Volume 30 Number 2

BioPharm International

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February 2017

The Science & Business of Biopharmaceuticals

NUVIA™ cPRIME™ RESIN

Development of an Efficient Manufacturing Process for Adenovirus

Affinity capture is usually not an option for the downstream processing of recombinant proteins, viruses, and second-generation mAb-like proteins. Process developers seek high recovery and purity during initial capture. Using a mixed-mode resin for capture enhances selectivity and delivers increased total protein recovery and purity.

We demonstrated mass capture of a recombinant human adenovirus using the hydrophobic cation exchange Nuvia cPrime Mixed-Mode Resin (Figure 1). This step of the purification achieved a tenfold reduction in processing volume and a reduction in feedstream contaminants.

This efficient two-step purification process, Nuvia cPrime Mixed-Mode Resin followed by Nuvia Q Anion Exchange Resin, provided approximately 54% overall recovery of virus particles (Table 1).

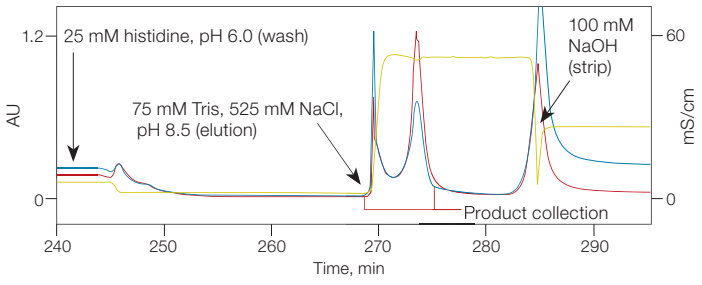

Fig. 1. Initial capture of recombinant virus. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units.

Table 1. Viral particle recovery and impurity clearance.

Sample	Total virus ($x10^{11}$ particles)	Impurity levels ($ng/10^{10}$ particles)	
		DNA	HCP
Bulk harvest	30.6	3,144	n/d
Nuclease-treated harvest	31.8	30	3,020
Nuvia cPrime eluate	18.4	n/d	58
Nuvia Q eluate	16.4	<0.02	2

HCP, host cell protein; n/d, not determined.

Learn more at **bio-rad.com/info/cPrime**

BIO-RAD

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MASS SPEC MEASURES UP TO BIOLOGICS DRUG ANALYSIS CHALLENGES

INTERPHEX

UPSTREAM PROCESSING

ENSURING THE BIOLOGICAL INTEGRITY OF RAW MATERIALS

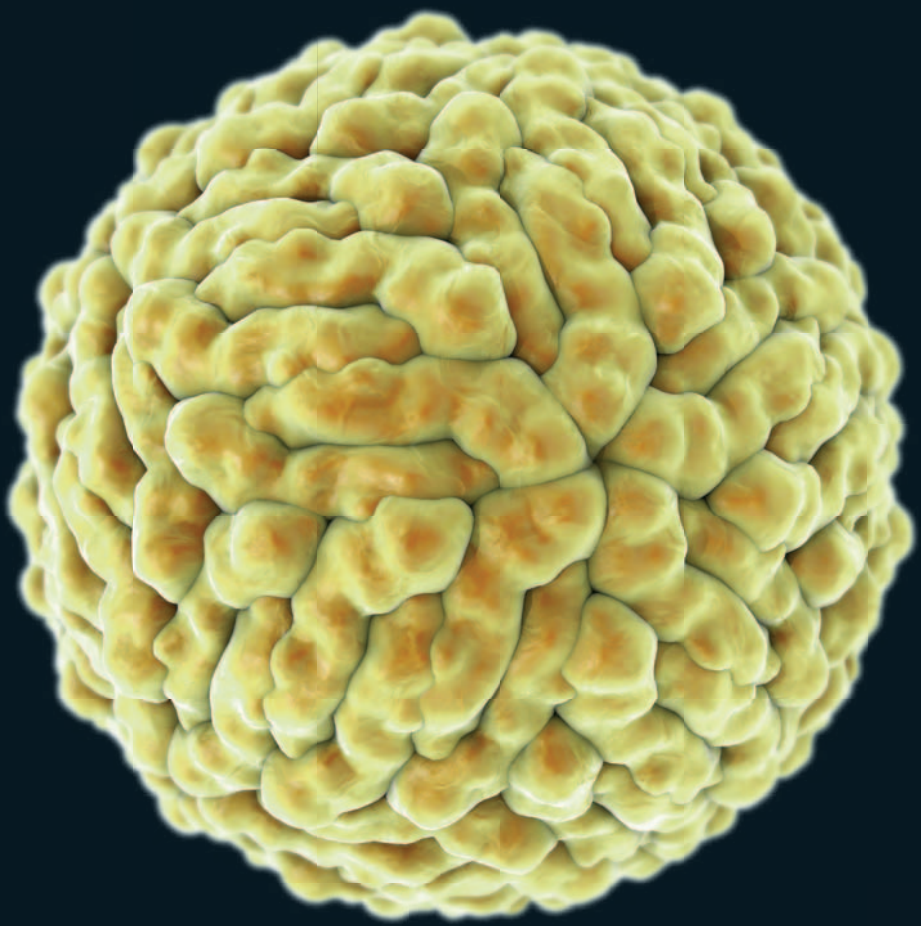
PEER-REVIEWED

IMPACT OF MANUFACTURING-SCALE FREEZE-THAW CONDITIONS ON A mAb SOLUTION

DOWNSTREAM PROCESSING

mAb PURIFICATION: RETHINKING THE INITIAL CAPTURE STEP

UBM



BIOPHARMACEUTICAL RESEARCHERS HAVE UNEARTHED TREATMENTS THAT ARE ERADICATING HEPATITIS C.

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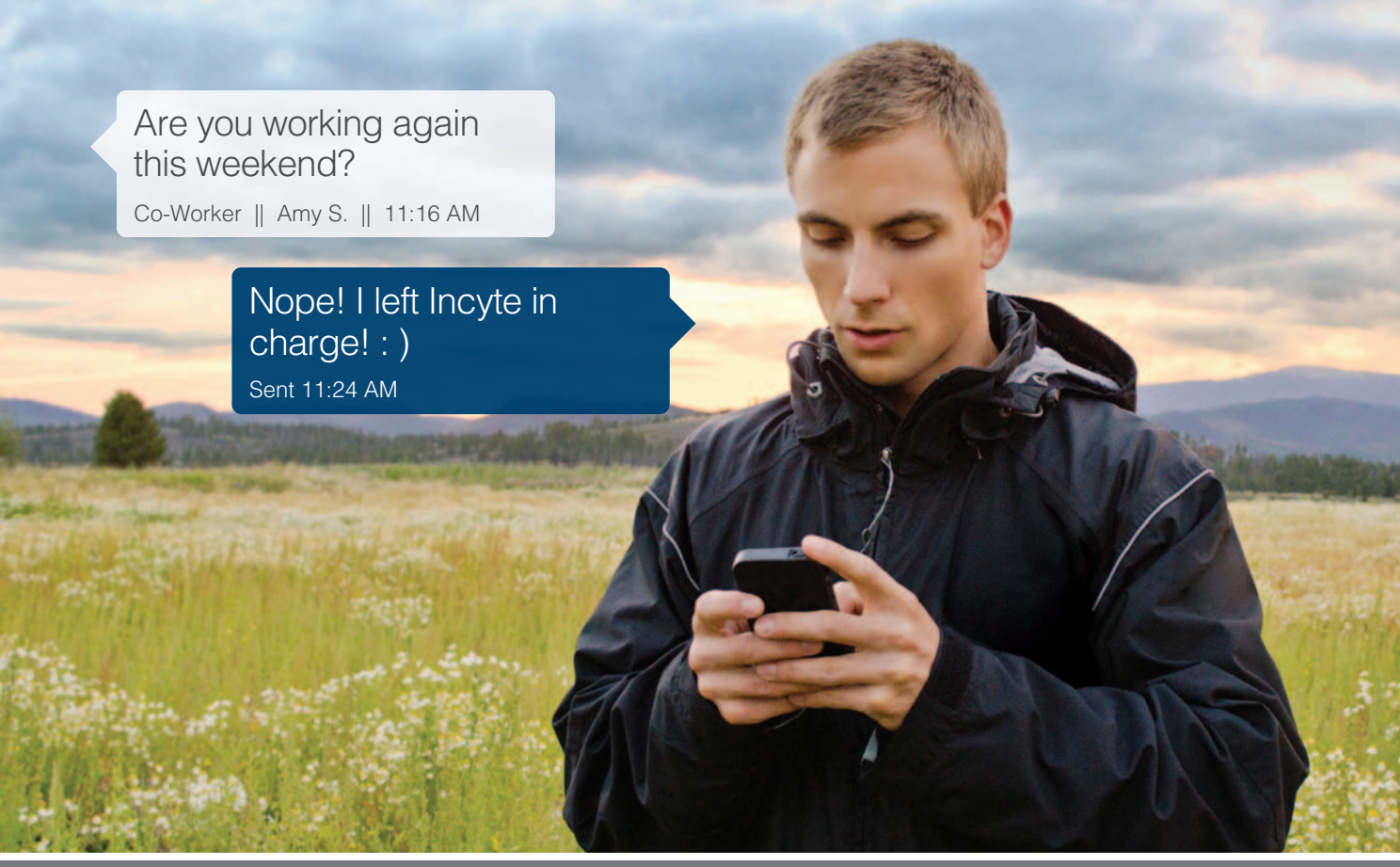

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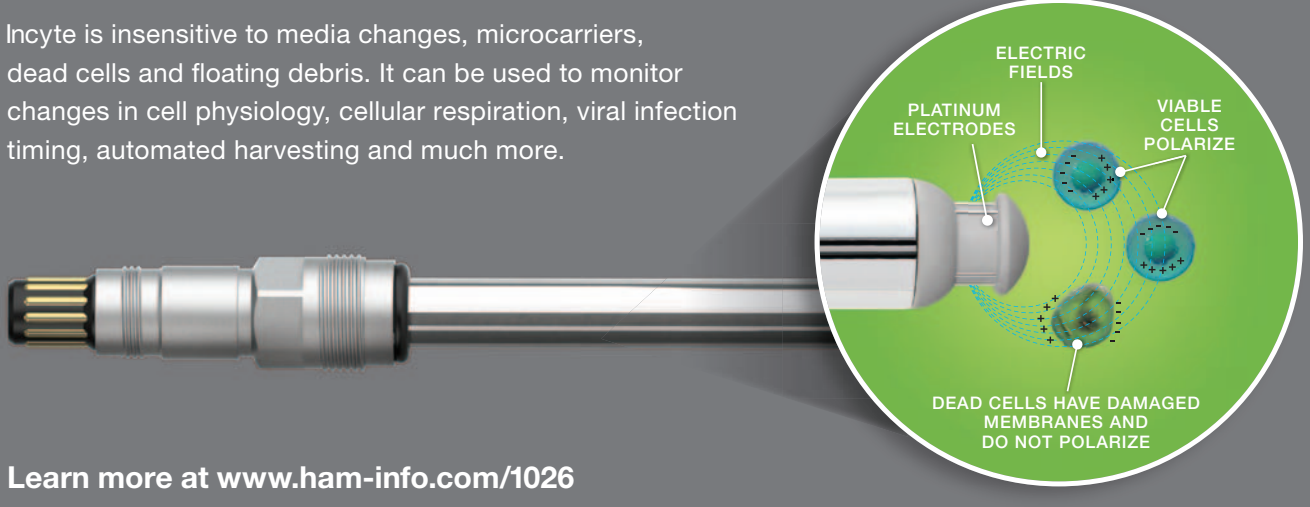
Incyte Measures Viable Cell Density in Real Time

Incyte is insensitive to media changes, microcarriers, dead cells and floating debris. It can be used to monitor changes in cell physiology, cellular respiration, viral infection timing, automated harvesting and much more.



