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Internal and External Challenges to Biopharma in 2019

Good news about a record-setting number of new drug approvals in 2018 was tempered by financial and political pressures external to the industry. Questions on Pharma's 2019 agenda include pressure to lower drug prices and contain costs, and deal with a volatile investment market and contentious federal government.

In the annual GlobalData outlook report (1), 51% of respondents said drug pricing and reimbursement constraints will have the greatest negative impact on the biopharmaceutical industry in 2019. Many drug companies kicked off 2019 with price increases for more than 100 drugs averaging 6.3%.

Investment options

The investor market in 2018 ended on a downward trend. The Vantage 2019 Preview report (2) said it was difficult to predict how much further the market may fall; those interviewed for the report expected a more volatile year.

Companies looking for funding will have to work harder in 2019, but the report says financing options are not expected to dry up. While there is enthusiasm over new therapies, these products must prove commercial success to maintain investor interest in biologic products, the report said.

A Deloitte report (3) said the majority of US companies were interested in investing in research and development (R&D), business operations, and capital projects, including some US operations, as a result of the corporate tax reforms of the Tax Cuts and Jobs Act enacted in December 2017. The biopharma executives surveyed said they were likely or very likely to invest in R&D (67%), capital projects (57%), general business operations (50%), share buybacks (50%), and M&A (42%). Lower priority investments were compensation/pension funding (40%) and hiring (40%).

The 2016 presidential election and tax reform debate of 2017 put some mergers and acquisition activity on hold, according to the Deloitte report; however, deals picked up in the first half of 2018.

In three weeks spanning the end of 2018 and start of 2019, Pfizer and GlaxoSmithKline announced a merger of their consumer healthcare products businesses; Eli Lilly announced the acquisition of Loxo Oncology for $8 billion; and Bristol-Myers Squibb announced it will acquire Celgene for $74 billion.

Restructuring FDA?

While the pharma industry—and the rest of the nation—awaited a resolution to the partial government shutdown, a white paper (4) based on input from seven former FDA commissioners, recommended FDA be reconfigured as an independent federal agency. A new structure is needed, they argued, to promote science-based decisions, increase transparency, streamline processes for developing regulations and guidance documents, and allow for more responsive and predictable decision making.

The paper stopped short of recommending a new model for agency operations but defined several major shortcomings of current operations. With federal government operations on hold over political squabbling, it was nice to see constructive efforts to address faulty practices.

References
It will be a challenge for FDA to match or exceed its success this past year in approving record numbers of innovative and generic drugs. These achievements reflect the ability of biopharma companies to capitalize on important advances in science, as well as strong support from regulators in clarifying requirements and R&D policies. While the Center for Drug Evaluation and Research (CDER) will strive to further enhance its operations, it also will support initiatives to ensure that all medicines are safe, effective, and of high quality.

Amidst these advances, CDER Director Janet Woodcock cites the importance of addressing the nation’s deadly opioid epidemic as FDA’s top priority for the coming year. The immediate need is to reduce the more than 200 million outpatient prescriptions for these drugs each year. Recently enacted legislation (1) instructs FDA to develop evidence-based prescribing guidelines and to explore how manufacturers can provide pain medicines in more secure packaging, Woodcock pointed out at the FDA/CMS Summit in December 2018. At the same time, FDA will develop guidelines on developing non-opioid medications for acute and chronic pain to improve treatment for patients.

ENSURING QUALITY
Woodcock also aims to advance the safety and quality of medicines. CDER has launched a two-year program to improve oversight of drug safety, featuring new methods to evaluate the more than two million adverse event reports received this year on marketed drugs. The agency is pressing drug companies to comply more fully with good manufacturing practices (GMPs) through more targeted inspections and recalls for those failing to meet standards. Drug compounding pharmacies and outsourcing facilities will remain in the spotlight in 2019, as FDA inspectors continue to find violations at these operations. A related initiative is to continue to implement requirements for tracking drugs through the supply chain to detect unauthorized medicines, an effort designed to have “a big impact on the gray market,” Woodcock commented. CDER’s Office of Pharmaceutical Quality (OPQ) will continue to seek more timely inspections of manufacturing facilities, a process that should be facilitated by a new inspection protocol for drugs, beginning with sterile drug manufacturing facilities.

These efforts may be enhanced by visible progress in industry implementing advanced manufacturing systems. Woodcock noted at the Summit that five firms have filed applications with continuous manufacturing components, and that generic-drug makers are moving in this direction. Other federal agencies support such efforts as a way to
Gene Therapy Holds Promise for Blood Disorders

Researchers at the University of Delaware (UD) have made a step forward in gene therapy by engineering microparticles that deliver gene-regulating material to hematopoietic stem and progenitor cells, which live deep in bone marrow and direct the formation of blood cells (1).

In a paper published in the journal Science Advances on Nov. 7, 2018, Chen-Yuan Kao, a doctoral student in chemical engineering, and Eleftherios T. (Terry) Papoutsakis, Unidel Eugene du Pont chair of Chemical and Biomolecular Engineering, describe how they used megakaryocytic microparticles to deliver plasmid DNAs and small RNAs to hematopoietic stem cells (2).

Some previously developed methods to target these stem cells deliver genetic material with help from a virus, risking side effects to the patient, Papoutsakis said. Instead, the research team developed a method that takes advantage of tiny particles that already float in the bloodstream called megakaryocytic microparticles. The researchers found that they could load these microparticles with gene-regulating material and that they would infiltrate only the desired stem cells because of distinctive properties on the surface of the microparticles.

According to the university, with more development, this technology could be useful in treating inherited blood disorders such as sickle cell anemia, which causes abnormally shaped red blood cells, and thalassemia, which disrupts the production of the blood protein hemoglobin. The methods developed by the researchers could also be used to deliver personalized medicine because the microparticles can be individually generated and stored frozen for each patient, said Papoutsakis.

“A lot of researchers are trying to deliver DNA, nucleic acids, or drugs to target hematopoietic stem cells,” said Papoutsakis in a university press release. “This is the right cell to target because it gives rise to all blood cells.”

In theory, altering those cells would allow for the prevention of the genetic defect for most or all of the patient’s life, as stated by the university.

Currently, Papoutsakis is collaborating with Emily Day, an expert in nanomedicine and assistant professor of biomedical engineering at UD, to explore more ways to deliver this material.

References

Brain Glial Cells Reprogrammed into Working Neurons

A new gene therapy can turn certain brain glial cells into functioning neurons, which could help repair the brain after a stroke or during neurological disorders like Alzheimer’s or Parkinson’s diseases, according to researchers at Penn State (1).

In a series of studies done in animals, the team, led by Gong Chen, a professor and Verne M. Willaman chair in Life Sciences, developed a new gene therapy to reprogram glial cells to turn into healthy, functioning neuron cells. In addition to neurons, the human brain is composed of glial cells, which surround each neuron, help support healthy brain function, and can be activated when neurons die. Chen said each glial cell contains neural genes that are silenced, or switched off, during early brain development.

By creating a new in-vivo cell conversion technology, Chen said he and his team were able to inject a neural transcription factor called NeuroD1, a protein that activates neuronal genes and silences glial genes, within injured parts of the brain to infect glial cells. NeuroD1 then binds with the glial cell’s DNA and activates the neuron genes, turning the glial cell into a functioning neuron.

“This is an economic way of internal neuroregeneration without the need to transplant external cells,” Chen said in a university press release. “Because glial cells are abundant throughout human brains, every patient is equipped with such potential for internal neuroregeneration that has not been fully realized yet.”

Chen said that in their animal studies, they were able to not only regenerate neurons with the new technique, but also restore motor and cognitive functions.

“Our new technique is different in that it actually regenerates neurons after they’ve already died, and can be used days, weeks or months after injury.”

While the technology has only been tested in animals, the team hopes to eventually test the technology in a clinical trial, although trials in humans may be years away. When a patient experiences an injury like a stroke, or develops a neurological disorder like Alzheimer’s, neurons in parts of the brain die, creating a decline in brain function. Chen believes that because adults do not have the ability to regenerate neurons on their own, developing a treatment to help patients make new neurons would benefit a large number of patients experiencing neurological disorders that are currently incurable.

“Neuronal loss is the common cause of these functional deficits in the brain and spinal cord,” Chen said. “Therefore, simply targeting cell signaling pathways affected by these neurodegenerative disorders without regenerating new neurons will not be most effective to restore the lost brain functions.”

Additionally, the researchers are also working on a drug therapy that converts human glial cells into neurons. The team has reported success with the drug therapy in-vitro in cell cultures, and Chen said they hope to move to animal studies in vivo and eventually to help human patients.

Reference
Biopharmaceutical industry-related indicators and trends are supporting continued incremental increases in outsourcing of pre- and commercial API and biopharmaceutical product manufacturing services to contract manufacturing organizations (CMOs). BioPlan Associates’ survey of bioprocessing professionals overall confirms that outsourcing to CMOs is being viewed increasingly as a desirable option (1). Routine services and products with smaller markets will be outsourced more frequently, and products requiring novel bioprocessing, where expertise or capacity remain limited, will also be outsourced more frequently, such as cellular and gene therapies. Most innovator drug companies continue to retain manufacturing and development of their prospective blockbusters in-house.

The financial outlook for CMOs is solid, with growth in revenue tracking that of the overall biopharmaceutical sector, which consistently grows at more than 12% annually. CMOs continue to expand capacity, staff, etc., to try to retain clients as their products advance in development. Total annual biopharmaceutical CMO revenue was approximately $3.4 billion in 2018 and is expected to grow to about $3.8 billion in 2019. Biopharmaceutical CMOs remain a relatively small niche, however, with chemical substance-based drug outsourcing revenue more than 10 times that of the biopharmaceutical sector.

Despite being just a small part of the overall biopharmaceutical industry, CMOs play important roles. CMOs currently commercially manufacture approximately 30% of marketed mainstream (recombinant) commercial products, although this remains concentrated among a few, large-capacity CMOs. Most CMOs primarily support R&D and early-phase support—and only a minority performing commercial manufacturing—so, in general, CMOs’ involvement in earlier-phase aspects of product development and manufacturing is even larger than with commercial manufacturing. CMOs also play an important role in new bioprocessing technology development and adoption, with CMOs often the first (compared with developer companies) to implement new technologies. CMOs often have much more technical expertise than developer companies, with CMOs having worked on more products/projects and using a wider variety of technologies.

A decade or more ago, Big Pharma and other well-established developer companies outsourced as much work as possible to CMOs, often without critical analysis. Most biopharmaceutical companies traditionally turned to outsourcing to control costs and/or manage their internal staff and resources. Biopharma companies are now increasingly taking a more strategic view of outsourcing and seeking to outsource as much as possible. Most companies periodically re-evaluate their core competencies and rationally decide how they will manage their R&D, manufacturing, and related outsourcing. As a result, almost every area of R&D and manufacturing is considered a candidate for outsourcing (1).

The number of products in development continues to grow, with this carrying over to CMO outsourcing. FDA approved approximately 25 new biopharmaceuticals in 2018 (1), and the number of annual approvals is expected to increase in coming years, as new classes of products receive approvals, particularly biosimilars and cellular and gene therapies. In the next five years, the number of approved biosimilars will exceed the number of approved mainstream products.
The evolving mix of biopharmaceuticals in the development pipeline is also expected to increase outsourcing to CMOs, because CMOs often have more capabilities and expertise in new areas of bioprocessing than developer companies. Product types, classes, and the underlying molecular structures of products in R&D continue to diversify. Rather than just familiar-type recombinant proteins and monoclonal antibodies, CMOs are often the pioneers in terms of manufacturing novel products, including antibodies with novel core structures/backbones, antibody-drug conjugates (ADCs), cellular therapies, gene therapies, RNAi, pegylated proteins, and other novel types of products. Also, lesser innovative classes of products are often outsourced; CMOs report a 15% increase in business in recent years from biosimilars projects.

Successful CMOs continually expand capacity and staff/expertise. The industry is starting to see a major trend for CMOs adding 1000–2000-L single-use bioreactor process lines for commercial manufacturing as products currently in development manufactured with single-use systems advance to approvals. In the past year, BioPlan has identified approximately 180,000 L of single-use systems with capacities greater than 1000 L added as CMO expansions or new facilities (2). As CMOs develop this ‘entry-level’ commercial scale single-use capacity, the number and percentage of marketed products commercially manufactured by CMOs will further increase, at the expense of stainless steel-based bioprocessing.

**SURVEY DATA AND TRENDS**

Developers generally outsource a higher percentage of projects/products involving mammalian vs. microbial expression systems. The historical picture of respondents to the annual BioPlan survey reporting that they do not outsource bioproduction is shown in Figure 1. In 2018, 30% of survey respondents reported no mammalian outsourcing, meaning that 70% outsourced at least some of their mammalian projects/products; 43% reported no (57% reported some) outsourcing of microbial work. The percent not outsourcing of any mammalian or microbial work has generally decreased (i.e., percent reporting some outsourcing has increased) since 2006. Comparable small portions, approximately 15%, report outsourcing the majority of their mammalian and microbial work. Growth and trends in outsourcing of microbial work remain

**Contin. on page 40**
Identifying Incentives to Adopt Advanced Manufacturing

Bio/pharma companies are successfully launching novel therapies; however, the industry still needs to work on manufacturing innovation.

RITA PETERS

A 2003 Wall Street Journal article noted a pharmaceutical industry “secret;” as the industry developed “futuristic new drugs, its manufacturing techniques lag far behind those of potato-chip and laundry-soap makers” (1). Since that article was written, the introduction of quality-by-design practices, new monitoring technologies, and advances in science and engineering improved some bio/pharma manufacturing processes. In too many cases, however, manufacturing and quality practices are still inadequate or too expensive.

The number of drugs approval by FDA—as well as innovative “firsts” designed to address patient needs—accelerated in the past two years. Following the 2017 approvals of the first cell therapies and gene therapies, 2018 saw the approval of new treatments for infections, the first novel antiviral treatment for the flu in 20 years, the first drug with an indication for treatment of smallpox, the first non-opioid treatment for the management of opioid withdrawal symptoms, and the first drug derived from marijuana.

