior technical editor
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BPA WORLDWIDE BUSINESS

Control of Single-Use System Supply Chains

Accelerate Adoption Into Commercial Production Facilities

by Jean-Marc Cappia

Single-use technologies are now dominant for the clinical production of biopharmaceuticals and are becoming more mainstream within commercial manufacturing facilities. They allow biologics manufacturers to decrease the footprint of their facilities by approximately 20% because of a reduced need for utilities that generate water, steam, and clean-in-place solutions. Engineers believe that the capital outlay for a single-use facility is 25–45% less than for a facility based on stainless steel equipment. Similarly, they estimate that such facilities need half the water and energy during operations and can be constructed in as little as 18 months. That is in contrast to the three years it can take to get a stainless steel facility up and running. Furthermore, the risk of product cross-contamination between batches is considerably reduced.

Biomanufacturers must be able to trust single-use technology as they implement it into increasingly critical bioprocessing process steps and applications. We have seen evidence that a single bag failure can cost manufacturers between US$100,000 and $1 million and that, each year, leaks in single-use systems have resulted in the loss of product valued at up to $20 million. Changes to the raw materials used in production of single-use systems must be analyzed by biopharmaceutical producers, a process that can cost €100,000 per change. What is worrying for process scientists is increasing evidence to show that some single-use materials are biologically active. A recent study by

Figure 1: Simplified schematic for development of a risk-assessment filing strategy — simple validation by connecting existing extractables with specific process conditions to simulate leachables

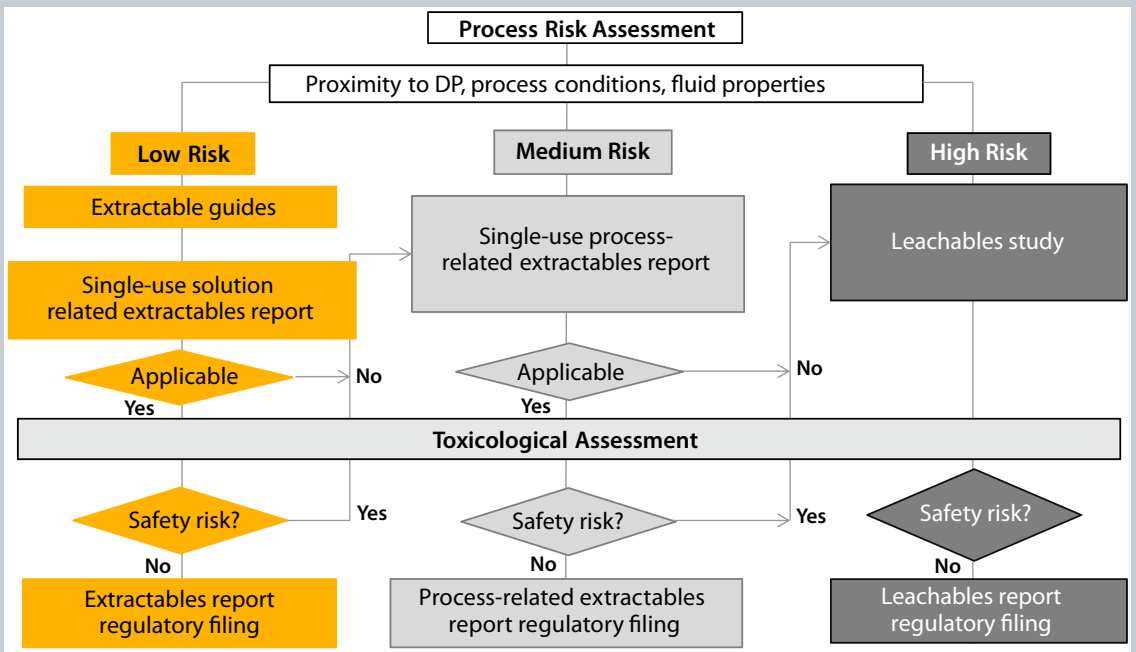
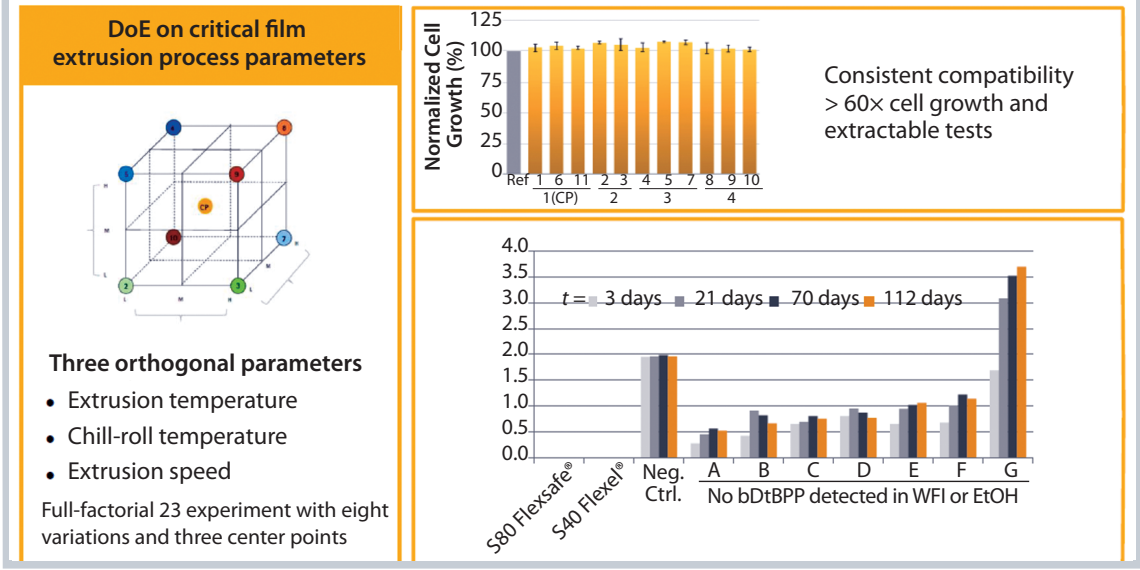


Figure 2: Film extrusion design of experiments (DoE) guarantee consistent compatibility in the entire design space.



the DECHEMA society for chemical engineering and biotechnology showed that 7 out of 11 readily available bags on the market inhibited or interfered with cell growth.

The industry must address four key challenges that biomanufacturers face when developing a single-use system: It needs to ensure that the technology does not interact with living cells or biological products, that it is a closed and integral system, that it does not leak, and that its supply is ensured. Finally, such systems must reduce complexity, improve quality, and minimize the burden of change controls through standardization. The solutions to these challenges are interrelated, but they depend on improving control of raw materials, components, and processes within the supply chain.