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Rita Peters is the editorial director of *BioPharm International*.

Reducing regulatory roadblocks requires more than the stroke of a pen.

Regulatory Reform Is Not That Simple

Professionals involved in the development and manufacture of new drugs understand that drug development is—by design—a slow, deliberate, detailed, scientific, and technical effort. Getting a drug to market quickly is crucial to meet business objectives and provide needed patient treatments. The goal: accelerate the delivery of safe, efficacious, and cost-effective drugs to patients and to keep a steady supply of safe, efficacious, and cost-effective drugs on the market.

While some complain the drug development and manufacturing process is over-regulated, others seek more direction and guidance from regulatory authorities. FDA has been promoting a "carrot" approach to compliance, encouraging drug manufacturers to implement quality measures and, in effect, self-regulate their operations. Unfortunately, due ongoing quality problems, the "stick" is still needed, in the form of regulations, guidance documents, and inspections, particularly offshore.

A new order for rules?

The Trump administration lost no time in pushing forward on its campaign promises including the reduction of regulations perceived to be roadblocks to innovation. Executive action, however, may reduce both regulations and regulatory explanations; impact the implementation of the 21st Century Cures Act, the Prescription Drug User Fee Act (PDUFA), and the Generic Drug User Fee Act (GDUFA); and hinder FDA's ability to approve new and generic drugs.

A Jan. 30, 2017 executive order, "Reducing Regulation and Controlling Regulatory Costs," set a regulatory cap for fiscal year 2017; for every proposed new regulation, at least two existing regulations should be identified for repeal. Based on language in the order, both regulations and guidance documents issued by FDA would be included in the "one in–two out" policy. Positioned as an effort to help small businesses, President Donald Trump described the order as "the largest ever cut by far in terms of regulation" in the signing ceremony. He noted that regulation cuts for large business would "be different" but did not specify the differences.

A freeze on the hiring of federal employees was put in place with a Jan. 22, 2017 memorandum. As of this writing, it was unclear if hundreds of vacant positions at FDA would be deemed necessary for public safety and exempt from the freeze.

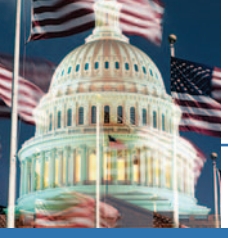
Promises vs. reality

Regulations are developed for many reasons, most notably to implement enacted legislation. Eliminating mandated regulations could require changes or repeals of existing laws. Guidance documents, which explain FDA's current thinking on regulations, do not have the force of law, but are used by pharma companies for guidance in complying with regulations and are frequently cited by FDA in warning letters to facilities that do not comply.

Writing federal regulations and guidance documents requires input and detailed consideration from knowledgeable professionals. FDA consults pharma industry experts, publishes drafts in the *Federal Register*, and solicits public comment.

The agency planned an ambitious guidance agenda for 2017; 26 guidance documents were slated for revision and 81 new guidance documents proposed. The implementation of the Cures Act and pending reauthorizations of PDUFA and GDUFA should generate the need for even more regulations.

A "one in–two out" strategy could result in the elimination of vital regulations and guidance documents that prescribe practices to ensure the quality of manufactured drugs for the US population here and abroad. The executive order, designed to fulfill a campaign promise, is shortsighted. At a minimum, it creates confusion and uncertainty for a complex drug development and manufacturing system. If followed to the letter, it could create the safety threats, drug shortages, and fraudulent products FDA was established to prevent. ◆



User Fees Needed to Help FDA Manage its Full Plate

User fee reauthorization is crucial to implementing the Cures Act and refining the approval process.

Congress surprised Washington policy makers in December 2016 by enacting the 21st Century Cures legislation after two years of deliberations. The new law shores up FDA operations, streamlines drug development, and funds National Institutes of Health (NIH) research programs, including the Obama administration's cancer "moonshot" and personalized medicine initiative. Support on Capitol Hill was near-unanimous after adding language promoting regenerative medicine and funding state programs to treat opioid addiction and bolster mental health services.

Now it's all hands on deck as FDA devises a plan for implementing the many Cures requirements for furthering patient-focused drug development, biomarker qualification, pediatric research, and the development of needed antibiotics and treatments for rare conditions. FDA has to issue many new guidance documents and reports within set timeframes and establish specified processes and procedures (1). FDA commissioner Robert Califf highlighted how the Cures Act will improve FDA's ability to hire and retain scientific experts, probably the most important provision for agency managers struggling to fill hundreds of vacant positions (2). Biopharma companies developing new drugs gained more flexibility to use novel clinical trial designs and to rely more on real-world evidence to expand indications on marketed medicines.

Jill Wechsler is *BioPharm International's* Washington editor, Chevy Chase, MD, 301.656.4634, jillwechsler7@gmail.com.

USER FEES CRITICAL

The Cures Act also provides an additional $4.8 billion over 10 years for NIH and $500 million for FDA. But these funds are not appropriated, opening the door to cutting these promised resources.

It's all hands on deck as FDA devises a plan for implementing the many Cures requirements.

Implementing the Cures Act, therefore, depends on timely reauthorization of user fee programs for drugs (Prescription Drug User Fee Act [PDUFA]), biosimilars (Biosimilar User Fee Act [BsUFA]), and generic drugs (Generic Drug User Fee Amendments [GDUFA]) before they expire on Sept. 30, 2017. Normally, enacting legislation as extensive as the Cures Act should smooth the path on Capitol Hill for user fee bills. The Cures Act already has tackled several controversial issues, such as greater reliance on real-world evidence in regulatory decision-making, expansion of priority review voucher programs to spur drug development, and more flexibility for manufacturers to present healthcare economic information on unapproved drug uses.

But drug user fees need to be enacted by late summer 2017 for FDA to avoid issuing pink slips to staffers supported by fee revenues. FDA officials and industry representatives spent the past two years negotiating the fee agreements and want to avoid changes at this point from Congress and Trump administration officials. Another wrinkle is that the Center for Drug Evaluation and Research (CDER) has been working with non-prescription drug manufacturers to devise a user-fee program to support modernization of the over-the-counter monograph process, which could take more time. And legislators on both sides of the aisle talk about curb-

ing high prices on new and old drugs by enacting proposals that were not included in the Act.

PRICING PRESSURES

Senate Democrats are pressing the new President to take more aggressive action on drug pricing. Trump has called for Medicare to negotiate drug prices and for FDA to approve competitive generic drugs more quickly to provide market competition. And they all propose broader disclosure of pharma R&D and manufacturing costs to clarify production outlays.

Exorbitant price increases on old off-patent drugs are targeted in a report from the Senate Special Committee on Aging, released in December 2016. It documents four such cases that generated huge profits for the manufacturers, while imposing prohibitive costs for patients and payers (3). Susan Collins (R-Me), chair of the Aging Committee, and colleagues seek to prevent such behavior by boosting competition in monopoly drug markets. Specific proposals would authorize targeted prescription drug importation, help generic-drug makers access therapies needed for bioequivalency testing, speed FDA review of generic versions of sole-source drugs and those in short supply, and offer priority review vouchers to firms that develop needed alternative products. The legislators also criticize manufacturers' patient assistance programs for steering patients to too-costly therapies and seek more transparency in negotiated drug prices and rebates.

Meanwhile, the outgoing Obama administration gave up on its plan to test a range of new pharmaceutical payment models for Medicare Part B, anticipating its sure demise under the Trump administration. Pharma companies and physicians had criticized the Part B demonstration as overly focused on saving money, as opposed to improving outcomes, and a threat to patient access to life-saving therapies. Despite growing interest in developing value-based payment models for medicines, the backlash to the proposal from the medical community illustrates the difficulties facing most drug price control strategies.

MANUFACTURING PROBLEMS MULTIPLY

While Congress enacted Cures, FDA officials were delivering the troubling news that fewer new medicines came to market in 2016 than in the previous two years. CDER approved 22 new molecular entities (NMEs), a significant drop from the near-record 45 new drugs in 2015, reported John Jenkins, just before he retired as director of CDER's Office of New Drugs (OND). Jenkins speculated that the 2016 decline may be a blip, as CDER continues to approve most novel drugs in the first review cycle and to provide expedited action on breakthrough therapies. He attributed the decline in new drugs to fewer new applications filed with the agency in the first place, plus an increase in incomplete or inadequate submissions.

That increase was evident in a sharp rise in Complete Response Letters (CRs)—14 in 2016 compared to three a year earlier—including five citing manufacturing difficulties. For example, in May 2016, AstraZeneca announced a CR from FDA for a new treatment of hyperkalaemia that had failed a pre-approval manufacturing inspection (4). FDA cited Valeant for GMP deficiencies with a new Bausch + Lomb ophthalmic solution (5), and in October 2016, Sanofi and Regeneron acknowledged that a CR citing problems with its fill/finish facility in France would delay launch of a potential blockbuster new treatment for rheumatoid arthritis (6). And Cempra was hit with a CR at year-end that cited safety concerns for its new antibiotic Solithera, along with manufacturing deficiencies at contract manufacturers Wockhardt and Hospira (7). Roche didn't get a CR but had to announce in December 2016 that problems with its commercial production process would mean a three-month delay in its user fee approval date for a much-anticipated new treatment for a serious form of multiple sclerosis (8).

Perhaps these developments will compel manufacturers to look more seriously at investing in modern pharmaceutical manufacturing processes to ensure reliable quality production of critical drugs and biologics. In reviewing FDA's achievements for this past year at the FDA/CMS Summit in Washington, DC in December, CDER director Janet Woodcock cited progress in ensuring drug quality, partly due to CDER development of an inventory of facilities all over the world that produce drugs for the United States. She anticipated further gains from implementing a risk-based inspection program through reorganization of FDA's field inspection force.

The Cures legislation supports these efforts by authorizing grants for research by academic institutions on continuous manufacturing methods for drugs and biologics. Woodcock also looks to expand agreements for mutual reliance on European GMP inspections and to extend such agreements to other regions and to additional types of inspections.

Contin. on page 12

Viewpoint: Challenges and Opportunities for CDMOs

The outlook for the CMO and CDMO industry may be affected by ever-changing politics.

In mid-2016, consolidation, IPOs, pharma-spinouts, and facility selloffs were the major themes in pharmaceutical outsourcing. In the aftermath of the US presidential election, pharma stocks (and other industrials) went up. This giddiness among pharma investors lasted less than a month, ending when President-Elect Trump told *Time* magazine, in his Person of the Year interview, "I'm going to bring down drug prices. I don't like what's happened with drug prices" (1).

There was no elaboration for several weeks, and industry, investors, and the general public alike were in the dark about what he meant and how he might achieve that goal. No one was prepared for his Jan. 11, 2017 press conference (2), in which he complained that pharma companies are "getting away with murder" and said that Medicare should be negotiating with drug companies for better prices. A few days later, he expanded that notion, calling for Medicaid to also have authority to negotiate prices, despite the fact that it has "best price" entitlement under current law.

THE FUTURE OF THE CMO/CDMO SECTOR

In recent months, the contract manufacturing and contract development organization (CMO/CDMO) industry has seen a major acquisition in Lonza–Capsugel; a significant one in Asahi Glass–CMC Biologics; a pair in which Pharma & Biopharma Outsourcing Association (PBOA) member Catalent acquired two potential PBOA members in Accucaps and Pharmatek; and a large IPO by Samsung Biologics, which seems to value that company as bigger than Catalent and Patheon's combined market cap.