The upswing in approvals of novel therapies indicates that bio/pharma companies are embracing innovation in R&D. However, analysts report that R&D efforts show diminishing returns. And, the ongoing pace of FDA warning letters, observations, recalls, and drug shortages demonstrates that the bio/pharma industry still has work to do to achieve efficient, cost-effective manufacturing, quality, and analysis to ensure a safe, sufficient drug supply.

A Deloitte/GlobalData study (2) reported that R&D returns at 12 Big Pharma companies hit 1.9% in 2018, the lowest level in nine years, down from 10.1% in 2010. Costs to bring a biopharmaceutical drug to market have almost doubled since 2010 from $1.18 billion to $2.18 billion while forecast peak sales per asset have fallen from $816 million in 2010 to $407 million in 2018, the report notes.

Four small, specialized biopharma companies analyzed for the Deloitte report fared better than the Big Pharma companies, posting returns on R&D of 9.3%; higher development costs ($2.8 billion) were offset by higher anticipated sales ($1.17 billion).

In light of ongoing criticism of the high cost of drugs, raising prices may not be the financial answer; bio/pharma companies need to find innovative ways to improve efficiencies.
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WARNING SIGNS
In 2016, the number of warning letters issued by FDA for good manufacturing practice infractions doubled and maintained that higher level in 2017 and 2018. The number of drug products recalled by FDA’s Center for Drug Evaluation and Research (CDER) has declined in the past two years; however, the number of recall events increased to the second highest level in the past 10 years (3). While efforts over the past five years have cut the number of new shortages from 251 in 2011 to 35 in 2017, as of Jan. 3, 2019, FDA listed 114 drugs as “currently in shortage” and 207 as discontinued (4).

Recalls of angiotensin II receptor blocker (ARB) drug products from multiple manufacturers—due to the presence of probable human carcinogens traced to API manufacturers—were launched in 2018 and continued in early 2019.

Frustrated with the high cost and chronic shortages of medicines used in hospitals, seven health organizations representing 500 US hospitals formed Civica Rx, a not-for-profit generic drug company; the new organization plans to manufacture 14 hospital-administered generic drugs either directly or through contract manufacturing organizations (5).

The Deloitte study authors stressed the need for a “transformational change” in R&D productivity that uses technology—artificial intelligence, robotic process automation, natural language processing and generation, and machine learning—to replace or augment work done by humans. Partnerships, collaborations, and non-traditional operational models will be necessary to compete in a digital environment and companies will have to compete with non-pharma organizations for the technical talent needed to operate a digitally-driven company.

Many of the predicted applications of information technology focus on drug discovery, clinical trial, and patient monitoring applications. There are, however, opportunities to adopt other new technologies for drug formulation, process development, manufacturing, and supply chain phases.

ADVANCED MANUFACTURING TECHNOLOGY NEEDED
While bio/pharma companies have demonstrated success at turning out new therapies, the uptake of advanced manufacturing technologies to produce these products, or better ways to manufacture existing products, has been slower.

Manufacturing issues, delays or capacity issues, and loss of manufacturing site account for two-thirds of the causes of drug shortages, FDA reports (6). Capital costs, lost production time for retrofitting, and the need for regulatory review for process changes deter some companies from investing in new technologies for established product lines. For new drug products, the race to get the drug to market is incentive to stick to proven technologies that may not be efficient or cost-effective in the long term.

Some advanced manufacturing technologies—such as continuous manufacturing for solid-dose drugs, 3D printing of drug products, and single-use bioreactors—have demonstrated their effectiveness. Recent FDA approvals of continuous manufacturing processes for oral solid-dose drugs has spurred interest and innovation for advanced manufacturing processes. The Engineering Research Center for Structured Organic Particulate Systems (C-SOPS) at Rutgers University (NJ) works with industry and FDA to modernize pharmaceutical manufacturing processes focusing on continuous processing with predictive control and the next generation of dosage forms.

“THERE is a lot of technology out there, but the largest challenge remains in bringing it all together in a timely and cost-effective manner,” says Douglas B. Hausner, associate director of C-SOPS. “This is a challenge with modern advanced methods that have greater complexity and require greater upfront effort and investment to then run smoothly thereafter. Much of this pertains to software, sensor, and control integration. Some companies are looking to form partnerships and ‘pre-integrate’ where possible to minimize this.”

Many technological advances and materials are needed to reduce the costs of biopharmaceutical manufacturing, says Ruben G. Carbonell, chief technology officer at the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), a public-private partnership that advances biopharmaceutical manufacturing innovation and workforce development. In-line process analytical technology (PAT) for product quality and process control is one of the areas of greatest need, to enable continuous and hybrid processes, he says.

Automated bioreactors and purification processes for cell-therapy manufacturing; rapid adventitious agent (AA) detection and novel materials for viral clearance; and other technologies to reduce release times for biotherapeutics are other areas of need. The availability of advanced technologies does not necessarily mean they are widely adopted, however. For example, “significant advances are being made in rapid AA detection utilizing next generation screening that are yet not being broadly throughout the industry,” Carbonell says.

THE FEAR OF BEING FIRST
Bio/pharma companies operate in a regulated environment and face pressures to control end-product prices while driving investor return on costly R&D efforts. The tendency to take a conservative approach to the adoption of new technologies or materials is not surprising.

“There are significant efforts within large biopharma companies and suppliers in the testing and evaluation of novel approaches to cost-effective manufacturing,” says Carbonell. “Adoption of these technologies has been slow because of perceived risks of not being approved for new products or processes.”

Hausner notes that when assessing new technologies or processes to advance
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Many new technologies are complex and require additional expertise often in the form of new hires or at least vendor/consultant relationships. There needs to be true organizational buy in for an advanced integrated technology. It can be much closer to buying or building a new plant than a new piece of hardware like a tablet press.

“The bottom line is this: drug makers won’t switch to these systems until we create a clear path toward their adoption and provide more regulatory certainty that changing over to a new manufacturing system won’t be an obstacle to either new or generic-drug approvals,” wrote Gottlieb. “The FDA recognizes that it’ll require additional investment in policies and programs that’ll provide regulatory clarity to enable these new methods to be more quickly and widely adopted.”

The fiscal year 2019 budget includes $58 million to accelerate the development of the regulatory and scientific architecture, Gottlieb wrote. In August 2018, the agency issued nearly $6 million in grants to study improvements to continuous manufacturing, monitoring, and control techniques (8). An additional $2.4 million in grants were awarded to six universities to investigate improvements to continuous manufacturing for biologic-based drugs (9).

MORE COLLABORATION AND RESEARCH

Although the complexities of drug manufacturing may exceed that of other industries—including snack foods and laundry soaps—can bio/pharma learn from other industries about adopting new technologies?

Pre-competitive efforts have paid off in other industries, explained Carbonell. “Years ago, the microelectronics industry created large, pre-competitive non-profit consortia of manufacturers, academic and research institutions, and government agencies to evaluate new technologies, such as SEMATECH, to standardize and harmonize equipment, connectors, and measurement devices and approaches,” he said. “These efforts played a key role in reducing costs and advancing the chip manufacturing industry in the US.”

“Pre-competitive industry organizations play a major role by helping to inform the public consensus on new technology. This aids in regulatory understanding, acceptance, and approval,” says Hauser. “These groups perform a soft harmonization on technology and nomenclature ahead of more official groups like regulatory bodies and standard-setting organizations.”

“Industry-university-non profit-government consortia such as NIMBL can play a key role in accelerating the adoption of novel manufacturing approaches. New technologies that show promise in the laboratory can be tested and de-risked in an industrial setting taking into account regulatory expectations,” said Carbonell. “These consortia reduce the costs of technology development and significantly reduce the risk to an individual company of developing a new approach that may not be approved because it is not broadly accepted.”

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Compensation and professional challenges are key pieces to solving the biopharma employee satisfaction puzzle.

RITA PETERS

The biopharma industry has found itself under political and societal microscopes in recent years, as executives search for answers to criticisms about high drug prices, shortages of vital therapies, and the industry’s role in the opioid epidemic. Meanwhile, a record number of new drug approvals and the emergence of innovative therapies demonstrate the potential of bio/pharma R&D efforts.

Bio/pharma development and manufacturing functions rely on skilled and knowledgeable workers. Hiring and retaining this expertise should be a top priority. With the US unemployment rate at record lows, the career opportunities for US-based bio/pharma employees should be promising. Insight provided by respondents to BioPharm International’s annual employment survey (1) suggests that employee satisfaction is tied not just to compensation, but also to the challenges presented by the work and the employer’s potential for success. (See the infographic on pages 18–19 for an overview of the survey results.)

In one indicator, salary ranked only seventh on a list of 12 factors contributing to job satisfaction. Intellectual stimulation and challenging projects were the leading “main reasons I come to work,” followed by a good work/life balance, the company’s potential for success, job security, and good benefits.

Nearly 75% of the respondents, however, said insufficient budgets and resources to accomplish a task contributed to job dissatisfaction. Nearly 70% said issues with management, timelines that are too short, and uncertainty about the company’s performance contributed to job dissatisfaction.

SURVEY RESPONDENT PROFILE

The annual survey, fielded in November and December 2018, represents more than 250 responses from the United States (72.2%), Europe (18.9%), Asia and India (10.4%), and other regions. Respondents predominantly were full-time, permanent employees (80.1% of respondents) at innovator biopharmaceutical companies (29.4%), generic-drug manufacturing companies (8.6%), academic institutions (10.2%), and contract research and manufacturing organizations (12.2%).

The respondents represented companies that develop or manufacture large-molecule drugs, vaccines, and cell therapies or gene therapies; more than half reported that their companies are also involved in small-molecule drug development.

Text Contin. on page 20
### Special Report: Employment Survey

#### How secure do you feel in your job compared with last year?

- **2018:** 54.5%
- **2017:** 43.8%

#### Does your current salary reflect a change over last year’s salary?

- **Increase:**
  - **2018:** 50%
  - **2017:** 50%
- **Decrease:**
  - **2018:** 6.1%
  - **2017:** 6.6%
- **No change:**
  - **2018:** 43.9%
  - **2017:** 37.4%

#### Please rate your satisfaction with your current salary.

<table>
<thead>
<tr>
<th>Response</th>
<th>2018</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am paid below market value, considering my level of expertise and responsibility.</td>
<td>18.1%</td>
<td>23.5%</td>
</tr>
<tr>
<td>I am paid within market value for my job function, but at the low end of the range, considering my level of expertise and responsibility.</td>
<td>39.8%</td>
<td>36.4%</td>
</tr>
<tr>
<td>I am paid fairly for my level of expertise and responsibility.</td>
<td>39.4%</td>
<td>37%</td>
</tr>
<tr>
<td>I am paid excessively for my level of expertise and responsibility.</td>
<td>2.7%</td>
<td>3.1%</td>
</tr>
</tbody>
</table>

Bio/pharma workers contemplate job and career changes.

What is your prediction for your company’s business prospects in the coming year?

- Improve: 35.1%
- Decline: 14.5%
- No Change: 41.5%

If it were necessary for you to change jobs this year, how would you assess the job market?

<table>
<thead>
<tr>
<th>2018</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>It would be straightforward to find a job comparable to the one I have now.</td>
<td>27.0%</td>
</tr>
<tr>
<td>It would take a while, but I would be able to find a job comparable to the one I have now.</td>
<td>46.1%</td>
</tr>
<tr>
<td>It would be straightforward to find a job, but it probably wouldn’t be as good as the one I have now.</td>
<td>13.7%</td>
</tr>
<tr>
<td>I would have to search hard and be prepared to take what I could get.</td>
<td>13.2%</td>
</tr>
</tbody>
</table>

In your career, how long, on average, have you stayed with the same employer?

- Less than 2 years: 14.8%
- 3 to 5 years: 31.9%
- 6 to 10 years: 20.1%
- 11 to 20 years: 17.3%
- More than 20 years: 10.2%
Respondents in the biologics sector work for companies of all sizes. Almost 36% of the respondents work for companies with more than 5000 employees; more than 46% work for companies with fewer than 500 employees.

The respondents were evenly split in tenure in the biopharma industry; 28.6% had fewer than 10 years of experience, 26.9% had 10–20 years, 34.8% had 20–35 years of experience; almost 10% of the respondents have worked in the industry for more than 35 years. About one-half of the respondents worked outside the bio/pharma industry for more than five years.

Fewer than half of the respondents held doctorate-level degrees or higher (43.8%). More than half of the respondents (54.8%) indicated that they were individual contributors versus managers of people.

Respondents to the 2018 survey reported slightly more job mobility than in previous surveys. More than 40% stayed with the same employer, on average, for less than five years. Fewer than one-third stayed for 11 or more years.

**SALARIES, BENEFITS, AND WORKLOAD**

As reported in previous surveys (2), respondents said the volume of work continued to increase year over year. Almost 38% said they used less than half of their allotted vacation, sick, or personal time. Fewer than 20% said they used all available paid time off.

However, the number of people reporting salary increases in 2018 (54.8%) dropped compared with 2017 (61.1%). More respondents reported no change in salary in 2018 (39.4%) compared with 2017 (33.3%).

Nearly 58% of the respondents said they were paid at the low end of the range for their job function, or below market value; a slight improvement over the 60% who reported the same ranges in 2017.

While employers may offer benefits and perks to retain employees, survey respondents noted little or no positive changes in benefits offered. Almost 43% said there were no notable changes. Only 6% reported an increase in company pension or 401K contributions; fewer than 4% said profit sharing increased. Health insurance costs were a pain point; 40% said costs for insurance increased and almost 17% said the coverage was reduced.

**EMPLOYMENT OUTLOOK**

Overall, respondents were more optimistic about job security. Only 19.8% said they felt less secure in their positions in 2018, compared with one-third of the 2017 respondents. The percentage of people who said there was “no change” in their job security year over year increased from 43.8% in 2017 to 54.5% in 2018; however, there is no indication if this “no change” represents a secure or insecure feeling.