How to Achieve Those Goals

How can suppliers support biomanufacturers and limit the impact of possible incompatibilities between production technologies and biological systems? First, we need to understand how plastics can interact with bioprocesses. Extractables are chemicals originating from single-use materials and manufacturing processes that can be extracted under worst-case conditions. Leachables are a subset of extractables that enter the manufacturing process under normal operating conditions. They can inhibit cell growth, alter protein expression, or change the structure of biological products through processes such as oxidation and unfolding. Particles also can find their way into bioprocesses from the external environment or from materials (such as plastics) that operators use during processing. Both leachables and particles can be immunogenic or thrombogenic, or they can lead to embolization in patients.

Extractables, Leachables: Biomanufacturers need to be certain that the exposure of cultures and products to extractable compounds will be consistent for every batch of biologic produced. Suppliers of single-use systems must characterize their materials and processes thoroughly, then ensure consistency through incoming material specifications, design spaces, process controls, and change control mechanisms. Sartorius Stedim Biotech has characterized the extractables present in all single-use system components according to the industry-accepted BioPhorum Operations Group (BPOG) methodology and the company's own methods, which it believes to be more stringent. Sartorius Stedim Biotech has developed a simplified schematic to guide customers when developing their filing strategy and help them determine whether vendor data on extractables will suffice, whether a simulation study is required, or whether full leachables testing is needed (Figure 1).

To achieve the necessary level of reproducibility, the company has thoroughly characterized its resins and established the design space of S80 film used in Flexsafe® bags by performing full-factorial 23 design of experiments (DoE) based on extrusion temperature, chill-roll temperature, and extrusion-speed process parameters. Films produced from across the design space gave consistent biocompatibility performance in over 60 tests. Furthermore, the studied films gave normalized cell-growth performance that was comparable to a reference and

showed no cell-growth inhibition. The supplier's knowledge of film manufacturing has allowed it to set resin specifications and design spaces that ensure Flexsafe® bags reproducibly will not release detectable amounts of the compound bDtBPP, which is a known cell-growth inhibitor, into solutions stored for up to 112 days at 40 °C (Figure 2).

Control of particulates in bioprocesses can be just as important as controlling extractables and leachables. Our primary concern is the control and reduction of visible particles, greater than 100 microns, which could enter the product formulation. It is our belief that the industry can reduce problems caused by particles by understanding the main mechanisms by which they are generated and then taking steps to eliminate those components and operations shown to be significant contributors to the overall particle burden. We increase the cleanliness of our components by our partnerships with suppliers that guide major improvements. Südpack, for example, extrudes our film under conditions that meet the particulate specifications of an ISO Class 7 environment under activity. We have also implemented incoming component inspections against specifications, perform regular supplier audits and we continuously improve our own cleanroom operations to reduce sources of contamination. All of our 2D and 3D bags and mixing containers undergo visual inspection during production while our finished product assemblies destined for drug substance and drug product processing applications receive an extended visible particle inspection before release.

Leaks: Suppliers of single-use systems have a vital role to play in minimizing the risks that biomanufacturers face from leaking single-use assemblies. In addition to applying quality by design (QbD) principles and improving the robustness of films, welds, and connections, the company has implemented integrity tests throughout the entire life cycle of an assembly. When making bag chambers, Sartorius Stedim Biotech performs an in-process pressure-decay test to the ASTM F2095 standard with a detection limit of 40–100 µm. In addition, the company can perform a helium-based supplier integrity test on entire, finished single-use assemblies for critical applications — a sensitive test can detect defects as small as 2 µm in size. For an extra level of integrity assurance, customers can benefit from a point-of-use leak test technology that allows the detection of defects in the range 10–100 µm before using a bag in their applications. Find more details of those tests elsewhere in this report.

Standardization: The biopharmaceutical industry often has highlighted problems with large numbers of nonstandard consumables within its supply chain. That leads to additional complexity and cost but also can have a real impact on quality, change control, and assurance of supply. The company has developed a large number of predesigned solutions using off-the-shelf standard components for a broad range of process steps and applications. By taking advantage of those predefined solutions, biomanufacturers can ensure that they receive notice of changes 24 months before they occur. We believe that standardization allows the highest levels of delivery performance, the greatest assurance of supply, and the most optimized inventory of parts.

Ensured Supply: Receiving an assurance of supply of single-use systems is critical to manufacturers of biopharmaceuticals for commercial supply. In addition to the two years guaranteed supply of unchanged components within its predesigned solutions, Sartorius Stedim Biotech has signed long-term supply and quality contracts with its own suppliers and implemented stocking policies that allow it to provide customers with four-year guarantees of unchanged films used in bag chambers. The company operates multiple manufacturing sites with >7,000 m^2 of cleanroom capacity located around the globe for full business continuity planning.

A Science-Led Approach

Biotechnology companies increasingly are implementing single-use technologies for the commercial production of biopharmaceuticals to improve speed, efficiency, flexibility, and costs. Biomanufacturers and their suppliers are combining expertise and using a science-led approach to gain end-to-end control of these technologies. Control of materials, production processes, and quality can reduce variability, ensure consistent compatibility with biological systems, and increase process integrity.

The following articles address the range of technological developments in single-use bioprocessing that will allow bioprocess engineers to develop more efficient and flexible processes without compromising on the robustness of single-use systems or associated supply chains.

Jean-Marc Cappia *is group VP of marketing and product management in fluid management technologies at Sartorius Stedim Biotech FMT.*

