Maximize Purity and Recovery with CHT™ Ceramic Hydroxyapatite

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Maximize Purity and Recovery with CHT™ Ceramic Hydroxyapatite

Among a selection of mixed-mode resins evaluated for the purification of mAb S, CHT provided the best monomer recovery at 83% in the smallest elution volume with a target purity of 99.5%. Using a bind and elute strategy provides enhanced purification power compared to flow-through modes. Purity, recovery, and elution volume are important factors and parameters when screening multiple resins for the development of efficient, robust, and economical production processes.

Bind and elute strategies allow enhanced selectivity compared to flow-through methods as both low- and high-binding impurities can be removed. Important factors to consider in addition to selectivity are the number of buffers, ease of buffer preparations, and overall elution volumes.

### mAb S Purification Performance Comparison

<table>
<thead>
<tr>
<th>Target Monomer Content</th>
<th>Chromatography Media (Resin)</th>
<th>Monomer Recovery, %</th>
<th>Eluate Volume, CV</th>
<th>10% DBC of mAb S, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5</td>
<td>CHT</td>
<td>83</td>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>99.5</td>
<td>Capto adhere</td>
<td>49</td>
<td>14</td>
<td>31.9</td>
</tr>
<tr>
<td>99.5</td>
<td>Capto adhere ImpRes</td>
<td>62</td>
<td>14</td>
<td>70.9</td>
</tr>
</tbody>
</table>

DBC, dynamic binding capacity

Capto is a trademark of GE Healthcare.

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**Highest Binding Capacity**

**Widest Affinity for mAb and Fab Classes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Subclass</th>
<th>TosoProtein A ligand (C-Domain)</th>
<th>Species</th>
<th>Subclass</th>
<th>TosoProtein L ligand</th>
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</thead>
<tbody>
<tr>
<td>Human</td>
<td>IgG, IgG_{s2}, IgG_{s3}</td>
<td>yes</td>
<td>Human</td>
<td>IgG_{s1}, IgA, IgD, IgE, IgM</td>
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</tr>
<tr>
<td>Mouse</td>
<td>IgG_{s1}, IgG_{s2}, IgG_{s3}, IgG_{s4}, IgG_{L}</td>
<td>yes</td>
<td>Mouse</td>
<td>IgG_{s1}, IgG_{s2}, IgG_{s3}, IgG_{L}, IgA, IgM</td>
<td>yes</td>
</tr>
<tr>
<td>Rat</td>
<td>IgG_{s1}, IgG_{s2}, IgG_{s3}, IgG_{s4}, IgG_{L}</td>
<td>yes</td>
<td>Rat</td>
<td>IgG_{s1}, IgG_{s2}, IgG_{s3}, IgG_{L}, IgA</td>
<td>yes</td>
</tr>
<tr>
<td>Chicken</td>
<td>IgY</td>
<td>-</td>
<td>Chicken</td>
<td>IgM, IgY</td>
<td>yes</td>
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<tr>
<td>Goat</td>
<td>IgG_{s1}</td>
<td>yes</td>
<td>General</td>
<td>Kappa light chain</td>
<td>yes</td>
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<tr>
<td>Rabbit</td>
<td>IgG</td>
<td>yes</td>
<td>Fab</td>
<td>scFv</td>
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<tr>
<td></td>
<td>scFv (25 kDa)</td>
<td></td>
<td>Dab</td>
<td></td>
<td>yes</td>
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</tbody>
</table>

Residence time (minutes)

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FEATURES

NEXT-GENERATION ANTIBODIES
Witnessing Major Growth in Next-Generation Antibodies
Cynthia A. Challener
Including next-gen antibodies in pharma pipelines is considered essential for future success.

UPSTREAM PROCESSING
Engineered Proteins as Tools in ADC Development and Manufacture
Adeline Siew
OrlaSURF technology can be used for the development of target-binding assays to monitor the binding of an ADC to its antigen.

DOWNSTREAM PROCESSING
Downstream Processing for Cell-Based Therapies
Qasim A. Rafiq and Fernanda Masri
For cellular materials, new ultra scale-down devices inform large-scale downstream processing techniques.

FILL/FINISH
Impact of PFS and Filling Process Selection on Biologic Product Stability
Wendy Saffell-Clemmer
The author discusses the impact of pre-filled syringe product contact materials and the filling and stoppering process on protein aggregates.

QUALITY
Critical Quality Attributes Challenge Biologics Development
Susan Haigney
The complex nature of biologics adds additional CQAs that must be determined to ensure the safe development of biologics.

ANALYTICS
FTIR Spectroscopy as a Multi-Parameter Analytical Tool Therapeutic Proteins
Allison Derenne and Erik Goormaghtigh
FTIR can successfully measure key characteristics of therapeutic proteins in a single step.

Mechanistic Modeling of Preparative Ion-Exchange Chromatography
Anurag Rathore and Vijesh Kumar
Chromatography modeling can enhance bioprocessing efficiencies.

RAW MATERIALS
Redefining Excipients for Advanced Therapies
Agnes Shanley
As regulators strive for balance in cGMPs for cell, gene, and tissue therapies, risk-management principles must guide decisions involving process media and additives.

BIOBURDEN TESTING
Kill the Bioburden, Not the Biological Indicator
James Agalloco
Understanding the purpose of the biological indicator can guide the development of an effective sterilization process.

COLUMNS AND DEPARTMENTS

6 From the Editor
Philadelphia plays host to CPhI North America.
Rita Peters

8 Regulatory Beat
Industry fears limited benefits as FDA readies data tracking program.
Jill Wechsler

10 Perspectives on Outsourcing
New study shows China biopharma companies face staffing shortages.
Vicky Qing Xia

14 NEXT-GENERATION ANTIBODIES
Witnessing Major Growth in Next-Generation Antibodies
Cynthia A. Challener
Including next-gen antibodies in pharma pipelines is considered essential for future success.

20 UPSTREAM PROCESSING
Engineered Proteins as Tools in ADC Development and Manufacture
Adeline Siew
OrlaSURF technology can be used for the development of target-binding assays to monitor the binding of an ADC to its antigen.

22 DOWNSTREAM PROCESSING
Downstream Processing for Cell-Based Therapies
Qasim A. Rafiq and Fernanda Masri
For cellular materials, new ultra scale-down devices inform large-scale downstream processing techniques.

28 FILL/FINISH
Impact of PFS and Filling Process Selection on Biologic Product Stability
Wendy Saffell-Clemmer
The author discusses the impact of pre-filled syringe product contact materials and the filling and stoppering process on protein aggregates.

33 QUALITY
Critical Quality Attributes Challenge Biologics Development
Susan Haigney
The complex nature of biologics adds additional CQAs that must be determined to ensure the safe development of biologics.

35 ANALYTICS
FTIR Spectroscopy as a Multi-Parameter Analytical Tool Therapeutic Proteins
Allison Derenne and Erik Goormaghtigh
FTIR can successfully measure key characteristics of therapeutic proteins in a single step.

41 Mechanistic Modeling of Preparative Ion-Exchange Chromatography
Anurag Rathore and Vijesh Kumar
Chromatography modeling can enhance bioprocessing efficiencies.

46 RAW MATERIALS
Redefining Excipients for Advanced Therapies
Agnes Shanley
As regulators strive for balance in cGMPs for cell, gene, and tissue therapies, risk-management principles must guide decisions involving process media and additives.

50 BIOBURDEN TESTING
Kill the Bioburden, Not the Biological Indicator
James Agalloco
Understanding the purpose of the biological indicator can guide the development of an effective sterilization process.

56 New Technology Showcase

57 Ad Index

58 Ask the Expert
The value of internal audits and how the information gained can be applied are discussed.

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In my role as editorial director of a bio/pharmaceutical industry publication, I attend many trade shows and conferences. The largest events, sponsored by CPhI, a sister brand of BioPharm International at UBM plc, have a long track record of gathering pharmaceutical development and manufacturing buyers and sellers in one location to hear about the latest trends, network, and conduct business. On May 16–18, 2017, the CPhI brand will host CPhI North America in Philadelphia, PA, bringing together bio/pharma experts from the United States, Canada, and beyond. The following are some highlights.

Educational sessions
The CPhI North America exhibition will be home to more than 600 global companies including API and excipient suppliers, packaging and drug delivery suppliers, small- and large-molecule contract development and manufacturing organizations, as well as equipment and other contract service providers. A co-located event, InformEx, features suppliers in fine and specialty chemical markets, including biopharmaceuticals, medical devices, and green chemistry.

In educational programming, CPhI Connect features keynote and master class sessions focused on drug development, drug manufacturing, outsourcing, and quality and regulatory issues. Topics include: accelerating product development; legal and policy strategies; improving drug R&D productivity; novel container/closure technologies; bioconjugates and biotherapeutics; economies of capability vs. economies of scale; track and trace in a post-serialized world; biophysical characterization of protein therapeutics; continuous biopharmaceutical manufacturing; pharmaceutical impurities; good supply practices; and quality metrics.

Industry insight
In Insight Briefing sessions, attendees can listen to updates on technical and business issues. Topics include formulation techniques for controlled-release powders to create patient-friendly products; liquid stick packs for unit dosing; process safety evaluations for contract development and manufacturing API operations; worker protection in life-science laboratory and production environments; and bio/pharma business opportunities in the Middle East–North Africa region. The briefings will be held in the Insight Briefings Theater during the three days of CPhI North America; admission is free to anyone with an exhibition, conference, or exhibitor badge.

During a briefing on May 17 at 2:30 PM the editors of BioPharm International and Pharmaceutical Technology will release the results of a study on quality practices in drug development and manufacturing organizations serving the US market. The briefing, based on a comprehensive survey and additional research, will reveal current quality-related practices in bio/pharma organizations and contract manufacturers producing drug products for patients in the US.

In another highlighted session, bio/pharma industry expert Jim Miller, president of PharmaSource Information Services, will share his perspective on the contract services landscape and suggest strategies for outsourcing companies looking to grow in this sector in the session The Contract Services Market: Status and Strategies. BioPharm International and Pharmaceutical Technology will host this special session on Wednesday, May 17, from 8:30–10 AM, in the conference area of the Pennsylvania Convention Center.

For more details on the exhibition, conference, briefings, or networking opportunities at the event, visit www.cphinorthamerica.com.

The editors of BioPharm International will be at CPhI North America, booth 2640. See you there!
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The agenda will include:

• Plenary sessions focusing on the role of QRM, risk-based approaches to system design and practical implementation
• Breakout groups that will conduct a risk-assessment and e strategies for given processes
• A breakfast session meeting of the Quality Risk Management Interest Group
• An evening Networking Reception in the Exhibit Area, where attendees can mingle with colleagues with similar interests and challenges and engage with exhibitors showcasing the latest products and services

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FDA plans to launch its quality metrics data initiative in January 2018 by opening an electronic portal (e-portal) to collect data on certain manufacturing processes electronically from biopharmaceutical companies. The aim is to identify and reward those firms able to demonstrate that their operations can consistently produce high-quality products and thus merit reduced regulatory oversight. Yet as the metrics program nears implementation, industry is pushing back, voicing concerns about the scope of FDA’s data requirements, the timing of the program’s launch, and what the agency will do with the resulting information.

Questions about the agency’s legal authority to require detailed reports on specific manufacturing operations prompted FDA to shift from a mandatory to a voluntary program in 2016, but that creates uncertainty about how many companies will participate and whether the results will be meaningful. Agency officials hope that many firms will submit data so that the program can better detect and prevent drug shortages and support adoption of a smarter regulatory approach by the agency.

The quality metrics program has been under development at the Center for Drug Evaluation and Research (CDER) for more than a decade, part of efforts to encourage industry adoption of advanced manufacturing systems able to produce high-quality medical products continuously and with minimal oversight. The aim is to avoid drug shortages and recalls, which largely arise from production issues, noted Lawrence Yu, deputy director of CDER’s Office of Pharmaceutical Quality (OPQ), at the Parenteral Drug Association’s (PDA) February 2017 Pharmaceutical Quality Metrics and Quality Culture Conference in Bethesda, MD. Yu added that advanced manufacturing methods based on robust quality programs are even more important with the rise of precision medicine, biosimilars, and complex combination products. And high-performing firms may be rewarded with fewer or shorter plant inspections and reduced oversight of postapproval manufacturing changes, as well as high placement on a metrics “reporters list.”

MOVING FORWARD
FDA’s current plan is to open the e-portal early next year for three months in which 2017 metrics data may be accepted. The agency published a technical conformance guide in 2016 with information on how to submit requested data electronically (1). A revised technical guidance will further clarify issues related to e-data transmission. FDA will announce in the Federal Register early next year when the portal is open and provide further updates on what information to submit. Manufacturers that plan to participate are collecting metrics data for 2017, even if only for six or nine months in this initial year.

FDA will evaluate the data and summarize how many firms reported which metrics and the number of products and facilities involved. A “reporters list” may identify participating firms, but not provide data on individual companies. Even so, the plan to list firms in tiers according to extent of reporting has raised concerns that this may discourage participation, especially by small companies.

This more scaled-back program reflects industry objections to FDA’s initial metrics data proposal, which was published in July 2015. A revised draft guidance issued in November 2016 makes the program voluntary,
slims down data reporting, and gives manufacturers the option for reporting metrics by products or by sites (2).

These changes “reflect your voice,” Wu told industry representatives at the PDA conference, noting that the revised program seeks data only on lot acceptance rate, invalidated out-of-specification (OOS) rate, and product quality complaint rate. The agency acknowledges that it would be useful to have information on process capability, corrective actions and preventive actions (CAPA) effectiveness, or right-first-time rate, but has agreed to limit the program’s initial scope for now.

The initial program involves only drugs and biologics regulated by CDER, and not yet vaccines, blood products, and cellular and gene therapies overseen by the Center for Biologics Evaluation and Research (CBER). CBER is actively involved in the metrics program and plans to extend it to its products as the initiative matures.

FDA and industry also acknowledge the importance of a company’s quality culture in ensuring reliable drug production, but recognize the need to delay specifying metrics to measure such criteria pending further study of methods for documenting that desired state. While a strong quality culture might be reflected in visible senior management support for quality production, there’s much debate over how to achieve and report such behavior.

**BENEFITS VS. COSTS**

Many attendees at the PDA conference expressed continued reservations about the scope and timing of the metrics program and the effort involved in revising data systems and establishing complex reporting programs. Deborah Autor, head of strategic global quality and regulatory policy at Mylan, described the considerable challenge in providing metrics on more than 600 finished products and APIs, noting that reports on the three requested metrics involve mapping 11 data points from many different information systems within the company. “Slow it down,” Autor advised, proposing that FDA “phase in the program gently” and drop the reporters list until program feasibility becomes clearer.