Gil Roth is president of the Pharma & Biopharma Outsourcing Association, gil.roth@pharma-bio.org.

Where is the CMO/CDMO sector headed in 2017 and beyond? Making predictions is pretty inane at the moment, but the industry should see more efforts at integrating "one-stop" CDMO concepts, along the lines of Patheon's OneSource model, and that may entail more mid- to large-scale acquisitions, along with strategic purchases for specific technologies.

> The industry should see more efforts at integrating "one-stop" CDMO concepts.

Regarding the new administration in the United States, the industry is in wait-and-see mode. With the Republican party controlling both houses of Congress and President Trump in the White House, the Republicans will be in a position to implement business, industrial, and healthcare policy that can trickle down to affect the CDMO sector.

Tax reform seems to be the top priority for the GOP (after repealing the Affordable Care Act, the process for which has turned out to be a minefield). Along with a drop in the corporate tax rate, they have pushed for a tax holiday that would allow US-headquartered companies to bring overseas profits into the country at a reduced tax rate, along the lines of the American Jobs Creation Act of 2004, which lowered the tax rate from 35% to 5.25% for a one-time repatriation.

What effect could a large-scale influx of funds by major pharma companies have on CDMOs? Those pharma companies may simply pay out higher dividends to shareholders, but this repatriation could also trigger a wave of mergers and acquisition activity among domestic players, which could lead to shifts in outsourcing allocation. In concert with the GOP's idea of a border adjustment tax, there may be greater investment

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in US R&D and manufacturing facilities by in-house pharma, as well as a potential shift from ex-US facilities to domestic CDMOs for products intended for the US market.

Anti-immigration sentiment could become problematic if it keeps high-value scientific personnel from coming to the US. More than one CDMO has expressed concern about potential visa restrictions and their impact on the labor pool.

FDA REFORM

Congress has talked about accelerating generic drug reviews to provide another outlet for bringing drug prices down. While more generic approvals could benefit the CDMO sector, based on this author's experience inside the Generic Drug User Fee Amendment (GDUFA II) negotiations, FDA does not have a lot of margin to shave from review times.

The appointment of a new FDA commissioner could certainly shape policy in ways that benefit or hinder pharma and CDMOs, but FDA is awfully big, and it's not the sort of organization that can change direction on a dime. Top-down policy decisions may impact discrete areas (will quality metrics survive the administration's push for deregulation?), but the day-to-day functions of the agency overall may not reflect the priorities of a new administration very quickly.

One can hope that the new administration recognizes the value that the pharma industry brings to the US healthcare ecosystem.

It's all too uncertain at this point, and no Magic 8-Ball is going to point out the correct path. But one can hope that the new administration recognizes the value that the pharma industry brings to the US healthcare ecosystem, the financial and innovative engines that they represent, and the importance of the CMO/CDMO sector. PBOA's members provide pharma and biotech companies with the advanced dose forms, regulatory-compliant manufacturing, and supporting development services that help them develop and manufacture drugs, biologics, vaccines, and other treatments safely and cost-effectively, and I hope we can work with the new administration to continue to bring value to American patients.

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Regulatory Beat —*Contin. from page 9*

More reliable drug manufacturing operations would help CDER remedy the approval slow-down. A priority for 2017, Woodcock said, is for CDER to establish a Pharmaceutical Platform for new drugs, along with clear data standards for drug submissions and effective IT policies, similar to the automated Panorama management system recently completed to manage generic-drug application review and approval.

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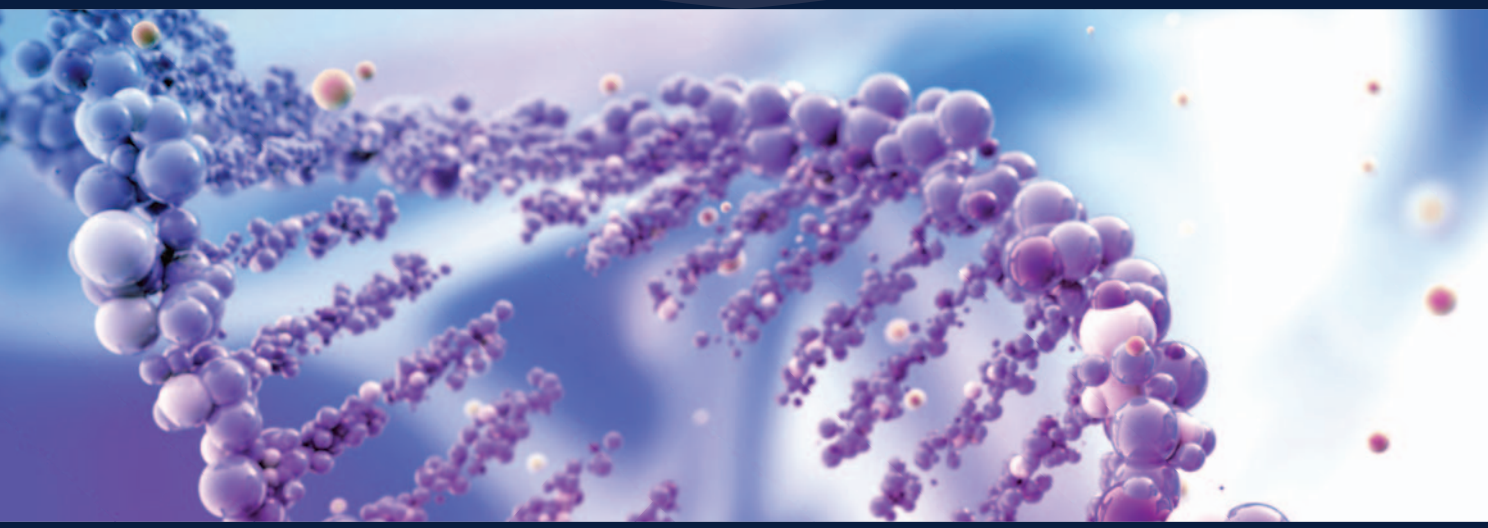

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Mass Spectrometry Measures Up to Analysis Challenges

Cynthia A. Challener

Despite limitations, mass spec is having an impact on biologic drug development and manufacturing.

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Mass spectrometry (MS) is a powerful and sensitive technique used to detect, identify, and quantify molecules separated by their mass to charge (m/z) ratio in the gas phase. In a recent publication by FDA reviewing MS usage trends over the past 10 years of biologic license application (BLA) filings, the agency determined that MS is now ubiquitous in filings and that the number of ways it is being used is increasing (1).

The demands of life-science applications have led to the improvement of MS technologies and rapid growth of new types of instruments that feature powerful analytical capabilities—sensitivity, selectivity, resolution, throughput, mass range, and mass accuracy, according to Gang Huang, vice-president of analytical development and regulatory affairs with WuXi Biologics. In the biopharmaceutical industry today, mass spectrometry finds applications at the earliest discovery stages (e.g., imaging of biomarker/protein binding in cells), during process development, and for product characterization throughout the development cycle, including for release testing.

Challenges with sample preparation and data processing remain, however. Biologics manufacturers and analytical laboratories are working closely with instrument suppliers to address these issues. Improvements are continually introduced, and as a result the potential for additional use of this analytical technology for biologic drug development and manufacturing is significant.

Cynthia A. Challener, PhD, is a contributing editor to *BioPharm International.*

EXPANDING APPLICATIONS

Mass spectrometry is widely used for the characterization of protein therapeutics in the development stage. More specifically, MS is a effective tool for intact protein analysis (measurement of the molecular weight of the protein), fragment analysis, peptide mapping, and identification of post-translational modifications (PTMs), according to Tiffani Manolis, segment manager for pharma at Agilent Technologies.

As a characterization tool, MS is used to assign the primary structure of biologic drugs, including amino acid sequencing for peptides/proteins, disulfide bond mapping, N-glycan profiling of monoclonal antibodies (mAbs), and nucleotide sequencing for oligonucleotides, adds Jie Ding, associate director of mass spectrometry services at PPD Laboratories' GMP laboratory.

Peptide mapping can also be applied for determination of other critical quality attributes (CQAs), such as physicochemical properties of oxidation, glycosylation, deamidation, and isomerization and the presence of N-terminal cyclization as well as confirmation N- and C-terminal groups, according to Hillary Schuessler, an investigator with GlaxoSmithKline (GSK) R&D Platform Technology & Science. She adds that characterization of product variants and higher-order structure (HOS) analysis via hydrogen–deuterium exchange (HDX) are also important applications of MS.

In addition, MS can be used for media and feed characterization, such as quantitative targeted metabolomics for amino acids, vitamins, lipids, central carbon metabolites, sugars and sugar bases, etc. In this case, the absolute quantities of these compounds are measured as they are depleted/excreted from cells. "A challenge is to differentiate extracellular from intracellular metabolites. It is possible, albeit challenging, to measure the flux of metabolites for use in predictive modeling of protein product quality attributes such as glycoform variants and amino acid misincorporation," says Greg Kilby, manager of biopharm analytical sciences with GSK R&D Platform Technology & Science.

Profiling of process-related impurities such as host-cell proteins (HCPs) is another application for MS. "The identification of the most abundant HCP species provides valuable information to process scientists for developing a tailored process for removal of these critical impurities," notes Huang. Sanofi uses MS to support process development and manufacturing, including impurity identification and tracking and the collection of detailed, high-level, advanced structural information to confirm that the product is as intended, according to Jianmei Kochling, director of analytical science and technology for the company.

Sequence variant analysis of production cell lines used for biologic production is an important part of process development. The potential presence of sequence variants, which can result from DNA mutations and amino acid misincorporations, is analyzed at the protein level using high resolution MS and data analysis software. From multiple candidate clones, the one without mutations or low level mutations will be chosen as the final clone for further development, according to Huang.

At early development states, MS is used in cell imaging applications, as well as for characterization of biomarkers and determining drug metabolism and pharmacokinetic (DMPK) profiles (clearance/lifetimes).

These applications are just the beginning, however. "The roles for MS are rapidly expanding to more hybrid qualitative/quantitative applications and the examination of higher-order biotherapeutic structure," notes Scott J. Berger, senior manager of biopharmaceuticals in the Waters Corporation's pharmaceutical business group.

Other newer applications include biosimilar development, where MS is an enabling technology to show that an innovator and biosimilar have identical sequence and comparable variant profiles. Similarly, Berger says, the rise of antibody-drug conjugates (ADCs) has required more advanced liquid chromatography (LC)–MS laboratory workflows for challenging separations, mass detection, and data processing to determine the average number of drug conjugates (DAR) on a molecule, their distribution across the many possible sites of reaction, and individual occupancy levels for each of these sites on these hyper-complex molecules.

WHY MS?

Mass spectrometry is a preferred analytical technique in many of these applications because it is a more targeted method that provides detailed information about protein structure/conformation, whether for the desired product or impurities like HCPs that are present at low concentrations.

The sensitivity and specificity, high mass accuracy at low part per million levels, and ability to return precise chemical information on the molecule of interest are main drivers for using mass spectrometry, according to Kilby. Additional attractive characteristics include compatibility with most chromatographic methodologies, the ability to gain both qualitative and quantitative [relative and absolute] information, and the lack of any theoretical limitations on the size of proteins that can be analyzed.

"MS is an ideal tool for supporting process development and a quality-by-design approach. For process impurity identification, it is much more specific than ELISA (enzyme-linked immunosorbent assay) test-

ing, which is the conventional method (and is still used for rapid product release)," Kochling says.