Survey respondents were divided on the level of competition for available positions. A similar number of respondents said competition for open positions was strong (29.5%) or employers must compete for qualified candidates (28.1%). The shortage of qualified candidates may be easing: In 2017, more than one-third of the respondents noted that there was a shortage of qualified candidates; one-quarter said there were more qualified candidates than positions.

However, individuals entering new biopharma positions may not be prepared for the requirements of the job; fewer than 7% of the respondents said new hires were extremely well trained.

Nearly 60% said they would like to leave their job, given the opportunity; however, two-thirds do not expect to leave in the coming year. More than one-quarter said they would like to leave the bio/pharma industry, a concern for bio/pharma companies worried about employee retention.

Salary was the most cited single reason to spur a job change, followed by work-life balance, professional advancement, and intellectual challenge.

Most respondents were confident they would be able to secure a job comparable to the one they currently hold; 27% said it would be straightforward to find a new position; 46.1% were confident in finding a new position but said it would take a while to locate such a position.

More than three-quarters of the respondents said their employer provides adequate training for basic skills. However, fewer (62.2%) said the company offers advanced training.

**BUSINESS OUTLOOK**

Biopharma company employees were more optimistic about business prospects than their counterparts in the pharma sectors (3). More than 17% of respondents from around the world queried in the *Pharmaceutical Technology* employment survey predicted a decline in pharma business; 22.6% foresee a drop for Europe-based companies. In comparison, fewer than 10% of respondents working in biopharma—those responding to the *BioPharm International* survey—anticipate a decline in business in 2019.

**REFERENCES**

Optimizing SEC for Analysis of Antibodies and Antibody-Drug Conjugates

ON-DEMAND WEBCAST  Aired December 10, 2018

View this free webcast at www.chromatographyonline.com/lcgc_p/optimizing

All attendees will receive a free executive summary of the webcast!

EVENT OVERVIEW:
Antibody and antibody-drug conjugate (ADC) analysis via size-exclusion chromatography (SEC) is a standard approach for characterization and purification under native physiological conditions. Monitoring and managing naked antibody aggregation is essential. Also, the hydrophobicity of ADCs, due to the payload-linker, increases the risk of aggregation, which can contribute to *in vivo* toxicity. Increased resolution and decreasing salt concentrations are desired for initial research and downstream development.

In this webcast, we will demonstrate the utility of SEC methods for analysis of antibodies and ADCs with varying salt concentrations, additives, and column lengths. A case-study triaging a set of novel antibodies based upon aggregate analysis will be examined, discussing how the analysis of the overall biophysical data of these biomolecules aids in the selection of the optimal candidates for further development towards the clinic.

Key Learning Objectives
- How to analyze ADC aggregation using SEC columns
- How to optimize methods for analysis of antibodies and ADCs
  How to save time in routine analysis by transferring the method from 300 mm column to 150 mm column

Who Should Attend
- Lab managers in pharmaceutical company
- Scientists working in biopharmaceutical development, particularly for antibodies and ADCs

Presenter
Edward Ha
Principal Scientist
Angiex

Moderator
Laura Bush
Editorial Director
LCGC

For Research Use Only. Not for use in diagnostic procedures.

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Agilent  LC|GC  BioPharm

For questions contact Kristen Moore at kristen.moore@ubm.com
The Role of Automation in Cell-Line Development

Automation in cell-line development and cell culture is leading to more consistent quality while improving efficiency, and, ultimately, speed to market.

FELIZA MIRASOL

A stable, high-expressing cell line is a must for commercial biopharmaceutical production, but cell-line generation and cell culture have traditionally been manual processes, requiring hours of repetitive, meticulous work. Not only are these processes low throughput, they are also vulnerable to human error (1,2). Nurturing living cells requires optimizing and controlling process conditions throughout the cell culture cycle, during cell growth, harvesting, reseeding, and analysis. This requires careful control of media conditions as well as freezing and thawing rates to ensure viable and consistent cell lines (1).

Automating mammalian cell-line development and cell culturing can not only speed up processes and offer consistent operation but can free up time for technicians to focus on other tasks. It also allows the date and time of cell growth as well as cell type to be scheduled, so that researchers and developers will know when the cells will be available for screening or for research projects (1).

For technicians, automation reduces the risk of injury caused by the repetitive strain that is often associated with continuous manual tasks, thereby improving overall efficiency. Robotics can achieve a level of consistency in procedure and sterility that is difficult for even the best-trained technicians to reach. This consistency results in less batch-to-batch variability, and removes the lag time typically experienced at the beginning of a week because cells are not usually cared for over the weekend. Automated systems also allow the production timeframe to be shortened, enabling quicker scale-up.

In order to be fully automated, however, a system should be able to provide both an environment within which cells will grow as well as the capability to monitor that growth without the need for human interaction. In addition, automated cell culture systems should be able to run unattended over the course of days or weeks while allowing technicians to evaluate pH, nutrient/waste levels, and cell concentration and viability (1).

ESTABLISHED AUTOMATION

A number of automated platforms for cell line development have been useful in reducing development costs and increasing throughout for biopharmaceuticals. One platform based
According to researchers involved in ADVANCEMENTS, one example of recent automation is the time required to screen cell-based assays. The integrated approach resulted in cell lines that were of equal quality to traditionally generated cell lines. According to researchers involved in that study (3), the automated system permitted a three-fold increase in the number of development projects that could be done, while maintaining the same manual workload.

Automation of cell assays facilitates quality cell-line development by improving the quality of assay results and enhancing throughput. Recently, biopharmaceutical developers have been focusing on compact and scalable automated low-to-medium-throughput systems that are flexible enough to adapt to changing needs (4).

Various factors can still limit productivity in cell-based automation, including:
- automation of the actual cell-based assay protocols
- maintenance and sub-cloning of cells
- the time required to screen cell-based assays.

No single factor predominates, so improving productivity may involve improving multiple parameters (4).

CELL CULTURE ADVANCEMENTS

One example of recent automation advancement has been the establishment of an integrated high-throughput, automated platform for developing the cell lines used to manufacture protein therapeutics. The integrated approach combines a cell sorter, a clone cell imager, and a liquid handling system, enabling high-throughput screening and a more efficient process for developing cell lines, according to a study (2). The integrated process can screen approximately 2000–10,000 clones per operation cycle.

According to the study, the integrated process has been used to manufacture high-producing CHO cell lines, which are then used to produce therapeutic mAbs and their fusion proteins.

Manual operations, no matter how efficiently carried out, will always be limited by throughput.

By using different types of detecting probes, the method can be applied to the development of other protein therapeutics or be used in other production host systems (2). The platform offers the advantages of significantly increased capacity, ensured clonality, traceability in cell line history with electronic documentation, and much less opportunity for operator error.

Automation is beneficial in that it allows biomanufacturers to take advantage of the latest cell culture technologies. Manual operations, no matter how efficiently carried out, will always be limited by throughput, according to Bhagya Wijayawardenena, senior applications specialist, Beckman Coulter Life Science, in a webinar (5).

“Most screening experiments require you to look at thousands of cultures at one time. It is impossible to achieve that throughput manually. Also, automation allows you to use low-region values, using 384-well plates instead of 96-well plates. This could drive your reagent cost down,” Wijayawardenena said in the webinar.

Another compelling reason to use automation in cell culture is to address the long workflows involved. “Cell-culture workflows take weeks, sometimes even months, to complete. Timing is very important,” Wijayawardenena affirmed. “Instead of coming during the weekend to check the confluence of your cell culture, for example, you can program the liquid handler to do that,” she said.

There is only so much that one can do manually, especially with a limited staff, she said, adding, “You want your staff members to [perform] more productive tasks than protein pipetting. Furthermore, by using automation, you eliminate the human-to-human variation in pipetting, thereby improving [overall] reliability and the quality,” Wijayawardenena said.

Among the advancements in automation for cell culture are 3D cell-culture techniques, which have been receiving much attention from scientists in recent years, Wijayawardenena noted in the webinar. These 3D techniques provide more accurate models of tissues. Most recently, the cell-culture field has been moving more toward continuous cell culture, which includes continuous feed addition, cell maintenance, and process control, she said.

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www.biopharminternational.com
January 2019 BioPharm International 23

Upstream: Cell Culture
A Look at the Affinity Chromatography Landscape

New ligands are being developed to meet the separation and purification needs of next-gen biologics.

CYNTHIA A. CHALLENER

Affinity chromatography offers the high selectivity required for biopharmaceutical manufacturing and analysis. Specific binding interactions between biologic molecules and immobilized affinity ligands allow for selective separations. With the advent of next-generation biopharmaceuticals, chromatography material suppliers have been challenged to develop affinity ligands and support systems that enable the separation and purification of not only novel protein-based biologics, but a wide range of other biomolecules, from viral vectors and nucleic acids to cells.

Beyond Impurity Removal for “Simple” Proteins

Affinity chromatography in biologics manufacturing has typically served as a method for removal of process-related impurities such as host-cell proteins and DNA, with Protein A the most widely used resin. Resin suppliers have in recent years been challenged to increase the productivity of the capture chromatography process to enable more efficient purification of increasingly high titers. They have also needed to develop resin chemistries more suitable for the growing range of protein-based biologics, such as fusion proteins and antibody fragments, and affinity ligands that can separate not just process impurities, but closely related protein products.

Protein A is the most sensitive resin to cleaning-in-place and sanitization processes, and one key issue has been the development of more robust versions that can withstand high pH washes to prevent biocontamination, according to Eva Heldin, section manager for bioprocess R&D at GE Healthcare. To meet this need, in 2017 the company developed a new Protein A ligand that offers both greater caustic stability (up to 1 molar sodium hydroxide) and increased binding capacity for monoclonal antibodies (mAbs). It also has the ability to bind molecules with a certain variant of the VH3 domain and thus can be used for the purification of antibody fragments and bi/multispecific antibodies.

In addition to more base-tolerant resins, the selection of Protein A-based affinity resins has expanded to include the growing range of protein-based biologics, such as fusion proteins and antibody fragments, and affinity ligands that can separate not just process impurities, but closely related protein products.

Cynthia A. Challener, PhD, is a contributing editor to BioPharm International.
BioPharm International integrates the science and business of biopharmaceutical research, practical peer-reviewed technical solutions to enable biopharmaceutical professionals to perform their jobs more effectively.

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resins with increased capacity and improved homogeneity of the chromatography beads, which helps to reduce elution volumes, enabling increased process intensification and throughput, according to Michael Murray, a director of downstream process development with Fujifilm Diosynth Biotechnologies. He points to engineered antibody affinity resins and mild-pH elution Protein A resins that enable elution of mAbs without the use of traditional, low-pH elution buffers as important examples.

“As the complexity of the medicines in pipelines increases, so do the processes required to purify them, and these affinity resins allow platform-like approaches to novel molecular entities, reducing the time to manufacturing biologics. The mild pH elution of these resins allows the traditional mAb purification processes to be applied to low-pH labile mAbs or proteins engineered with Protein A binding domains,” Murray notes.

The binding capacities of these new resins are, however, typically lower than state-of-the-art Protein A products. “Increasing binding capacities while retaining unique selectivity continues to be a development opportunity for resin manufacturers,” he adds. There is also a continual demand to increase the productivity for Protein A resins in order to achieve faster purification cycles and higher binding capacities, according to Heldin. She does add, however, that as knowledge increases about different affinity ligands, there is an improved opportunity to tailor future Protein A resins for specific needs regarding affinities, pH elution, and alkaline stability.

Significant effort has in fact been directed at designing different libraries of affinity ligands so that structures of choice can be retrieved based on the application, according to Mario DiPaola, senior scientific director at Charles River Laboratories. “More common synthetic chemical ligand libraries include triazine biomimetic structures, multicomponent Ugi reaction libraries, and peptoids. Libraries of ligand peptides have been used for the purification of fibrinogen and immunoglobulins. Other libraries consist of polypeptides produced by means of phage display and/or ribosome display and aptamers, short single-stranded RNA or DNA oligonucleotides that spontaneously fold into structures with high affinity for protein targets,” he observes.

Thermo Fisher Scientific has taken the approach of developing product-specific affinity chromatography ligands based on its camelid VHH antibody fragment technology, according to Laurens Sierkstra, affinity purification business lead in the BioProduction Division.

A separate area of development is the use of membrane adsorbers for capture of mAbs and other smaller biomolecules.

“We offer products targeting other domains on antibodies that support the development of novel antibody fragments and formats for which Protein A does not work, such as those binding to the Fc region or CH1 region of antibodies. The technology has also been used to develop chromatography resins for manufacturing of non-antibody biosimilars—such as FSH [follicle-stimulating hormone] and hCG [human chorionic gonadotropin]—and viral vectors,” Sierkstra says. The technology is being used in both commercial and clinical manufacturing.

A separate area of development is the use of membrane adsorbers for capture of mAbs and other smaller biomolecules, for which limitations in capacity have previously restricted their use, according to Volkmar Thom, head of membrane modification at Sartorius. “There appears to be a renaissance occurring in this field. Several parties are working on new membrane materials and formats, aiming to overcome capacity issues, especially in affinity applications. We believe new technology developments will enable this step change, delivering high productivity in ready-to-use devices,” he says. Thom notes that while increased binding capacity is key, the importance of scalability, maintaining permeability and operating conditions suitable for manufacturing, and device design are equally critical.

AFFINITY CHROMATOGRAPHY

FOR BISPECIFIC ANTIBODIES

Due to the molecular diversity of bispecific antibodies, a general purification platform is not available. A toolbox approach is needed, in which purification is based on molecular design, according to Heldin. Many process development scientists are using alternative (non-Fc) Protein A antibody-binding domains to aid purification, according to Jonathon Haigh, head of R&D for downstream processing operations with Fujifilm Diosynth Biotechnologies.