The difficulties may be even greater for Johnson & Johnson, which is supposed to provide metrics data on hundreds of non-prescription medicinal products that fall under the program, but raise little risk of patient harm from production problems. And Barbara Allen, senior director of global quality systems at Eli Lilly, was skeptical that data on a few production indicators can really inform on risks associated with specific products or sites.

Many biotech manufacturers recognize the importance of implementing continuous improvement systems, but still may find it challenging to meet metrics requirements, said Melissa Seymour, vice-president for global quality control at Biogen, at the PDA meeting. She was optimistic that the initiative could lead to greater trust in industry and fewer inspections, but concerned that many small companies will decide that falling to the low tier of the reporters list might be worse than not being on the list at all. Hovione CEO Guy Villax agreed with “start small” proposals, predicting that participants may “game the system” to spin data to a company’s advantage.

In launching the program now, explained Ashley Boam, acting director of OPQ’s office of policy for pharmaceutical quality, FDA aims to test and improve data collection through the e-portal, to better assess which incentives encourage firms to participate, and to gain insight into how to use the resulting data to improve inspections and oversight. Data from multiple sites and firms, she noted, is key to detecting signals of possible drug shortages that often involve more than one company or operation. And FDA wants to be sure that collection of metrics data does not create barriers to manufacturers adopting new technology and continuous manufacturing systems.

FDA will continue to partner with manufacturers in defining and assessing measures of quality operations and culture. Industry organizations have sponsored multiple conferences, white papers, pilot programs, and task forces on this initiative. An International Society for Pharmaceutical Engineers (ISPE) conference in April 2017 will further discuss quality metrics and quality culture assessment, and PDA plans to publish a report on its current metrics pilot in September. Boam expects a voluntary metrics program to continue for more than a year and that it will help the agency focus resources on facilities and products that raise the highest risks to patients. FDA also intends to provide opportunities for companies to provide feedback and share knowledge from ongoing, industry-driven quality metrics programs.

**REFERENCES**


The past decade has witnessed robust growth in China’s biopharma industry due to rise in gross domestic product (GDP), expansion of China’s middle class, greater coverage of the national healthcare, as well as a rapidly aging population and massive urbanization. In 2014, the market was approximately US$5.0 billion, projected to be the second largest biologics market globally by 2020 (1). This represents a compound annual growth rate (CAGR) of approximately 20%.

Although the past couple years have shown signs of a cooling domestic economy, China’s biopharma industry has remained strong, as manufacturers both domestic and abroad make long-term investments. BioPlan Associates’ study of domestic Chinese bioprocessing capacity shows that both domestic Chinese and multinational pharma investments have dramatically grown since 2008 (2).

Analysis of the largest facilities indicate growth in both liters capacity and staffing for production of biologics. In addition, global companies continue to invest in construction of biomanufacturing facilities. Boehringer Ingelheim’s contract manufacturing facility in Shanghai and Pfizer’s biologics center in Hangzhou (3) are two prominent examples. Many more domestic companies, however, are moving in the same direction. Many of the established biologics makers, including China National Biotech Group Company, the biggest vaccine maker in China, are expanding and upgrading their existing biomanufacturing facilities. The group is also supporting one of its subsidiaries, the Chengdu Institute of Biological Products Co., Ltd., to become the first in China to get World Health Organization (WHO) prequalification with a patented Japanese Encephalitis vaccine. Newcomers in the biopharma industry also broke ground for new bio-manufacturing facilities. Teruosi Pharma, for example, is building its 24,000-L antibody manufacturing facility, and WuXi Biologics began construction on a new $150-million biologics manufacturing facility. When complete, this new facility will be the largest mammalian cell-culture manufacturing facility using disposable bioreactors in the world. This makes the facility the largest biomanufacturing facility of any kind in China. In addition, several Chinese biologics facilities are under active construction.

Both domestic Chinese and multinational pharma investments have dramatically grown since 2008.

The growth in market size also requires a more innovative biologics pipeline. According to data from China’s regulatory authorities, as of February 2017, China had 171 monoclonal antibody (mAb) therapeutics under clinical development, from 82 biologics manufacturers. In 2016 alone, 32 new projects entered clinical trials (3). Though China started commercialization of antibody drugs relatively late compared with the United States and Europe, and currently has only granted a new drug application (NDA) to fewer than 10 ‘made-in-China’ mAb therapeutics, more launches are expected in the next few years.
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STAFFING CHALLENGES
Over the past decade, China has been fixing problems related to funding, pipeline development, quality management, and facility upgrades. But what’s more difficult to fix is the looming shortage of talented staff to support the increase in capacity and quality production. This is likely to become a real bottleneck, as domestic biomanufacturers move ahead and target international, more regulated markets.

And, capturing market share internationally is clearly something Chinese manufacturers expect to be doing over the next decade (4). In a BioPlan Associates’ study, nearly 90% of responding Chinese biologics managers indicated their company currently plans to target global distribution of GMP-produced biologics within 10 years. Further, nearly half of respondents indicated the need for more ‘scientific/technical expertise’ to succeed (4).

In another recent analysis of training in China, BioPlan interviewed more than a dozen experts and high-level biopharma industry managers in China. Many industry insiders lament that current degree programs from academic organizations in China are relatively out of touch with industry and are not designed to meet the evolving demands of biomanufacturers.

While multiple bioprocessing vendors (including GE, Sartorius, and Pall) currently offer product training seminars, many core bioprocessing staff regard these as too general, or not comprehensive enough to meet their operational training needs. Vendor courses may also not fit systems provided by competitors. Those who need training most are new/entry level staff in bioprocessing; yet due to high turnover rates in China, many companies are reluctant to make investment in training. As in the US/EU, many companies must rely on existing experienced staff to train entry level new staff. But industry insiders agree that such real expertise in bioprocessing—those capable of training new core staff—can only be found in the EU/US or in returnee scientists.

Some companies are hiring consultants overseas to give training courses, and others have developed internal courses to meet needs. Manufacturers find quality control (QC) and formulation to be two specific areas where in-depth training is needed the most, and they are willing to make investments. Currently, there are training companies offering courses in drug formulation, QC, etc. Others are considering sending staff to US universities.

OVERCOMING TALENT SHORTAGES IN CHINA
With almost 100 Chinese companies developing mAb therapeutics at some stage, training staff and attracting returnee scientists to enable efficient, GMP-grade biomanufacturing in the near future may be just too challenging for some smaller and early-stage biotechs. A short-term alternative may be to avoid building biomanufacturing facilities, and training internal bioprocessing teams. Outsourcing manufacturing to contract manufacturing organizations (CMOs) may be the only option for some. China is already home to several technically advanced CMOs, such as JHL Biotech, WuXi Biologics, and Boehringer Ingelheim in Shanghai. Each has in-depth technological know-how in biomanufacturing and a team of bio-processing professionals with industry experience from Western countries. JHL Biotech, which kicked off operation in 2016, has already entered agreements with BeiGene, Ltd. for a new biologic. The company also announced a strategic alliance with Sanofi to collaborate on the commercialization of biologics in China. In 2016, Zai Lab of Shanghai and Boehringer Ingelheim (BI) also signed a cooperation agreement under which BI will provide manufacturing services in its Shanghai facility for a mAb from Zai.

China is currently reforming its rather restrictive CMO regulatory environment with a pilot program in Market Authorization Holder (MAH) in certain provinces, to allow a drug developer to outsource manufacturing to a CMO. These changes are likely to expand in the future.
Although recent regulatory reforms have opened the door for CMO operations, many domestic biomanufacturers plan to build internal manufacturing capability. To staff such facilities, employers are actively seeking standardized programs applicable across multiple systems and multiple vendors; for both entry level and core staff. Potentially a supplier/industry association collaboration from Sino-US joint education programs to develop certificate programs in bioprocessing to address current and future needs.

**Hiring Difficulties in China vs. US/EU**

There are no significant differences in the challenges faced by US/EU facilities and their Chinese counterparts in terms of staffing challenges (2). Of the 222 qualified global industry executives, over a third cannot fill upstream or downstream process development positions (5). The magnitude of the problem is likely to be even greater in China as facilities rapidly expand (see Figure 1).

This ongoing hiring challenge will further confound the growth of manufacturing capacity to levels needed to support China’s domestic manufacturing. The industry can expect a capacity crunch due to hiring challenges as more biologics facilities compete for a limited pool of staff talent. While CMOs and others will likely alleviate some of this demand, manufacturing capacity may be at a premium in coming years, as available staff keep busy.

**REFERENCES**

3. Biologics Industry, mAb therapeutics under development in 2017, original data from Center for Drug Evaluation, CFDA.
Next-generation antibodies are designed to be more specific and are often more potent than traditional monoclonal antibodies (mAbs). As a result, their commercial potential is significant. The global market for next-generation antibodies, including antibody drug conjugates (ADCs), engineered antibodies, bispecific antibodies, antibody fragments, antibody-like proteins and biosimilar antibody therapies, is estimated by Vision Gain to reach $11.6 billion in 2020 (1). Both large and small pharmaceutical and biotech companies are pursuing these newer therapies. A few have already been approved, and many more are in preclinical and clinical development. There are numerous challenges that need to be overcome first, however, and the full impact of next-generation antibodies will likely not be observed for many years.

MANY TECHNOLOGIES

Next-generation antibody formats include ADCs, other bioconjugates, bispecific (multispecific) antibodies, nanobodies, engineered antibodies, antibody fragments, antibody-like proteins and biosimilar antibody therapies. Based on discussions with partners, Catalent sees ADCs, other bioconjugates, and bispecific antibodies as being the most prevalent technologies, according to Mike Riley, vice-president and general manager at Catalent Biologics.

The various formats can be placed into four main categories. One involves technologies designed to improve the effector function and/or extend the half-lives of traditional mAbs through modifica-
tion of the back end, or Fc (fragment crystallizable) region. “This approach aims to optimize the killing potential (i.e., impart more antibody-dependent cell-mediated cytotoxicity [ADCC] activity), so that target cells are killed more effectively,” notes Tony de Fougerolles, chief scientific officer with Ablynx. Two products developed using glycoengineering have been approved (Gazyva and Poteligeo). MacroGenics is taking an alternative approach with the substitution of amino acids in the Fc region.

The second category consists of antibodies that have additional specificities for targeting. Most are bispecific, and thus have two targets, but tri-specific and other multi-specific therapies are being explored, according to de Fougerolles. Various technologies are being used to develop these products, such as the joining of two different heavy and two different light chains together to produce a substance that can attack two different targets. Two bispecific antibodies have been approved to date (Removab and Blincyto).

The third category includes drugs made from smaller antibody fragments or other protein-based scaffolds. These encode much smaller target-binding molecules (in the case of Nanobodies, these are 1/10th the size of a traditional antibody) and as such can be easily genetically linked together to encode for bi- and multi-specific drugs.

The fourth category is conjugates, including ADCs and others. While the potential of ADCs has been widely touted, to date only two products have been approved (Adcertis and Kadcyla). Drug developers are, however, improving linker technologies and the specificity of newer ADC products, such as the targeting of dividing cells vs. non-dividing cells, and Scott Koenig, president, CEO, and director of MacroGenics expects to see a new wave of products reach the market in the coming years.

Some of these technologies, such as ADCs and bispecific antibodies, were initially proposed decades ago but, at the time, the technical capabilities required to make them commercially feasible were lacking, according to Koenig. “Today there is a whole set of technologies in preclinical and clinical trials with tremendous promise,” he notes.

**Many Therapeutic Targets**

Oncology continues to be a key focus for next-generation antibody therapies, but Catatals is also seeing an interest in new modalities, such as conjugates, for other indications, according to Riley. “Novel antibody platforms have primarily been applied to cancers because immunogenicity and potential unforeseen clinical effects that might be better tolerated for cancer treatment than for chronic treatment in non-oncologic diseases,” agrees Andrew Chan, senior vice-president of research biology at Genentech. He notes that bispecific and multispecific antibodies have also been broadly applied in oncology for the neutralization of multiple antigens, the fusion of binding proteins to antibodies, and cell recruiting therapeutics in cancer immunology to recruit T cells to facilitate tumor killing (e.g., T cell-directed bispecific antibodies).

The development of next-generation monoclonal antibodies has expanded beyond oncology into all therapeutic areas. “For instance, ADCs were initially focused primarily on cancer, but Genentech has applied the lessons learned to infectious diseases, leading to the discovery and development of THIOMAB antibody conjugates (TACs) for the treatment of methicillin-resistant Staphylococcus aureus (MRSA),” says Chan. In addition, he observes that the use of fragment antigen-binding (Fab) and other antibody fragments and nanotechnologies have expanded to ophthalmologic indications, where intravitreal injection volumes are limited requiring small molecular mass therapeutics with high potency. Application bispecific antibodies are also being applied to diseases of the central nervous system with the development of brain-penetrant antibodies.

“Hence, next-generation biotherapeutic technologies have potential in virtually all human diseases,” Chan asserts.

**Real Advantages**

These new antibody formats are attracting attention because they have several advantages over current monoclonal antibody therapies. “These new modalities very often can be more targeted and potent than traditional technologies, and in some cases can access new targets or combinations of targets,” Riley explains.

“With multispecific antibodies in particular,” adds de Fougerolles, “it is possible to block multiple pathways with a single molecule, which provides development cost and cost of goods advantages in addition to enhanced therapeutic effects. In some cases, synergistic results have been obtained, with bi-specific drugs providing better results than even the use of both mono-specific drugs in combination.”

For instance, MacroGenics has shown preclinically that a bispecific antibody containing two checkpoint inhibitors has an even more dramatic effect than the use of two separate antibodies, according to Koenig. He adds that molecules that interact in multiple ways with the same target cell, such as bispecific antibodies with components that are physically close together in the same molecule, can result in unmasking biological functions that cannot be activated with conventional mAbs. He also notes that Fc-engineering can lead to mitigation of the body’s natural checkpoint mechanisms, which modulate
Next-Generation Antibodies

the expression of T cells, resulting in amplification of cytolytic effects.

“The key is that these next-generation antibodies are offering new biological activities that cannot be achieved with traditional mAbs while at the same time adding greater specificity to their targeting mechanisms for improved potency and reduced potential toxicities and side effects,” Koenig states.

COMMERCIAL POTENTIAL

Typically, it takes 10–12 years or more between the advent of a new technology and the first approval, sometimes several decades for complex technologies, according to Paul Carter, senior director and staff scientist of antibody engineering at Genentech. For example, the concepts of antibody drug conjugates and bispecific antibodies were first described in 1960 and the early 1970s, respectively.