Mass spectrometry is also preferred in these applications because it offers superior sensitivity and specificity without the need for a large volume of samples, according to Ding.

Berger also notes that MS provides more confident qualitative mass analyses and makes it possible to identify a single peptide or modified peptide in the presence of the many other peptides that are generated in the digest of a biotherapeutic protein, even if that peptide is fully or partially co-eluting with other components. "Monitoring of multiple mass channels simultaneously allows for monitoring of several components at the same time, and in many cases without the need for optimizing the separation conditions to get valuable quantitative information," he says.

In addition, the ability to detect components at levels lower than optical detection-based assays expands the dynamic range of detection of an assay for the peptide mappeak of a peptide or its variant, according to Berger.

Overall, observes Manolis, MS is an indispensable tool for peptide and protein analysis due to its speed, sensitivity, and versatility. "MS is particularly useful for gaining knowledge about the location of disulfide bonds and amino acid sequences," asserts Kochling. Adds Mario DiPaola, senior scientific director for Charles River Laboratories: "No other analytical technique can provide the extent of information obtained by mass spectrometry, nor the selectivity or sensitivity. Previously it would have taken months to determine or confirm the entire sequence of a protein by first collecting enzymatic digests and then performing Edman degradation, but now the same analysis can be performed in a matter of days with mass spectrometry while using micrograms of product rather than milligrams."

CONTINUOUS IMPROVEMENT

Several advances in technology have been enabling the wider use of MS. Examples include developments in high-resolution mass spectrometry, such as quadrupole time-of-flight (QTOF) and orbitrap technology, and workflow-driven software development, according to Ding. Ion mobility methods have made MS useful for HOS analysis, cysteine variant determination, and N-Glycan profiling, while top- and middle-down analyses, which have been made possible through the introduction of electron transfer dissociation (ETD) fragmentation, are useful for determination of CQAs and identification of product variants, says Schuessler.

While not very recent, DiPaola points to the introduction of tandem mass spectrometry and the electrospray ionization (ESI) interface as key advancements in the field of mass spectrometry. ESI has allowed for easy coupling of a high-performance LC (HPLC) system to a mass spectrometer, as a key advancement because it permits separation of species followed by direct in-line mass analysis. Hybrid mass spectrometry has made it possible to obtain very detailed information on PTMs and protein primary structures.

In addition to ETD, the introduction of a variety of other alternate methods to collisional induced dissociation (CID), including electron capture dissociation (ECD), higher-energy collisional dissociation (HCD), electron transfer in the higher-energy collisional dissociation (EThcD), ultraviolet photodissociation (UVPD), and surface induced dissociation (SID), among others, have led to significant improvements in fragmentation technology, according to DiPaola. Some of these fragmentation methods can be used independently or in-series to garner as much information about protein analyte as possible.

For Huang, one of the most powerful developments in the evolution of MS technology is the commercialization of hybrid instruments. "Hybrid MS instruments are made by combining two different types of mass analyzers together in tandem; one can choose almost any combination of quadrupole, time-of-flight, or ion-trap hybrids. These hybrid instruments promise the ability of combining the best features from the different components and allow tandem mass spectrometry experiments and unique scanning modes that are not possible on a single instrument," he explains.

Berger adds that the increasing focus on hybrid quantitative/qualitative workflows favors TOF-based platforms that do not suffer the lower-end dynamic-range limitations of automatic gain control (AGC)/orbitrap-type instruments. "Time-of-flight and quadrupole time-of-flight MS technology has been the primary high-resolution MS tool used for biopharmaceutical characterization and monitoring due to its ability to maintain high resolution and sensitivity independent of the mass of a species, and the ability to do so with increasingly rapid LC and CE [capillary electrophoresis] separations on the front end of these analyses," he explains.

Various bioinformatics tools associated with MS analyses, such as pathway mapping/analysis, network association, and ADC calculators, have had an impact on the use of MS as well, notes Kilby. "Each major instrument vendor has software that works specifically with its instrument and uses complex algorithms to process its proprietary data files. Vendors constantly seek feedback from users to improve software features, such as chemical intelligence, batch data process, automation,

report generation, Title 21 *Code of Federal Regulations* Part 11 compliance, and so on," agrees Ding.

More specifically, Manolis notes that dedicated data analysis software has been developed for biopharma applications, and the workflow-specific design this software has streamlined the process. "In addition," she says, "walkup software has been developed to enable MS novice users, such as biologists, to have access to high-end MS instruments. Special consideration has also been given to providing total workflow solutions to address sample preparation all the way to reporting."

Overall, Berger believes that the expanded use of MS in more targeted biotherapeutic CQA monitoring experiments, even in later (regulated) development and quality control (QC) environments, is related to the increased robustness of the instrumentation, growing usability of these systems, and deployment on informatics platforms designed for regulated environments. "The ability to follow specific product variants in a peptide map, intact mass profile, or released glycan profile enables targeted quantification of the amount of that modification as an organization develops and matures its manufacturing processes or sets specific limits of a variant in a QC release test," he explains.

The other area of great expansion has been in HOS analysis, according to Berger. The development of ion mobility MS has introduced the ability to measure collisional cross-section (CCS) data for molecules, bringing an added level of separation to MS analysis and generating data based upon gas-phase cross-sectional area and shape, in addition to the traditional mass and charge characteristics measured by a mass spectrometer. Biologic folding interactions and stability can be screened and assayed using this type of ion mobility information. In addition, Berger notes that more resolving HOS information can now routinely be provided by hydrogen deuterium exchange MS (HDX–MS), which measures the accessibility of backbone amide hydrogens to exchange with deuterated water in solution.

WORKFLOW LIMITATIONS

With current MS technology, there is a disconnect between the practical and actual time it takes to complete complex analyses, according to Kochling. "The analysis of proteins is very complicated, and even with current mass spec instrumentation and software, a significant amount of manual labor is required, and in some cases it can take up to one month to complete an analysis, which is not practical in an industrial setting," she states. "Although mass spec technology has been ever improved in sensitivity and dynamic range, the hardware capability is ultimately limited by the complexity of samples. Sample preparation technology and procedure improvement can compensate for the instrument capability in sensitivity and dynamic range," she continues.

Current mass spectrometers were designed and optimized for the analysis of smaller molecules, and as a result large protein molecules often suffer from poor data quality resulting from limited resolution, sensitivity, and mass range, adds Manolis. She notes that additional alternative and optimized fragmentation methods for large molecules for top-down and middle–down analysis are desired. On the other hand, Berger believes that MS systems are designed to be general-purpose platforms for both large- and small-molecule studies, with compromises on some specific performance attributes for large molecules to accommodate a wider range of applications in the lab.

At GSK R&D Platform Technology & Science, poor parallelization is an issue; currently it is not possible to highly multiplex MS analyses without buying extra mass spectrometers (compared with genomic and microarray technology, for example). In addition, as resolution and scan speed increase, files sizes are getting very large such that current informatics suites struggle with data analysis, especially for large experiments, according to Schuessler. "With the enormous volume of data being generated, data processing and analysis become increasingly important and remain a bottleneck," agrees Huang.

Furthermore, according to DiPaola, the analysis of these files requires sophisticated and expensive software, as well as highly skilled and knowledgeable users. "Both of these scenarios present recruiting and financial challenges for laboratories and companies," he asserts.

Kilby also points to the incompatibility of MS with commonly used biologic matrices/buffers/detergents, issues with samples that have extended dynamic ranges (HCPs, serum proteomics, etc.), integration in process analytical technology and continuous flow manufacturing, and the fact that response factors are not universal (unlike for UV, charged-aerosol, and evaporative-light-scattering detectors).

The need to label or spike analytes with an appropriate isotopically labeled species to obtain highly quantitative results due to the ionization variability of species is one issue for DiPaola. Another is the difficulty of detecting low-level impurities (<1–2% in abundance) in biological samples without some prior knowledge of their type. A third issue for DiPaola is the need to confirm isobaric amino acids when conducting sequencing by MS/MS using Edman degradation, which becomes a bottleneck because the peptides containing such isobaric amino acids must be collected and individually sequenced.

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Ensuring the Biological Integrity of Raw Materials

Catherine Shaffer

A multi-pronged approach to raw materials testing can help mitigate the risk of future contamination events.

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Contamination with microbes, mycoplasma, viruses, and other adventitious agents can be a significant problem in biopharmaceutical manufacturing. Although contamination can occur from the cell culture itself or from labware and the laboratory environment, raw materials are the most significant source of contamination. That can lead to false research results and a serious health risk to patients receiving the product.

Contamination of biologic drugs and vaccines by adventitious agents is extremely rare. However, when contamination incidents do occur, they can be costly in terms of time and resources.

In 2010, Eric Delwart, PhD, researcher and adjunct professor of laboratory medicine in the Blood Systems Research Institute at the University of California San Francisco, tested eight viral vaccines using polymerase chain reaction (PCR) and DNA sequencing, and found that three of the vaccines contained unexpected viral sequences (1). One affected vaccine was Rotarix, a rotavirus vaccine manufactured by GlaxoSmithKline. Porcine circovirus was detected in the vaccine, a discovery that led to a halt in the use of Rotarix, which is given to babies at two, four, and six months of age. The contamination traced back to the use of raw materials originating from animals in the production of the vaccine, and highlighted the need for better procedures to eliminate viral contamination and for better tests to detect adventitious agents.

Catherine Shaffer is a contributing writer to *BioPharm International*.

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Protocols for control of contamination in raw materials rely on cleaning and decontamination procedures combined with rigorous testing. These procedures are effective for most agents, but some organisms, particularly viruses and prions, have evaded standard prevention and testing methods. New technologies in biosafety testing target those previously undetectable contaminants.

TYPES OF ADVENTITIOUS AGENTS

Raw materials can be contaminated with a variety of adventitious agents. Those include bacteria, yeast, molds, viruses, and sometimes prions.

Mycoplasmas are the smallest of free-living organisms, and are frequent contaminants of mammalian cell cultures. They can alter the metabolism and properties of cells and change product yield, cause false assay results, and generally wreak havoc in the culture.

Viruses are some of the simplest of all organisms. They are very small and are generally comprised of a small amount of DNA or RNA surrounded by a lipid envelope. They rely on the host for reproduction, and sometimes incorporate their genetic material into the host cell's genome. Viral contamination is generally the greatest contamination risk because of the ability of viruses to evade detection and cause silent infections in cell cultures. There is no universal, one-size-fits-all method for treating materials that will eliminate all viruses.

STOPPING CONTAMINATION

The most common type of contamination incident happens when a media component is contaminated. For example, bovine serum can be contaminated with reovirus, epizootic hemorrhagic disease virus, Cache valley virus, or *Vesivirus* 2117. Porcine circovirus is sometimes found in porcine trypsin. Minute virus of mice (MVM) is a common source of raw material contamination of various media components due to infestations of mice in facilities where products are manufactured (2, 3).

Global regulations, including those from the United States Department of Agriculture, the European Medicines Agency, and the FDA's Center for Biologics Evaluation and Research (CBER) set standards for minimizing viral contamination, particularly spongiform encephalopathies (4).

Standard procedures for inactivation of adventitious agents include the use of heat, filtration, pH, and gamma irradiation. Thorough cleaning of equipment, testing, and review of material sources are also important steps to take. Bovine serum, for example, should be sourced from a country with a negligible risk of bovine spongiform encephalopathy. Animals should be less than 30 months old, designated for human consumption, and test free of all forms of transmissible spongiform encephalopathy. And there should be a quality assurance system in place with a system for delineation of specific batches. The supplier should have a regular audit routine (2).