“Engineered recombinant Protein A resins with smaller bead sizes are often suitable for bispecific antibodies with Fc regions, while for bispecific molecules without Fc regions, other affinity resins can provide good options,” Heldin comments. In the latter case, protein L affinity chromatography appears to be a suitable replacement for Protein A, according to DiPaola. In addition, resins with affinity for many VH3 domain regions are often a good choice for bispecific antibody fragments, while resins with affinity for the kappa light chain may be better alternatives for other bispecific molecule designs.
Thermo Fisher has taken this approach as well, developing a portfolio of new affinity products that target different epitopes on antibody structures and are suitable for the purification of bispecifics, according to Sierkstra.

Depending on the technology used to produce bispecific antibodies, removal of product-related variants can be a challenging hurdle that needs to be addressed, according to Haigh. He does note that the use of standard and multimodal resins and high-throughput screening technologies greatly aid in defining separation techniques.

Most importantly, Heldin adds that affinity resins for bispecific molecules must provide selectivity and a toolbox for the capture step, reducing the need for costly process development. She notes, too, that for some of these affinity ligands there is still room for improvement in terms of both binding capacity and alkaline stability.

**AFFINITY CHROMATOGRAPHY FOR DNA-BASED BIOLOGICS**

Methacrylate monolithic columns have been an enabling tool for developing fast and efficient separation processes for DNA-based biologics with low back pressure, high capacity, and high stability, according to DiPaola.

He points to the use of conventional, non-porous particles in amino acids-DNA affinity chromatography for the purification of super-coiled plasmid DNA (sc pDNA) as an example. “Using amino acid affinity ligands such as arginine and lysine with monolithic supports, it is possible to achieve high flow rates along with high binding capacity and productivity, resulting in a final sc pDNA product suitably pure for therapeutic applications,” he explains.

GE Healthcare has increased the window of operation for the separation of open-circular versus sc pDNA by improving an existing, flexible ligand based on thiophilic and hydrophobic interactions. The new ligand uses a more rigid high-flow agarose base matrix to decrease the flexibility while retaining the same selectivity.

Oligonucleotide purification has traditionally been achieved via anion exchange, hydrophobic interaction, and reversed-phase chromatography, but interest is increasing in the use of novel affinity ligands for this application, according to Sierkstra.

**Traditionally, vectors have been purified using ultracentrifugation, but there has been a move toward chromatography in recent years.**

**AFFINITY CHROMATOGRAPHY FOR CELLULAR PRODUCTS**

For cell purification, expanded bed chromatography has been shown to be a more favorable mode of affinity chromatography compared to packed bed chromatography, according to DiPaola. “Expanded bed chromatography is characterized by a high interparticle porosity and surface area and consequently lower shear forces,” he observes. It also provides an efficient and gentle affinity chromatography-based technique for cell separation.

Another option is the use of monolithic, convective chromatography supports, which are widely used for the purification of macromolecules such as viruses, virus-like particles, plasmid DNA, and cells. “The transport of the target molecule through these monolithic supports is based on convection, which allows quick separation of molecules of large sizes, rather than diffusion, which favors separation of small molecules,” DiPaola explains.

**AFFINITY CHROMATOGRAPHY FOR VIRAL VECTORS**

Viral vectors are required for the production of gene therapies and the gene modification of cells used in various cell therapies. The greatest demand is for lentiviral and adeno-associated viral (AAV) vectors, with sourcing of lentiviral vectors becoming a bottleneck for gene and cell therapy manufacturing.

Traditionally, vectors have been purified using ultracentrifugation, but there has been a move toward chromatography in recent years, according to Michael Baker, a director of downstream process development at Fujifilm Diosynth Biotechnologies. “Use of affinity chromatography has removed some of the burden on the purification steps downstream of the affinity step, allowing the focus to be directed toward specific attributes such as enrichment of full capsids, rather than on removal of gross impurities from the product stream,” he says.

The interest in chromatographic solutions has led to the development of several new affinity ligands for viral vector purification. Baker points to ligands for AAV vector purification with both broad and specific serotype selectivities. “These developments have made the purification of certain vectors much more efficient and consistent than previously achievable with traditional methods,” he observes. In addition, Baker notes that manufacturers are starting to release second-generation products that are more amenable to scale up and high-throughput applications.

Affinity columns with specific antigens to each AAV serotype had been used for purification of AAV-based gene therapy products, and more recently a new resin has been introduced that binds all AAV serotypes with enhanced capacity, according to DiPaola. A new affinity purification procedure using cell surface heparan sulfate proteoglycan and the non-ionic gradient medium iodixanol has also resulted in improved viral infectivity,
good recovery rates, and faster, more efficient purification of AAV vectors. Lentiviral vectors have also been purified using heparin/heparin sulfate affinity chromatography and immobilized metal affinity chromatography (IMAC), according to DiPaola.

“Following the successful development of resins enabling the scale-up of AAV production for commercial supply, Thermo Fisher has now set its sights on developing similar products for purification of adeno and lentiviruses to be offered as off-the-shelf products,” notes Sierkstra. Similar to its improvement of an affinity resin for DNA, GE Healthcare has improved an existing affinity resin for AAV vectors by using a much more rigid, high-flow agarose base matrix to increase the window of operation for vector purification, according to Heldin.

Affinity chromatography is also being used for the analysis of viral vectors, according to Thom. Specially designed kits specific for lentivirus or adeno-virus count total viral particle load in rapid, no-wash assays.

AFFINITY CHROMATOGRAPHY FOR VIRUSES

Membrane adsorbers with large pores and a fast, convective mode of operation have been a preferred tool for the purification of large biomolecules such as viruses, according to Thom. A new, sulfated cellulose affinity membrane specifically designed by Sartorius for virus capture provides maximum virus access with increased selectivity and product recovery. It is useful for the capture of several virus types including influenza, Japanese encephalitis, measles, and viral antigens from Hepatitis B.

“This sulfated cellulose membrane adsorber exhibits 10–20 times higher binding capacity for various strains of Influenza virus than commercially available resins, while offering comparable recovery and purity,” Thom asserts. In a pandemic situation, he notes that production of 40 million doses of flu vaccine is possible in one day with 54 L of membrane.

A number of different technologies are being explored as supports for affinity chromatography in biomanufacturing.

NANOFIBERS FOR AFFINITY CHROMATOGRAPHY

A number of different technologies are being explored as supports for affinity chromatography in biomanufacturing. One that is receiving significant attention is the use of nanofibers within membrane systems. Membranes based on nanofibers are interesting and could have advantages in processing, but the technology is still being developed and a successful prototype that matches resin chromatography hasn’t been demonstrated yet, according to Sierkstra.

The recent acquisitions of Natrix Separations and Puridify by MilliporeSigma and GE Healthcare, respectively, show that the industry is looking at alternatives to traditional chromatographic methods, according to Baker. “Both of these firms have great potential to achieve the higher flow rates of membrane technology, but with the ligand density and binding capacity of a traditional resin. In addition, when combined with their macroporous structures, these technologies could represent a significant improvement over the current offerings for viral vector purification,” he says.

GE Healthcare is commercializing a fiber-based chromatography platform that it obtained via the acquisition of Puridify, a UK-based bioprocessing company, in 2017. The fiber format has an open pore structure that allows for convective mass transport, leading to flow rate-independent binding capacities and cycle times of minutes compared to hours for traditional resin chromatography, according to Heldin. “The technology opens new opportunities, where protein A fibers can be used for rapid cycling many times across a single batch in a repetitive manner before disposal, enabling use of the protein A capture format for mAbs to its full extent,” she asserts. The larger surface area may also make the fiber technology suitable for the purification of viral vectors.

The new resin will be produced at GE’s biotechnology manufacturing center at the Stevenage Bioscience Catalyst (SBC) Open Innovation Campus, in Stevenage, UK, which was opened in November 2018.
Single-pass tangential flow filtration (SPTFF) has generated a lot of interest over the past years. Despite the wide use of SPTFF in wastewater treatment and dairy whey manufacturing (1–5), it was just recently applied to biopharmaceutical processes. A reason for this shift is the current trend of the biopharmaceutical industry to investigate continuous processing and ways to achieve higher final concentrations for the injection of recombinant proteins into the human body. Also, the increase in titer capacity in current processes due to the improvement of cell-culture efficacy has opened an opportunity for SPTFF to reduce volumes in-line and debottleneck plant limitations (6, 7). In these cases, SPTFF offers some unique advantages over the familiar batch operation used to date.

In batch tangential flow filtration (TFF), product concentration is reached while the retentate is recirculated through a set of TFF devices. During every pass through the membrane, a small portion of the feed is removed as permeate. Over time, the retentate concentration in the tank rises until the final target concentration is reached. An overconcentration of protein is usually targeted to allow for dilution during recovery by buffer flush and finally obtain the target concentration with high yield.

In SPTFF, there is no retentate recirculation. The feed is passed once through the TFF devices as enough feed is converted into permeate to reach the target retentate concentration or a slightly higher concentration to allow for recovery flush. Compared to batch TFF, the feed flow rates in SPTFF are much lower and the cassettes are set up in series to provide a longer flow path. This set-up and operation gives the liquid feed a longer residence time under pressure in the cassettes to generate more filtrate. The
simplicity of the SPTFF flow path and its steady-state operation for processing make it a useful operation for several applications, including in-line volume reduction to reduce tank requirements, in-line concentration to reduce subsequent step sizing, in-line concentration with in-line buffer addition for desalting, and in-line concentration after diafiltration to facilitate high final concentrations with high yields.

TFF devices used in batch mode are commonly cleaned and reused to reduce costs. For SPTFF, cleaning and reuse are also expected unless single use is deemed desirable. The cleaning procedures developed for batch systems employ high, recirculating flows compared to the low feed flows used in SPTFF. It would be useful to extend the processing simplicity of SPTFF to device cleaning by achieving reduced flows and avoiding recycle during cleaning. For this purpose, a static cleaning method was developed that ensures that every section of the cassettes is exposed to the cleaning agent at a target concentration, temperature, and time. The strategy described herein involved arranging the cassettes to conveniently alternate between processing in-series and cleaning in-parallel configurations using valve switching. With this new method, continued flow through the cassettes (single pass) during cleaning was not required as the cassettes were cleaned and flushed in a parallel configuration to reduce fluid consumption. This cleaning process can be easily implemented in a practical way at production scale using a process holder (Pellicon, MilliporeSigma).

In this study, a model monoclonal antibody (mAb) feedstock was processed using regenerated cellulose membrane cassettes in SPTFF mode to monitor the performance of the static cleaning method over multiple processing and cleaning cycles to ensure consistency and reproducibility, while avoiding batch-to-batch carryover.

**OBJECTIVE**

The objective of this study was to extend previous cleaning results (7) to a static hold method with additional performance monitoring. A mAb concentration process was repeated over 20 cycles to qualify the proposed static cleaning process. For the cleaning procedure, only a feed pump, operating at relatively low flow rates, was used; no recirculation loop, vessel for recirculation of cleaning agent, or other equipment was used.

Before the first run and after each succeeding run, the normalized buffer permeability (NBP) of each cassette used in the SPTFF setup was measured as well as the total organic carbon (TOC) in the retentate flush after cleaning. In addition, the consistent filtration performance (conversion, flow path resistance, and product retention as yield) of the SPTFF system was monitored over the 20 runs.

The overall goal of this study was to prove that the static cleaning procedure enables 20 reuse cycles with consistent system performance and minimal product carryover.

**MATERIALS AND METHODS**

**Materials**

Table I lists materials and equipment used in this study, along with supplier information.

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<td>Measuring cylinder–250 mL</td>
<td>Iwaki Pyrex</td>
<td>Thermo Fisher Scientific</td>
<td>± 2.5 mL</td>
<td></td>
</tr>
<tr>
<td>CT0100P</td>
<td>Measuring cylinder–100 mL</td>
<td>Kimble Kimax</td>
<td>Thermo Fisher Scientific</td>
<td>± 1.0 mL</td>
<td></td>
</tr>
<tr>
<td>P3C030C01</td>
<td>Pellicon 3 Ultracel 30 kDa C-Screen</td>
<td>MilliporeSigma/ C0MA84132, C0PA10356</td>
<td>MilliporeSigma</td>
<td>Germany</td>
<td></td>
</tr>
<tr>
<td>XX42PMINI</td>
<td>Pellicon Mini holder</td>
<td>MilliporeSigma</td>
<td>Germany</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS-1200G-FLML-02A</td>
<td>Caustic compatible pressure transducers</td>
<td>Senzpak</td>
<td>Senzpak</td>
<td>US</td>
<td></td>
</tr>
</tbody>
</table>

**Table I.** Equipment and materials.
Methods

Three cassettes (Pellicon 3 Ultracel 30 kDa C-Screen, MilliporeSigma) were separately installed in three holders (Pellicon Mini holders, MilliporeSigma). The holders were connected using size 16 tubing (Masterflex Tygon LFL, Cole-Parmer). Luer lock valves (Cole-Parmer) 1-way (C, F, I, J, K, L), 3-way (D, E, G, H), and 4-way (A, B) were used to simultaneously arrange serial and parallel TFF cassette configurations, as shown in Figure 1.

Disposable pressure transducers (MPS-1200G-FLML-02A, Senzpak) were installed at the feed inlet port as well as three retentate outlets to capture pressure profiles during the concentration process. Four balances (PB3002-S/FACT, Mettler Toledo) were used to measure the volume of the retentate and permeates from the three different sections. The pressure transducers and balances were connected to a system for data collection (DAQ Gen. 1, MilliporeSigma). A hose pinch clamp was attached to the retentate line after each section for retentate pressure adjustments.

Twenty liters of 15±1 g/L immunoglobulin G (IgG) feed material (SeraCare) were prepared in phosphate buffered saline (PBS) solution (MilliporeSigma). The feed material was filtered through a 0.22 μm Stericup (Durapore, MilliporeSigma) before each cycle. Concentrated feed material and permeate after each cycle were stored, combined, and reused to prepare a new 15±1 g/L IgG feed solution for the next day. After every five cycles, fresh PBS solution was used instead of the recycled permeate.