“Industry interest in next-generation antibodies has grown substantially in recent years, fueled by the commercial success of first-generation antibodies and the widespread goal of developing best-in-class antibodies. Many of the more recent next-generation approaches are, however, still early in clinical development at the proof-of-concept stage,” he observes.

Each of the next-generation antibody technologies has its pros and cons, according to de Fougerolles.

“Improving the Fc region, for instance, is a useful step in improving on existing products, but isn’t helping to uncover new target biology. They could, however, be very commercially interesting as second-generation products. Multi-specific antibodies, on the other hand, have the potential to unlock new mechanism of action and afford significantly improved efficacy. As a new technology with many different approaches, commercial success will need to be proven on a product-by-product basis,” he notes.

Of technologies currently in research and development, Carter believes that bispecific and multi-specific antibodies have perhaps the broadest range of potential clinical applications across many areas of medicine, and for this reason greatest potential for success. “Having said that,” he comments, “only two bispecific antibodies have been approved with 50+ in clinical development. It will be years, perhaps another decade or more, before we realize the full potential of bispecific and multispecific antibodies.”

One bispecific technology of interest is bispecific T-cell engaging antibodies (BiTEs), which was developed by Micromet based on research conducted at the University of Munich. The company has since been acquired by Amgen. BiTEs are designed to direct the body’s T cells to attack tumor cells.

Ablynx’s Nanobody technology is another. Nanobodies are based on naturally occurring heavy-chain-only antibodies first discovered in the serum of camels and, according to de Fougerolles, upon sequence optimization and humanization maintain their extremely robust physical properties, making them excellent therapeutic drug candidates that can be delivered via multiple routes of administration, including inhalation. The company recently submitted a marketing authorization application to the European Medicines Agency for aplacizumab, its anti-vWF Nanobody for the treatment of acquired thrombotic thrombocytopenic purpura. De Fougerolles expects Ablynx to report results of an on-going Phase III study in the second half of 2017 and be at the approval stage in 2018.

“There are very few limitations when it comes to the use of smaller fragments, particularly with our Nanobody platform,” de Fougerolles asserts. The company also has a drug in development (ALX-0171) for the treatment of respiratory syncytial virus (RSV) infection in infants. This product is attractive because it is sufficiently stable for administration via inhalation directly into the lungs, and offers the potential to be the first drug aimed at treating ongoing RSV infection.

Nanobodies also have manufacturing advantages over other bispecific technologies, according to de Fougerolles. Because they involve a simple single heavy-chain immunoglobulin (Ig) domain, there are no heavy/light chain pairing issues seen with other mAb-based bi-specific platforms. It is possible to genetically string together any number of different Nanobodies to form multispecific antibodies that have well-behaved, simple structures. From the discovery perspective, he adds that a combinatorial approach can be used to readily construct and screen large numbers of different bispecific combinations.

MacroGenics’ dual-affinity re-targeting (DART) technology, which can target multiple disease-causing cells or different disease-causing pathways with one antibody, is also a bispecific antibody approach. DARTs have a proprietary minimal linker size and a content that reduces the potential for immune reactions, according to Koenig. In January 2017, FDA granted orphan drug status to the company’s candidate MGD006 (also known as S80880), a DART molecule for the investigational treatment of acute myeloid leukemia.

The company is also developing trispecific candidates, including one product that targets two different antigens on the same cancer cell and also modulates the activity of immune cells, recruiting them to help fight the targeted cells. “There is no reason we cannot combine out bispecific antibodies with other elements, such as Fc engineering and incorporation of ADCs. All of these approaches are being explored today,” Koenig comments.
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Riley concludes that “there is potential across a variety of next-generation antibody formats. ADCs, bi-specifics and other novel formats have already been commercially approved and preclinical and clinical pipelines continue to expand. As technologies for creating these types of molecules continue to advance, companies are able to develop the right molecule for a given target.”

NOT WITHOUT HURDLES
The key is the continued advancement of technologies from the discovery to commercial manufacturing stages. “The development of next-generation antibodies typically involves more uncertainty, complexity, and risk than well-established IgG (immunoglobulin G) technology on both the clinical side and with respect to commercial manufacturing. For these reasons, it is highly desirable preclinically to demonstrate some significant benefit of the next-generation approach versus a traditional IgG,” Carter explains.

For instance, for bispecifics consisting of multiple heavy and light chains, it is essential to ensure that the right components are connected together, according to de Fougerolles. The synthesis of these molecules can be complex and involve extensive purification to obtain the desired structure.

“The further removed these molecules are from conventional antibody structures, the more challenging their development and commercialization can be. As they get more structurally complex, there are concerns about expression, protein folding, etc.,” Koenig notes. It is necessary, he adds, to be able to show both scalability and manufacturability, particularly that these products can be produced in large quantities with relative ease in order to meet the treatment needs of large patient populations. “These issues also create opportunities for companies that can develop effective solutions.”

Delivery of next-generation antibodies is also an important issue for consideration, according to Koenig. “Traditional mAbs are administered via injection or subcutaneously. For next-generation products, delivery methods that give the best therapeutic effects, safety profiles, and convenience for the patient, the latter becoming increasingly important, should be the goal,” he asserts. That will require the use of new technologies. The very high potency of some next-generations antibodies—on the nanogram level—may provide opportunities to explore alternative delivery methods. “Because only very small quantities of the active drug substance must be delivered to the patient, there is the possibility of using newer delivery devices such as micro delivery systems,” says Koenig.

ADDITIONAL ANALYTICAL AND REGULATORY DEMANDS
Next-generation antibodies also have their own set of analytical and regulatory challenges. With respect to analytical methods, each format/target combination can require novel methods.

As mass spectrometry techniques have advanced in sensitivity over the years, researchers have the capability of pinpointing modifications to determine the location of conjugation sites for ADCs and ensuring the correct pairings of subunits with novel formats, according to John Joly, senior director of analytical development and quality control with Genentech. He adds that formats have been engineered to engage T cells directly through the use of CD3 binding domains and analyze extremely low levels of these actives at the site of action and wherever they end up in the body,” he notes.

On the regulatory front, there are additional requirements for next-generation antibody treatments that involve modulation of immune responses. Problems observed with early CAR T-cell therapies with respect to overly aggressive immune responses have led regulators to require treatments that involve immune responses be started at very low doses, with the dosage then increased slowly until the actual therapeutic level is reached, according to Koenig. “It is necessary to be able to detect and analyze extremely low levels of these actives at the site of action and wherever they end up in the body,” Joly asserts.

The high potency of next-generation antibodies is also creating the need for the development of analytical methods with much higher sensitivities, down to picomolar levels, according to Koenig. “It is necessary to be able to detect and analyze extremely low levels of these actives at the site of action and wherever they end up in the body,” he notes.

MORE GROWTH EXPECTED
Most large pharmaceutical and biotechnology companies have recognized the importance of next-generation antibody therapies and have multiple products under development, whether ADCs, multispecific antibodies, or engineered products, according to Koenig. “Some of this work is being con-
ducted in-house, while other technologies are being accessed through relationships with emerging/smaller pharmaceutical companies focused on next-generation antibodies,” he notes.

There are, for instance, roughly a dozen well-established companies in the bispecific antibody space, according to de Fougerolles. He notes that a few like Ablynx have multiple products in advanced-stage clinical trials, but most are earlier stage companies with just one or two products in man. Many of these smaller companies are funded by venture capital firms.

“Recent years have witnessed major growth in the variety of next-generation antibody approaches. We expect this trend to continue with no single next-generation antibody strategy in particular becoming dominant over the next few years,” concludes Carter.

Following FDA’s approval of seven new therapeutic biological product approvals in 2016 (1), life-science commercial intelligence firm Evaluate estimated that 8–16 monoclonal antibody (mAb)-based drugs could be approved in 2017 (2). That prediction appears to be on track based on results of the first quarter of 2017, when four antibody-based drugs received marketing authorization from FDA.

Valeant Pharmaceuticals International received FDA approval on February 16 for Siliq (brodalumab) for subcutaneous use for patients with moderate-to-severe plaque psoriasis. The novel human monoclonal antibody binds to the interleukin-17 (IL-17) receptor and inhibits inflammatory signaling by preventing the binding of several types of IL-17 to the receptor. Siliq is indicated for the treatment of moderate-to-severe plaque psoriasis in adult patients who are candidates for systemic therapy or phototherapy and have failed to respond or have lost response to other systemic therapies, according to a company statement. Valeant reports that it expects to begin selling the therapy in the United States in the second half of 2017 (3).

On March 23, FDA approved Bavencio (avelumab) injection 20 mg/mL for the treatment of adults and pediatric patients 12 years and older with metastatic Merkel cell carcinoma (mMCC). Developed by EMD Serono, the biopharmaceutical business of Merck KGaA, in the US and Canada, and Pfizer Inc., the therapy was approved under FDA’s breakthrough therapy designation and priority review programs and was available for order upon FDA approval, the sponsors report. A human programmed death ligand-1 blocking antibody, Bavencio is the first FDA-approved therapy for patients with mMCC, a rare and aggressive skin cancer, according to a statement by the drug sponsors (4).

On March 28 Genentech announced FDA’s approval of Ocrevus (ocrelizumab), a mAb-based treatment that targets both progressive and relapsing-remitting forms of multiple sclerosis. The humanized mAb is designed to selectively target CD20-positive B cells, a specific type of immune cell thought to be a key contributor to myelin and axonal damage. The therapy will be available to patients in the US within two weeks of the approval date, the company reports (5).

Also on March 28, FDA approved Dupixent (dupilumab) injection to treat adults with moderate-to-severe atopic dermatitis whose disease is not controlled with topical therapies or where those therapies are not advisable. The human mAbs designed to inhibit overactive signaling of proteins IL-4 and IL-13, which are believed to be major drivers of the underlying inflammation in atopic dermatitis. The therapy, available in a pre-filled syringe, can be self-administered after an initial loading dose, according to the drug developers, Regeneron Pharmaceuticals and Sanofi. The drug was expected to be available to US patients at the end of March 2017 (6).

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References

—The editors of BioPharm International
There has been a growing interest in antibody-drug conjugates (ADCs) as cancer treatments because of their ability to enhance the selectivity of chemotherapeutic agents while limiting systemic exposure and adverse effects. The development and manufacture of these complex molecules, however, can be a challenge.

“There has been a growing interest in antibody-drug conjugates (ADCs) as cancer treatments because of their ability to enhance the selectivity of chemotherapeutic agents while limiting systemic exposure and adverse effects. The development and manufacture of these complex molecules, however, can be a challenge.”

“The typical manufacturing process for a monoclonal antibody (mAb) begins with a production cell line and progresses through filtration and chromatography steps to capture and polish the final drug substance,” says Deepan Shah, team leader at Orla Protein Technologies. He explains that an ADC manufacturing process, however, involves additional steps of linker coupling and toxin coupling, followed by ultrafiltration/diafiltration to remove excess toxin and linker. “The antibody, linker, and toxin each must be characterized both structurally and in terms of impurities before they can be added to the process,” he adds, stressing that it is crucial to ensure the absence of antibody-degradation products, critical contaminants, and drug/linker-related impurities such as degradation products, residual solvents, and heavy metal ions. ADC development begins with the selection of target antigen and antibody, mAb production, payload-linker synthesis, followed by ADC optimization of conjugation and purification conditions, analytical method development, and formulation and stability studies. Production is then scaled up for GMP manufacturing.

“Therapeutic antibodies are generally targeted to antigen with high abundance in tumor cells and low expression on normal tissue to limit toxicity and maximize efficacy,” Shah explains. He adds that linkers with attachment sites for both the antibody and drug are used to join the two components. “Numerous techniques and linker chemistries targeting differ-
ent sites on antibodies have been developed,” he notes. “Most methods rely on conjugation to exposed cysteine or lysine residues resulting in populations with heterogeneous drug-antibody ratios (DARs).” Shah points out that because low drug loading reduces potency and high drug loading can negatively impact pharmacokinetics (PK), DARs have a significant impact on clinical efficacy. “The linker must remain stable in systemic circulation to minimize adverse effects, yet rapidly cleave upon endocytosis,” he says. “Once inside the cell, the drug is released through hydrolysis or enzymatic cleavage of the linker or via degradation of the antibody. Typically, the unconjugated drug should demonstrate high potency, ideally in the picomolar range, to enable efficient cell killing upon release from the ADC.” Shah highlights that method development to optimize all these characteristics is, therefore, vital prior to manufacture and clinical use of ADCs. Advances in bio-orthogonal chemistry and protein engineering have facilitated the design of optimal ADCs. Shah spoke to BioPharm International about the tools used in ADC development and manufacture, including the application of the OrlaSURF protein engineering platform.

ASSAYS FOR ADC PRODUCTION

**BioPharm: What are the different assays used in ADC production?**

**Shah:** There are several important quality attributes that require careful monitoring during the design of an ADC production process.

- The DAR and the drug distribution (i.e., the number of antibody molecules containing 0, 1, 2, 3, etc... drug molecules) are key parameters since individuals in a heterogeneous population may have differing PK properties. These parameters are most commonly monitored by ultraviolet-visible (UV/VIS) spectroscopy and mass spectrometry; but many other methods (1) are available depending of the specific properties of the antibody, linker, and drug. DAR and drug distribution can be driven to narrower distribution ranges by careful optimization and control of the reaction conditions, such as temperature, solvent, reagent ratio, pH, and reaction time.

- The ability of the ADC to bind to its target antigen, become internalized, and release the toxic cargo are all crucial steps that determine the efficacy of the ADC. The first step in this process (antigen recognition and binding) is the most likely to be adversely affected by the treatments necessary for ADC production. Organic solvents, pH, and temperature shifts can all cause damage to an antibody. Therefore, the ability of the antibody to recognize and bind to its target needs to be monitored throughout the development. Commonly used assays are dot blots and enzyme-linked immunosorbent assay (ELISA) to assess binding, and cell kill assays that test the ability of the ADC to recognize and kill the target cell.

In addition to these key attributes, further downstream in the production process, the purity and stability of the ADC are also important. The presence of contaminants such as buffer excipients, unreacted toxin, and endotoxin must be tested. Stability in circulation must be investigated. Tolerance to freeze thaw and other ambient temperature shifts during storage must be determined.

The industry, therefore, requires cost-effective analytical tools that allow rapid decision-making during the development process to facilitate progress of the ADC to the clinic and beyond. In particular, the ability to monitor the binding of antibody to its target during ADC method development and manufacture is key to its success.