Exploring the Science Behind Single-Use Container–Closure Integrity Assurance

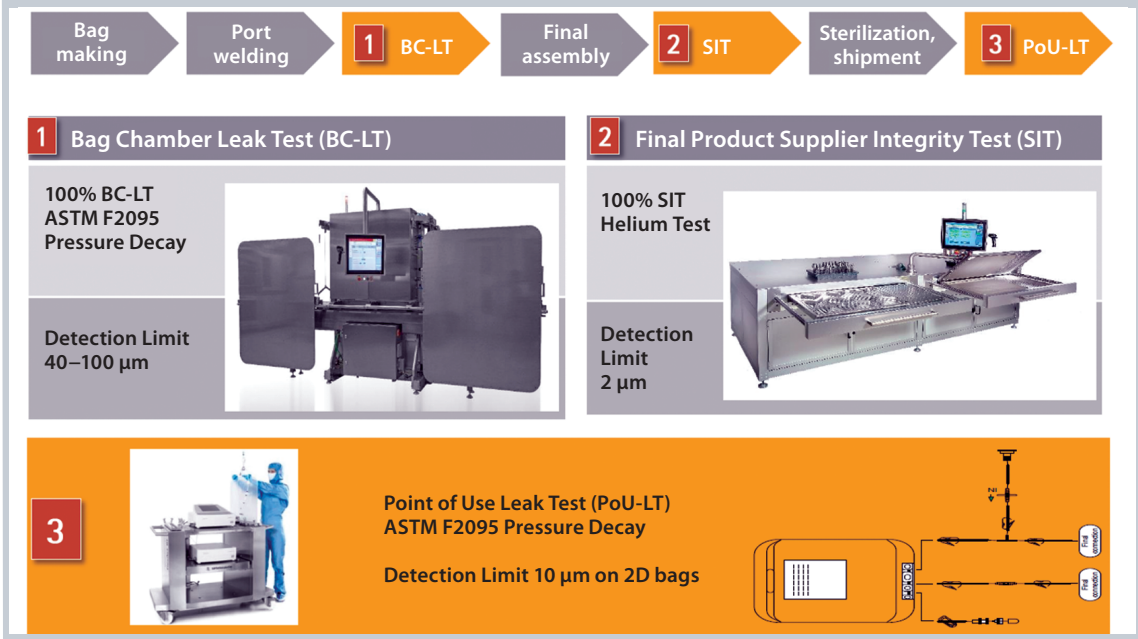
by Carole Langlois and Marc Hogreve

Failures in the integrity of single-use systems during commercial manufacturing can cause a number of serious problems for biomanufacturers. A loss of system integrity during processing can allow environmental contaminants that can be dangerous for patients (e.g., microbes) to enter a process. Biopharmaceuticals and their intermediates can be highly potent or even infectious agents, so an integrity failure can jeopardize the safety of operators. In severe cases when biomanufacturers cannot ensure the quality of drug products for fear of a contamination, the supply of life-saving medicines to patients can be restricted. Finally, significant costs can be associated with product losses and quality investigations that arise because of leaks within systems.

It is perhaps unsurprising that single-use container–closures are coming under increasing scrutiny from regulators. At many industry meetings, participants from suppliers, end users, and regulators discuss the topic.

For example, the US Food and Drug Administration (FDA) and the American Society for Testing and Materials (ASTM) ran a workshop in 2016 exploring issues relating to using single-use systems in fill–finish applications. The outcomes of that workshop reflected much discussion happening more broadly such as the recommendation that development of physical integrity tests should be correlated to microbial ingress. Suppliers and biomanufacturers must share the responsibility for container–closure integrity assurance. Reflecting that shared responsibility, workshop participants concluded that packaging integrity validation is needed at both the supplier site and (following shipment and installation) at the biomanufacturing location. It was also proposed that defect sizes that could allow bacterial ingress under process

Figure 1: Integrity testing along the entire life cycle improves process integrity



conditions into the critical flow path downstream of sterilizing-grade filters should be identified by microbial challenge testing — thereby allowing a physical integrity test to be developed.

Despite the increasing regulatory scrutiny, the industry lacks a general understanding about the size of defects in single-use systems that can allow for liquid leaks or microbial ingress under processing conditions. Biomanufacturers often are confused about commercially available integrity-testing technologies for single-use systems and the meaning of the results that they generate. Here we describe our current understanding of these subjects with a view to advancing discussion within the industry regarding how best to ensure the integrity of single-use systems.

Understanding Liquid Leaks and Microbial Ingress Mechanisms

Liquid leakage and microbial ingress both depend on process conditions, fluid attributes, and the size of container defects. The *maximum allowable leakage limit* (MALL) is defined by USP <1207> for maintaining the microbiological integrity of sterile packaging as the largest defect that is tolerable and poses no risk to product safety. Determining the MALL for single-use systems is fundamental to development of physical test methods with detection limits that are correlated both to liquid leaks and microbial ingress.

We have reviewed literature in this field, concluding that there is strong relationship between the size of defects that allow leaks and those that allow microbial ingress. Some scientists have found that ingress could not occur without liquid flow through a defect. Whether fluid flows through a defect depends on pressure and surface tension. Water in a representative bioprocess bag (e.g., a hanging Flexboy® 20L or Flexsafe® 500L installed in a Palletank) has a liquid height of ~630 mm. The pressure of water under static conditions does not exceed 65 mbar at that liquid height. Using mathematical models from the literature, we can predict that leaks will occur under liquid-storage conditions with defects of 15 µm or larger.

That situation becomes more complex when we consider critical applications. Our analysis shows that pressure pulses of ±0.3 bar can occur during transportation of bags by truck or by air. However, because the headspace in liquid-filled bags usually is insignificant, and the liquids they contain are noncompressible, the differential pressure during transportation does not affect pressure in the bags to significant extent. It is our belief that pressure pulses during shipping do not significantly affect the MALL. What should be of greater concern to bioprocess engineers transporting liquids in single-use systems, however, are accelerations and shocks during shipment, which can be up to 20*g*. The MALL for bags exposed to such aggressive conditions decreases to 2 µm. Our conclusion is consistent with empirical data based on microtube and immersion data published by academics and other researchers in the industry.

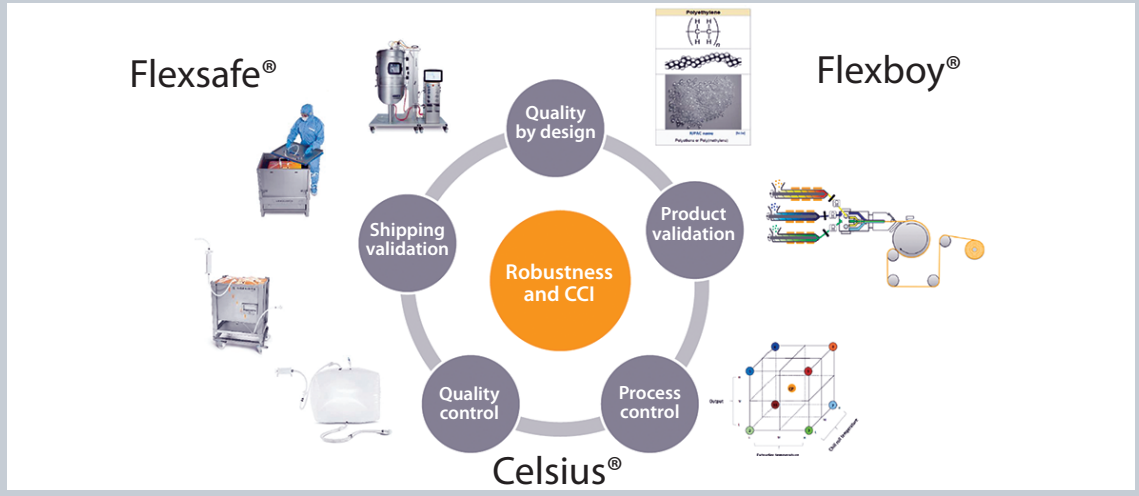
Sartorius Stedim Biotech has developed and validated a robust bacterial aerosol challenge test to further support its integrity testing program. We believe that this test will provide the most useful and relevant data available for correlating microbial ingress to a physical integrity test. Holes of 2–100 µm were laser-drilled into samples of S70 and S80 films. Those samples then were exposed to aerosolized *Bacillus atrophaeus* spores with a size distribution of 0.2–0.3 µm and a concentration of 10^6 cfu/cm^2 for 3 hours. Tryptone soya agar (TSA) medium from the downstream side of the film then was incubated for 7 days at 30–35 °C. No colonies were observed from films containing defects of 40 µm or less for either film. That result falls in line with existing published data from aerosol tests performed on 20-µm to 50-µm diameter microtubes.