The 20-L feed tank was set on a stir plate to ensure proper mixing. Multi-way valves were used to direct the flow either in a parallel or in serial flow path configuration. A graduated cylinder and calibrated stopwatch were used to measure pre- and post-use NBP.

Initial and final feed samples were taken for conductivity, pH, and protein concentration measurements using a conductivity meter (Schott/Lab 960, ITS Science & Medical), a pH meter (Schott/Lab 850, ITS Science & Medical), and a UV spectrophotometer (GEN10BIO, Thermo Fisher).

UV measurement for mAb concentration analysis

To generate the UV standard curve, five different IgG concentrations between 0.1 and 1 g/L were analyzed at 280 nm. Equation 1 was obtained from the standard curve and used for concentration determination of the samples in this study.

\[
\text{IgG Concentration (g/L) = 1.2605 (g/L) /AU * (AU – 0.0076 g/L)}
\]

where \(AU\) is measured absorbance.

Process Conditions

The conditions shown in Table II were used in the IgG process for each cycle.

PROCEDURE

Installation

Three Pellicon 3 cassettes with 0.11 m² of Ultracel 30 kDa membrane were individually installed in a holder and tightened with a calibrated torque wrench at 180 in-lbs. The cassettes were flushed with deionized (DI) water in parallel at a feed flow rate of 1.2 L/min (3.64 L/min/m² [LMM]—normalized to the total area). A total of 13.6 L (123.6 L/m²) of DI water was flushed through the retentate channel, and 27 L (81.8 L/m²) of DI water was flushed through the permeate channel. Details are discussed later in this article.
Pre-use cleaning cycle
Pre-use cleaning was done in parallel configuration as shown in Figure 1A. Three liters (10 L/m²) of 0.5 N sodium hydroxide (NaOH) were pumped into the system for 7.2 min at a feed flow rate of 460 mL/min (1.4 LMM) with a retentate pressure of 10 psi. The three-way valves E and H (between sections 1 and 2 and sections 2 and 3, respectively) were switched for about 10 seconds for caustic exposure. The system was left to stand to allow the cassettes to soak in caustic solution for 50 minutes before flushing with DI water.

Post-caustic water flush was done in parallel configuration. Flushing through the system was done with 9.9 L (30 L/m²) of DI water at a feed flow rate of 460 mL/min (1.4 LMM) with a retentate pressure of 10 psi. A permeate sample was taken at the end of the flush for pH and conductivity measurements. Permeate samples (~5 mL) were taken from each section at the end of each flush for total organic carbon (TOC) measurement. Blank system samples were collected after every five cycles when the system was stored over the weekend (more than two days).

Integrity testing
Integrity testing of each cassette was done manually before use (new cassettes) and after use (after completing the 20 cycles). The test specification of the cassettes at test pressure of 30 psi was consistently passed with less than 14 mL/min air diffusion flow rate.

NBP measurement
NBP was used instead of normalized water permeability (NWP) for practical reasons. The NBP value was measured before and after each concentration cycle at 460 mL/min (1.4 LMM) of feed flow with 10 psi retentate pressure in total recycle mode to determine the cleaning efficiency.

Twenty liters of 15±1 g/L IgG solution were used as the feed stock for this study. The concentration process ran in series at a feed flow rate of 75 mL/min (0.23 LMM) with a retentate pressure of 10–15 psi for four hours. Figure 1B highlights the flow path for in-series configuration. The required feed flow rate was determined during a flux excursion before the static cleaning experiment. At the end of the four hours, the system was left to stand for 10 minutes before PBS buffer (3× system hold-up volume; about 200 mL) was pumped through the system for product recovery.

Storage
Flushing through the cassettes was performed with 2.2 L (6.67 L/m²) of 0.1 N NaOH at a feed flow rate of 460 mL/min (1.4 LMM) and 10 psi retentate pressure for overnight storage before the start of the next cycle.

RESULTS AND DISCUSSION

Concentration process
A measure of consistent process performance for the reuse of TFF devices is the consistency of the retentate concentration (CR) over multiple runs with intermediate cleaning. By a steady-state mass balance, the retentate concentration is calculated in Equation 2:

\[ CR = \frac{CF \cdot JF}{JR} = \frac{CF}{1 - Y} \]  

where \( CF \) is the feed concentration in g/L, \( JF \) is the normalized feed flow rate in L/min/m² (LMM); \( JR \) is the normalized retentate flow in LMM; and \( Y \) is the conversion or \( J/JF \), where \( J \) is the permeate flux in LMM. For constant run-to-run feed concentrations (CF) and conversion (Y), and for consistent times to achieve steady-state polarization and mass hold-up in the cassette, the retentate concentrations remain constant.

Process time (t) is determined based on the time it takes to pump the batch

### Table II. Conditions used in the immunoglobulin G (IgG) process for each cycle.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Target</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial volume</td>
<td>20 L</td>
<td></td>
</tr>
<tr>
<td>Initial IgG concentration</td>
<td>15 g/L</td>
<td>14.5–16 g/L</td>
</tr>
<tr>
<td>Final concentration</td>
<td>130 g/L</td>
<td>92.4–132 g/L*</td>
</tr>
<tr>
<td>Feed flow</td>
<td>75 mL/min</td>
<td>74.9–79.2 mL/min</td>
</tr>
<tr>
<td>Retentate pressure</td>
<td>10 psi</td>
<td>10–16.5 psi</td>
</tr>
<tr>
<td>Process temperature</td>
<td>24 °C</td>
<td>12–25 °C</td>
</tr>
<tr>
<td>Process loading</td>
<td>909 g/m²</td>
<td>897–962.4 g/m²</td>
</tr>
</tbody>
</table>

*Runs 2 and 4 at 15 °C

### Figure 2. Concentration, conversion, and feed temperature versus cycle.
through the cassettes, as defined by $t = \frac{V}{JFA}$, where $V$ is feed volume in L and $A$ is filter area in m$^2$. As a result, the process time is fixed by the feed flow rate ($JF$) and determined by the feed pump. Similarly, the conversion ($Y = \frac{J}{JF}$) is fixed, as $Y$ is mainly dependent on the permeate flux ($J$).

When the process temperature remained constant (runs 1, 5–12, 14–20), the conversion was very consistent, averaging $88\pm1\%$ from cycle to cycle (see Figure 2). The retentate concentration during these runs ranged from 122–133.2 g/L. Measurement errors of $1\%$ in the graduated cylinder were consistent with this cycle-to-cycle variability. When the temperature dropped, the viscosity increased, and the protein diffusivity, mass transfer, and flux decreased. Cassette properties that impact flux (e.g., permeability, mass transfer coefficient, and effective membrane area) were re-established by a successful cleaning procedure. The applied transmembrane pressure (TMP) had a minor effect on conversion when kept above the osmotic pressure of the retained protein at the polarization concentration. SPTFF mAb conversion has been reported to be stable above 20 psi [6]. Further, osmotic pressures of 8–10 psi are typical for mAbs up to 300 g/L (5,8). Therefore, maintaining the retentate pressures above these values should avoid reverse flow across the membrane.

Figure 3 shows the consistency of the permeate process flux throughout the 20 runs for each of the three sections. Because no trend was observed with cleaning cycles, this result implies that each section was cleaned equally well despite being subjected to different process conditions.

**Retention**

The protein retention was determined by measuring the product concentration in the permeate pool in relation to the total mass used for the run. The high average product retention of 99.9% over the 20 SPTFF cycles shows a stable and reproducible process with effective cleaning (data not shown). The variability of $\pm 0.1\%$ is mostly attributed to systematic errors from ultraviolet (UV) measurement (0.5%) and dilution (0.8%); therefore, product retention is considered to be consistent from cycle to cycle.

**NBP, TOC, cassette resistance**

Cassette cleaning efficiency in batch TFF operation is commonly monitored using water or buffer permeability, normalized to temperature. The NBP value was measured post-use for each section over 20 cycles, averaging 100% of the initial value (see Figure 4). The average NBP did not decline with repeated use, showing that the cleaning cycle is effective in maintaining membrane permeability. This result implies that no significant amount of protein that would foul the membrane was carried over from the previous run. NBP values fluctuated between 92–113% of their initial value, reflecting test variability consistent with errors in pressure and flow measurements. The conductivity after 30–45 L/m$^2$ water-for-injection flush was 0.6–0.9 μS/cm and the pH between 6 and 7.9.

The post-flush TOC levels in the permeate were below 1 ppm for all runs and sections (data not shown). This result indicates that after the static cleaning, no significant amount of protein was left in the system from the previous run that could be carried over and released from the cassette into the subsequent batch.

Changes in the feed-to-retentate channel flow resistance (psi/LMM) in each cassette can potentially alter the flow distribution among cassettes in parallel for a particular section or between sections in series. Flow resistance values averaged approximately
0.45 psi/LMM for all sections over 20 cycles (see Figure 5). The average resistance did not decline with repeated use, showing that the cleaning cycle is effective in maintaining flow resistances. This result implies that no significant amount of protein was carried over from the previous run that would plug the feed channel screens. Resistance values fluctuated at 0.18–0.72 psi/LMM or ±60% from their average, reflecting test variability. Although temperature fluctuations and trapped air bubbles can contribute to this variability, the low operating flows and pressures characteristic of SPTFF increase measurement error.

Comparison of static cleaning to single pass and recycle loop
Static cleaning as applied in this study consisted of a single pass-to-drain period, where 10 L/m² caustic solution was pumped through at 1.4 LMM. This cleaning step took 7.2 min. A 50-minute static hold (soak) with caustic followed to complete the cleaning cycle.

Table III compares the applied static cleaning strategy with cleaning by using single-pass only (without a recirculation loop or static hold step) and with cleaning by using a recirculation loop.

Table III. Comparison of cleaning strategies for single-pass tangential flow filtration (SPTFF).

<table>
<thead>
<tr>
<th>Cleaning method</th>
<th>Static</th>
<th>Single pass</th>
<th>Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>System configuration</td>
<td>No recirculation loop</td>
<td>No recirculation loop</td>
<td>Recirculation loop</td>
</tr>
<tr>
<td>feed flux (LMM)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Step 1</strong></td>
<td><strong>Flush to drain</strong></td>
<td><strong>Flush to drain</strong></td>
<td><strong>Recirculation</strong></td>
</tr>
<tr>
<td>Objective</td>
<td>Clean retentate and filtrate side dynamically, flush out product residuals</td>
<td>Clean retentate and filtrate side dynamically, flush out product residuals</td>
<td>Clean retentate side</td>
</tr>
<tr>
<td>Operation mode</td>
<td>Single pass, retentate valve partially closed</td>
<td>Single pass, retentate valve partially closed</td>
<td>Recirculation, retentate valve open</td>
</tr>
<tr>
<td>Volume (L/m²)</td>
<td>10</td>
<td>83.6</td>
<td>5</td>
</tr>
<tr>
<td>Time (min)</td>
<td>7.2</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td><strong>Step 2</strong></td>
<td><strong>Static soak</strong></td>
<td>N/A</td>
<td><strong>Recirculation</strong></td>
</tr>
<tr>
<td>Objective</td>
<td>Clean membrane and system</td>
<td>–</td>
<td>Clean filtrate side</td>
</tr>
<tr>
<td>Operation mode</td>
<td>Static cleaning</td>
<td>–</td>
<td>Single pass, retentate valve partially closed</td>
</tr>
<tr>
<td>Volume (L/m²)</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Time (min)</td>
<td>50</td>
<td>–</td>
<td>45</td>
</tr>
<tr>
<td>Total time (min)</td>
<td>56.8</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Total volume (L/m²)</td>
<td>10</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>System complexity</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Process complexity</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Cleaning chemicals usage</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

Figure 5. Feed channel flow resistance over 20 cycles.
Understanding Absolute Stoichiometry of Oligomeric Protein Complexes Using SEC-MALS

LIVE WEBCAST: Tuesday, January 29, 2019 at 11am EST | 8am PST | 4pm GMT | 5pm CET

Register for this free webcast at www.biopharminternational.com/bp_p/complexes

Event Overview
Nucleosome assembly proteins (Naps) are histone chaperones that interact with histones H2A-H2B and/or H3-H4 in the regulation of chromatin architecture. Determining the stoichiometry of Nap-histone complexes has been challenging due to two major factors: Naps undergo dynamic oligomerization that is influenced by salt concentration; and H3-H4 forms a hetero-tetramer. Using SEC-MALS, researchers have determined the absolute stoichiometry of Nap-histone complexes containing Nap orthologs, a new Nap paralog, and relevant deletion constructs. In this webcast, the researchers will discuss the main findings including:

- The oligomeric state of Nap-histone complexes
- A comparison of Nap binding to H2A-H2B and H3-H4
- The determination of the role of Nap acidic regions in histone binding

Who Should Attend
- Protein biochemists and biophysicists
- Biologics discovery and characterization scientists

Key Learning Objectives
- Using SEC-MALS to study oligomeric states of proteins under different buffer conditions
- Designing SEC-MALS experiments to determine stoichiometry of protein-protein interactions
- Analysis and interpretation of SEC-MALS data

Presenters
Dr. Sheena D’Arcy
Assistant Professor
Department of Chemistry and Biochemistry
University of Texas at Dallas

Prithwijit Sarkar
Graduate Student
D’Arcy Laboratory

Moderator
Rita Peters
Editorial Director
BioPharm International

For questions contact Kristen Moore at kristen.moore@ubm.com
For cleaning using single-pass only, the system configuration and the process complexity were as low as those for the static cleaning strategy. However, the consumption of cleaning agents was much higher for single-pass only ($83.6 \text{ L/m}^2$) than for static cleaning ($10 \text{ L/m}^2$) as the pump ran continuously to pump the cleaning liquid to the drain.