**PROTEIN ENGINEERING PLATFORM**

**BioPharm:** How does the OrlaSURF protein engineering platform work?

**Shah:** The immobilization of poly-peptide target molecules on microtiter plates, biosensor matrices, and micro-carriers and resins is key to the sort of binding assays described previously. Current methods rely on adsorption or chemical coupling of peptides or proteins directly to a surface; it is difficult to control the structure and orientation of proteins when immobilized. In many cases, this leads to inaccuracies in the analytical determination of functional product. OrlaSURF technology enables the production of binding proteins that can be used to develop ELISAs, analytical biochips, and other tools for rapid in-process assays. The basic technology involves the fusion of the ADC target antigen to a proprietary surface-binding unit (SBU). The SBU is a small protein entity that has inherent ability to self-assemble as a monolayer on gold surfaces but it also binds tightly to many other substrata such as glass and plastic and biological polymers. The SBU is highly amenable to modification by fusion of other proteins and peptides. Orla has constructed a toolkit of vectors to enable a variety of fusions to be generated (N- or C-terminal, within loops), allowing the display of the antigenic target moiety in a structural milieu that most closely resembles that of its natural state. Subsequent immobilization via the SBU results in the presentation of the antigen in the correct orientation with near 100% preservation of its structure and function. Once a fusion of the ADC target antigen with an OrlaSURF SBU has been created, it can be attached to ELISA plates, surface plasmon resonance (SPR) biochips, quartz-crystal microbalance (QCM) biochips, bio-layer interferometry tips, and any other substrata by a simple apply-and-wash process requiring no chemical reactions.

*Contin. on page 48*
Downstream Processing for Cell-Based Therapies

Qasim A. Rafiq and Fernanda Masri

For cellular materials, new ultra scale-down devices inform large-scale downstream processing techniques.

With increasing numbers of advanced therapies—including cell and gene therapies—entering early- and late-stage clinical development, significant focus has been directed toward the development of scalable, robust manufacturing processes. Much of the research and development activity in the sector has addressed the upstream challenges with particular emphasis on large-scale cell expansion; however, equally important is the development of large-scale harvesting and downstream processing operations. This paper presents and summarizes some of the challenges associated with the downstream processing of these advanced therapies and highlights recent advancements in the technologies suitable for such applications.

The unavoidable transition towards an older aging population has become a significant medical challenge faced by many developed and developing economies. The increased demand for hospital and social care, coupled with complex medical interventions, has led to spiralling costs for healthcare systems globally. Advanced therapies, including cell- and gene-based therapies (CGTs), have the potential to address previously unmet patient needs, improve overall healthcare costs, as well as tackle the social and economic effects of chronic and age-related conditions. However, before this potential can be realized, it is crucial that robust, scalable, and cost-effective manufacturing processes are developed. Unlike the traditional manufacture of monoclonal antibodies (mAbs) and other biologics, the produc-
If the threat of unknown compounds lurking in your product is keeping you up at night, our Extractables & Leachables team will eliminate the nightmare of uncertainty.

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tion and processing of cellular material forms the basis of the product for CGTs (1). As such, manufacturing processes need to be adapted to ensure that the cellular material retains its functionality and the critical quality attributes (CQAs) are maintained throughout the entire process (2, 3).

As with the production of mAbs, significant research and development for CGTs processes has focused on the intensification of upstream production. For example, several studies have investigated the optimization of cell-expansion processes of adherent human cells. Significant progress has been made in recent years with human mesenchymal stem cells (hMSCs) cultured on microcarriers now reaching densities of >1x10^6 cells mL^-1 (4) and human pluripotent stem cells (hPSCs) reaching densities of >2-3x10^6 cells mL^-1 (5). Although R&D activity should continue focusing on improving cell density, the shift of the manufacturing bottle-necks is inevitably leaning towards downstream processing (DSP) (6).

The DSP activities for cell therapies are those unit operations and processes that arise after the cellular cultivation, or increased flow rates depending on the culture vessel employed. For example, the harvesting of adherent hMSCs cultured in stirred-tank bioreactors on microcarriers requires a combination of enzymatic dissociation and mechanical force (through impeller agitation) to aid in the successful detachment of cells from the microcarriers in situ (7).

The detachment procedure has been employed successfully for a range of anchorage-dependent cell types in a range of bioreactor systems, and in all cases (Figure 1), the CQAs of the cells were retained (8) despite concerns that the increase in agitation would result in cell-damaging shear forces. This harvesting method has demonstrated that it is possible to increase impeller agitation without compromising quality. This aids in the rate of cell detachment, while ensuring that the Kolmogorov scale remains greater than the size of the cell once it has been detached, making it unlikely to cause cell damage. Such an approach led to greater than 95% harvesting efficiency with the cells demonstrating retention of CQAs, despite a five-fold increase in the impeller agitation (compared with the original culture agitation) and a Kolmogorov scale of approximately 30 μm, larger than the average diameter of the cells (approximately 15 μm) (7). This in-situ detachment procedure should be scalable to larger, industrial-scale bioreactors, having been established on well-understood theoretical mixing principles. Moreover, given the detachment procedure is undertaken in situ, it eliminates the need for an additional processing step outside of the culture vessel, reduces contamination risk, and decreases time between process steps.

Although the procedure is likely to involve the use of a recombinant dissociation reagent, the harvest of a hollow-fiber or packed-bed bioreactor culture is likely to be different than the aforementioned procedure for stirred-tank bioreactors and may involve extended contact with the dissociation reagent, increased flow rates, and/or rotation of the culture vessel. In all instances of the harvesting procedure, it is important—particularly where mechanical forces are used to dissociate cells—that no
additional debris or particulates arise due to the microcarrier/cell-substrate damage. Such an outcome would result in additional burden on the DSP operations to remove such particulates. Some groups have demonstrated successful culture of cells on biodegradable microcarriers (9) to overcome this concern, yet the presence of particulates may still pose a concern for final formulation and patient administration.

Advances in biomaterial formulation have facilitated the development of alternative approaches for cell harvesting, with multiple studies demonstrating the potential to remove the need for enzymatic dissociation reagents and mechanical agitation to facilitate successful harvest. Such approaches include the use of environmentally responsive polymers to coat microcarriers, which enable both the culture of anchorage-dependent cells and the subsequent detachment of the cells through a change in the culture temperature and/or pH (10, 11). Although promising, there is a concern that non-enzymatic methods for cell dissociation will result in the formation of cell sheets rather than a single-cell suspension; this may be inappropriate for applications that require intravenous administration.

**VOLUME REDUCTION, WASH, AND FORMULATION**

CGTs to date generally use swing-bucket rotor centrifugation for cell washing and concentration. A biological safety cabinet usually is needed for the subsequent resuspension step, which involves discarding spent medium and resuspending in a suitable buffer. This process may be repeated multiple times to ensure the complete removal of any unwanted reagents and/or particles. However, centrifugation has proven to be a difficult step to automate while maintaining sterility (1) and can pose a problem to achieve the required cell numbers for the manufacture of highly concentrated CGTs (12). Alternatives to swing-bucket rotor centrifugation include continuous counterflow centrifugation systems and filtration systems, which can be more amenable to large-scale, contained and automated operations (6).

Counterflow centrifugation systems, such as kSep (Sartorius Stedim) and Elutra cell separation system (Terumo BCT), can keep the cells in suspension while washing them, clearing out residuals from the supernatant and exchanging the buffer. kSep, in particular, can be fully automated and contained, uses a disposable flow path, is scalable from 0.1 L to >1000 L, and can achieve high cell recoveries (>80%) and high cell viabilities (>90%). The capital investment needed, together with the cost of disposables, demands careful consideration when carrying out process development (6).

Tangential flow filtration (TFF) offers an alternative that can also be scaled, automated, fully contained and, generally, involves a lower capital investment and disposables costs, which may make it more amenable to process development activities (6). For instance, for the anchorage-dependent cells, after the cells are enzymatically dissociated into a single-cell suspension (whether that is detachment from microcarriers, culture surfaces, colonies, or embryoid bodies), these are generally quenched with culture media to preserve cell health by preventing the enzyme from further digesting cell components. More specifically, for the hMSC microcarrier-based expansion outlined previously, cell detachment from the microcarrier is only one aspect of the harvesting procedure. Assuming the microcarriers are not biodegradable and not part of the final product formulation, the next DSP step is likely to be the separation of cells by size-exclusion techniques. For example, dead-end filtration using a vacuum as a driving force was used in the aforementioned hMSCs studies (7, 8). However, this technique, although suitable for the purposes of the study, is not a scalable technique like TFF. Other groups have used TFF and expanded-bed chromatography (EBC) as continuous, integrated alternative techniques for the
Table I: Overview of ultra scale-down tools reported in literature as predictive tools for several different large-scale downstream processing steps and/or processing parameters.

<table>
<thead>
<tr>
<th>Downstream processing unit operation</th>
<th>References</th>
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<tbody>
<tr>
<td>Continuous centrifugation</td>
<td>Bocyn et al. 2001 (24)</td>
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<tr>
<td></td>
<td>Hutchinson et al. 2006 (18)</td>
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<tr>
<td>Batch centrifugation</td>
<td>Delahaye et al. 2015 (25)</td>
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<td></td>
<td>Hutchinson et al. 2006 (18)</td>
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<tr>
<td></td>
<td>McCoy et al. 2009 (28)</td>
</tr>
<tr>
<td>Chromatography</td>
<td>Titchener-Hooker et al. 2008 (27)</td>
</tr>
<tr>
<td></td>
<td>Hutchinson et al. 2006 (18)</td>
</tr>
<tr>
<td>Membrane separation</td>
<td>Masri et al. 2017 (21)</td>
</tr>
<tr>
<td>(tangential-flow filtration)</td>
<td>Ma et al. 2010 (22)</td>
</tr>
<tr>
<td>Depth filtration</td>
<td>Jackson et al. 2006 (19)</td>
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<td></td>
<td>Lau et al. 2013 (20)</td>
</tr>
<tr>
<td>Fill/finish (capillaries)</td>
<td>Acosta-Martinez et al. 2010 (23)</td>
</tr>
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</table>

improved washing and concentration of hMSCs (13, 14). Using TFF and EBC, Cunha and colleagues showed high protein clearance (98%), high recovery of viable hMSCs (70%) with no impact on cell viability (95%) or other CQAs (morphology, immunophenotype, proliferation, adhesion capacity and multipotent differentiation potential) (13, 14). Furthermore, these studies show shorter processing times when using continuous filtration techniques as opposed to discontinuous operations.

Another recent technique that is showing promise for the DSP of CGTs is acoustic filtration (15, 16). Acoustic filtration uses standing waves to capture, separate, and concentrate particles in a fluid without having a physical barrier (membrane or filter) like traditional filtration systems. It is, therefore, considered suitable for shear-sensitive cell lines and potentially allows for higher throughput. It also features fewer clogging issues than traditional membrane-based systems, extending the lifetime of the disposables.

**ADDRESSING THE DSP CHALLENGES**

Due to the relative infancy of the CGT DSP space, a large proportion of the research and development activity needs to focus on effective process development and understanding of the critical process parameters (CPPs) at the small scale. The development of bench-scale technologies that better mimic the full scale will be paramount to the rapid development of robust DSP processes and allow cost-effective screening of the susceptibility of cell lines to operating conditions for CGTs (17). To date, some efforts have focused on the development of ultra scale-down (USD) tools to mimic various large-scale operations, such as continuous centrifugation (18), dead-end filtration (19, 20), and membrane separation (21, 22) (Table I). USD devices allow the investigation of several manufacturing hydrodynamic environments (21) and geometries (23) with little material, increasing the throughput of the experimental phase in a cost-effective and time-efficient manner and ultimately, expediting process development of DSP operations.

Delivering effective DSP technologies and robust processes with the capacity to target specificity and throughput is required to ensure that advances in upstream processing are maximized. Effective scale-down models, adequately characterized, may facilitate a thorough understanding of the CPPs and expedite process optimization and parameter screening studies. Moreover, further process understanding and knowledge of the product’s CQAs will be key to develop effective and suitable DSP solutions that have the capacity to scale and are capable of maintaining CGT product quality.

**REFERENCES**

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Impact of PFS and Filling Process Selection on Biologic Product Stability

The market for pre-filled syringes (PFS) is expected to grow to reach sales of $6.6 million by 2020 (1). Nine out of the top 10 products produced in PFS are biologics (2). The use of PFS as a primary container and delivery system offers significant advantages for biologic products. These advantages include reduced risk of medication errors, simplified and faster preparation and administration, and reduced overfill requirements—which can reduce batch volumes and cost of the drug substance. While advantageous compared to vials, the use of PFS as a primary container adds additional complexity to what are already considered complex products. The PFS product contact materials, and filling and stoppering process, may have an impact on the formation of protein aggregates and particulate matter. As the link between immune response and protein aggregation has been understood over the past decade, FDA has urged product developers to employ risk reduction strategies to better understand the susceptibility of products to aggregation and to use orthogonal techniques to characterize final product (3, 4). PFS product developers must understand the impact the syringe manufacturing process can have on product contact materials and the influence the filling process can have on particle formation and final drug product quality.

Though vials and PFS are composed of similar materials, such as pharma-
Pharmaceutical-grade glass and rubber, the manufacturing process for syringes and requirements for functional performance increase potential product contract materials. PFS container compatibility issues have been determined to be a root cause of protein aggregation in multiple products (5, 6, 7). The PFS system consists of the barrel, which, on one end has a tip with staked needle, luer lock, or slip tip. The tip or needle is closed with a rubber tip cap or rubber needle cover, often with a rigid plastic cover. The opposite end of the barrel has a flange and is sealed with a rubber plunger stopper. A plunger rod is inserted into the plunger stopper but does not come in contact with the product (Figure 1).

**Syringe Barrels with Luer-Lock Tip**

**Syringe Barrels with Staked-Needle**

The use of PFS as a primary container and delivery system offers significant advantages for biologic products.

**Differences in Materials**

The production of glass syringes differs significantly from vials. Until recently, most syringe barrels were made from glass, but new options that are growing in popularity include polymers such as cyclic olefin co-polymer (COC) or cyclic olefin polymer (COP). Glass vials are typically made from both Type IA and Type IB borosilicate glass, while glass PFS use only Type IB. Type IA and IB glass differ in chemistry and in thermal properties, which allows Type IB to be formed at lower temperatures, allowing the syringe barrels to be shaped from long tubes of glass. Additionally, the lower temperatures (less than 1200 °C) used for syringe forming versus vial forming (up to 1400 °C) reduce the risk of delam-
The glass tubing is produced through several processes that involve heating the glass mixture, pulling the glass, and blowing through a hollow tip to form a tube. The tube is cut into sections, and a flange is formed first using heat and forming tools. The flange region of the barrel has no product contact. A cone is formed at the opposite end by heating the glass while inserting a tungsten probe and compressing the glass around the probe. The tungsten probe is removed, resulting in a fluid path through the syringe tip. If a staked needle is used, it is placed and glue is applied, which is then cured with ultraviolet light.