We used that analysis to guide development of a three-stage single-use system integrity-testing strategy. Based on the analysis above, we defined a detection limit of 2 µm for bags that our customers intend to use in critical applications, including liquid shipping. To reach that limit, we implemented an integrity test based on helium detection. That test is performed on final assemblies before their sterilization and shipment. Before assembly, however, all bag chambers are subjected to an ASTM F2095 pressure-decay test that can detect defects in the range 40–100 µm as part of our process control strategy (Figure 1).

Biomanufacturers can ensure that no defects have been introduced into 2D and 3D bags during shipping and handling with our point-of-use leak test. It is the third test in the single-use system life cycle. The leak tester will detect defects as small as 10 µm in 2D bags, which is smaller than the 15-µm MALL that allows leaks and microbial ingress in storage bags used under controlled conditions.

We are working to correlate those detection limits to aerosol bacterial challenge tests, which are more representative of actual process conditions. Our patented aerosol bacterial

Figure 2: Robustness and closure integrity by design



challenge test allows more tests to be performed and thus provides for greater statistical validity.

Robust Single-Use Systems

The ability to identify when defects exist within single-use systems is, of course, only part of the problem. Suppliers of single-use technologies must take steps to reduce the number of defects in the first place. Designing robust single-use bags is the foundation for providing assurance of container–closure integrity (Figure 2). Sartorius Stedim Biotech has applied quality by design (QbD) principals to ensure that its bags will be robust across the manufacturing design space. The bags have been validated using the most stringent standards such as ASTM D4169 for shipping. And the company has introduced technology such as self-deploying bags to prevent mishandling and thereby reduce the risk of bag failures.

The risk of defects in films, seals, welds, and connections are reduced by stringent process controls. Sartorius Stedim Biotech carefully controls many process parameters, such as film-extrusion temperature and speed, weld and seal temperatures, and times. Quality control protocols confirm the absence of leaks in all single-use systems. For instance, the company routinely performs an ISO 15747 immersion bacterial-challenge test to confirm the microbial integrity of representative final products. A detailed understanding of failure modes that cause leaks from single-use systems helps focus continuous-improvement efforts on further reducing the number of bag defects.

Our detailed records are continuously updated, showing that of the million bags Sartorius Stedim Biotech produces each year, ~400 have defective bag chambers, most of which are identified before leaving the production facilities. Only 20 of those million bags have defects that lead to leaks at customer site — however, that is still too many. Our target for continuous quality improvement is to eliminate all leaks caused by production failures at Sartorius Stedim Biotech locations. A similar number of defects come from damage that occurs because of bag transportation or handling problems. Again, we see it as our responsibility to help reduce the number of such defects to zero. Point-of-use leak testing will help identify single-use systems that are not integral, but the defects themselves can be reduced by thorough package and liquid-shipping training, self-deploying technologies, and operator expertise.

For the benefits of single-use technologies to be realized fully within commercial biomanufacturing facilities, the industry must takes steps to understand container–closure integrity fully. We continue to conduct research into the links between bag defects, liquid leaks, and microbial ingress. This research is helping Sartorius Stedim Biotech to develop sophisticated integrity-testing strategies at its production facilities and for biopharmaceutical production sites. As part of these strategies, we will correlate our integrity tests to bacterial aerosol challenge tests. The ability to identify potential leaks before use is important for biomanufacturers; however, Sartorius Stedim Biotech is working hard on continuous quality improvements to further improve the robustness of its single-use systems.

Carole Langlois *is senior FMT product manager at Sartorius Stedim Biotech FMT.* ***Marc Hogreve*** *is senior engineer in integrity testing solutions at Sartorius Stedim Biotech.*

Single-Use Production Platforms for Biomanufacturing

by Miriam Monge

The pipeline of biopharmaceuticals remains strong, and the market for biologics could exceed US$450 billion by 2025. Analysts predict that sales within segments such as regenerative medicine and antibody–drug conjugates (ADCs) will grow faster than 20% each year. Yet considerable challenges remain for biopharmaceutical companies to overcome if they wish to be successful. First, they must reach the market quickly. Analysis by the Boston Consulting Group shows that the proportion of available value that a newly launched product can capture is a function of both the therapeutic advantage that it provides and its position in the launch of competing products. Being first to market is critical for commercial success of a new biologic.

Commercial risks inherent in developing new biological products have not gone away. Only a small proportion of such products that enter clinical assessment will make it through to a commercial launch. Although speed to market is important, companies must be careful to balance that need for speed against the risk that investments in process development activities will yield no return. It is a difficult balancing act. Cutting corners on development activities may allow companies to reach clinical phases quickly but also could result in them launching with poorly optimized production processes. Optimizing those processes after commercial launch is a very costly exercise because of regulatory obstacles that firms must overcome in making postapproval changes to manufacturing operations. When product sponsors enter the market with high operating costs, their profitability is reduced, making product sales vulnerable to attack from low-cost biosimilars. That is no longer a hypothetical situation: Regulators have been approving such products since 2013.