Use of a recirculation loop can reduce the cleaning chemical usage to the same amount required for static cleaning. However, addition of a recirculation loop eliminates the advantage of simplicity and straight-through flow path of a typical single-pass setup, thus adding system complexity. The recirculation cleaning method consisted of two steps: first, the retentate side was cleaned with an open retentate valve; second, the filtrate side was cleaned with fresh cleaning liquid and a partially closed retentate valve. Overall, this procedure using recirculation is in line with the typical practice applied in batch TFF processes, which has increased process complexity compared to single-pass processes.

There was no significant difference in the time requirement among all three cleaning methods. The method using static cleaning kept the system simplicity of a straight-through flow path without a recirculation loop and did not require more cleaning chemicals than cleaning with a recirculation loop.

CONCLUSION
Static cleaning was effective in providing consistent conversions and process fluxes for consistent retentate concentrations over 20 runs. The consistent feed flows also kept a steady process time. To maintain a reliable conversion performance, consistent process temperature and flushing to remove air bubbles before the run were also required.

Static cleaning was effective in maintaining consistent, high NBP values and consistent, low feed channel resistance in each section over 20 runs. Variability was ascribed to measurement errors at low flows. Low TOC values demonstrated that static cleaning prevented batch-to-batch carryover after cleaning and flushing.

This study qualified static cleaning as a viable, robust cleaning method for an Ultracel membrane running in SPTFF mode after protein concentration. Other SPTFF applications with more fouling feeds, such as clarified harvest concentration, may form precipitates upon concentration and will require more extensive cleaning regimens. In addition, polyethersulfone membranes are, in general, more fouling than regenerated cellulose membranes and may require more extensive cleaning regimens as well. Thus, biopharmaceutical manufacturers should test their process to qualify any cleaning procedures for manufacturing.

Static cleaning offers advantages over traditional methods used for cleaning batch TFF systems (9). A caustic cleaning solution can be introduced into the system at the low flows used in a SPTFF process. Retentate and permeate flows can be directed to drain until the caustic solution exits the final section permeate. At that point, no further flow is required and no recycle is needed. Further, no clean-in-place station or separate tank is needed for cleaning. These attributes maintain the simple, straight-through flow path without the need for a recirculation loop. Additionally, the static operation limits the amount of caustic cleaning volume needed, while the cleaning time is comparable to batch TFF.

Reductions in cleaning and flushing volumes may be useful for large systems. Valves could be installed between successive sections to allow feed flow to all sections when the valves are open (parallel flow), or to prevent feed flow to the successive section when the valves are closed (serial flow). During serial flow, the flushing fluid tends to preferentially flow through the first section; thus, more volume and time is required for the latter sections to be adequately flushed. Using closed valves (parallel flow) for flushing out extractables or cleaning agents and distributing caustic cleaning chemicals throughout the system before switching to open valves (serial flow) is advantageous to SPTFF processing and recovery. This strategy further reduces fluid volume usage and the need for waste disposal.

Cassette cleaning is recommended to enable reuse and cost savings over many batches for a more economical operation. For operations in which single use of SPTFF devices will increase throughput and productivity, the devices may only be used once. Cycling two parallel SPTFF systems could be used to allow continuous operation while cassettes of the unused system are cleaned off-line to facilitate continuous processing.

ACKNOWLEDGMENTS
The authors would like to acknowledge Joseph Parrella, Elizabeth Goodrich, Jonathan Steen, and Yanglin Mok.

REFERENCES
The Impact of Expedited Review Status on Biomanufacturing Facility Design

For rapid scale-up, facility design must better integrate product development and manufacturing lifecycle activities.

JEFF ODUM

Consider this situation: data coming out of a Phase II trial is better than expected. An application for Expedited Review has been submitted and accepted by FDA. Exciting times, no doubt, but what does this mean for planning and executing a facility design program moving forward?

WHAT IS EXPEDITED REVIEW?
In 2014, FDA defined four programs to facilitate and expedite development and review of new drugs that show significant promise in addressing and treating serious or life-threatening conditions (1):

• Fast-track designation
• Breakthrough therapy designation
• Accelerated approval
• Priority review designation.

These programs provide a number of advantages during the clinical trial phase of drug development that include:

• More frequent meetings with FDA to define the clinical trial program to ensure proper data collection
• Acting on drug applications in a shorter time frame than the standard review

• Increasing FDA review/guidance as early as Phase I
• Basing approval on an agreed surrogate marker instead of a defined clinical endpoint.

Once a drug has been granted any of these designations, a number of actions will be set in motion that will eventually have an impact on the planning and execution of facility design.

REGULATORY REQUIREMENTS OF EXPEDITED REVIEW
In a traditional approach to drug development, a manufacturing process is defined for early-stage clinical materials, and those materials are often manufactured, at small scale, in pilot or development facilities or by contract manufacturing organizations (CMOs) that are set up specifically for that type of manufacturing program. Quite often these facilities have some of the following attributes:

• Small-scale manufacturing equipment, often at a bench-scale level

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Figure 1. Product development and manufacturing lifecycle. Activities above the dashed line are performed in the lab under good laboratory practice (GLP), and activities below the dashed line are performed in manufacturing facilities under good manufacturing practice (GMP). Red lines indicate integration points. QTPP is quality target product profile; CQA is critical quality attribute; UO is unit operation; PD is product development; PV is process validation; DoE is design of experiments; MCB is master cell bank; WCB is working cell bank.

- Open process characteristics
- Significant reliance on procedural controls in lieu of facility controls
- Less restrictive implementation of GMP protocols; often a focus on good laboratory practice (GLP)
- More manual-driven operations instead of automated approaches.

Figure 1 represents a visual overview of the relationship between development and commercial manufacturing activities that highlights the challenges that must be addressed by the manufacturing enterprise when expedited review status becomes a time-driven reality.

In Figure 1, the integration points between clinical and commercial manufacturing (shown by red arrows) become easy to see. But why are these integration points important?

For the example of Accelerated Approval, FDA may focus on evidence of the drug’s impact on a surrogate endpoint (1). Here, the surrogate endpoint is defined as “a marker, such as a laboratory measurement … that is thought to predict clinical benefit …” (1). Thus, a potential to accelerate advancement of the product into later-stage clinical manufacturing at a more rapid pace becomes the new driver for process development that would lead to potential product launch upon final FDA approval.

When a company receives expedited drug development designation from FDA, the guidance sets in motion numerous requirements around process and facility attributes that would support the eventual commercial launch of the product to meet the anticipated market demand (1). The guidance is clear on the information that is expected to support manufacturing development. This information includes timelines for manufacturing capabilities and validation approach, as well as relative information from the chemistry, manufacturing, and controls (CMC) section.

For a biopharmaceutical, the biology license application (BLA) identifies the information that will drive facility design planning and execution. From the specific instructions for completion of Form 356h submission as part of the BLA (2), the CMC section addresses manufacturer information that includes:

- Method of manufacture; visual representation of the manufacturing process
- Detailed process description
- Process controls
- General layouts and floor diagrams
- Contamination precautions
- Bioburden control
- Container and closure systems.

The establishment description goes on to identify specifics such as:

- General information that includes product, personnel, equipment, waste, air flows
- Water system design basis
- Heating, ventilation, and air conditioning (HVAC) system design basis
- Contamination control measures such as cleaning and cleaning validation
- Computer systems for manufacturing.

Once expedited review designation becomes reality, the details of process and facility design for the commercial product, whether by the manufacturer or a designated third-party CMO, should be in place.

PLANNING FOR EXPEDITED REVIEW

Under expedited review, timelines for the development of key design documents will become a crucial factor. Planning for commercial launch capabilities will need to begin earlier. Development focus must be on achieving synergy between clinical and commercial manufacturing sooner rather than later.

Manufacturing output is crucial, driving design decisions on equipment platforms, influencing physical size and adjacencies in facility architec-
Figure 2. A schematic of the product manufacturing lifecycle demonstrates the need to design a facility to meet supply at different scales.

This strategy has five key focus elements:

- **Risk management:** Early in the development phase, risk identification and mitigation strategies (assumptions) must be implemented. Strategies should include a clear understanding of the quality-by-design aspect of the product-process relationships.
- **Seamless process and automation:** Move quickly to define control strategy to enhance quality engineering design and productivity.
- **Integrated project delivery:** Produce deliverables in a timely manner to support rapid review and implementation.
- **Innovation tools:** Enhance early reviews for operations, maintenance, constructability, and key drivers around regulatory compliance (e.g., layouts, segregation, validation strategy) using tools such as building information modeling, virtual reality, and process modeling.
- **Lean project delivery:** Focus on “right the first time” execution; time is of the essence.

Expedited review also encourages frequent communication with FDA during development and supports enhanced efforts to ensure that manufacturing equipment and facilities are ready for inspection during review of the clinical section of the application (1). As a result, process and facility design must take on an expedited execution approach. For example, under breakthrough therapy designation, FDA promotes many interactions that include type B and C meetings, critical milestone meetings, and advisory committee meetings (4).

**CASE STUDY**

During the planning for conceptual design of a new product launch facility, one of a company’s monoclonal antibody (mAb)-based products in early-stage clinical trials received breakthrough designation, and an expedited review approach was implemented. The design project changed its focus to move the planned development facility into a development and launch-capable asset.

Using the lifecycle framework described in Figure 1, the new project scope and focus were defined. For this project, the following design themes were identified:

- Eliminate tech transfer when transitioning from clinical to commercial manufacturing
- Develop synergy between operational execution of late-stage clinical and commercial launch capabilities
- Optimize site material supply-chain management to support manufacturing
- Perform risk mitigation focused on clinical/commercial success
- Optimize key in-process manufacturing operations to remove bottlenecks and improve efficiency.

The following were the key design assumptions and considerations for the project:

- Designed specifically for the defined mAb-based platform at 2000-L scale
- Ability to address clinical uncertainty (success/failure)
- Facility to be used for clinical supply and commercial launch
- Incorporate a strategy for transition to long-term commercial manufacturing
- Provide expansion flexibility (2X) within the facility without building modifications.

To meet expedited review scenarios as a “future-case” solution, the developed design solution included:

- Multi-product capability beyond the defined mAb platform with scale-out
- Incorporated product development capability (minimizing development facility cost)
- Faster product development timeline
- Minimization of development investment and operational cost
Facility Design

- Full operational modularity.
- Flexibility to address rapid scale up/down depending on clinical uncertainty via a high level of segregation.
- Adaptability to different process configurations (e.g., multiple 2x2000-L configurations).
- Simple process transfer in/out.

One of the key challenges in this effort was to address how to support the rapid execution of product development timelines, when the product development function has a large number of potential integration points associated with accessing important manufacturing assets necessary for product launch. The number of integration points can increase significantly as the launch effort requires access to larger-scale manufacturing assets. Although a great deal of process characterisation and optimization is done at small scale, product launch support will require rapid scale-up to support downstream development as well as assure upstream operations perform as required in larger bioreactor systems. The optimized solution allowed performing process scale-up within the “launch” facility to eliminate tech transfer issues for rapidly moving from process development and preclinical manufacturing into early clinical manufacturing and again into the commercial launch manufacturing phases. Early understanding and resolution of scale-up and equipment problems can facilitate producing clinical material on an accelerated schedule as required for supporting clinical trials and product licensure.

CONCLUSION
Expeditied review designation brings with it challenges to facility planning, design, and project execution. The traditional timelines and methodology will require a new approach to conceptual planning, process-facility attribute definition, and design implementation and execution. With the high level of activity around new advanced therapy medicinal products, planning for the challenges presented by expedited review designation is becoming a crucial need for manufacturing organizations.

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somewhat unclear, with the largest CMO market, the United States, relatively lacking in microbial CMOs and GMP capacity greater than 100–200 L; Europe is the clear leader in microbial CMOs and GMP capacity.

When developer respondents to the 2018 survey were asked about expectations for outsourcing any work (any expression system) in five years, 72.3% projected at least some mammalian and 58.3% said the expected to outsource at least some microbial work. More than 61% of respondents are currently outsourcing at least some API manufacturing. But this percentage was lower than for many tasks outsourced to contract research organizations, including 77.8% outsourcing some analytical testing/bioassays, and 72.6% outsourcing at least some toxicology testing. More than one-quarter of the respondents (27.7%) cited expectations/plans to outsource more biopharmaceutical API manufacturing in the next two years, increasing from 12.4% in 2010.

The US continues to be the destination for the largest portion of outsourcing to foreign CMOs, with 30.1% of respondents citing US facilities as likely being considered for CMO work within five years. Figure 2 shows the top 10 countries, other than their own, respondents expected to be a destination for outsourcing of their international expansions in the next five years. Among US respondents, China was the top destination, cited by more than 50%.

Despite these industry expectations of more outsourcing to China, CMOs are not fully permitted in China; just a few select companies currently participate in government-run CMO pilot programs (3). Once China turns on its domestic bioprocessing industry, China may become a major off-shoring destination, even if these CMOs are hired to only manufacture products for China’s massive domestic market. BioPlan expects Chinese CMOs to capture business, potentially doing this at the expense of Indian CMOs. India appears to be focusing on serving its own and international markets for lesser-regulated biogenerics, while nearly 85% of the Chinese biopharmaceutical industry targets GMP manufacture for their own domestic and Western markets (3).

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www.biopharminternational.com
Modeling the Degradation of mAb Therapeutics

Ensuring the stability of biotherapeutic products, which has an impact on their safety and efficacy, continues to be a major challenge for the biopharmaceutical industry. This is particularly true for monoclonal antibodies (mAbs), which account for most commercial biopharmaceuticals today. Both physical effects such as aggregation and chemical modifications (e.g., fragmentation) are the two leading paths for mAb degradation. Kinetic modeling can be an effective tool for establishing product stability, offering a deeper understanding of these processes and allowing developers to mitigate the risk of product degradation during the early stages of process and product development.

PROTEINS POSE STABILITY RISKS

Monoclonal antibodies are currently the leading class of protein therapeutic molecules due to their ability to treat a wide range of lethal and life-threatening diseases, including cancer, Ebola (1, 2), multiple sclerosis, rheumatoid arthritis, psoriasis, and asthma. FDA has approved a number of these antibody drug molecules, which are in various stages of clinical and pre-clinical development. But since the major constituent of the final drug product is protein, there is a significant risk associated with the stability of these drug molecules (3–5).