**ADVERSE EVENT REPORTS**

Since June 2012, five recalls have been issued as a result of glass delamination in vials, while there have been no reports of delamination in syringes during the same time period. A greater concern resulting from the production process of syringe barrels is residual tungsten, which has been identified as a root cause for the formation of protein aggregates in epoetin alfa and an unidentified alpha-helical protein. Risk of tungsten-induced aggregation may be greater in formulations with a pH less than seven. Additionally, the use of vacuum stoppering may increase the likelihood of tungsten-protein interactions because it may remove the air gap at the tip of the syringe, increasing the contact of the drug product with the “tungsten-rich” cone of the syringe.

The incidence of tungsten-induced aggregation is formulation-specific and may be molecule-specific. In internal studies, a monoclonal antibody (mAb) solution was spiked with sodium tungstate at levels of 1, 10, and 100 ppm and then analyzed by size-exclusion chromatography (SEC), turbidity, and imaging particle analysis. No patterns were observed that indicated measurable interaction of the mAb with sodium tungstate.

Most PFS are supplied ready-to-fill in a nest or tub format. All glass and most polymer syringe barrels require some level of silicone application to ensure that the plunger stopper moves smoothly during administration. Stoppers and needles may also be siliconized. An advantage of coating barrels with silicone is that it can protect the glass from etching processes resulting from higher pH formulations and further reduce risk of delamination. The application of silicone is typically performed by the syringe manufacturer and is usually performed by atomizing viscous silicone fluid using high-pressure air. The silicone may be applied using a fixed needle, which is located at one position during the application process, or a diving needle which applies the silicone while moving within the barrel. The use of the diving needle has been demonstrated to improve the consistency of the silicone coating over the syringe barrel interior and improve the gliding force profile. Silicone may be applied as a non-reactive oil, a non-reactive emulsion, or as a reactive or curable silicone fluid. A high-temperature baking process is required to cure silicone emulsions or silicone fluid. Cross-linked silicone is also available in specific low-silicone PFS products.

Silicone has also been implicated in the formation of protein aggregates and protein-silicone complexes. Silicone oil-induced aggregation is more likely to occur in formulations lacking in surfactants, at high protein concentrations, and at formulation pH near the protein’s isoelectric point (pI). The occurrence increases with rising silicone oil concentration. Fewer silicone-related particles can be expected in products filled in PFS with baked-on silicone than with silicone oil. Also, agitation of syringes with headspace resulted in greater particle counts than in containers without headspace.

**United States Pharmacopeia** Chapter <1787> classifies silicone particles as intrinsic, but cautions that “careful design, control, and application of the silicone oil are recommended to obtain proper functionality with the minimum amount needed for the shelf life of the product.” The chapter warns that protein may be absorbed into silicone oil droplets. The mixture of particle sizes of silicone oil droplets and silicone oil-protein complexes may mimic those of inherent protein particles. Fortunately, silicone oil droplets are easily differentiated by their morphology and optical properties when particulate matter analysis is performed by imaging particle analysis.
Laboratory studies completed at Baxter Biopharma Solutions have indicated that silicone oil-induced aggregation is product-specific. In a typical study, a mAb formulation was spiked with silicone oil levels of 0.5 mg/mL and 1.0 mg/mL and then agitated on a shaker table for four hours at room temperature. Analysis by SEC, turbidity and imaging particle analysis identified no measurable change in aggregation resulting from the addition of silicone oil. In one internal study, the silicone spiked formulation was placed on stability for nine months at real time and accelerated conditions without indication of impact to product quality. Silicone-sensitive products can be filled in low-silicone syringes or a silicone-free polymer syringe. However, even low-silicone syringes can have lot-to-lot variability. In one such case, high particle counts were observed in a batch of syringes filled with product. Micro-flow imaging was used to identify the particles as silicone droplets based on their morphology. The issue was isolated to one syringe manufacturing lot. In an internal laboratory study, syringe samples from a previously used acceptable lot, the suspect lot, and a new lot were filled with placebo solution, allowed to sit for 30 minutes, and then the solution was drained, pooled, and tested for particles by light obscuration. As suspected, the solution from the suspect lot of syringes contained a higher level of particles than the solutions stored in the other two lots of the same syringe assembly.

PFS filling lines are typically equipped with rotary piston pumps or peristaltic pumps. Historically, piston pumps offered greater accuracy and speed, but advances in peristaltic pump head design, drive motors, and calibration algorithms have narrowed differences in performance for most formulations (16). Accuracy may be decreased when peristaltic pumps are used to dispense viscous formulations greater than 100 cP. Rotary piston, time-pressure, peristaltic, and rolling diaphragm pumps were evaluated for their impact on particle formation in a purified mAb solution (17). The study concluded that while the measured amount of aggregates by SEC was unchanged, use of a piston pump increased levels of proteinaceous particles observed by imaging particle analysis. The author theorized that the increase in particles could be the result of sheer denaturation and concluded that potential for the increase in aggregates may be product-specific (17).

A Baxter product development study provided additional evidence that sensitivity to denaturation during pumping is not only molecule-specific but formulation-specific. Sheer sensitivity was evaluated by stressing a formulated mAb solution at a rate of 1500s-1 for up to three hours using a rotary cone viscometer followed by analysis by SEC and micro-flow imaging. A slight increase in aggregate concentration (< 0.2%) was observed through SEC, but it was well within assay variability and a fragment peak increased (<1%) after three hours. Particle counts by imaging particle analysis also increased within expected levels based on prior studies. The same solution was subjected to 25 cycles of repeated circulation through a piston pump without increases in particle count, aggregates, or fragments. After receipt of a new lot of API, however, small visible particulates were observed in process development samples subjected to piston pumping after storage for seven days. An evaluation using imaging particle analysis and microscopic magnification revealed that the particles appeared to be proteinaceous. A comparative study was initiated in which formulated material using the new API was cycled three times through a peristaltic or piston pump, filled in syringes, and placed on stability at accelerated and real-time conditions for three months. Samples exposed to piston pumping developed visible particulates in as little as seven days at accelerated storage temperatures. A significant increase in 1–2 μm subvisible particles was observed in the piston pump cycled samples stored at accelerated conditions throughout the duration of the study. Following the pumping, a follow-up study was performed comparing different buffer systems. All other formulation parameters remained equal, including the pH, the protein concentration, and the

**Advances in peristaltic pump head design, drive motors, and calibration algorithms have narrowed differences in performance for most formulations.**
concentration of surfactant. The two formulations were exposed to three cycles through a piston pump and placed on stability. An immediate increase in 2–10 μm and 10–25 μm particles was observed in pumped samples of both formulations relative to control. After storage at 5 °C for six weeks, the number of particles counted in the range of 2–10 μm and 10–25 μm increased significantly in formulation two samples exposed to pump cycles. A corresponding increase was not observed in formulation one samples (Figure 2) relative to control. After six weeks of 25 °C storage, both the formulation two control and exposed sample had increased particle counts, indicating that the product was not stable in the selected buffer component. No changes were observed by SEC.

The development of biologic PFS products must be undertaken with a thorough understanding of the impact product contact container materials and filling processes may have on the potential for protein aggregation and product quality. While potential risks can be generalized, the impact of such variables as syringe type and pumping method on the product is both molecule- and formulation-specific. Product-specific studies can allow for the design of specific risk reduction strategies and allow product developers to make scientifically sound decisions while meeting other requirements related to container selection and filling technology such as cost, availability of equipment, dosing accuracy, end user safety, and ease of use.

REFERENCES


17. A. Nayak et al., J. Pharm. Sci. 100 (10), 4198–4204 (October 2011).◆
Critical Quality Attributes Challenge Biologics Development

Susan Haigney

The complex nature of biologics adds additional CQAs that must be determined to ensure the safe development of biologics.

Critical quality attributes (CQAs) are used in biopharmaceutical development to gain an understanding of a product and the processes used to make that product. The International Council for Harmonization (ICH) Q8 (R2) defines CQAs as “a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug product” (1). While CQAs of small-molecule drugs can affect the purity, strength, drug release, and stability of a drug, the complex nature of biologics can create additional quality attributes that can affect the safety and efficacy of the biologic (2). Identifying CQAs for biologics can, therefore, be a complicated process.

*BioPharm International* spoke with Tapan Das, head of stability, methods and analytical development at Bristol-Myers Squibb and Finance Committee member and past-chair of the BIOTEC Section at the American Association of Pharmaceutical Scientists (AAPS), about how CQAs for biologics differ from CQAs for small-molecule drugs and how they can be determined.

**CHALLENGES FOR BIOLOGICS**

**BioPharm:** Are there specific challenges to identifying CQAs in biologics when applying quality by design (QbD)?
Das (BMS): Knowledge-based design, as opposed to a heuristic approach, is a key principle of QbD. Determination of criticality of process and quality attributes improved significantly in the past decade with the advancement of knowledge in protein engineering, biology, and cell culture engineering. However, challenges remain, specifically in establishing criticality, when an attribute has the potential to impact a multitude of factors in the broad category of safety and efficacy. Protein modifications (such as post-translational modifications) and degradation products may affect potency as well as secondary pathways such as altered clearance or induction of immunogenicity.

BioPharm: How are CQAs in biologics defined?

Das (BMS): For recombinantly produced biologics, special attention is paid to the cell culture and purification process parameters as well as storage conditions because of their influence on heterogeneity commonly observed in biologics. It is the heterogeneity in biologics that makes the process of defining criticality of each heterogeneous species extremely challenging. Therefore, consistency of product quality becomes even more important for biologics.

BioPharm: What is the process for determining risk, specifically for biologics?

Das (BMS): Risk assessment is an evolving process from the discovery phase to clinical stages to product approval and commercial manufacturing. In the early discovery and development stages, criticality determination of each process parameter and quality attribute is not meaningful, rather the emphases are on establishing safety and biological function of the product and producing them in a consistent manner. When developing a commercial process and formulation, appropriate analytical, structural, and biological assays are developed that are capable of studying the components in a heterogeneous biologics product, and understand its impact on function, safety, stability, microbiological safety, and dosage form integrity.

TOOLS FOR DETERMINING CQAS

BioPharm: What tools can be used to determine CQAs for biologics?

Das (BMS): Criticality determination relies on understanding probability of occurrence and relevance of a given attribute or process parameter; identifying product variants, understanding its biochemical/biophysical properties, and establishing its link to process parameters; and studying impact on function (e.g., binding potency, clearance, antibody-dependent cell-mediated cytotoxicity [ADCC], etc.) and safety (toxicity, immunogenicity). As noted above, it is impractical to study all possible attributes of process and product in early stages to understand, at an individual level, its impact on function and safety.

BioPharm: Do some studies/tools work better than others? Why?

Das (BMS): This is an important point. Establishing appropriate analytical/biophysical/functional tools that are fit-for-purpose for sensitivity and specificity is key to ensure the known/hypothesized impacts (on structure, function, and safety) can be studied objectively. For example, during commercial development, enrichment studies can be conducted to study impact (and hence, criticality) of one variant at a time. Another example is use of state-of-the-art higher order structure tools, when needed (not suitable for routine use), to deconvolute structural impact from multiple degradations (e.g., oxidation, isomerization) occurring at the same time.

REGULATIONS AND RISK ASSESSMENT

BioPharm: Are the regulations for CQAs and QbD different for biologics? If yes, how?

Das (BMS): The ICH guideline [(ICH) Q8 (R2)] is commonly followed for CQA approaches and its use toward establishing a robust manufacturing process. Adoption of QbD differs greatly across regions including within the United States. However, the underlying principles of QbD (i.e., a systematic approach for product and process understanding and process control, based on sound science and quality risk management) towards developing robust process and product are increasingly used across regions.

BioPharm: As biologics become more popular in the industry, are companies paying more attention to risk assessment and QbD for these products?

Das (BMS): Generally, yes. Risk assessment still differs significantly across organizations. The past decade has experienced a tremendous growth in the biologics portfolio of the biopharmaceutical industry. With the advancement of knowledge in analytical sciences, protein engineering, biology, and cell-culture process, it is now possible to assess quality attributes more objectively than before. The awareness for the importance of CQA assessment and its critical role in developing robust process/product is also increasing.

REFERENCES

1. ICH, Q8 (R2) Pharmaceutical Development (ICH, August 2009).
Rapid commercialization of therapeutic proteins remains a challenge notably due to their chemical and physical instabilities. Yet, the inherent complexity of proteins requires the development of new analytical strategies to characterize and ensure the quality and safety of these products. Harnessing the strengths of Fourier transform infrared spectroscopy (FTIR) and recent improvements in chemometrics, new analytical methods have been developed to study the stability and perform comparability studies of therapeutic proteins. In this article, the authors demonstrate the feasibility of simultaneously obtaining information regarding four key characteristics of therapeutic proteins: structural integrity, overall protein concentration, quantification of glycosylations, and quantification of phosphorylations. Depending on specific situations, gathering these data would typically require leveraging three to four distinct techniques and protocols. Moreover, infrared spectroscopy allows researchers to analyze these parameters in a precise, quick, and direct manner (i.e., giving results in a couple of minutes), with limited sample volume (<50μL) and without the need for extensive sample pretreatment or the requirement to recalibrate on each measurement day.

The aim of this work was to develop an innovative methodology to analyze the stability of therapeutic proteins based on FTIR spectroscopy.
With this technique, the attenuation of an infrared light beam is measured when it passes through a sample. This attenuation is due to the interaction between the light and vibrational transitions in the covalent bonds of the molecules present in the sample. FTIR spectroscopy has already been widely used to analyze protein structure and was shown to be very sensitive to tiny structural changes (1–6). This technique confers a series of other advantages, which are described in subsequent paragraphs.

As illustrated in Figure 1, the authors used FTIR to investigate four key characteristics of therapeutic proteins: (i) structural integrity, (ii) overall protein concentration, (iii) quantification of glycosylations, and (iv) quantification of phosphorylations. The procedure does not require any labeling or separation.

Aggregation is a common source of protein instability; this phenomenon and its triggers are not fully understood. Moreover, misfolding or alterations in the three-dimensional structure of proteins can be responsible for loss of activity and elicit immune responses (7–10).