The implication is that senior executives from biopharmaceutical companies need to consider their approach to commercialization of biologics very carefully to reach the market quickly with efficient processes that deliver necessary product quality

Figure 1: Needs within a standardized platform process

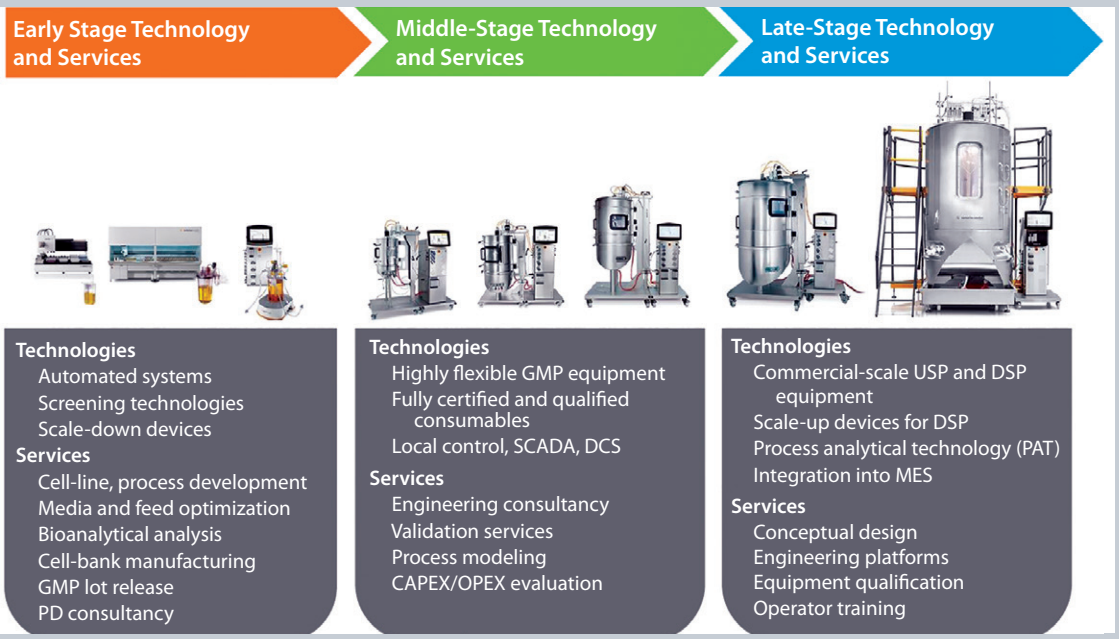


Figure 2: Implementation of single-use MAb platform from PD to commercial (EXCERPT FROM PRESENTATION AT 2017 ISPE ANNUAL MEETING IN BARCELONA, SPAIN, COURTESY OF SYNTHON)

Highlights

Multiproduct GMP facility to manufacture a pipeline of products

Flexible process design for performing either 500-L or 2,000-L batch sizes

Entirely end-to-end, single-use process with integration of chromatography systems from a third party

Key Success Factors

Collaboration between engineering teams

Engineering expertise and process know-how

Face-to-face meetings

Understanding limitations and drivers for decisions

Scalable platforms from development through to commercial scale

Timing: Order October 2014, delivery August 2015, first engineering run October 2015

with as low a cost of goods (CoG) as possible. They should find ways to harness the skills and expertise that their suppliers have accumulated over many diverse projects from different clients. Biomanufacturers can feed that knowledge into their own activities in early, middle, and late development and augment their own resources with those of their supply partners. A critical factor when adopting such an approach is understanding how the range of available technologies and services can be brought together to achieve a product sponsor's objectives successfully (Figure 1).

Biomanufacturers' Needs Change During Development

During early stage process development, high-throughput screening technologies can accurately predict the performance of large-scale equipment and thus be used to rapidly develop and optimize bioprocesses. In some cases, automation can allow engineers to generate large amounts of process data and accelerate development time lines. Our process development consultants work with clients to identify work packages that make efficient use of laboratory-scale tools. They apply process platforms for antibodies, vaccines, and ADCs that we have put together based on many years of experience working with clients involved in those segments. Process platforms save considerable time by largely defining unit operations and their sequence in advance. More important, however, is that adopting platforms significantly increases a drug developer's line of sight with detailed information on equipment requirements and likely costs of scaling up. By predicting future production needs, Sartorius Stedim Biotech process development consultants understand how to integrate critical services such as cell-line development and banking, media and feed development, and bioanalytical analysis into platform development to give customers a highly productive process in as little time as possible.

During middle-stage development, biopharmaceutical companies typically install flexible manufacturing capacity. Single-use technologies have been adopted widely for production of clinical supplies. The flexibility offered by such systems is invaluable to engineers fine-tuning production processes. Yet to prevent that flexibility from turning into costly complexity, biomanufacturers should configure their processes from predesigned consumables with robust supply chains. It is critical to ensure that technologies selected at this stage will be fully scalable to what a company anticipates its commercial scale of production to be. Sartorius Stedim Biotech single-use systems are scalable up to 2,000 L. Very often at that development stage, single-use systems will be controlled locally through SCADA systems.

As biomanufacturers prepare to launch their biologics onto the market, integrated production solutions become more important than the need for flexibility. Engineers must perform conceptual engineering designs and plan optimal layouts of production platforms within their facilities. Conceptual designs can be supported by process modeling programs such as BioSolve software from BioPharm Services. It helps

biopharmaceutical companies optimize the likely capital and operating costs of a facility. Integration of processing equipment into manufacturing execution systems (MES) is more common during preparations for commercial manufacturing, requiring suppliers to have automation expertise. They can provide additional support through system qualification, operator training, and maintenance services.

Single-Use Platforms for Commercial Manufacturing

Sartorius Stedim Biotech believes that customers who implement its end-to-end process platforms benefit significantly from investments the supplier has made in ensuring biocompatibility, integrity, and assurance of supply for its single-use technologies. Its range of standardized consumables reduces supply chain complexity and improves quality assurance. Recently, the company has invested in a data-analytics platform that allows customers to make the best use of both laboratory and process-scale data, gain additional insight, and improve process control.

In April 2017, Nienke Vriezen of Synthon reported at ISPE Europe on her company's implementation of a Sartorius Stedim Biotech single-use platform for production of monoclonal antibodies (MAbs) (Figure 2). The platform allows for production of different products, and its capacity is flexible depending on their production requirements. When product demand increases, Synthon can bring into operation two BIOSTAT® STR 2,000-L bioreactors. When smaller batches are required, the company can harvest from a BIOSTAT® STR 500-L bioreactor.

Vriezen described the full scalability of that Sartorius Stedim Biotech upstream platform from small-scale to the 2,000 L production scale. She also explained how much of her company's downstream process requirements could be met from a single FlexAct® system from Sartorius Stedim Biotech. It allows Synthon to perform depth filtration, adjustment, nanofiltration, and ultrafiltration/diafiltration (UF/DF) steps during antibody purification.