Instability associated with various critical quality attributes (e.g., aggregation, fragmentation, charge variants, and glycosylation) continues to be a major concern for the biopharmaceutical industry. It can, either directly or indirectly, affect mAbs’ biological activity as well as toxicity (4).

The instabilities associated with mAbs can be broadly classified as either physical or chemical (3, 4). Aggregation is one of the major physical instabilities that is believed to impact the immunogenicity of a therapeutic (5).

Different types of chemical instabilities include deamidation, oxidation, fragmentation, and hydrolysis. Although the effects
of these instabilities (both physical as well as chemical) may differ among various classes of mAb molecules, biopharmaceutical manufacturers desire to minimize them (2). Aggregation and fragmentation are the two most significant degradation pathways for mAbs (6–7). They result in instabilities that can occur at various stages of product development, including upstream processing (during protein expression in cell culture), downstream processing (purification), product formulation, storage, and transport (6, 7).

FACTORS THAT AFFECT MAB STABILITY

Factors that affect the stability of an mAb therapeutic can relate either to its structure (primary, secondary, or tertiary), or to its environment. In the primary structure, a protein is more susceptible to instability if there is change in its surface charge distribution or hydrophobicity. Alterations in the secondary structure, especially an increase in beta content of the protein molecule, can make it significantly more vulnerable to aggregation. Environmental factors that can have an impact on the protein include pH, temperature, salt concentration, buffer type, protein concentration, ionic strength, mixing, shear, metal ions, pressure, freeze thawing, freeze drying, and reconstitution (4, 8).

A protein may change in response to these factors, altering its physical structure either by physical association (aggregation) or chemical degradation (fragmentation). This article in the Elements of Biopharmaceutical Production discusses the kinetics of these degradation pathways as well as the insights they reveal that can help developers ensure the stability of biotherapeutic products.

CASE STUDY I: AGGREGATION OF MAB PRODUCTS

Modeling the kinetics and thermodynamics of protein aggregation can prove valuable in understanding its mechanism (9). Mathematical modeling of protein aggregation correlated along with kinetic data can help developers gain both qualitative and quantitative insights into the mechanism behind aggregation. Armed with this knowledge, developers can better predict and control aggregation by optimizing timeframes and environmental conditions (10).
A number of protein aggregation models already exist (9), including the Lumry–Eyring model, which is one of the most commonly used models to predict aggregation kinetics. According to this model, aggregation occurs in two steps: conformational reversible unfolding of the protein molecule followed by irreversible assembly into aggregates, which are either physically or chemically linked to each other.

Many researchers have modified the Lumry Eyring model to account for nucleation as well as higher order aggregation (10). Examples are the Extended Lumry Eyring (ELE) Model and the Lumry Eyring Nucleated Polymerization (LENP) Model (11). In addition, alternative models (9) have proposed alternate aggregation models for proteins such as prions (12), amyloid β (13), and insulin (14).

**LUMRY EYRING NUCLEATED POLYMERIZATION (LENP)**

The Lumry Eyring model introduces the concept of nucleation to aggregation. The kinetic reaction scheme presented in this model depends on:

- Order of reaction
- Starting protein concentration
- Size distribution amongst different aggregate species (6).

The parameters which are considered in this model are nucleus stoichiometry (x), monomers added in each growth step (δ), and the inverse rate coefficients for nucleation and growth (10). These rate coefficients denote the corresponding time scales (τ, and τr) and the details of the model can be found in the literature (10).

**EXTENDED LUMRY EYRING MODEL (ELE)**

Another model used for kinetic analysis of aggregation is the extended Lumry–Eyring (ELE) model. With this approach, unfolding is considered as a single reversible rate-limiting reaction with both folded native and reactive unfolded monomer species in thermodynamic equilibrium with each other (11, 15, 16). This model takes into consideration both reversible and irreversible conformational changes that occur during aggregation (17). It also considers the solution's conformational and kinetic colloidal stability. Concentration-based experimental data are fitted into model equations to calculate the apparent rate constants and predict the monomer degradation rate (17).

**Factors that affect the stability of an mAb therapeutic can relate either to its structure (primary, secondary, or tertiary), or to its environment.**

**AGGREGATE SAMPLE PREP AND CHARACTERIZATION**

To monitor and understand the aggregation mechanism and its behavior in proteins, a model protein (mAb) was chosen with an isoelectric point (pI) of 8.5. Buffer exchange was used to monitor the aggregation kinetics of this mAb under the conditions that are prevalent in the biopharmaceutical industry during downstream purification.

Detailed experimental procedures and conditions have been described elsewhere (6). In brief, buffers examined included those commonly used for Protein A chromatography, cation exchange chromatography, and anion exchange chromatography.

Samples were kept at three different temperatures (4 °C, 15 °C, and 30 °C) at a concentration of 10 mg/mL. Sampling was performed over 120 hours at intermittent time points. The samples were analyzed for aggregation content using size-exclusion high performance liquid chromatography (SE–HPLC) and types of oligomers using dynamic light scattering (DLS) (7) (Figure 1A).

All the experiments were done in triplicate to ensure reproducibility. Aggregation data obtained by SE–HPLC were analyzed using MATLAB R2011a and fitted into ELE and LENP models. Sets of ordinary differential equations (ODEs) were then solved using Gauss-Newtonian algorithm to estimate the model parameters (18).

**FACTORS AFFECTING MAB AGGREGATION**

Aggregation was found to be at its maximum at low pH and accelerated upon increasing temperature and salt concentration (9). Aggregation at low pH has been primarily associated with changes in the fragment crystallizable (Fc) domain of the antibody (19). These changes include partial unfolding of the Fc domain of the mAb, which leads to the exposure of the hydrophobic residues. These hydrophobic residues, which were previously buried inside the molecule, became attracted to each other. This resulted in the enhancement of aggregation at low pH. Figure 1B illustrates that aggregation is minimal in cation and anion exchange (CEX and AEX) buffer conditions (high pH) as compared to protein A purification conditions (low pH). Further, higher temperature resulted in partial or complete unfolding of mAb structures and resulted in aggregation due to instability of the protein molecule at higher temperature (Figure 1B).

Presence of salt also affects protein stability and the extent depends on the type of salt, its concentration, protein charge, and protein and salt interaction (Figure 1B) (6). An increase in the rate of aggregation is seen with increasing salt concentration. Apart from these factors, the type of buffer species also seems to have a profound effect on the stability of protein products.

Higher stability is seen in glycine buffer, as compared with citrate and acetate buffers (Figure 1B) (6), gov-
mAb Stability

Figure 2. Fragmentation: characterization, analysis, and modeling data.

(A) Chromatograms showing change in $\lambda_{280}$ for initial, intermediate and final day sample

(B) Change in % area under chromatogram via SEC

(C) Fragments Mass determination using RPLC-MS

(D) Fragments Size determination by DLS and SDS-PAGE

(E) Pooled fragments analysis on 0th day and 6th day

(F) Temporal Change in Fragments Concentration as compared to model predicted values

cerned by the interaction of the $Fc$ domain of the mAb molecule with different buffer species (20). Results obtained from circular dichroism (CD) spectroscopy and DLS measurements support these observations as well (6). To summarize the key findings, it can be said that pH is the most important factor in protein aggregation, followed by temperature, salt concentration, and buffer species.

KINETIC MODELING OF mAb AGGREGATION

Data obtained from SE–HPLC analysis were fitted using both ELE and LENP models, and the accuracy of the models was judged by doing a comparative evaluation of the experimental values with the model predicted values. Curve fitting for computing the coefficient of regression ($R^2$ value) was performed using both models. A comparison of the models found that the LENP model did a better job of predicting the aggregation kinetics than ELE (6).

Results were then used to compute the time scales of nucleation ($\tau_n$) and growth ($\tau_g$) in order to predict aggregation kinetics (Figure 1C). The modeling parameters of nucleation and growth time scales were compared across different incubation conditions. It was noted that, as the temperature is increased from 4 °C to 30 ºC (in the case of citrate buffer at pH 3.0 with 100 mM NaCl), the nucleation and growth time scales decrease, implying faster aggregation (6) (Figure 1C).

There is also a linear relationship between nucleation time scale and monomer half-life as temperature is increased from 4 ºC to 30 ºC. The steeper monomer loss at high temperature is attributed to the increase in the collision frequency due to an increase in protein diffusion.

A similar effect was observed upon increasing the salt concentration from 0 mM NaCl to 100 mM NaCl (Figure 1C). This is likely due to salt ionization, which weakens protein structure and decreases stability because of hydrophobic interactions and weakening of electrostatic repulsions as the salt concentration increases.
Various contaminants (e.g., metal ions) (6) (hydrolysis) at the mAb's hinge region leads to the formation of Fc–Fab and Fab fragments and corresponding to the published results (7).

**Table I.** Kinetic rate constants (obtained using the constructed mechanism for the same sample stressed at three different temperatures).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>k_1 (day^-1)</th>
<th>k_3 (10^2 \cdot ml^{-1} \cdot mol^{-1} \cdot day^{-1})</th>
<th>k_4 (10^3 \cdot ml^{-1} \cdot mol^{-1} \cdot day^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.0006</td>
<td>0.0002</td>
<td>0.0063</td>
</tr>
<tr>
<td>45</td>
<td>0.0001</td>
<td>0.0043</td>
<td>0.0288</td>
</tr>
<tr>
<td>50</td>
<td>2.22E-14</td>
<td>0.0049</td>
<td>0.0362</td>
</tr>
</tbody>
</table>

**Table II.** R^2 value of the fit of linearized Arrhenius plot of ln(k) vs (1/T). (The table includes the activation energy values as well as the fit equations).

<table>
<thead>
<tr>
<th>Rate constant (k)</th>
<th>Linear Arrhenius equation (ln(k) vs (1/T))</th>
<th>Activation Energy (kcal/mol)</th>
<th>Comments (R^2 value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_1 (day^-1)</td>
<td>ln(k) = -8782 (1/T) - 786.5</td>
<td>2.1</td>
<td>Not following Arrhenius law</td>
</tr>
<tr>
<td>k_3 (10^2 \cdot ml^{-1} \cdot mol^{-1} \cdot day^{-1})</td>
<td>ln(k) = -64610 (1/T) - 453.3</td>
<td>15.38</td>
<td>0.77</td>
</tr>
<tr>
<td>k_4 (10^3 \cdot ml^{-1} \cdot mol^{-1} \cdot day^{-1})</td>
<td>ln(k) = -64610 (1/T) - 453.3</td>
<td>15.38</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Overall, for the system under consideration, the citrate buffer at pH 3.0 showed the steepest monomer loss as compared to acetate and glycine buffers (6) (Figure 1C). There is a linear correlation between nucleation time scale and monomer half-life across the different conditions, with slope between 0.4–0.5, indicating that nucleation dominates aggregation in mAbs and hence that the LENP model offers a better fit than the ELE model (6) (Figure 1C).

**CASE STUDY II: FRAGMENTATION OF mABs**

Fragmentation involves the breaking down of the mAb into smaller units, fueled by thermal or chemical energy (21). Fragmentation results in the breakage of bonds between the amino acids, transforming the primary structure of the molecule and, ultimately, other higher order entities. Cleavage of the peptide bond (hydrolysis) at the mAb's hinge region leads to the formation of Fc–Fab and Fab species (22).

Responses from different classes of the mAb molecules vary with respect to the fragmentation phenomena, depending on each one's inherited primary structure. Various contaminants (e.g., metal ions and proteases) in the formulation buffer systems significantly affect the extent of fragmentation (22), and degradation can occur at various stages of mAb production and processing (23). Size-based monitoring tools such as SEC, DLS, mass spectrometry (MS), and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) are commonly used to detect fragmentation and identify the various species that are formed (24).

**FRAGMENTATION SAMPLE PREP AND CHARACTERIZATION**

The mAb sample was diafiltered in the formulation buffer (15 mM phosphate, pH 6.5 with 150 mM NaCl, and 0.02% Polysorbate 80) at a final concentration of 5 mg/mL. This sample was then incubated at 50 °C, and the extent of fragmentation was monitored by SEC–HPLC at regular time intervals (Figure 2A and 2B).

Various other analytical techniques (e.g., DLS and SDS–PAGE [7]) were used to confirm the sizes of the fragments that were formed (Figure 2D). Reverse–phase high-performance liquid chromatography with mass spectrometry (RP–HPLC–MS) was used to analyze the intact mAb samples as well as the fragmented mAb sample and the purified and enriched fragment species to locate the site of fragmentation in the mAb molecule (Figure 2C).

Results of the SEC, DLS, SDS–PAGE, and MS analysis found that two different fragment species were formed upon fragmentation of intact monomer (148 KDa) mAb molecule—Fragment 1 (96.9 KDa) and Fragment 2 (47.3 KDa) (Figures 2C and 2D). This shows that cleavage occurs at the hinge region of the mAb, leading to the formation of Fc–Fab and Fab fragments and corresponding to the published results (7).

**KINETIC MODELING OF mAB FRAGMENTATION**

Upon observing the fragmentation trend via SEC, it can be inferred that the monomer mAb molecules (M) break down into two species (F1 and F2) with time. Following the temporal trend in concentration change of these three species (M, F1, and F2), it was noticed that, initially, for a period of 72 hours, there is a slow decrease in the concentration of M followed by an abrupt rise in the slope. F1 and F2 fragments also follow this trend in incremental fashion (7) (Figure 2A). This time-based analysis was performed at different temperatures to confirm our hypothesis, upon which a fragmentation model was based. The authors suggest that the phenomenon of fragmentation is autocatalytic in nature based on the assumption that a threshold concentration of fragments is needed to react with intact monomer molecules to further accelerate its degradation (7). To confirm that the proposed model is general in nature, it was tested on another buffer system at 50 °C (15mM phosphate, Ionic strength -29mM, pH 6.8, and 0.015% PS80) (Figure 2B) (22).