Post-translational modifications such as glycosylations and phosphorylations are also important issues. Phosphorylation is the reversible addition of a phosphate group on a polypeptide chain. It is often involved in signalling pathways and generally influences structural properties, dynamic, and binding (11). Glycosylation is defined as the attachment of polysaccharides at specific sites of the amino acid chain in proteins. This modification is involved in product solubility; stability; half-life; pharmacokinetics and pharmacodynamics (PK/PD); and bioactivity and safety (e.g., immunogenicity). In the context of protein production, protein glycosylation is subject to a high degree of heterogeneity, depending on the manufacturing conditions (cell lines, culture parameters, protein purification, etc.). It is, therefore, important to evaluate the proportion of sugar that is present in a commercial protein product (12–15).

As these four characteristics significantly affect the efficacy and safety of protein products, they all must be carefully and systematically monitored. Regulatory agencies worldwide require robust, information-rich, reproducible, and preferably orthogonal methods to analyze the aforementioned parameters and ensure accuracy and consistency of the final drug product (16). Therefore, a single analysis for these four parameters with a short measurement time and a low sample volume is of great interest.

**MATERIALS**

Albumin from human serum (A3782, Sigma-Aldrich, Bornem, Belgium) was used to demonstrate the possibility to assess the four parameters by FTIR spectroscopy. The protein was purchased powderded and used without further purification.

Five peptides ordered from Eurogentec (AS-61329, AS-61334, AS-61332, AS-20292 and AS-24537, Liège, Belgium) were also used in this work. The peptide AS-61329 is derived from the human mucin MUC5AC gene sequence. AS-61334 and AS 61332 are glycopeptides with the same sequence as AS-61329, with respectively one site (Thr3) or two sites (Thr3 and Thr13) labeled with a N-Acetyl galactosamine. AS-20292 and AS-24537 are derived from the insulin receptor tyrosine kinase. AS-24537 is the unphosphorylated form of the peptide and AS-20292 has one phosphorylation.
All samples were purchased powdered and diluted in purified water at a concentration of 5 mg/mL. Powders were conserved at 4 °C and solutions at -20 °C.

**METHODOLOGY**

**FTIR spectroscopy**

All measurements were carried out on a Bruker Tensor 27 FTIR spectrometer (Bruker, Karlsruhe, Germany) equipped with a liquid N₂-refrigerated mercury cadmium telluride detector. All spectra were recorded by attenuated total reflection (ATR)—for a review, see (17). A diamond internal reflection element was used on a Golden Gate Micro-ATR from Specac (Orpington, UK). The angle of incidence was 45 degrees. A 0.5-μL amount of the proteins was deposited on the diamond crystal. The sample was quickly evaporated in N₂ flux to obtain a homogeneous film of proteins. The FTIR measurements were recorded between 4000 and 600 cm⁻¹. Each spectrum was obtained by averaging 128 scans recorded at a resolution of 2 cm⁻¹.

**Spectral pre-processing**

All the spectra were pre-processed as follows. The water vapor contribution was subtracted as described previously (18, 19) with 1956–1935 cm⁻¹ as reference peak. The spectra were also baseline-corrected. Straight lines were interpolated between frequencies corresponding to local minimum and were then subtracted from the spectrum. Normalization for equal area was finally applied between 1724 and 1480 cm⁻¹. In the case of the overall protein concentration, the normalization for equal area was applied between 2080 and 2000 cm⁻¹.

**RESULTS**

The results presented describe the development and the validation of the methodology for each parameter separately. However, it should be noted that all these data are extracted from the same spectrum.

**Structural integrity**

Albumin at 5 mg/mL was exposed to 16 successive temperatures from room tempera-
ture to 90 °C. After the heating and before the measurements, all samples were cooled at room temperature. For each temperature, between three and six independent samples were prepared. For each sample, between three and five FTIR spectra were recorded.

Figure 2A displays all the preprocessed spectra recorded for albumin. Proteins were progressively heated from room temperature (blue color) to 90 °C (red color). Significant variations can be observed with the naked eye, especially for the highest temperature (red color). In the present case, the absorbance at the maximum of this band (1622 cm⁻¹) was monitored. The mean of this absorbance and the standard deviation is calculated for each temperature and presented in Figure 2B. A typical denaturation curve is observed on this figure and underlines the possibility to use attenuated total reflection (ATR)–FTIR spectroscopy as a relevant tool to monitor the structural stability of protein.

It must be noted that FTIR spectroscopy is an interesting orthogonal technique for assessing aggregation, which is almost always associated with a structural change. Whereas size-exclusion chromatography characterizes aggregates according to their size, FTIR spectroscopy provides structural information characteristic of intermolecular interactions. Moreover, this technique is especially attractive in the case of high protein concentration, as no dilution is required to perform the analysis.

**Protein concentration**

With ATR–FTIR spectroscopy, determining absolute concentrations remains a challenge because the film thickness is not known accurately. To measure the quantity of protein in a sample, the authors have added a molecule that serves as an internal reference. This molecule is added at the same concentration in all samples. The normalization step is then realized on a specific absorption peak of this reference. The molecule used as an internal reference is ferrocyanide.

The listed methodology was followed:

1. Preparation of protein samples: Seven concentrations of albumin were prepared: 9.5; 7.5; 5; 2.5; 1; 0.5; 0.1 mg/mL. These samples also contained 0.1 mg/mL of ferrocyanide as internal reference. They were prepared three times on a different day with a new solution of albumin at 10 mg/mL.
2. Recording and preprocessing of FTIR spectra of these samples
3. Calibration of the model: Using the partial least square (PLS) regression, a quantitative model can be built to predict the protein content.
4. Validation of the model with unknown samples (not included in the calibration step): the same protocol is used to record and preprocess the FTIR spectra. The statistical model is then applied to predict protein concentration.

Figure 3 presents the results of the validation of the model. It reports the predicted protein concentration as a function of the true protein concentration. Each point corresponds to the mean of all the spectra recorded at one concentration. The error bars indicate the standard deviation associated with this concentration. With a correlation coefficient of 0.97, the predicted values are highly correlated to the true values underlining the performance of this model. This correlation can be up to 0.99 using other internal reference (data not shown). The results demonstrate that ATR–FTIR spectroscopy can successfully be used to measure protein concentration.
It must be underlined that for most of the techniques currently used to assess protein concentration (UV absorption spectroscopy, Bradford or Bicinchoninic assay, etc.), the measurement depends on the presence of specific amino acids. For example, in the case of UV absorption at 280 nm, the measure relies upon the presence of aromatic amino acids. Conversely, FTIR spectroscopy relies on the presence of the amide bond, which is always present in a protein.

**Glycosylation**
The goal was to develop a method to quickly predict the carbohydrate content, which is defined as the ratio between the mass of carbohydrate and the total mass of the sample (carbohydrate and polypeptide chain).

Glycosylated peptides were mixed to albumin to obtain a range of carbohydrate content from 0.43% to 7.03% (w/w). Ferrocyanide was added in all samples as an internal reference at a concentration of 0.1mg/mL to use the model built previously to predict protein concentration. It must also be noted that all samples were prepared six times on a different day with new albumin solution and new ferrocyanide solution. The FTIR spectra of the first three preparations were used to build the quantification models based on PLS regression for glycosylations. The FTIR spectra of the other three were used to validate these models. For the validation samples, new vials of peptides were ordered and new solutions of peptides were prepared.

The calibration (and validation) samples contain both glycosylated and phosphorylated peptides. As carbohydrate and phosphate absorb in the same zone of FTIR spectra, the absorption bands associated with these post-translational modifications overlap. Due to this overlapping, the prediction of the carbohydrate content in the presence of phosphate was challenging and unexpected (20).

**Figure 4A** presents the results of the validation of these models and reports the predicted carbohydrate content as a function of the true carbohydrate content. Each point corresponds to the mean of all the spectra recorded for one carbohydrate content. The error bars indicate the standard deviation.

**Figure 4B** presents the results of the validation of these models and reports the predicted phosphate content as a function of the true phosphate content. Each point corresponds to the mean of all the spectra recorded for one phosphate content. The error bars indicate the standard deviation.
mass spectroscopy (MS) or fluorescence detection, coupled with chromatography techniques. These procedures generally involve several preliminary steps of sample preparation such as cleavage from the protein backbone, labeling, etc. Moreover, to obtain quantitative information, internal standards such as stable isotopes should be used (12, 14).

Phosphorylation
The goal was to develop a method to quickly predict the ratio between the mass of phosphate and the total mass of the sample (carbohydrate and polypeptide chain). It is called below the phosphate content.

The same samples prepared to calibrate and validate the model to quantify glycosylation were used. The range of the phosphate content obtained was from 0.1% to 2.63% (w/w). Figure 4B reports the predicted phosphate content as a function of the true phosphate content. The predicted values are highly correlated with the true values (correlation coefficient superior to 0.99), underlining the performance of these models. The results underline the feasibility of quantifying simultaneously glycosylation and phosphorylation in protein samples containing both phosphate and carbohydrate. It was particularly challenging to obtain such measurements, as carbohydrate and phosphate absorb in the same zone of FTIR spectra (20).

Most of the current procedures to quantify phosphate in a protein sample are also based on mass spectroscopy and generally involve several preliminary steps of sample preparation (11).

MULTI-ATTRIBUTE METHOD
For all the four parameters, the information obtained is extracted from the same FTIR spectrum. The methodologies described previously can be combined in a data interpretation automated process. Moreover, only a few minutes are required to record the spectra. The full process (measurement and data interpretation) is thus very quick.

The inherent complexity of therapeutic proteins requires the development of new analytical strategies to characterize and ensure the quality and safety of these products.

Furthermore, two other practical advantages of FTIR spectroscopy need to be underlined: There is no (or there is limited) sample preparation (no dilution, no probe, no label) required; and very few materials are required to perform the analysis (maximum 50 microliters per condition).

Another advantage of FTIR should be mentioned—FTIR spectra analyses of proteins, as a global fingerprint of all the molecules present, also allow the detection of a series of unexpected deviations or problems in the production and purification process.

In conclusion, considering the benefits of FTIR spectroscopy and its high sensitivity to tiny changes in a sample, a multi-attribute technique is an attractive and innovative tool for stability testing (including stress studies such as heating, shaking, pH, freeze-thaw cycles, etc.), long-term stability studies, and the comparison of formulations.

REFERENCES
Among the various downstream processing unit operations that are used for purification of biotech products, chromatography is uniquely capable of providing resolution between a product and other closely related product variants and impurities. This specificity is of particular relevance to the production of biotech therapeutics, which require high standards of purity. However, development and optimization of a process chromatography step presents a major challenge due to the large number of process parameters that can affect the performance of the step. For example, for the commonly used ion-exchange chromatography, pH, ion concentration, gradient, and stationary phase can affect the recovery step as well as the resulting product quality.

For complex unit operations such as process chromatography, mechanistic modeling can assist in developing a systematic approach for process development, monitoring, and control. Models can also be judiciously used to reduce lab experimentation, thereby significantly reducing the time required and the cost incurred during process development. With the advent of quality by design, there have been renewed efforts toward gaining a deeper process understanding through mechanistic modeling of key bioprocess unit operations.

Researchers have attempted a variety of approaches to chromatography modeling. The basic requirement for a good model is for it to be predictive and be able to explain at least most of the variability in the data. In addition, the model should be feasible for imple-
mentation in an industrial environment. Modeling approaches that are commonly used can be broadly classified into two categories. The first category, empirical modeling, is where the model is built using experimental data based on approaches such as the design of experiments (DOE). The second category is mechanistic modeling, where the model is built on the underlying fundamental processes of the system. DOE-based approaches dominate modeling efforts in industry and can be quite effective at offering process knowledge and determining the design space. However, because they do not incorporate the underlying mechanisms, empirical models suffer with respect to accuracy and robustness. In contrast, mechanistic models provide excellent predictability. While they are preferred over empirical models, the complexity of most chromatography operations does not lend itself to mechanistic modeling and hybridization of the mechanistic model. Traditional approaches of mechanistic modeling require so much experimental data that their use in industry is severely limited.

In general, models are used for prediction and optimization of the process outputs such as breakthrough or elution profile. A detailed investigation is often required to examine how the retention behavior and capacity vary as a function of the different process variables. Apart from the prediction of the retention and peak profile, mechanistic models can also be used to develop an efficient optimization strategy and for robustness testing of the gradient elution in chromatography.

PREREQUISITES FOR MODELING
With the recent advancement of efficient numerical methods, it is feasible to create a complex and detailed mechanistic model that is capable of describing mass transfer at the bead level for a chromatography resin and can be executed using a personal computer. Ion-exchange chromatography (IEC) is a major workhorse for purification of biotherapeutics. Development of a mechanistic model requires three major steps: modeling of mass transfer, modeling of binding, and finally, validation of the mechanistic model. The choice of details depends on the desired predictability.

Mass-transfer models
The transport of solute through the various phases inside the packed bed column has been described using mathematical models, albeit with different level of details (1). Mechanistic modeling requires the solution of mass balance equations, which are typically achieved using numerical methods (2). As desired accuracy increases, resource requirements for the numerical solution of the partial differential equations obtained for the model also increase. Broadly, there can be four boundaries for mass transfer limitations at the bead level, namely, external mass transfer through the film layer ($k_{film}$) around the bead particle, diffusion through the pore ($D_{pore}$) and surface ($D_s$) of the bead, and reaction rate at the binding site. A complete description of mass transfer models is available in the form of the general rate model (GRM), which covers $k_{film}$, $D_{pore}$, $D_s$, and reaction rate (1, 3–4). However, a numerical solution of the GRM is computationally intensive. With the availability of new, optimized numerical solvers and ready-made tools, such as CADET and chromX, the past decade has seen an increase in usage of GRM researchers (5, 6). Many researchers have instead chosen to use less complex models, such as the transport dispersive model (TDM) (7–11). In this model, the internal and external mass transfer resistances are lumped into one effective transfer coefficient. The next simplest model in the hierarchy is the lumped rate model (LRM) (12–16). In this model, rate kinetics and mass transport are lumped in a single parameter and diffusion in pore and solid is assumed to be infinite. LRM is the simplest model that has been widely used to model IEC. The commonly used equations and their associated conditions are depicted in Figure 1.
**Binding models**

A key aspect in modeling of IEC is that of the choice of model for protein binding. There are dozens of adsorption models available in the literature. Most of the models are based on either the Langmuir model or the stoichiometric displacement model (SDM) (17). Among the Langmuir models, the mobile phase modulator model for salt-based ion-exchange systems are more common (7, 8). The steric mass-action model is a common choice for a stoichiometric displacement model (4, 9–10, 12–13). Thermodynamic-based adsorption models that are based on Mollerup’s formulation have also been used by many researchers (18). Recently, pH and temperature dependence have also been incorporated into this model (13). A more detailed model based on molecular structure has also been applied recently (14). If none of the binding models is able to sufficiently model the data, then empirical models can also be used (16).