It is impractical to test all raw materials for every possible adventitious agent. Two testing approaches may be used to screen materials for most types of viruses and other contaminants. One is based on identifying the characteristics of the contaminant, such as cytopathic effects of viruses. Another option is to test using immunoassays or PCR for a panel of viral antigens or sequences.

Archie Lovatt, biosafety scientific director of SGS Vitrology, advocates an active risk mitigation strategy incorporating multiple strategies and approaches for managing contamination risk. "Essentially, it's about knowing your manufacturing process, knowing your raw materials, and going deep. Understand exactly what the risks are, then try and mitigate the risk," Lovatt says. That would include preliminary testing of materials and process monitoring. "If there is a contamination, you catch it early—before you send the batch for purification."

Trending strategies for testing raw materials are included in GMP practices, quality by design, and process analytical technology. Single-use manufacturing devices, disposable consumables, and ready-to-use reagents and media are also reducing rates of contamination in the industry.

Faster, more accurate tests are being introduced to the market to address the problem of contamination. "Traditional test methods require up to seven days for reliable results," Theresa S. Creasey, MilliporeSigma's head of applied solutions strategic marketing and innovation tells *BioPharm International*. To reduce testing delays, MilliporeSigma offers its Milliflex Quantum system, a fluorescence-based test method that gives results in one-third of the time of traditional media methods.

Negative test results do not guarantee that there is no contaminant in the material, according to Mark Plavsic, chief technology officer of Lysogene. "Assuming that all sourcing of raw materials has taken place in a controlled manner, assuming the components are well selected and examined, assuming that all of the testing has been done by the letter of the law, what is left is treatment for viral inactivation and removal. Not every company is doing this. Not every supplier is doing this," says Plavsic.

Ray Nims, a consultant at RMC Pharma, advocates a multi-pronged mitigation strategy. Nims explains, "Where testing fails is this. You

typically test one bottle. And out of the bottle, you test a small amount, maybe 100 mLs. These lots of serum can be 3000–5000 bottles, so the serum company may test from one or two bottles. The company procuring the serum typically will test 100 mL from another bottle. If the testing passes, the lot is declared released and then used. The assumption that if you tested it clean the entire lot is clean fails sometimes."

DISINFECTION APPROACHES

Barrier technologies complement testing. The most common barrier disinfection method is gamma irradiation, according to Nims. Gamma irradiation is standard for manufacturers of bovine serum, however; it's not normally an option for other raw materials such as media. Two alternatives are an in-line treatment called high-temperature short-time processing (HTST) and ultraviolet irradiation, a technology that has a great deal of potential applicability, but is has not yet been taken up by the industry.

Ultraviolet disinfection is a powerful technique for neutralizing living microorganisms. Exposure to the UV light causes the formation of dimers between neighboring nucleic acids in the genome, which prevents the organism from reproducing. Ultraviolet (UV) disinfection is commonly used to treat waste water and drinking water in the United States. UV disinfection has some support from the EMA, which recommends it as one of two complementary virus reduction steps. Combining inline UV disinfection with other barrier methods for preventing contamination, such as filtration, is a "belt and suspenders" approach that would be more effective than either method alone.

NON-ANIMAL-BASED MATERIALS MAY NOT BE THE SOLUTION

There is a trend in the industry away from animal-based sources of raw materials. Use of serum-free media can instantly eliminate the most common contaminants, including virus risk and most mycoplasma risk. Animal serums are considered rather old-fashioned in the production of biological drugs and vaccines. Most processes can be adapted to use serum-free media. However, many legacy processes currently still make use of animal-derived materials, particularly fetal bovine serum.

Non-animal sources are not a panacea. Plant source materials can be exposed to soil, animals such as field mice, bird feces, human contact, and other environmental contaminants. Human handling can also introduce adventitious agents to source material.

Adam Elhofy is chief scientific officer at Essential Pharmaceuticals, which manufactures an animal-free media supplement for cell culture called Cell-Ess. He points out that reliance on Chinese hamster ovary (CHO) and other non-human cells is more of a problem. "Cross-species contamination for a virus is fairly low," Elhofy said. "There's still the risk. The problem is people are using cells that are not human cells. Those cells can be infected by animal origin viruses."

Strategies for preventing contamination include upfront testing of materials, barrier disinfection methods, and adhering to best practices in processing and sanitation. Avoidance of animal-based raw materials eliminates the most common and problematic sources of contamination, as well as careful sourcing of any materials used. Contamination risk can never be fully eliminated, but with vigilance, it can be minimized.

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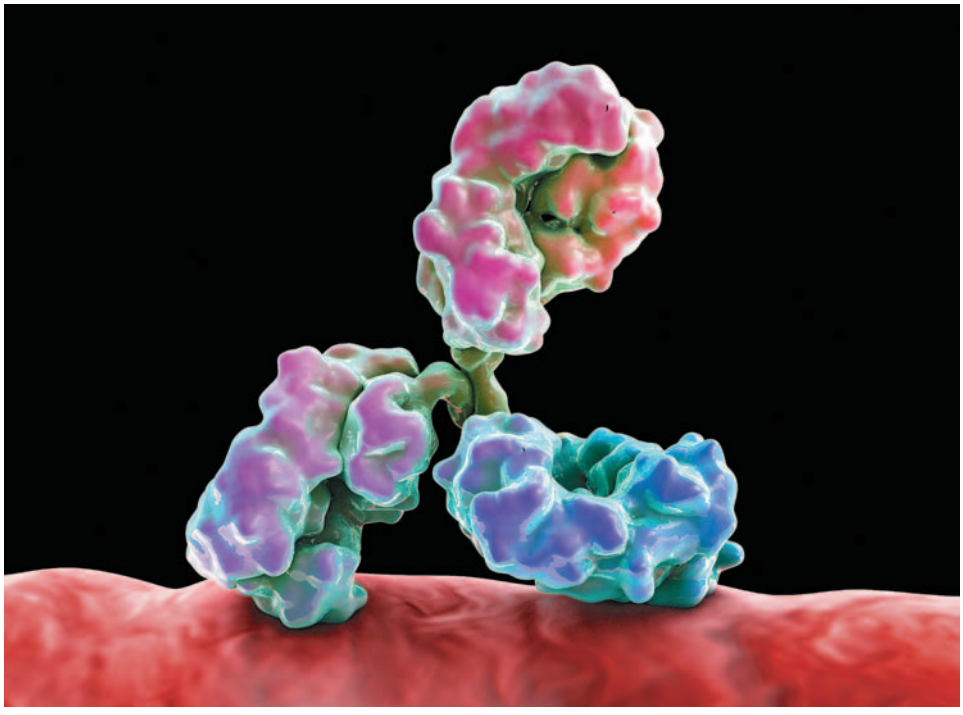
mAb Purification: Rethinking the Initial Capture Step

Angelo DePalma

Although Protein A remains a top technology for monoclonal antibody purification, the industry continues to look for new approaches to improve conventional capture chromatography.



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Capture of target proteins from raw harvest defines the remaining downstream processing steps. The more efficient and selective the capture, the less involved will be subsequent chromatography, filtration, formulation, and finishing operations.

For monoclonal antibodies (mAbs), capture invariably means affinity chromatography with Protein A resins. Yet emerging molecular classes—and the desire to reduce mAb capture to its essential, most economic components—drive the search for alternatives.

Thus, in one sense, the technical and economic arguments for using Protein A resins become less obvious. Thermo Fisher Scientific (Longmont, CO), for example, has adapted its CaptureSelect resin technology towards non-Protein A affinity ligands. The resins employ immobilized single antibody domains to provide full functionality and high-affinity binding; the ligands' compact structure provides robustness under diverse chromatographic conditions.

"CaptureSelect products are proving to be generally useful for antibody fragments, bispecific antibodies, fusion proteins, and many other non-antibody therapeutic proteins. They can also be used to purify antibody molecules which, for structural or stereochemical reasons, Protein A cannot access. In addition, in some instances the target protein may not withstand low-pH elution conditions that are typically required for Protein A chromatography," says Kevin Tolley, senior field applica-

Angelo DePalma, PhD, is a contributing writer to *BioPharm International*.

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tion scientist for purification at Thermo Fisher Scientific.

Protein A has higher, better-characterized affinity for antibodies than cationic exchangers.

Protein A binds at the interface of the CH2 and CH3 Fc region. One Thermo Fisher resin, CaptureSelect FcXL, binds lower down on the CH3 part of the Fc region and releases product under mild elution conditions, making it suitable for Fc-fusion molecules. Additional products are selective to the CH1 domains and the antibody light chain, offering a platform solution for Fab fragments.

AN ATTRACTIVE OPTION

The economics of biosimilar antibodies underscore the need for downstream process efficiencies and Xuemei He, R&D manager for chromatography media chemistry at Bio-Rad (Hercules, CA), relates that biosimilar sponsors "are looking for less-costly alternatives to Protein A capture."

An attractive option is cation-exchange chromatography, a recognized cost-effective capture modality that delivers higher binding capacity, faster run times, and greater stability under clean-in-place conditions. The knocks on Protein A are cost and leaching of the potentially immunogenic ligand during column regeneration. In discussing the relative costs of Protein A and cation-exchange media over the years, however, the specific cost advantages of the latter have been difficult to pin down.

Binding and elution onto ion-exchange media is sensitive to feedstock conductivity. "Low conductivity is required for binding and that is sometimes an issue," He admits. "If your product feed is 10,000 L, adjusting the conductivity could be time-consuming." Where Protein A bind/elute has been standardized to near-platform status, cation exchange still requires tweaking to establish selectivity for binding the product and washing out impurities. "It takes more effort, but it's doable," He says.

The suitability of cation exchange for mAb purification arises from the proteins' generally high isoelectric points, which renders them positively charged near pH 7 and allows species that are negatively charged at relevant acidity—for example—DNA, to pass through. This creates a near-perfect purification storm for antibody feedstocks.

Make no mistake: Protein A has higher, better-characterized affinity for antibodies than cationic exchangers. Host-cell proteins (HCPs) would present a purification challenge if they were present at significant levels in typical feedstocks. Since production cells secrete mAbs but generally not HCPs, this is not the case. "SDS-PAGE [sodium dodecyl sulfate polyacrylamide gel electrophoresis] analysis finds aggregates and antibody fragments in the purified fraction, but negligible levels of HCPs," He says.

Media developers are also investigating mixed-mode cation exchangers for mAb purification. Adding a hydrophobic moiety to the ion-exchange ligand renders a chromatography resin that more closely approximates the affinity to mAbs of Protein A. "Mixed-mode ion exchangers also tolerate broader conductivity ranges in the feed compared with ion exchange," He says.

ENHANCED UTILIZATION

Puridify (Herts, UK), founded in 2013, addresses protein capture from the angle of resin utilization rather than reinventing the proverbial wheel. "Our technology involves understanding the limitations of capture technology based on porous beads," says Oliver Hardick, CEO.

The proliferation of antibody-derived therapeutics and biosimilars provides the stimulus to rethink the initial capture step.

Protein A has flourished up to now because, expensive as it is, its contribution to cost of goods was never much of a concern. Given that process in large part defines product, the industry has stuck with what it knows will work rather than assuming potentially risky technologies. The proliferation of antibody-derived therapeutics and biosimilars, however, provides the stimulus to rethink the initial capture step.

"We focus on overcoming risk by retaining familiar purification modalities, ligands, adsorptive surfaces, and materials of construction, Hardick continues. "We're offering significant performance enhancements but not anything radically new."