After analyzing the experimental results of the two datasets, it was found that, although the fragmentation trend was identical in both the buffer systems, the rate of fragmentation was a bit different because of the different ionic strengths of the two buffers. Approximately 57% of the monomer mAb transformed into fragment spe-
Implementation of PAT at the Bioreactor: On-Line Sensor Technology for Improved Bioprocessing

The FDA’s process analytical technology (PAT) initiative is a framework for innovative pharmaceutical development, manufacturing, and quality assurance. Hamilton Bonaduz AG recently published a 20-page whitepaper that clarifies process parameters, key performance indicators, and process analysis devices for bioreactors. In this interview, Giovanni Camplongo, Market Segment Manager Biopharma at Hamilton Bonaduz AG Process Analytics, talks about what drug manufacturers need to know about applying PAT at the bioreactor.

**BioPharm International:** Hamilton recently released a whitepaper discussing how the FDA’s Process Analytical Technology (PAT) initiative can be applied to the pharmaceutical industry, especially at the bioreactor. What were the findings?

**Camplongo:** The PAT initiative establishes the value in identifying and monitoring critical process parameters as well as defining key performance indicators, both with the goal of improving consistency and optimizing the critical quality attributes present in biopharmaceutical processes.

While this seems logical and straightforward, implementation in the bioreactor can be challenging. That is because fermentations themselves are complex processes based on living organisms with a wide variety of methodologies, cell lines, and conditions. Because of this complexity, PAT is an important step forward for all biopharma companies.

To control such complex processes, and to do it automatically, data collection of the critical process parameters and key performance indicators should be collected in real time. That is what PAT is all about. Controlling is best in real time and in-line.

**BioPharm International:** You mentioned critical process parameters, key performance indicators and critical quality attributes in general, but what are the most important of the parameters and attributes addressed by PAT? What tools are available for monitoring them?

**Camplongo:** With respect to biopharma and the contents of the whitepaper, the most important parameters that can currently be monitored include pH, dissolved oxygen (DO), temperature, and, in regards to key performance indicators, cell density. In-line sensors are available for all of these parameters.

Once again, PAT stresses that it is crucial to monitor process parameters and key indicators in real time because they can trigger automated control loops, which can improve productivity and build the so-called quality by design (QBD).

Let me give a quick example. The most classical example of PAT applied to bioprocesses is the maintaining of culture pH. Mammalian cells vary between 6.8 and 7.4 pH. Tight control of this parameter within predefined limits is crucial because it directly affects the therapeutic effect of products such as forming monoclonal antibodies (mAbs). Low pH negatively influences the protein’s glycosylation pattern resulting in a loss of their bioactivity. And as we know, glycosylation is one of the most important critical quality attributes for monoclonal antibody production.
Other parameters such as metabolite concentration and protein titer are important parameters as well. However, the accuracy, robustness, and repeatability of current technologies are not yet commonly accepted for in-line process controls. Meanwhile, viable cell density is a parameter that can be measured in-line.

**BioPharm International:** Why are the cell density measurements you mentioned an improvement over the more common off-line methods for viable cell counting?

**Campolongo:** Viable cell density is a strong example of the value of PAT application in bioprocessing. While pH and DO can provide information related to cell viability, cell density is measured with capacitance-based probes, which is the only type that can measure with consistent robustness in real-time. We then have data on the actual cell density in real time, and it can be used to monitor the bioprocess. This data can be used for automated feed control in fed-batch or perfusion processes. This is a huge benefit over the labor-intensive and error- and contamination-prone methods that are considered state-of-the-art.

For example, a glucose feed can be controlled off of this data so that lactate production (if we are talking about mammalian cell cultures) remains within limits and doesn’t disturb the growth or final yield. With alternative methods, you have to do that off-line. If it’s a manual process, you will only have defined points in time, and cannot enable real-time control.

If anyone wants to have more information about this application of PAT, including cell density, and the whitepaper, go to [www.hamiltoncompany.com](http://www.hamiltoncompany.com) where we have many applications about on-line/in-line sensor solutions.

**BioPharm International:** PAT favors on-line sensing over off-line measurements, but why are some of the desired attributes not yet established for on-line measurements?

**Campolongo:** Ideally, all these parameters would have an in-line or on-line sensor to monitor them. However, some important measurements, such as nutrients or metabolite concentration or the protein titer or glycosylation, mentioned before, are not yet possible to measure in real time—at least not with the robustness that you would expect. This is much more the limit of the current technology than it is a limit of need or value of such measurement. But, I imagine in the next few years, we will see these technologies develop. As we at Hamilton know very well, innovation never stops in process analytics.

**BioPharm International:** Process analytics is a field of constant innovation, but there is still a gap between the technology that is available and the ideal array of sensing options. What innovations have been made in this field to help close this gap?

**Campolongo:** Many, and especially when we talk about a connection with process control and optimization as indicated by the PAT initiative. Let’s just consider two of the most prominent trends in the biopharmaceutical industry. On one side, we have the increasing use of single-use solutions and on the other side we have a renewed push toward continuous processing. Both require sensors to be accurate, precise and reliable.

Hamilton, for example, has brought single-use pH and optical DO to the industry, which can guarantee the same measurement performance as our state-of-the-art reusable sensors. With regards to continuous measurement, you must have consistent performance across long processes that can last three weeks, even one month or more. In this case, Hamilton was the first to introduce optical dissolved oxygen sensors, which, compared with the ones that existed before, solved problems like drift issues caused by CO₂ fouling. But most of all, what we are proud of is our Arc intelligent sensor technology for simplified workflows and data management.

**BioPharm International:** How is the Arc technology beneficial to biopharma processing, especially PAT?

**Campolongo:** As I just mentioned PAT, is about collecting a lot of data. But, this data is only important if it is reliable across the entire process and consistent between processes. Arc intelligent sensors have a micro-transmitter in the head of the sensor to provide a digital signal right from the source. This minimizes the risk of receiving wrong data as a result of weak signals.

With Arc technology, calibration data can be stored in the sensor head. Therefore, for example, you can calibrate the sensors in the laboratory, and store them ready for use in production. Arc technology also provides real-time sensor diagnostics so you don’t have to wait until the end of a run to determine the health of the sensor. This is especially important for long, continuous processes. We think that Arc has been a real breakthrough innovation in regard to in-line and on-line measurement.

**BioPharm International:** What other requirements are there for sensors in the bioreactor?

**Campolongo:** In the case of reusable sensors, for example, process sensors need to be robust enough to handle the vigorous cleaning procedures that take place in the bioprocess environment, along with the aforementioned need of a reliable signal and diagnostics. All of Hamilton’s reusable sensors can withstand clean-in-place (CIP), steam-in-place (SIP), and autoclaving, so sensors can be sterilized with the bioreactor.

On the other hand, single-use sensors do not need CIP or SIP, but still have the need for high accuracy and low drift after long periods of dry storage. The main goal of the single-use probe is a ready-to-use sensor offering the opportunity to start up the system without manipulation prior to the process. Hamilton’s single-use pH sensor, the OneFerm, for example, can be stored dry up to 18 months after gamma radiation with an accuracy of ±0.15 pH units. And the sensor is ready to use within 30 minutes after the first wetting.

At Hamilton Process Analytics, we like to think that we pioneer open sensing solutions to enhance the understanding and control of critical process parameters, as well as key performance indicators. Independently, if we are talking about a reusable or single-use sensor, we push the right technology to perform what PAT requires as far as in-line measurement.
SUDDEN INCREASE IN FRAGMENTATION RATE SEEN
In addition, the fragmentation rate was initially gradual but increased suddenly on the fourth day until the sample had completely degraded. Further, it was observed that the peak next to the monomer peak is the first fragment species (F1 or Fc–Fab), and the second peak is the second fragment species (F2 or Fab).

The concentration of F1 species increases in the beginning and then decreases (7). The concentration of F2 species continuously increases throughout the study. On examining these observations, the following mechanism is proposed:

\[
\begin{align*}
M & \xrightarrow{k_1} F1 + F2 & [1] \\
M + F2 & \xrightarrow{k_1} F1 + 2F2 & [2] \\
F1 & \xrightarrow{k_2} 2F2 & [3]
\end{align*}
\]

Moreover, this model was verified experimentally at three different temperatures (40 °C, 45 °C, and 50 °C). The results obtained from kinetic analysis showed that reaction [1] is a non-Arrhenius reaction with negative activation energy. This was also checked at further two temperatures (30 °C, 35 °C). At all the five temperatures, the trend is non-Arrhenius with negative activation energy (Table I).

The reaction [3] was confirmed by performing purifying and enriching F1 and F2 species and incubating them at 50 °C separately. F1 species (Fc or Fc–Fab) dissociate into Fc and Fab fragment species and F2 fragment was found to be the final end product after which the sample degrades completely (Figure 2F) (7).

Amongst all rate constants, while \( k \) did not follow Arrhenius kinetics, \( k_1 \) and \( k_2 \) did with \( R^2 \) greater than 0.75. Further, \( k_1 \) and \( k_2 \) exhibited a linear relationship with temperature corresponding to increased fragmentation (Table II).

MATLAB R2015b was used to analyze fragmentation data obtained by SE–HPLC, which was then fitted into the proposed model equations to obtain the rate constants (Figure 2F). For all the fragment species, the \( R^2 \) value was found to be greater than 0.9, proving that the model is statistically significant (7).

Aggregation and fragmentation are the two most significant degradation pathways for mAbs. They result in instabilities that can occur at various stages of product development.

SUMMARY
Because they are proteins, mAbs are highly sensitive labile molecules and degrade easily via aggregation and fragmentation. This article has discussed kinetic modeling and shown how it can be used as a tool for the early assessment of protein stability.

Insights presented in this article and the accompanying references can help readers in the development of plans for examining and controlling product degradation during production and storage. Such studies open the door for better understanding of mAb degradation pathways, which can only help efforts aimed at increasing the shelf life of mAb drug products.

ACKNOWLEDGMENTS
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REFERENCES
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the time of the inspection. As with all inspections, courtesy and professionalism will go a long way.

To answer your question: be prepared by having a sound understanding of the regulations and the regulatory inspection processes, including the unwritten expectations. Regulatory intelligence is essential as regulations change continuously and so do the inspection processes.

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enhance surge capacity when additional treatments are needed to manage infectious disease outbreaks or bioterrorism attacks. OPQ also aims to launch a structured approach to the manufacturing supplement review process to better manage product changes through the drug lifecycle.

ACCELERATING APPROVALS

An important goal for Woodcock is to complete the overhaul of the new drug review process to better manage CDER’s growing volume of applications. She recently named Peter Stein director of the Office of New Drugs (OND), and long-time CDER guru Bob Temple will become OND senior advisor, positioned to address the more controversial and difficult drug development and review issues. Woodcock hopes to finalize the OND reorganization by next summer, but it has been delayed by difficulties in gaining Congressional approval of a new user fee program for improving the regulation of over-the-counter drugs.

Modernizing the review process will involve implementing new automation tools for managing drug applications, study data, and review documents under a “multi-disciplinary, issue-based review document” system. CDER also will continue to carry out provisions of the 21st Century Cures Act and reauthorized user fee programs to further advance patient-focused drug development, expanded use of real-world evidence, novel clinical trial design, and added authorities to hire more experts needed to carry out these multiple drug regulatory programs.

REFERENCE
Q: We are a medium-sized company in Europe, specializing in gene-therapy products. So far, we have only marketed our products in Europe, but we are planning a global expansion. By now, we are familiar with the European inspectors and how they inspect. How can we prepare for inspections from overseas agencies? We hear that there is no harmonization in expectations?

A: Congratulations on your plans and being proactive in preparing for these inspections. To some degree, inspectorates from around the world are making attempts at harmonizing how they inspect. Regulatory agencies that belong to Pharmaceutical Inspection Co-operation Scheme (PIC/S) follow a harmonized inspection process. It is sensible to familiarize oneself with these procedures, which are freely available on the PIC/S website (1).

Other drivers for harmonization are mutual recognition agreements (MRAs), which require agencies to recognize each other’s competence and equivalence. Currently, the United States and the European Union (EU) are in the process of finalizing such an MRA (2). As you will already be aware, inspectorates of the EU’s member states, the National Competent Authorities, are all peers (i.e., recognizing each other’s inspections as completely equivalent) (3).

That said, you may still encounter differences in inspection process and style, and moreover, differences in opinion from your inspectors. Why might this be? There are three main contributions:

- The law: different national regulations
- The approach: country-specific inspection processes and requirements
- The human factor: personal preferences of the inspectors.

To make your inspections as smooth and successful as possible, you need to acquaint yourself with all three aspects and prepare for them. A pharmaceutical firm is legally required to comply with the national regulations in any country it wishes to market its products. Therefore, you must be familiar with the regulations. For example, your products will have to comply not merely with the pharmacopeia in your native country, but also with those of the country the overseas inspector is from. Do not be surprised if an inspector wants to see that you do possess a copy of that document and that you can read it if it isn’t in your native language.

Many agencies publish details on how they inspect and often also provide additional information on the inspection process and the agency’s expectations. The United Kingdom’s Medicines and Healthcare products Regulatory Agency (MHRA), for example, has a blog (4). FDA publishes guidance for industry documents (5), and Australia’s Therapeutic Goods Administration (TGA) updates information on their inspection approaches regularly on their website (6). It is essential to stay informed on the inspection process, as some agencies may have specific requirements. Russia’s Roszdravnadzor, for example, requires you to provide certified third-party translators with good manufacturing practice expertise for its inspection; or Colombia’s National Food and Drug Surveillance Institute (INVIMA) expects you to have all observations resolved by end of inspection. Here, the difficult part is that not all the expectations are included in the guidance documents. The best way to get access to such detailed information is to use the help of professional service providers and to interact with peers (e.g., at conferences or through industry associations).

The last point, namely an inspector’s personal opinions, is one you simply have to address at
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