Intensified Platforms of the Future

The biopharmaceutical industry must continue to develop production platforms that bring down CoG so medicines can reach patient populations around the world. Fed-batch processing in single-use systems are very costly when final production quantities of product exceed 1,000 kg/year. Traditional six-pack stainless steel facilities are a risky investment because companies must start their construction before evidence of the likely product demand can be understood fully. Such facilities require engineers to scale processes up to 12,000-L or even 15,000-L bioreactors — with unpredictable results.

The ultimate goal might be to move to fully continuous processing, but such technology has yet to be demonstrated fully especially at large scales. Sartorius Stedim Biotech is investing in upstream development tools such as ambr® systems and BIOSTAT® B-DCU benchtop bioreactors to help customers design continuous and intensified upstream processes. Those can be scaled up to the BIOSTAT® STR range of single-use bioreactors that allow for cell cultures of very high density through excellent mixing and mass-transfer characteristics. The Integrated Solutions team can support customers implementing high–cell-density cell banking, $n - 1$ perfusion systems, and concentrated fed-batch production. KSep single-use centrifuges are ideal for robust clarification of high-density cultures.

Conclusion

Challenges remain for biopharmaceutical companies bringing products to market. Product sponsors must manage uncertainty while accelerating biologics into the clinic and then onto market. Bioprocesses must meet stringent quality requirements with efficiency to provide the lowest possible overall costs. Sartorius Stedim Biotech is helping clients address those challenges through end-to-end single-use production platforms. Those platforms are underpinned by a robust single-use supply chain to ensure that customers always can meet their commitments to healthcare providers and patients. Sartorius Stedim Biotech has incorporated data analytics to enable clients to make sense of the process data they collect and use the resulting knowledge to improve quality and efficiency further. With these industry leading tools, we can help bring treatments to patients with unmet clinical needs and supply safe medicines around the world.

Miriam Monge *is director of marketing for integrated solutions at Sartorius Stedim Biotech.*

Control of Critical Process Parameters Using In-Situ Analytics

by Dan Kopec

Biopharmaceutical companies can develop entire end-to-end single-use production platforms and use them for commercial manufacture of their biological products. Single-use facilities are flexible, can be implemented quickly, and do not require the large up-front capital investments needed for stainless steel equivalents. However, single-use facilities must be supplied with a large quantity of high-quality consumables. Biomanufacturers should pay close attention to their supply chains for those consumables to ensure that they are robust, integral, and fully compatible with biological expression systems and products.

Simply attempting to ensure that single-use manufacturing platforms provide equivalent performance to stainless steel facilities is a missed opportunity. Innovations in the fields of process analytical technologies (PAT) and data analytics allow engineers to gain greater understanding and control of single-use processes. Those innovations deliver the combined benefits of more consistent production of high-quality product with improved productivity. Here I explore innovative PAT tools for control of critical process parameters (CPPs).

Scientists and engineers can apply a number of methods for understanding conditions within bioprocesses. The approach a company adopts should reflect the context in which it wishes to implement a given analytical method. For example, off-line multiparameter analyzers can be linked to autosampling systems and provide significant insight into conditions inside bioreactors. Such a set-up is particularly useful during process development, when sampling and analysis often cause bottlenecks that reduce a company's ability to reach the clinic quickly. Autosampling and off-line analysis can be used to monitor nutrients and metabolites, automate cell counts, and provide some level of nutrient feed control.

Sartorius Stedim Biotech has integrated the Bioprofile® FLEX2 from Nova® Biomedical into its ambr® 15 automated, high-throughput microbioreactor system. This allows customers to monitor multiple process parameters with cell cultures during design of experiment (DoE)

Figure 1: Comparing BioPAT® Trace data collected in-situ with off-line glucose and lactate measurements; blue dot points to fixed-volume bolus dosages of Feed A.

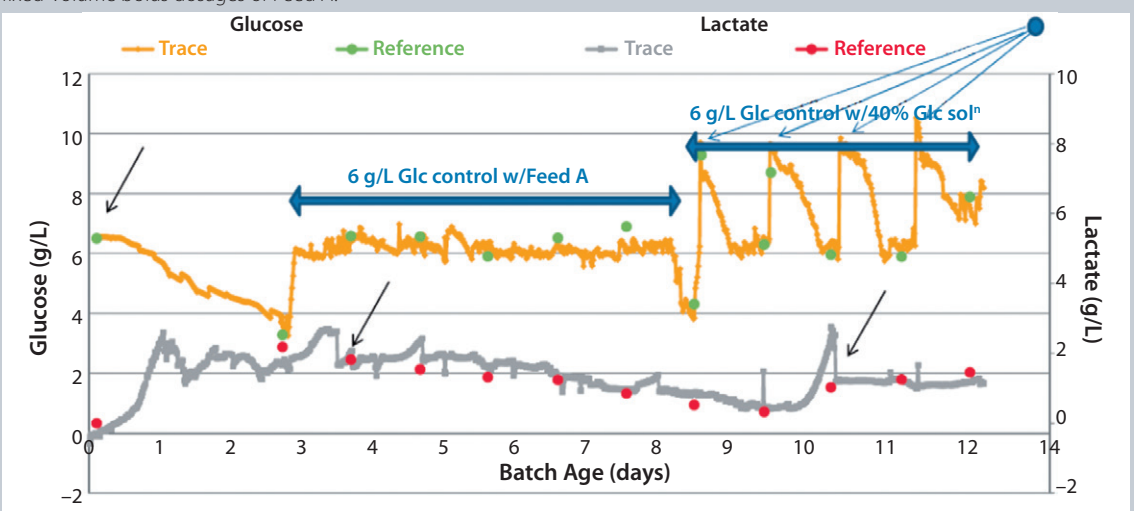
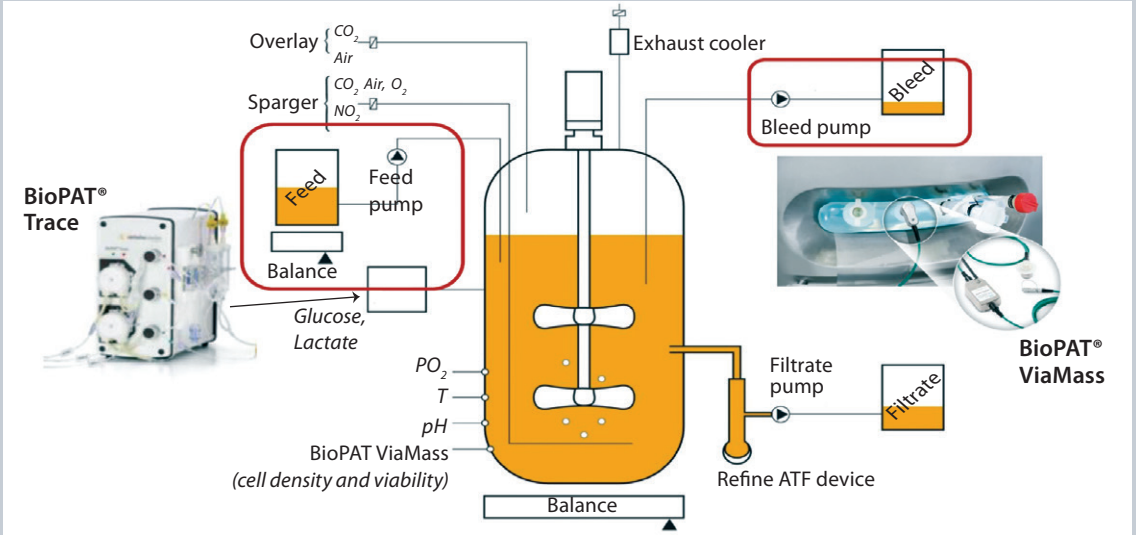