Puridify immobilizes Protein A on nanofibers rather than porous beads. Although the theoretical binding capacity is significantly lower

than for beads, reliance on diffusion into pores is reduced and bind/elute occurs much more rapidly, so much more so that, according to Hardick, downstream productivity improves a hundredfold.

Bioprocessors should instead look for efficiencies in process scheduling, the number and type of polishing steps, and optimization of buffer systems.

In addition to matching the approximate improvement in protein titers that Chinese hamster ovary cultures achieved during the past 15 years—and avoiding capacity mismatch—super-fast capture opens up a realm of novel possibilities in the utilization of what is arguably the costliest unit operation in mAb manufacture.

"Large columns take many hours over several shifts to run," Hardick noted. "With super-efficient resins you can run a similarly-sized column in a tiny fraction of that time, or recycle a much smaller column repeatedly for the same elapsed time, with favorable economics. This provides bioprocessors with unprecedented flexibility in terms of process time and column size." It also enables true single-use or single campaign use of Protein A columns for industrial manufacture.

Puridify is developing its novel Protein A resin, FibroSelect, through key industry collaborations. Together with GlaxoSmithKline, Hardick's team recently won an award for a single-use capture evaluation project based on the new resin.

BACK TO REALITY

The industry has seen how utilization can improve Protein A economics, as it enhances productivity gains for other downstream operations. Emerging from the background noise is the contribution from improvement in conventional capture chromatography. "The doubling of Protein A resin binding capacity over the past five or six years is an example where downstream has evolved to match the needs of upstream," notes Jonathan Royce, senior product manager at GE Healthcare's Life Sciences business.

Nicolas-Julian Hilbold, bioprocess R&D engineer at Novasep (Lyon, France), sums up the current thinking within biomanufacturing: "Protein A capture chromatography is difficult to avoid due to its unparalleled selectivity, ease, and efficiency of implementation at industrial scale." Although chromatography is generally viewed as a complex unit operation, Protein A purification simplifies the process while providing flexibility. "When deadlines are tight and time-to-market is critical, people know that Protein A will always provide the expected result," he says.

While biomanufacturers have shown interest in such alternative non-chromatographic methods such as aqueous extraction or precipitation, these unit operations do not integrate as easily into platform processes as chromatography. The logical extension to this thinking leads to considering non-Protein A resins. Alternatives within the existing arsenal of chromatography media have a tall order to fill. But because they fail to provide equivalent selectivity, the process becomes "un-intensified." Says Hilbold, "these [non-Protein A resins] would probably require an additional chromatography step—four instead of the typical three—to achieve quality specifications." Even if such resins were significantly cheaper, extending processing time would likely affect plant productivity and product quality negatively, while requiring additional development time as well.

Hilbold does not believe Protein A will be replaced, particularly for standard mAb processes. Bioprocessors should instead look for efficiencies in process scheduling, the number and type of polishing steps, and optimization of buffer systems. Another alternative, continuous processing, has the potential to mitigate the costs of Protein A while reducing process footprints and infrastructure costs. ◆

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Pumping Fluids in Biopharmaceutical Processing

Jennifer Markarian

Pump systems must be designed to meet the needs of specific processes, including preventing cross-contamination and damage due to shear forces.

In a biopharmaceutical process, pumps are needed to move fluids (e.g., buffer, media, and water for injection) through tubing and deliver them to the process equipment. Several types of positive displacement pumps are used, including peristaltic, diaphragm, rotary lobe, and gear pumps. In hygienic or sanitary uses such as in biopharm, pumps must prevent contamination and be able to be validated. Pumps may use single-use components or, if multi-use, be designed to be easily cleaned. Pumps must be designed to meet appropriate standards for biopharmaceutical processing, such as the American Society of Mechanical Engineers (ASME) Bioprocessing Equipment (BPE) standard (1). *BioPharm International* spoke with experts at manufacturers of peristaltic and diaphragm metering pumps about some of the considerations for fluid handling in biopharmaceutical manufacturing.

DIAPHRAGM PUMPS

There are different types of diaphragm pumps. Air-operated double-diaphragm (AODD) pumps are used for transferring fluids from one place to another and for ultrafiltration or diafiltration, notes Gary Gaudet, technical leader of bioprocessing at LEWA-Nikkiso America. AODD pumps, however, do not have volumetric control. Diaphragm metering pumps, which have highly accurate volume control, are used in processes and for dosing. Applications include: chromatography, buffer inline dilution, homogenization, injection of fluids (e.g., liposomes) into extruders, coating operations, filling, caustic dilution, and aseptic transfer of proteins, cells, and other materials, says Gaudet.

A diaphragm pump uses the reciprocating movement of a flexible diaphragm that decompresses to draw fluid into the pump chamber and then compresses the pump chamber to push fluid out. The diaphragm separates the pump drive from the product-wetted side. This separation means that mechanical seals are not needed, which ensures product safety, simplifies maintenance, and allows the pump to run dry (i.e., without fluid) without being damaged, says Andreas Frerix, product manager Quattroflow, at PSG, a Dover company.

Multiple-use pumps have housings made from stainless steel that can be reused after cleaning in place. Multiple-use diaphragm pumps are still commonly used and are meeting customers' needs, says Gaudet. Single-use diaphragm pumps have chambers made of plastic that have been designed for one process or batch. "After the process, they are replaced with a new chamber and the new process can begin. Single-use pump chambers save money and time by avoiding cleaning and associated cleaning validation and eliminating the risk of cross-contamination between batches or products. Single-use pumps are most valuable if products are changed frequently and fast product changeovers are needed," says Frerix.

PERISTALTIC PUMPS

In a peristaltic pump, the fluid is contained in a single-use tube that is compressed and decompressed by a moving rotor to move the fluid. Tubing is made from biocompatible materials that meet requirements for purity, and the single-use tubing is disposed of after each process to prevent cross-contamination. Because the fluid is completely contained within the tubing and con-

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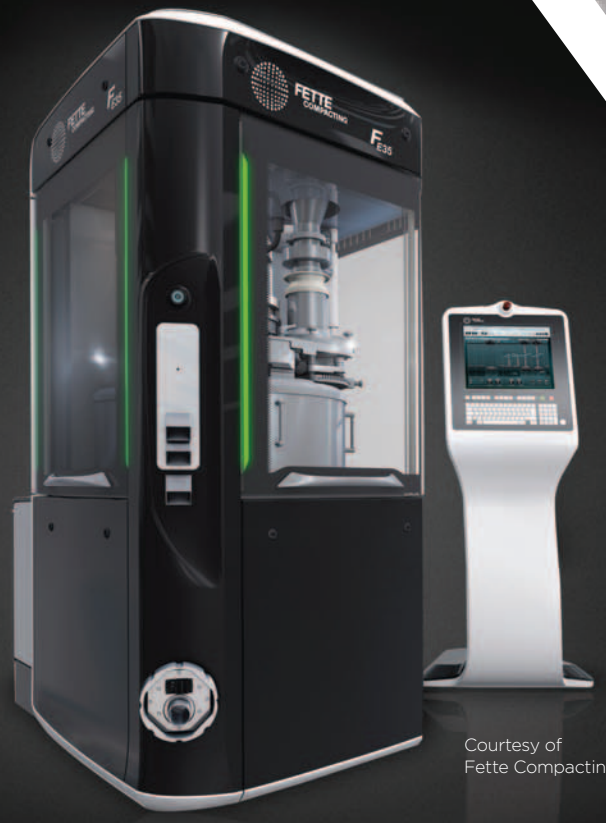

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nectors, process validation is simplified, notes Russell Merritt, marketing manager at Watson-Marlow Fluid Technology Group, "Once the pumps are within a cleanroom environment and remain there, no regular cleaning and little maintenance of the pump itself is required."

Although tubing could be treated as multi-use (i.e., cleaned and sterilized between batches), most tubing is treated as "single-use" because this ensures that no cross-contamination occurs and provides new performance for every batch, says Merritt. "The need to maintain fluid path sterility is a key consideration for handling/changing any component in the fluid path. Sterility is another advantage of single-use tubing. The fluid path is provided assembled and sterile, which minimizes the number of connections which need to be made; each connection presents a risk of the introduction of contamination."

Peristaltic pumps are easy to install and set-up, says Gregg E. Johnson, global senior product manager for the Cole-Parmer peristaltic pump product lines. Because they don't need to be primed, they are flexible in where they can be installed in the fluid path, explains Johnson.

"Monitoring the tubing performance is probably the greatest concern," says Johnson. "A catastrophic failure can cost the loss of an entire campaign, but it is easily prevented by monitoring the tube life, use of new long-life tubing materials, and a preventive maintenance program to replace or move the tubing to new position," (See **Sidebar**).

HANDLING SHEAR-SENSITIVE FLUIDS

Biopharma fluids vary in how sensitive they are to shear forces due to flow. Peptides and small proteins, for example, are relatively insensitive to shear, but mammalian cells can be very sensitive. "Even if the product is not shear-sensitive, using a low-shear [diaphragm] pump also reduces the temperature increase of the fluid and thus reduces the need for cooling (e.g., for tan-

Preventive maintenance of single-use tubing for peristaltic pumps

Single-use tubing can be used for the length of a biopharmaceutical product campaign and changed when a new product is to be manufactured. To prevent failure during lengthy use, tube life should be monitored and a preventive maintenance program enacted. *BioPharm International* spoke with Gregg E. Johnson, global senior product manager for the Cole-Parmer peristaltic pump product lines, about these concerns.

BioPharm: How long does single-use tubing typically last?

Johnson (Cole-Parmer): The lifespan for tubing is dependent upon pumping conditions— speed, pressure, fluid, and fluid temperature. Some new biopharm formulations of silicone tubing can last 200 to more than 1000 hours. For the Pharmed materials (made from a thermoplastic elastomer), life expectancy can be several thousands of hours.

BioPharm: How does a company go about monitoring the tube life?

Johnson (Cole-Parmer): In our testing lab, we monitor the tube life by recording the hours between failure. Once we have a pattern, we can set up preventative maintenance based on the specific installation. Each one is different due to different fluid, piping systems, and other devices in the fluid path.

BioPharm: What are some rules of thumb for performing preventive maintenance?

Johnson (Cole-Parmer): Preventative maintenance (PM) and the frequency at which it is performed is determined by the pump operator. A simple recommendation is to review the tube-life data provided by the pump manufacturer. A good place to start may be at 75 to 80% of the manufacturer's data until a reliable pattern is defined. Each installation is different. Once this pattern is established, and longer PM cycles are desired, the operator can elect to extend the cycles to fit needs.

BioPharm: How can tube life be extended by moving the tubing to a new position?

Johnson (Cole-Parmer): If an operator is performing a long-term campaign and is concerned about tube life, he or she can extend the run and prevent the possibility of tubing failure by moving the tubing to a new position. This is accomplished by setting the system up with excess tubing on the discharge (outlet) of the pump. Once the campaign is running, the operator can, on a periodic basis—once a week or once a month—pause the pump rotation, open the pump head, and slide a section of unused tubing (from the excess at the start of the campaign) and move the used section from the pump head to the suction (inlet) side. The pump is then closed and restarted.

gential flow filtration). If the product is shear sensitive, then using a larger-sized pump helps, because the pump can be run at low speed to achieve low velocities and thus minimum shear," explains Frerix.