Table I presents a summary of the different models and their use in recent publications. The choice of the binding model is mostly driven by how easy and accurate it is in determining the model parameters.

**Estimation of model parameters**

The greatest challenge in development of a model lies in gathering accurate estimates of the various parameters used in the model. Model parameters can be divided into three broad categories, namely, column parameters, mass transfer coefficients, and binding parameters. Column parameters are mainly reaction-independent and are easy to determine. They consist of column and pore porosities. Typically, these porosities are estimated using a non-binding condition of protein sample or a suitable tracer. The second category is that of mass transfer coefficients. These consist of column axial dispersion ($D_{ax}$), $k_{film}$, $D_{pore}$, and $D_s$. If GRM is not used, then a separate estimation of $D_{pore}$ and $D_s$ is not needed. The rest of the mass transfer coefficients can be estimated using the height equivalent to a theoretical plate (HETP) method or the Yamamoto linear gradient elution (LGE) method (12–13). There is no specific method for estimating $D_{pore}$ and $D_s$ for the GRM. In most cases, a theoretical estimation or inverse fit method is used. The binding parameters are estimated using off-column uptake batch experiments or on-column pulse injection and elution through step or gradient of the mobile phase modulator. The rough estimation can be made from retention of peak maxima and can be further refined using the inverse fit method.

Both the methods of off-column and on-column estimation of binding parameters have their benefits and shortcomings. While the uptake batch experiments are easy to perform and do not interfere with the mass-transfer parameters, this approach lacks accuracy in samples with a very low concentration of solutes. The measurements are accurate for the on-column methods, but these methods suffer from interference towards measurement of the mass transfer parameters. A summary of the different, recently used mass-transfer and binding models is tabulated in Table I. In the following section, a case study of of a monoclonal antibody (mAb) in an IEC model is presented.

### Table I: Recently used mass transfer models and their respective binding models.

<table>
<thead>
<tr>
<th>Mass transfer model</th>
<th>Binding model</th>
<th>Primary method for estimation of isotherm parameters</th>
<th>Recent publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>General rate model</td>
<td>Modified Langmuir model</td>
<td>Inverse method</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>SMA/SDM</td>
<td>Inverse method, on-column breakthrough</td>
<td>(3, 4)</td>
</tr>
<tr>
<td>Transport dispersive model</td>
<td>Mobile phase modulator Langmuir</td>
<td>Inverse method</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>SMA/SDM</td>
<td>Batch uptake</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>Thermodynamic-based model</td>
<td>Inverse method</td>
<td>(9, 10)</td>
</tr>
<tr>
<td></td>
<td>Inverse method, LGE inverse method</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Lumpied model</td>
<td>SMA/SDM</td>
<td>LGE method</td>
<td>(12, 13)</td>
</tr>
<tr>
<td></td>
<td>Statistical thermodynamics model</td>
<td>Prediction method</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>Empirical, linear driving force</td>
<td>System knowledge, batch uptake</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>Batch uptake</td>
<td></td>
<td>(16)</td>
</tr>
</tbody>
</table>

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**MECHANISTIC MODELING FOR DEVELOPMENT OF AN ION-EXCHANGE CHROMATOGRAPHY STEP TO PURIFY mABs**

In this section, the authors provide a step-wise method to build an ion-exchange mechanistic model using open-source chromatography design and analysis tool (CADET, https://github.com/modsim/CADET). The mechanistic model used in this approach is GRM with an extended Langmuir model that has salt as mobile phase modulator (3). The parameters are deter-
mined using inverse method (3). It is important in this method that all the dead and delay volumes be included in the model to depict the actual retention time and account for dispersion in the chromatograph and column band broadening separately. For example, tubings and detector dead volumes can be modeled as a series of dispersed plug flow and continuous stirred-tank reactors. A typical setup is shown in Figure 2 and has been described in more detail in the literature (3). Once the model has been set along with CADET, steps for determination of the model parameters are as follows.

### Step 1: Estimation of Äkta chromatograph parameters
A zero-volume connector should be used to determine the flow parameters for the external volume of the Äkta chromatograph (GE Healthcare Life Sciences), in place of the column. Protein samples can then be injected to generate the output flow profile without the column. This profile can be used to calculate dispersion and the residence time of the tubing, the mixture, and the detector for the system.

### Step 2: Estimation of column flow parameters
Once system parameters are estimated, a packed column of a bed height of 5 cm can be connected. Protein samples then can be injected with high Na+ content so that the protein does not interact with the resin (non-binding condition). The resulting data can be used to calculate column porosities and dispersion by fitting the output profile with the estimated profile using CADET simulation with trial values of the parameter.

Alternatively, a suitable tracer—one that is a smaller size than the bead pores and another that is bigger than the pore size—can be injected, and porosity can be directly calculated from the retention time of the tracers. The axial dispersion coefficient can be estimated using HETP (height equivalent to a theoretical plate) plots at different flow velocities.

### Step 3: Estimation of mass-transfer parameters and binding parameters
The binding parameters and internal mass-transfer coefficients can be estimated via injection of the protein samples. The outlet profile can be obtained with combinations of step and gradient elution. Because a mAb product may contain different variants, it is important to isolate the individual variants peak, or peaks can be reconstructed for individual variants using the high-performance liquid chromatography (HPLC) analysis from fractions collected (Figure 3). The reconstructed peak can then be used to fit the simulated profile with the trial parameters values fed in CADET. The adsorption and transport parameters trial values can be estimated using Henry's plot for step elution at various salt concentrations. The pore diffusion can be estimated using correlations available in the literature between free diffusion and pore diffusivity.

Alternatively, batch uptake experiments can be done for the isolated

Table II: Typical values for model parameters with extended Langmuir isotherm. $\tau_i$ are four continuous stir-tank reactors (CSTR) as shown in Figure 2.

<table>
<thead>
<tr>
<th>System and column parameters</th>
<th>Binding parameters for $q_{max}$ 2.0 mol/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column porosity $\epsilon_c$ (-)</td>
<td>0.350</td>
</tr>
<tr>
<td>Particle porosity $\epsilon_p$ (-)</td>
<td>0.440</td>
</tr>
<tr>
<td>Tubing dispersion (m$^2$/s)</td>
<td>1.00E-12</td>
</tr>
<tr>
<td>Column dispersion $D_{ax}$ (m$^2$/s)</td>
<td>2.00E-06</td>
</tr>
<tr>
<td>$\tau_1$ (s)</td>
<td>0.004</td>
</tr>
<tr>
<td>$\tau_2$ (s)</td>
<td>1.000</td>
</tr>
<tr>
<td>$\tau_3$ (s)</td>
<td>0.004</td>
</tr>
<tr>
<td>$\tau_4$ (s)</td>
<td>8.000</td>
</tr>
</tbody>
</table>

$\tau_i$ = four continuous stir-tank reactors (CSTR) as shown in Figure 2. The DPFR has length and dispersion coefficient as parameters. The system captures the extra column volume as well as the dispersion effect of the system, thus allowing the measurement of the accurate protein and salt profile that enters and leave the column.

![Figure 2](image-url)
peaks, and adsorption isotherm data can be fit to determine the parameters. The problem with this method would be obtaining sufficient quantities of pure isolated variants, as some of the variants may be present in a small amount and merge with other variants.

**Step 5: Model validation**

Model validation can be performed by using a lab-scale column of the same bed height as in a commercial column and running it at various protein loadings to cover the likely range of operation. The elution gradients should also be tested for a range of shallowness. At very shallow gradient or very low step, the model may not offer accurate predictions, because diffusivity coefficients may not be constant at extreme ionic concentrations.

**Step 6: Process optimization**

Once the model and model parameters are established, process optimization studies can be performed to achieve optimal selectivity and yield. The typical values for the model parameters calibrated from inverse method are listed in Table II for reference. Reconstructed peak shape for variants and its fit are also shown in Figure 2. The fit shows excellent agreement to the experimental data. Prediction of elution of the main component is more accurate than that of the smaller variants. The overall residual error was less than 0.01.

**USE OF MECHANISTIC MODELING IN THE BIOTECH INDUSTRY**

As seen in the aforementioned case study, predictions for a complex, four-component system can be achieved with high accuracy using the inverse method for determination of model parameters. This approach is feasible for current models due to the availability of efficient numerical methods. A typical simulation can be completed in less than 5 minutes (on an Intel i7 Desktop PC), even for detailed general rate models. Simulations enable the parameters to be estimated directly from the process chromatograms. Most of the older approaches were tedious and largely dependent on batch uptake data. Older approaches, coupled with the relatively inefficient numerical approaches, are viewed as too resource- and time-intensive to be useful in an industrial environment.

**CONCLUSION**

The need for new developments to understand adsorption behavior at extreme load capacity exists. Resin structures and chemistry will continue to evolve, and many advances have been made in the past decade that have made the use of mechanistic modeling for achieving efficient manufacturing possible.

Mechanistic modeling can also be used to perform sensitivity analysis to assess the impact of various input process variables on outputs. Modeling can make process robustness studies more efficient, aid process monitoring efforts, and can facilitate implementation of process analytical technology (PAT). The authors believe the industry will see a rise in modeling during the next decade as the biotech industry attempts to institutionalize the principles of QbD and PAT and moves towards continuous processing.

**Figure 3:** Modeling of separation of charge variants following ion-exchange process chromatography. The graph in (A) shows the reconstruction of a peak from high-performance liquid chromatography analysis, and (B) illustrates a typical simulation. A1 and A2 are acidic variants; B1 is basic variant.

**REFERENCES**

Cell, tissue, and gene therapies challenge the world’s regulators as few concepts have. There is little precedent for regulating these dynamic, variable systems, which require a radical redefinition of such established terms as “formulations,” “active ingredients,” and “excipients.”

As FDA has noted (1), GMP issues come into play, not so much with autologous materials, whose quality depends mainly on the method used to obtain the cells, but with allogeneic materials, which must be stored and processed, increasing the potential for contamination. In the United States, current good manufacturing practice (cGMP) regulations, including Code of Federal Regulations (CFR) Parts 210, 211, and 1271, are being applied (2). Although guidance documents for excipients and findings from research into vaccines and biopharmaceuticals are being adapted for use with advanced therapies, there are few insights into how processing affects the materials, and what impact it has on efficacy and overall safety.

Regulatory agencies must find a balance between overly rigid and prescriptive guidance, which could prevent investment into research and promising new treatments, and insufficient requirements, which could leave patients vulnerable to safety problems. In Europe, where only eight advanced therapies are currently licensed (3) (compared with 14 in South Korea [4] and 14 in the US [5]), this conflict between flexibility and inclusivity is playing out in full force. In February 2017, the Pharmaceutical Inspection Co-operation Scheme (PIC/S), a group of national pharmaceutical inspection authorities, complained that the European Commission’s
(EC’s) proposed cGMP guidance for advanced therapies was too lax and threatened patient safety (6).

The EC issued draft guidance in 2015 asking for industry comments, and reissued the draft guidance in June 2016 (7). The agency’s main aim was to provide a framework based on risk management that would allow manufacturers some flexibility to account for the extreme variability that these therapies present. Scientists from a number of organizations and companies outlined passages where they felt that more clarity was needed (8). After receiving the PIC/S letter, the EC stood firm on its decision (9).

No doubt, debate will continue, but one fundamental area in need of clarification is the potential impact of raw materials that are used to process advanced therapies. A number of different excipients (e.g., buffers and salts, polymers, and preservatives) may be used to stabilize cells or alter their permeability, or to improve delivery. In addition, cells will come into contact with culture media and media degradation products, trace amounts of which may remain in cells after harvesting. As Fouad Atouf, PhD, vice-president of Biologics and Biotechnology, US Pharmacopeial Convention, and colleagues noted in a paper published in The AAPS Journal in 2016, there is a need for good assays and efficient washing practices, as well as an effective way to account for residues in toxicity assessments (10).

Researchers are using established excipients (e.g., water-soluble poloxamer hydrogels) in some advanced therapy development programs. Safety profiles for well-characterized materials can be extrapolated for advanced therapies, Atouf wrote, but the impact of the materials on cells, and cells on materials, must still be studied (10).

At this point, relatively few of the materials being used to process advanced therapies have been thoroughly evaluated. In addition, some research groups may be using materials that were not made in GMP-compliant facilities, or using materials developed for research use only, which have not yet been evaluated for manufacturing or that do not have complete certificates of analysis.

USP has been developing standards and working on testing procedures and references to help manufacturers of advanced therapies prioritize and manage potential risks posed by ancillary materials. USP General Chapter <1046> (11), published in 2000, summarizes general best practices for advanced therapies. Work is now underway to revise Chapter <1043>, “Ancillary Materials for Cell-, Gene- and Tissue Engineered Products”, and USP plans to release the revised draft for industry comments this summer. The chapter presents a risk-based approach that should be used to evaluate the quality of ancillary raw materials (12). Fouad Atouf discussed risk issues, and evolving compendial standards, with BioPharm International.

Prioritizing Risk

BioPharm: What are the areas of greatest potential risk today regarding the use of ancillary materials in advanced therapy processing?

Atouf: In the highly specialized field of cell therapy, when qualifying ancillary materials, anything that is not an active ingredient would be called a raw material, including cell culture media and wash solutions, as well as process materials and aids. Unintended materials that may end up in the finished product should be considered as excipients.

Information, documentation, and selection criteria for these materials are needed from the very earliest stages of research. Another important aspect is the risk assessment, because some materials may be more critical, or pose greater potential risk to patients, than others. We are comparing upstream vs. downstream in the process. Basically, a material’s use earlier in the process reduces its level of risk. We’re also comparing animal derivatives with synthetic products. Another important consideration is the amount of material involved, whether measured in grams, micrograms, or kilograms.

USP Chapter <1043> for raw materials specifies the use of pharmaceutical grade materials, which are, generally, the lowest risk raw materials to use for any biopharmaceutical process. In some cases, though, these may not be available, or can be very expensive for researchers to use. In another common scenario, a raw material may be made in a GMP-compliant environment, but intended for a different use. Developers and manufacturers must be sure that they have adequately qualified the material and its supplier. With materials that have already been used in the same application, one can be more confident that they can be safely used.

BioPharm: Which materials pose the highest level of risk?

Atouf: The highest risk is involved in excipients that are made by suppliers that sell to multiple industries, since pharmaceutical manufacturing may be only a small part of their overall business. These materials need to be carefully evaluated and vendors need to be qualified. Generally, ancillary materials used in advanced therapies are not common raw materials with monographs that are made in GMP environments, and there are no compendial documents for these materials.