Figure 2: Enhanced control of a perfusion culture with the BioPAT® Trace and BioPAT® Viamass



studies and obtain data every six hours. Such an approach works well in laboratories but is difficult to implement into good manufacturing practice (GMP) environments because of the complexity associated with running these systems without increasing process risk. Furthermore, process parameters are not being monitored between samples, which limits the degree to which a process can be controlled and automated successfully. For example, controlling glucose at a concentration <1 g/L would be unachievable with this type of approach for the duration of a batch.

Bioprocess engineers historically have made good use of "soft sensors" such as glucose uptake rate, biocapacitance-based glucose control, and pH-based feed control to automate equipment even in GMP settings. Soft sensors provide an indirect, inferential, and predictive method of monitoring cell cultures when no direct method for measuring a specific parameter currently exists. These can provide visibility of a process to operators and allow some limited control. Soft sensors are particularly useful for verification of more advanced analytical approaches, which can be important in developing automation strategies. The disadvantage of soft sensors is that the models they rely on to estimate process outputs often are highly complex and susceptible to process variation.

There is considerable room for innovation in bioprocess PAT technologies that would overcome the limitations of off-line analyzers and soft sensors in GMP settings. Sartorius Stedim Biotech believes that the best way to control CPPs is to measure them directly and integrate such methods into equipment control systems with smart feedback algorithms for automated control loops. Bioprocess engineers need to scale and adapt technologies that are fully capable of measuring CPPs to all necessary bioreactor formats. New PAT tools should provide real-time sensing capability with an in situ analyzer that can provide rapid feedback and maximum control. They must be installed into single-use systems without breaching integrity and causing system sterility loss. Finally, the integration of such sensors into plantwide control systems or data historians should be as simple as possible for customers through plug-and-play technologies.

Sartorius Stedim Biotech has developed two new PAT technologies that meet those criteria: The BioPAT® Trace allows on-line measurement and control of glucose and lactate, and the BioPAT® Viamass capacitance probe measures viable biomass within cultures.

BioPAT® Trace: Controlling glucose and lactate levels in bioreactors maintains the health of cell cultures and ensures desired glycosylation profiles of biopharmaceutical products. Figure 1 compares BioPAT® Trace data collected in-situ with off-line glucose and lactate measurements. Companies can use the BioPAT® Trace with 500-mL to 100,000-L single-use or stainless steel bioreactors based on rocking motion or stirred-tank designs. It can allow nearly constant glucose feeding at concentrations <0.5 g/L. Control in that range is ideal for perfusion cell cultures, in which excessive glucose availability can lead to efficiency loss and diminished yields.

Investigators at Biogen Idec have increased product titers by >30% with a fed-batch Chinese

hamster ovary (CHO) cell culture process by installing a BioPAT® Trace with automated feedback control into 3-L glass bioreactors. The process had given low yields previously because of high lactate levels. The BioPAT® Trace monitored glucose and lactate concentrations every 15 minutes, a frequency of sampling that was not possible using an off-line analyzer, with which sample-volume requirements would have been prohibitive at such a small scale. The BioPAT® Trace provided more responsive control than would have been possible with a pH-based strategy because the medium buffer delays the pH response to lactate change, making it difficult to control around a lactate set-point.

Researchers at the Massachusetts Institute of Technology (MIT) scaled up an FRhK-4 roller-bottle culture to a microcarrier culture perfusion system using a BIOSTAT® B Univessel benchtop bioreactor from Sartorius Stedim Biotech. They used a BioPAT® Trace for the real-time monitoring of glucose concentration. Spent media were removed from the culture once glucose concentrations dropped <1 g/L. A level probe detected the resulting loss in volume and initiated feeding of the culture with fresh media.

BioPAT® Viamass: Viable biomass is a parameter that cell culture specialists use to visualize the trajectory of cell growth. It allows operators to monitor processes and detect deviations from an expected batch trajectory. Biomass levels also can be used to control feed addition, monitor and control perfusion cultures, and detect the optimum point for harvesting bioreactors. For downstream processing, engineers can use biomass levels in their bioreactors to size clarification technologies such as diatomaceous earth filtration.

The BioPAT® Viamass works with a broad range of bioreactor scales and formats. It helps engineers improve the productivity of their bioprocesses by controlling bioreactor bleeding when implementing continuous bioprocessing. A control loop limits cell densities to a desired value and prevents overgrowth. A peristaltic pump removes cells through a dip tube. An increase in viable cell density, as measured by the BioPAT® Viamass, defines the flow rate at which that occurs. For enhanced control of perfusion cultures, engineers can install both a BioPAT® Trace and BioPAT® Viamass for controlling feed pumps and bleed pumps together (Figure 2).

Conclusion

Here, I have shown how bioprocess engineers can improve control of their bioreactors using dedicated, real-time, on-line sensors. These will allow the industry to develop more controlled and automated single-use platforms that deliver consistent product quality and improve productivity. Novel PAT tools set the foundation for advanced process control strategies using multivariate data analysis. The next article explores this subject in greater detail.

Dan Kopec *is technology expert in process analytics at Sartorius Stedim North America Inc.*

Prescriptive Analytics for Bioprocessing Platforms

by Chris McCready

The preceding articles have shown how biopharmaceutical companies increasingly are adopting single-use manufacturing technologies for commercial production facilities as their confidence grows in the material science, supply chains, and robustness of single-use systems. Single-use suppliers can support adoption of end-to-end process platforms in commercial manufacturing settings with dedicated teams of experts in process development, engineering, and regulatory support. Advances in process analytical technology (PAT) are providing engineers with greater information on conditions within their single-use bioprocess platforms to allow for increasing levels of control.