Protecting fluids from damage is a complex issue that is affected by the pump as well as by the design of the entire fluid handling system, says Gaudet. For example, fluid damage can occur from squeezed flow (e.g., due to fluid exposed to crevices or dynamic seals) or from impact when flows come together as pipes join up. Shear is also created by cavitation at the gas/liquid interface. If the diameter of a pipe changes too quickly, for example, air can come out of the liquid and cause cavitation. "Gentle conveying means minimizing or avoiding these effects. The less resulting energy induced in the fluid, the better overall for the fluid. We design systems based on piping designs to ensure the operating conditions of the pump minimize shear and cavitation," says Gaudet. "The absence of any dynamic seal in the pressure chamber of diaphragm pumps allows gentle conveying of process fluids. The only areas of possible damage are at the product valves during closing and possibly opening. One must note, however, the extremely short closing or opening times (around 1% of the total time), resulting in low integral damage potential. In comparison, the shearing effect at the plunger seal of a plunger pump or the impeller are present during the complete stroke cycle." Gaudet adds, "Similar problems apply to other pump types (basically any rotating positive displacement, gear, rotary piston, peristaltic hose, or eccentric screw pumps)."

Peristaltic pumps are inherently low shear, which means that they will circulate or pump cell suspensions without damage, says Johnson. "The acceptable shear level for a cellular suspension typically can be determined by measuring cell viability after passing through the fluid-path system," explains Johnson. "Excessive shear is caused in a fluid path mostly by two surfaces that are moving against each other, which is particularly evident in centrifugal, gear, or other types of rotating pumps or mixers. Tubing occlusion can still cause cell death, but it can be mitigated by reducing the pump speed and increasing the tubing diameter."

"Peristaltic pumps ensure product cannot be damaged by high fluid velocities or contact with mechanical parts," explains Merritt. "The achievable flow rate is determined by the bore size of the tube and the speed at which the pump is run. Fluid viscosity plays an important part in the speed at which the pump is run and the deliverable flow. Higher viscosity products require a large bore size tube, but a low running speed. In downstream processing, such as tangential flow filtration and high performance liquid chromatography, flow linearity with only trace pulsation and over a wide pressure range is the ultimate requirement. The acceptable level of shear is product dependent, and the suitability of any pump type can only be determined through specific testing. With peristaltic pumps, the pumping concept naturally provides low shear; through the correct sizing of the pump this can be kept to a minimum to maintain product integrity. General good practice to ensure low shear is to minimize fluid velocity.

With peristaltic pumps, low shear is achieved through lowering the pumps operating speed and increasing the peristaltic tubing bore size."

TRENDS IN FLUID HANDLING

The move to continuous processing is evolving and will affect the requirements for pumps and other process equipment, says Frerix. Gaudet agrees, adding that there is a trend to have more self-contained processes, such as combining buffer dilution, clean-in-place systems, and chromatography skids rather than having separate skids. Steam-in-place systems are also being requested more than in the past, he notes.

Both multiple-use and single-use manufacturing systems are employed in biopharmaceutical manufacturing. "Biopharm is naturally risk averse; innovations, therefore, rarely take hold overnight, and the advent of single-use continues to expand into different process areas due to the benefits offered," says Merritt. "We will continue to see both single-use and hybrid (mixed single/multi use) processes being developed. Peristaltic technology is therefore well positioned to continue to meet customers' requirements to provide a low-risk fluid handling solution through accurate pumping technology and high quality peristaltic tubing."

Another trend, says Johnson, is an increase in the need for companies to be able to monitor fluid-path processes from remote locations. "The ability to monitor a process remotely allows the process to be run unattended and to notify users when they are complete, so the next campaign can be set up, which helps maintain productivity and improves efficiency."

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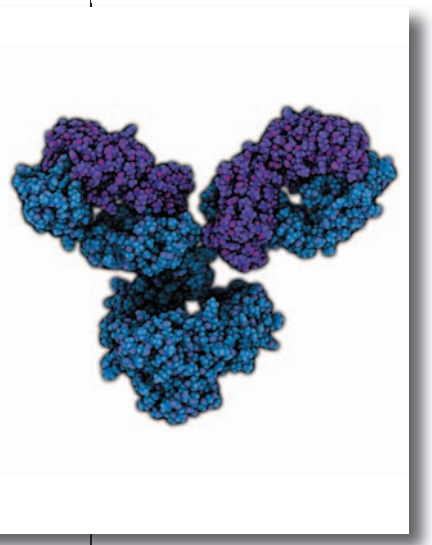

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Impact of Manufacturing-Scale Freeze-Thaw Conditions on a mAb Solution

Kashappa Goud Desai, W. Aaron Pruett, Peter J. Martin, James D. Colandene, and Douglas P. Nesta

ABSTRACT

The objective of this study was to assess the impact of manufacturing-scale, freeze-thaw conditions on aggregation and subvisible particle formation of a monoclonal antibody solution (mAb-A; IgG1) using a small-scale model. The temperature-time profiles of manufacturing-scale samples under different freezing and thawing conditions (i.e., slow, medium, and fast freeze-thaw conditions) were generated and used to simulate similar conditions for small-scale samples. Soluble aggregates and subvisible particle counts were measured by size-exclusion chromatography and micro-flow imaging, respectively. Thermal analysis of protein samples was performed by modulated differential scanning calorimetry. The freezing rate in a single freeze-thaw cycle had negligible impact on protein aggregation when fast-thawing conditions were used to thaw. Slow thawing led to higher protein aggregation and subvisible particle formation, which was exacerbated by fast freezing. These effects became more extreme when the number of freeze-thaw cycles was increased from 1 to 3. These trends were found to be similar in large-scale (6.2 L) and small-scale (30 mL and 100 mL) assessments, with the total magnitude of degradation higher in the small-scale system. The systematic small-scale model employed in the current study was predictive of manufacturing-scale freeze-thaw conditions.

Kashappa Goud Desai, PhD*, is investigator, Biopharmaceutical Product Sciences, GlaxoSmithKline, kashappa-goud.x.desai@gsk.com; **W. Aaron Pruett** is drug product process engineer, Novavax; **Peter J. Martin** is investigator, Biopharmaceutical Product Sciences, GlaxoSmithKline; **James D. Colandene, PhD**, is manager, Biopharmaceutical Product Sciences GlaxoSmithKline; and **Douglas P. Nesta, PhD**, is director, Biopharmaceutical Product Sciences, GlaxoSmithKline.

*To whom all correspondence should be addressed.

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Therapeutic proteins, peptides, and antibodies have emerged as highly effective modern medicines for numerous diseases and disorders (1). Therapeutic monoclonal antibodies (mAbs) represent a rapidly growing area in the biopharmaceutical sector (2). Enormous success achieved with mAbs can be attributed to their distinctive beneficial properties and advantages, which include high binding specificity and affinity, availability of humanized forms that can attenuate immunogenic responses, and robust manufacturing processes (3, 4). Poor stability of monoclonal antibodies outside their natural environment, however, is one of the major challenges in product development (2–6). A number of non-optimal formulation, manufacturing, or storage conditions often cause instability of mAbs, which in turn can affect the bioactivity of proteins (7).

Freezing and thawing are integral steps in the manufacturing of most biopharmaceutical products. Storage of biopharmaceuticals in the frozen state has distinctive advantages. It minimizes the risk of microbial growth, increases product stability with extended shelf life, eliminates agitation and foaming during transportation, and increases flexibility during manufacturing (7–11). Freezing and thawing stresses (e.g., cold-denaturation, cryo-concentration, ice formation, crystallization of buffer or non-buffer components, phase separation, redistribution of solutes, pH fluctuation, and thawing time), however, can induce complex physical and chemical changes in the solvent/solute conditions, which in turn can potentially lead to denaturation and aggregation of proteins (10, 12–16).

Formation of protein aggregates and subvisible particles during the manufacturing of drug products are a major concern due to the potential immunogenicity of protein aggregates in patients (17–22). There have been studies to determine the impact of potential freeze-thaw factors on aggregation of proteins. A number of potential formulation variables (e.g., buffer composition, pH, ionic strength, and cryoprotectants) and freeze-thaw stresses (e.g., crystallization of excipients, ice formation, and freeze concentration) have been evaluated (12, 15, 19, 23–35). There is also a growing interest in the pharmaceutical industry to develop small-scale models to study the potential impact of large-scale freeze-thaw process variables on protein stability. The small-scale models are cost-effective and less time consuming (36). A decision regarding freeze-thaw parameters must often be made prior to having sufficient amounts of the product available to assess process characterization at manufacturing scale, thereby necessitating use of small-scale studies.

One approach to scale-down freeze-thaw process characterization studies is to use a temperature-controlled chamber to expose small samples of a formulation to time-temperature profiles that simulate and/or bracket that of the manufacturing-scale process. This approach offers an improvement over uncontrolled freeze-thaw of small samples, which tend to occur quickly and may suppress stochastic phenomena such as ice nucleation or kinetic phenomena such as full crystallization of eutectic phases (37). A small-scale version of controlled freezing-thawing systems is useful for scale-down assessment of freeze-thaw process characterization (37). The authors, therefore, sought to assess the feasibility of a small-scale model to determine the impact of large-scale freeze-thaw conditions on aggregation and subvisible particle formation of a monoclonal antibody (mAb, IgG1).

MATERIALS AND METHODS

Monoclonal antibody (mAb-A, IgG1) formulation

The mAb-A formulation (64 mg/mL protein concentration) was prepared in a formulation that contained a buffering agent (10 mM), surfactant, cryoprotectant (disaccharide) and bulking agent (glycine). The formulation samples were filtered through a 0.22 µm filter into ~8.3 L, 100 mL, and 30 mL bags (Sartorius Stedim) with thermowells. Excess air was purged from the bags to minimize air-liquid interfaces. A thermal surrogate solution (64 mg/mL of disaccharide in formulation buffer) was used to minimize the amount of protein solution needed to add thermal mass for the large-scale experiments and reach different freezing profiles. Custom designed stainless-steel cases lined with foam padding (designed to protect the bags while in the frozen state) were used as containers for the ~8.3 L bags during storage and handling.

Size-exclusion chromatography (SEC)

Soluble aggregates in protein samples were measured using a high-performance liquid chromatography (HPLC) system attached with a 7.8 mm × 30 cm column (Tosoh BioScience). Approximately 350 µg protein was loaded onto the column. The detection wavelength was 280 nm. The column equilibration (65 minutes) and sample elution (35 minutes) was done with the mobile phase (50 mM sodium citrate, 450 mM sodium chloride, pH 6.5) run at 0.5 mL/min. Monomer, aggregate, and low-molecular weight species levels were calculated as a percentage of the total protein peak areas. Only aggregate levels are reported here because low-molecular weight species were unchanged in all conditions. Based on the qualified intermediate precision of the method, changes in percent aggregate greater than 0.1% were considered significant.

Micro-flow imaging (MFI)

The concentration of subvisible particles in 1–100 µm size range was measured by a

Table I. Methods used to create different freezing and thawing rates in manufacturing-scale cycle (6.2 L sample in 8.3 L bag).

Process	Freezing or thawing rate/ cycle	Method used to create intended environment
Freezing	Slow freezing	Two bags of mAb-A were placed on a single shelf of -80˚C freezer. Four bags of thermal surrogate solution were stacked above and below the mAb-A bags.
	Medium freezing	Two bags of mAb-A were placed side-by-side in covered stainless steel cases in -80˚C freezer.
	Fast freezing	A single bag of mAb-A was placed in an uncovered stainless steel case in -80˚C freezer.
Thawing	Slow thawing	Two bags of mAb-A were placed in a 2–8˚C cold room. Two bags of thermal surrogate solution were stacked above and below the mAb-A bags.
	Medium thawing	A frozen bag was placed in an uncovered stainless steel case on a room temperature bench top until completely thawed.
	Fast thawing	A frozen bag was removed from the stainless-steel case and placed in a 30˚C, 60% RH environmental chamber until completely thawed.

mAb-A: monoclonal antibody (mAb-A; IgG1)
RH: relative humidity

micro-flow imaging (MFI) system (Brightwell) equipped with a 100 µm/1.6 mm flow cell. The measurement involved flushing of the flow cell with a 0.25 mL sample, followed by imaging analysis of a 0.65 mL sample. Reported values are the average of two samples, with an acceptance criterion of <30% difference between the two values.