In such cases, guidance from the International Organization for Standardization (ISO) and International Council for Harmonization (ICH) is extremely relevant, as are the principles of pharmaceutical quality by design, in that one needs to have control over processes and to document all incoming materials. We have started to work on
tools that will help manufacturers do this, and are ranking different materials based on the potential risk that they pose to patients.

**BioPharm:** When did you start this work, and what materials have you studied so far?

**Atouf:** Efforts started around eight years ago with cell culture supplements and fetal bovine serum (FBS). We developed identification, functionality, and a reference standard for FBS. We also developed similar data for the enzyme, trypsin.

Another goal is doing work on cell culture media, a crucial raw material, but one that can be made up of 50 or more components. We are in the process of getting information and prioritizing efforts. USP already has compendial standards in place for amino acids, carbohydrates, and vitamins. We need them for these ancillary biopharmaceutical processing materials. We need to be able to calibrate their performance.

**BioPharm:** What’s the timeline for this work?

**Atouf:** By early summer, we hope to begin begin the characterization of two to three materials. After early characterization, we will then study their performance to develop standards. We invite suppliers and manufacturers to share their data, test their materials, and work with us. Our goal is to develop more standards like the ones that we already have for FBS and trypsin. It can take up to six months to get data for raw materials, and overall, the standards-setting process for other materials has generally taken 1—1.5 years.

We also plan to produce reference standards and make supporting documentation available. This work could also be supported by monographs. In some areas, such as media, we may have to work one on one with some suppliers. The approach to qualification of raw materials (e.g., Chapter <1043>) was written for cell therapies, but is applicable for use in bioprocessing in general.

**REFERENCES**

4. FDA, Cellular Gene Therapy Products, Marketed Products, Vaccine, Blood

**APPLICATIONS OF ORLA SURF**

**BioPharm:** What are the applications of OrlaSURF in ADC process development? Can you provide some examples or case studies?

**Shah:** OrlaSURF technology can be used for the development of target-binding assays to monitor the binding of ADC to its antigen during method development and optimization, and also for process monitoring.

One example is the development of a binding assay for Roche’s Herceptin (trastuzumab) in collaboration with Glythera, an ADC company, to facilitate their design of experiment process for evaluation of reaction conditions in ADC production. We created a fusion of the binding site for Herceptin on Her2 protein into a loop structure of an OrlaSURF SBU. The new protein, named ORLA254, was produced as inclusion bodies in E. coli, purified, and refolded in vitro using proprietary redox refolding techniques. The binding of trastuzumab to ORLA254 immobilized on ELISA plates was superior to that on native Her2-Fc fusion. The ORLA254 assay was identical on cheap, untreated polystyrene plates and also on more expensive treated plates (Nunc Maxisorp and Polysorp), whereas the “native” Her2-Fc fusion did not show binding at all on untreated polystyrene. ORLA254 protein and ELISA protocols were transferred to Glythera where they have been in routine use for the method development for ADC production. Glythera have demonstrated a clear correlation between binding to ORLA254 and cell kill ability of treated antibodies and this correlation adds a valuable analytical tool to inform and speed up their ADC development process.

**REFERENCE**

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Sterilization processes are used to ensure the safety of patients treated with products and materials expected to be sterile at time of use. The objective is to eliminate microorganisms in and on products that are introduced into the body in a manner that defeats the ordinary protections of skin, intestines, and other safeguards present. In considering patient safety with respect to sterility, a minimum requirement of one contaminated unit in a million units is considered acceptable for sterilized materials (1). The original term for this value, sterility assurance level (SAL), is non-intuitive and defining it usually entails the use of the word ‘probability’. Increasingly, this value is being called the probability of a non-sterile unit (PNSU). In routine practice, additional precautions are taken so that this minimum expectation is substantially exceeded.

The calculation of PNSU uses Equation 1, in which the lethality delivered, D-value, and initial population of the microorganism are inserted.

\[ \log N_u = \frac{-F_0}{D} + \log N_0 \]

where:
- \( N_u \) = Probability of a non-sterile unit (PNSU)
- \( D \) = D-value of the microorganism
- \( F_0 \) = Equivalent time, in minutes at 121 °C (lethality)
- \( N_0 \) = Initial population

The equation is simple enough; however, there is a common misconception in its use. The problem lies in the incorrect use of values for population and resistance from the biological indicator rather than for the bioburden. The safety expec-
tation relates to the routine use of a sterilizer where the bioburden is present, rather than the initial or periodic validation of the sterilization process when a biological indicator is employed. In the majority of instances, materials sterilized in conjunction with the validation exercise are not intended for patient use. The minimum PNSU as derived from the bioburden present is the critical concern. Equation 2 estimates the PNSU for a 3-minute process at 100 °C with a starting population of 100 CFU/unit and an estimated D100 of 0.0003 minutes (2).

\[
\log N_u = \frac{-3}{0.0003} + 2 = -9,998
\]  

[Eq. 2]

It should be immediately evident that this extremely short and low-temperature sterilization process provides an overwhelming margin of safety that is nearly 10,000 times greater than the minimum expectation. The moist heat resistance of the bioburden is so minimal at these conditions that there is essentially no chance for its survival (3). This is true even though the process is 3 minutes at 100 °C, not the more commonly (and wrongly expected) process performed in excess of 121 °C. The lethality of this low temperature process cannot be established with the conventional biological indicator of *Geobacillus stearothermophilus*, whose resistance is such that the assumed process would have no meaningful impact on its population.

Requiring destruction of a 106 population of *G. stearothermophilus* to the minimum PNSU expectation of 6 would require a process at 121 °C and an \( F_0 > 10 \) minutes. Such a process offers no benefit to the patient because the bioburden will already have been killed well beyond minimum expectations at the lesser condition. If a 121 °C process delivering an \( F_0 = 10 \) minutes were utilized instead, the PNSU would be as shown in Equation 3.

\[
\log N_u = \frac{-10}{0.000003} + 2 = -3,333,331
\]  

[Eq. 3]

The estimated PNSU in this example would be extreme: not more than one positive in more than three million times the minimum requirement. The only justification for using such a cycle is to destroy a bioindicator that has no resemblance to the native bioburden, is present at a concentration that exceeds any reasonable real-world situation, and has extreme moist heat resistance. Killing the bioindicator is certainly safe, but this approach arbitrarily increases the adverse process impact on the product. The real target in sterilization is always the bioburden, which is generally far easier to kill. Therefore, the sterilization process should be developed with that as the objective.

The purpose of the biological indicator in sterilization is not to define the process, but rather to measure it. The steps involved in sterilization process development are outlined in Figure 1.

**DEFINE AND VALIDATE**

The activities needed to define and validate a sterilization process focused on reliable destruction of the bioburden follow a simple sequence.

**Selection of a bioburden model**

The resistance of the bioburden can be obtained from experimental data collected on materials prior to sterilization or based on assumptions regarding the expected bioburden. Resistance information can be obtained from the literature or experimentally determined. The *United States Pharmacopeia* includes a boil test that can be used to estimate microbial resistance (1,3). The boil test can be adapted to estimate bioburden D-values at the appropriate temperature if a temperature other than 121 °C is used. The population determination or estimation is straightforward.

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**Figure 1. Establishing a bioburden-based sterilization process.**
Calculation of process duration
Inserting the population and resistance information for the assumed bioburden along with the desired minimum PNSU into Equation 1, the minimum process dwell time (F) can be determined.

Selection of the biological indicator
With the process duration established, a biological indicator with appropriate population and resistance can be identified that is appropriate for the determined process duration. The biological indicator should not be so resistant as to completely survive the process, but it should represent a meaningful challenge to confirm the required process conditions have been achieved. Partial kill of the biological indicator is most definitive as it confirms that the biological indicator possesses adequate resistance to support the process condition. Surprising as it may seem, complete destruction of the biological indicator does not provide that confirmation. Appropriate biological indicator options could include mesophilic sporeformers such as Bacillus megaterium or Bacillus subtilis (3,4).

Physical and microbiological confirmation of sterilization process
Use a combination of physical measurements and microbiological challenges to confirm that the required lethality is delivered.

Throughout this exercise, worst-case assumptions can be made to increase the confidence in the sterilization process. The typical assumptions include:
- Assuming a higher initial bioburden population
- Assuming a higher bioburden resistance
- Increasing the required minimum PNSU
- Arbitrarily increasing the minimum process dwell time.
- Increasing the temperature set-point for the process

All worst-case assumptions need not be utilized, because doing so can result in a final process that is overly harsh to the quality attributes of the materials being sterilized.

There are many reasons why the bioburden should be understood as the focus of the sterilization and the bioindicator relegated to a supportive role in the validation of the process:
- The bioburden is present during routine processing and its destruction to a safe level must be understood as the intent of the sterilization process.
- Controls over the bioburden are an essential consideration in GMP operations producing sterile products. Attention must be directed to its removal to safe levels.
- The biological indicator is used only during the validation exercise, and in the majority of instances, the materials from the validation cycle are never used with patients.
- Determining the sterilization process based upon biological indicator destruction extends the process duration unnecessarily, with negative impact on the sterilized materials (1).
- Changes in the biological indicator resistance can create problems in periodic revalidation activities.

The validation of sterilization processes must balance the often competing considerations of increased process safety and the negative impact of over-processing. The biological indicator should be chosen to support a sterilization process that provides a reliably stable and efficacious product with an adequate margin of safety. Extending process dwell and increasing temperature merely to kill biological indicators beyond what is necessary for patient safety is never appropriate. The correct use of a biological indicator is as a measurement tool confirming sterilizing conditions have been attained within the load items sufficient to render the process sufficiently safe. Sterilization and sterility assurance need to consider bioburden destruction to safe levels as the only true objective.

REFERENCES
1. USP, USP–NF 39, General Chapter <1229>, “Sterilization of Compendial Articles.”
3. I. Pflug, Microbiology and Engineering of Sterilization Processes (Environmental Sterilization Laboratory, Otterbein, IN, 14th ed. 2010), Table 13.7, p. 13.18.

FDA cites API manufacturer for cleaning validation failures
In a Feb. 3, 2017 warning letter, FDA noted that Resonance Laboratories Pvt. Ltd. did not provide sufficient information about how it planned to improve validation procedure deficiencies discovered during a May 2016 inspection of the company’s Bangalore, India facility. During that inspection, FDA officials found that the company had failed to validate the water system used in sterile manufacturing operations, and the distilled water used to clean equipment in cleanrooms had an unacceptable level of bioburden. The FDA investigator discovered that 105 cleaning verification samples taken between 2015 and the May 2016 inspection failed the firm’s specification for residual drug. The company repeated cleaning until it obtained passing verification results, however, it failed to investigate recurring cleaning procedure ineffectiveness and did not remediate the deficient procedures, FDA reported.

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ReNu SU cartridge technology enables the pump’s aseptic fluid paths to be positioned accurately. The package adds tetrahydrocannabivarin to the target analyte list, with a 10-minute analysis time. The package presents full baseline resolution for all 11 compounds in less than 30 minutes and offers the ability to expand the target list as regulations change.

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explaining why they are asking particular questions and providing the regulatory citation for the inquiry. Another important behavior is the ability to listen to the answers to the questions and refrain from judging. The auditor should adopt a proactive approach to the audit and look at items that are infrequently assessed. Above all else, the auditor needs to be friendly.

**Internal audits are a valuable tool for identifying issues before others identify them.**

The exact same behavior defined for the auditor should also be the exact same behavior displayed by the auditees. Auditees should be direct and avoid deflecting or obfuscating answers. They also need to be instructive and take the time to explain why they do things the way they do them. They need to listen to the auditor’s concerns and not overreact to the question being asked. They should be proactive and point out things of concern and seek advice on how to remediate them. Both parties need to remember they are not the enemies, rather they are the partners.

Internal audits are a valuable tool for identifying issues before others identify them. The information obtained during the audit can be used to improve your processes, and the audit process itself can be another tool to help train employees. If you consider the internal audit as a gap analysis for your processes and set a tone of partnership and cooperation, you will find that the audit program and the information obtained from it will become a valuable resource for the organization rather than an unwanted intrusion into operations.

**REFERENCES**

1. 21 CFR 820.20, Management Responsibility
2. European Commission, EudraLex, Volume 4, Chapter 1, Pharmaceutical Quality System.
3. ICH, Q10 Pharmaceutical Quality System (ICH, April 2009).
Susan Schniepp, distinguished fellow at Regulatory Compliance Associates, discusses the value of internal audits and how the information gained can be applied.

Q: I am in charge of the internal audit program at my company and am wondering if you have any suggestions on how I can make this activity more valuable for my company?

A: Internal audits are part of management responsibilities (1–3) and can provide valuable information and offer many benefits to an organization. The information obtained during the audit can be used in many ways to help your organization grow and continually improve its operations. How you approach the internal audit function will help the organization understand the advantages of supporting an effective, well-run internal audit program.

If designed and implemented appropriately, internal audits can provide valuable information that can be used to prevent issues before they become compliance concerns during a regulatory inspection. Issues can be identified and corrected before the regulatory authorities or current/potential clients identify them. If these issues can’t be completely remediated before an external audit, a plan to correct them can be established and action taken to mitigate them. Having corrective actions in place before others identify the issue may lessen the impact of the observation and instill confidence that your quality system is under control and there is a process in place for continuous improvement. In addition, the internal audit can be used for training staff and communicating valuable information to the organization.

The object of an internal audit is not to pretend to be the regulatory authority and show up unannounced but rather to work in cooperation with your colleagues to identify and solve potential issues. An effective program establishes a partnership between the audit function and the departments being audited. The ideal tone for an internal audit should be a team-oriented activity that is instructive, informative, open, honest, and inclusive. There are several factors that help contribute to establishing this tone. One way to set the proper tone is to publish the audit schedule in advance and make sure the functional areas are informed of the schedule. The audit itself should be forward-thinking and unlimited in scope. The auditors should work with the functional area and talk with as many employees as possible to identify the issues of concern. Individuals who are responsible for performing the day-to-day activities often have the best insight as to what is working and what needs to be improved. Excluding them from participating in the audit process might result in overlooking a serious issue that could come up during a regulatory inspection. To be able to get the most valuable information about the potential compliance issues facing the organization, internal audits should not be judgmental or antagonistic, or have a ‘check the box’ mentality in execution. They should also avoid looking retrospectively in lieu of looking forward.

The behavior of the auditors during the audit is also important to obtaining valuable information. The behavior of the auditors during the audit is also important to obtaining valuable information. The auditors should be direct and avoid asking questions designed to stump people. The auditors should take this opportunity to teach by

Contin. on page 57
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