Engineers can define set points for important parameters such as pH, temperature, and dissolved oxygen to provide consistent operation, use PAT tools to measure necessary parameters such as glucose and cell density, and then use regulatory controllers to maintain those parameters at the predefined set points. Some problems come with using this approach in isolation. First, each parameter is controlled independently from the others. Controlling to set points does not necessarily ensure optimum quality or productivity outcomes, nor is the control mechanism updated with new information process variability. More sophisticated control strategies could bring more productive processes and more consistent product quality. The biomanufacturing sector needs the ability, not just to understand what is happening in a bioprocess (triggering an action), but also to understand what will happen in the future based on current conditions. If needed, interventions can be made to modify this future state.

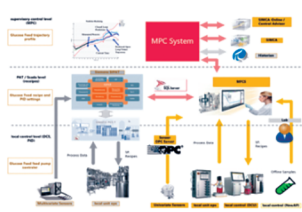
Model predictive control (MPC) is a supervisory control method used to determine optimal set points for regulatory controllers described above for optimizing qualities and yields. For example, a regulatory controller can be used to adjust nutrient flow to maintain glucose at a desired set point, and an MPC controller can be used to determine the optimal glucose set point to maximize cell productivity or final titer. As the name implies, MPC requires a process model relating the

Figure 1: Stepwise implementation of model predictive control for a cell culture process

Experimentation	Data Analysis	Deployment
Automated DoE for Process Definition	**Model Development**	**Comprehensive Automation Platform**
Generate data sets for model predictive control design	Recipe optimization Define optimal nutrient and other parameter profiles	Base regulatory control
Multiple simultaneous experiments run in parallel	Build supervisory models for MPC Forecasting	Advanced process analytical technology (PAT) control
Scalable geometries	Predictive analytics	Recipe control
Fully automated at small scale		Supervisory model predictive control

ambr system for automated DoE

SIMCA with consulting services



influence of regulatory control set point adjustments to cell productivity (or any other control objective).

As Figure 1 illustrates, Control Advisor software in the SIMCA-online program from Sartorius Stedim Biotech Data Analytics (formerly Umetrics) uses a patented imputation method based on partial least squares regression to provide that predictive ability for batch type systems. Using such an MPC set-up, it is possible to predict the future trajectory of a batch based on data from the current time point and change parameter set points to deliver required quality and performance.

Take, for example, the case of a 14-day cell culture process running in a bioreactor. It is possible to ascertain within the first couple days how the growth profile is likely to look over the remainder of the culture. This is because cells reveal their nature quickly, so it is possible to understand how they are growing and responding to the bioreactor environment right at the start of a run. With that information, a predictive model can calculate final conditions (e.g., titer and viable cell density) in the bioreactor. It then uses an iterative process to explore the effects of changing variables that can be easily manipulated (e.g., set points) until it identifies values for them to give the optimal outcome. Such a forecasting method allows for process deviations to be diagnosed and detected before they occur so the system can make corrective interventions.

MPC control systems are most effective when users "train" them with information-rich data. The ambr® 15 and 250 systems are ideal tools for generating cell culture data sets for MPC design. They enable rapid and straightforward generation of 16-run training data sets by running multiple cell cultures in parallel with design of experiments (DoE) methodologies to introduce structured variation. The ease with which those systems can be scaled up to a commercial BIOSTAT® STR platform ensures that data generated at small scales will be relevant at large scale.

Conclusion

The biopharmaceutical industry is becoming increasingly confident in single-use bioprocessing technology, and this confidence allows it to implement such systems into the most critical applications within the commercial biomanufacturing environment. In the future, by combining data-driven analytics with mechanistic modeling, we will be able to improve significantly the "observability" of key quality parameters in single-use process platforms. We are seeing a real pull from the bioprocessing community to consider new ways of thinking about data and implement deep learning, machine learning, and artificial intelligence into Sartorius Stedim Biotech platforms. The future is exciting indeed.

Chris McCready *is lead data scientist at Sartorius Stedim Data Analytics.*

Final Thoughts

by Nick Hutchinson

The development and launch of new biopharmaceutical products is a very challenging and risky process. Sponsors must get their products into clinical testing as quickly as possible to beat their competition and take the greatest share of the market. Yet that need for speed must not lead to companies launching products with inefficient manufacturing processes that ultimately will leave them vulnerable to attack from low-cost competition. Single-use systems have been a significant enabling technology for companies developing new biologics because of their inherent flexibility and low upfront costs, which help mitigate financial risk during process development.

As the technology has matured, more companies are becoming interested in widespread adoption of single-use systems even in commercial production settings. That would allow them to take advantage of the same flexibility of single-use technology while creating agile manufacturing networks that can respond to variations in demand, readily produce multiple products with fast changeovers, and facilitate new product introductions in the future.

Over many years, Sartorius Stedim Biotech (SSB) has demonstrated leadership in single-use technologies for biomanufacturing. We have gained considerable expertise from working with the world's foremost biopharmaceutical companies and have obtained considerable insight into the needs of the bioprocess industry. This has driven us to invest in detailed characterization of the materials and processes we use to provide our clients with highly robust single-use systems underpinned by equally robust supply chains.

As you can see from this report, the analyses we have performed are helping to ensure that the materials we use are compatible with our customers' biological products and cell lines. The original research we have conducted into the mechanisms by which microbial ingress can occur is helping us detect, avoid, and eliminate leaks within single-use platforms to provide customers with the highest levels of confidence when they install our systems into applications that are critical to patient safety.

We are proud to have the industry's most comprehensive offering of single-use technologies and services. They allow for end-to-end processing of monoclonal antibodies (MAbs), vaccines, antibody–drug conjugates (ADCs), and regenerative medicines at commercial scales — from out-of-freeze through final fill and finish. SSB will support you during every phase of the development and implementation of these standardized platforms with teams of experts such as our process development consultants, application specialists, process engineers, and validation services. Biomanufacturers that install our single-use platforms will benefit from an unprecedented ability to control critical process parameters thanks to our novel process analytical technologies (PAT) that allow for real-time, on-line process monitoring and our data analytics software for predictive bioprocess control.

Advances in single-use production platforms are expediting their adoption into commercial biologics facilities. Biopharmaceutical companies are gaining a competitive edge with highly optimized and flexible processes that they can implement rapidly, secure in their knowledge that the supply of necessary production technologies and materials will be assured. When combined with SSB's initiatives in intensified and continuous bioprocessing, these will allow the bioprocess industry's single-use facilities of the future to provide the highest production throughputs at the lowest costs.

Nick Hutchinson *is technical content manager at Sartorius Stedim Biotech.*