Low-temperature differential scanning calorimetry (mDSC)

Thermal events were measured in frozen solutions using a low-temperature differential scanning calorimeter (mDSC) (TA Instruments). A mechanical cooling accessory (RCS90) was used for cooling the sample chamber to temperatures as low as -80 ˚C. Dry nitrogen was used as a purge gas at a flow rate of 50 mL/min. A volume of 20 µL of solution was placed in an aluminum sample pan with a capacity of 40 µL. An aluminum lid was placed on the sample pan and was hermetically sealed using a crimping press. An empty aluminum pan with lid, identical to that used for the sample, was used as the reference. The sample and reference pans were cooled at a controlled rate of 5 ˚C per minute for fast freezing conditions and equilibrated at the lowest programmed temperature for 10 minutes. The slow freeze rate was at a controlled rate of 0.1 ˚C per minute, and sample and reference pans were equilibrated at the lowest programmed temperature. The modulation amplitude was adjusted to allow cooling during temperature modulation to amplify weak heat capacity signals. The melting and crystallization events are reported at the onset of the thermal event using the non-reversing signal. A smoothing region width of 1.000 ˚C was applied to the non-reversing heat flow signal.

Large-scale freeze-thaw cycling

Approximately 6.2 L mAb-A formulation were filled into 8.3 L bags (Sartorius Stedim). Prior to freezing, the bags were stored at 2–8 ˚C, and once frozen, they were stored at -80 ˚C. Modified freezing and thawing cycles were designed and used to achieve a broad range of temperature profiles (**Table I**). The freezing was conducted in -80 ˚C freezers (bags reached ≤70 ˚C), which were underpowered for freezing large volumes of aqueous solutions, causing the freezing temperature profiles to be load-dependent. Surrogate sample bags were utilized for adding thermal mass for the large-scale freezing experiments at -80 ˚C, to ensure reliability of freezing temperature profiles. The temperature profiles were recorded with multiple type-T thermocouples placed in the thermowells and attached to the upper and lower surface (**Figure 1**). The endpoint of the thaw was complete melting of visible ice. At the end of each thaw, the bags were mixed by multiple inversions. The bags were exposed from 1 to 3 cycles (1x–3x) of either fast freeze/fast thaw, fast freeze/slow thaw, medium freeze/medium thaw, slow freeze/fast thaw,

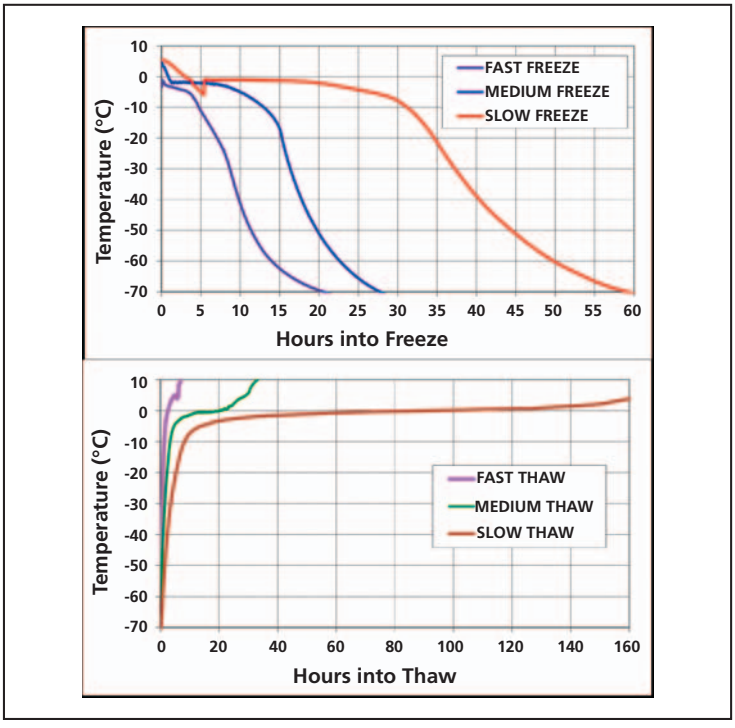
or slow freeze/slow thaw. A liquid control was placed at 2–8 ˚C during the entire duration of the study. A -80 ˚C control was also generated (this was exposed to one medium freeze-medium thaw cycle). Samples from each test condition were taken after the first and third freeze/thaw cycle (1x and 3x), and analyzed by size-exclusion chromatography (SEC) (along with the control samples). The 3x freeze-thaw samples were also analyzed for subvisible particles by MFI.

Scale-down freeze-thaw simulation

Approximately 30 mL and 100 mL of 64 mg/mL mAb-A were filled into 30mL and 100mL bags (Sartorius Stedim), respectively. The 6.2 L fill was a convenient volume (6-7 L) that manufacturing used for BDS (logistically ~6-7 L per bag was a suitable volume). Regardless, the surface area to volume ratio is higher in the 100 mL and 30 mL bags. Scale-down process simulation was done by using a programmable temperature control system (Sartorius Stedim) to apply a series of linear temperature ramping profiles to a sample chamber. The bags were installed in the chamber in such a manner that both side edges were in contact with the heat transfer surfaces. The sample chamber was insulated during operation to minimize environmental heat transfer.

For each large-scale freeze-thaw cycle (6.2 L in 8.3 L bag), the four thermocouple trends were averaged to generate a target freezing and thawing temperature profile (**Figure 1**), and a scale-down simulation program was developed to mimic each manufacturing-scale profile (a separate profile with custom-made temperature-time steps to mimic each manufacturing-scale profile was created and used to run small-scale processes via a custom-built software from Sartorius). The cycles were adjusted for heat loss in the control system. Supercooling steps to induce ice nucleation were added only for the medium and slow freezing rates. Time required for freezing to ≤ -40 ˚C ranged from 10–40 hours. The time required for completion of thawing, as indicated by thermocouple rising above 0 ˚C, ranged from approximately 3–125 hours. Samples subjected to the scale-down simulation were analyzed by SEC (after 1x and 3x freeze/thaw cycles). The 3x samples were also analyzed for subvisible particles by MFI.

Figure 1: Temperature profiles based on average of four thermocouples for freezing (top) and thawing (bottom) of 6.2 L aliquots of mAb-A in 8.3 L bags.



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RESULTS AND DISCUSSION

Effect of freeze-thaw process variables on aggregation and subvisible particle formation of mAb-A

Robust freezing and thawing processes are essential for manufacturing and storage of biopharmaceutical products. Optimal conditions ensure improved protein stability during these stages. Logistically, small-scale models are ideal to assess the impact of freeze-thaw process variables on protein stability. Small-scale models, however, may not reflect the actual freezing or thawing rates, or the effect on product, that occur at manufacturing scale. Small-scale freeze-thaw systems have been introduced that are capable of more accurately mimicking freezing and thawing rates that occur at manufacturing scale (37). Using these systems, the large-scale freezing and thawing environments can be simulated in small-scale models. In this study, the authors sought to evaluate and compare the impact of freeze-thaw process variables using both large-scale and small-scale models.

Large-scale freeze-thaw temperature profiles were successfully simulated in scale-down stud-

Figure 2: The effect of small-scale controlled freeze-thaw process parameters (e.g., rate of freezing and thawing, number of cycles) and scaling (simulated small-scale controlled freeze-thaw vs. large-scale uncontrolled freeze-thaw process) on aggregation (% soluble aggregate by size-exclusion chromatography [SEC]) of mAb-A. FF: fast freeze; MF: medium freeze; SF: slow freeze; FT: fast thaw; MT: medium thaw; ST: slow thaw. *The frozen control was exposed to one MF/MT cycle with extended storage at -80˚C.

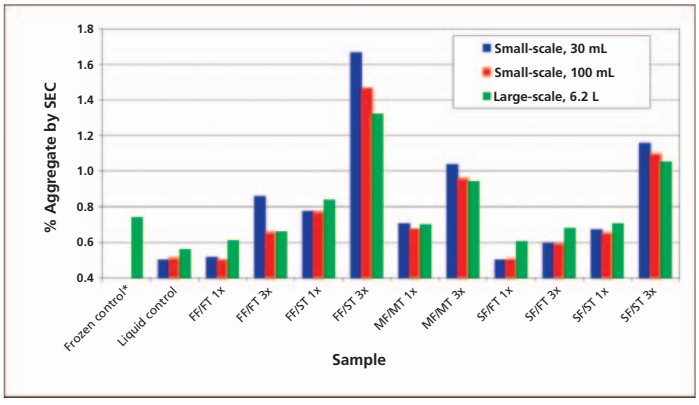
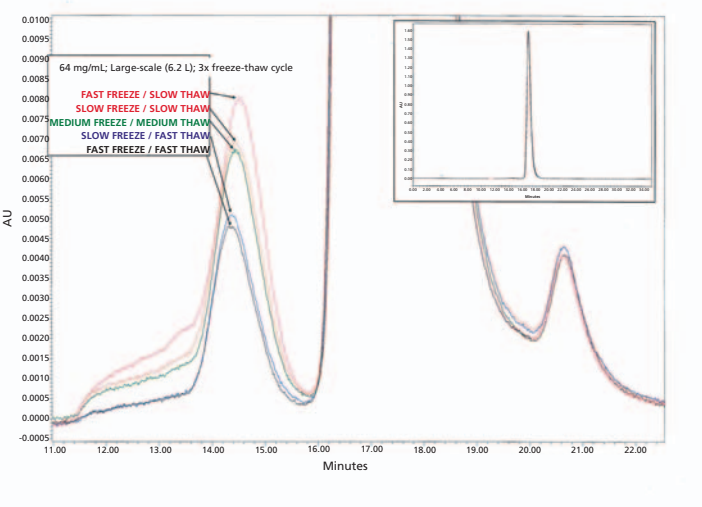


Figure 3: Size-exclusion chromatography (SEC) chromatogram of 3x fast-freeze/fast-thaw, fast-freeze/slow-thaw, medium-freeze/medium-thaw, slow-freeze/fast-thaw and slow-freeze/slow-thaw samples.



ies using a freeze-thaw unit (Sartorius Stedim) (applied programmed temperature profiles to freeze and thaw 30 mL and 100 mL bags) (**Figure 1**). The impact of freeze-thaw process parameters (e.g., freezing and thawing rates, number of cycles) on protein aggregation, as generated from the small-scale and large-scale models, is shown in **Figure 2**. A frozen control had a similar level of aggregate to that of the 1x medium freeze-medium thaw sample, indicating that the storage parameter alone did not influence the aggregation of mAb A. For a single freeze-thaw cycle, the freezing rate had a negligible impact on protein aggregation when followed by fast thawing conditions (percent aggregates for 1x slow, medium, and fast-freeze samples were ≤ 0.1% different, relative to that observed for the liquid control), and was similar for both large-scale and small-scale sample sizes. Slow thawing, however, led to significantly higher protein aggregation (percent aggregates for 1x slow-thaw and medium-thaw samples of small-scale and large-scale studies were >0.1% different when compared with the liquid control or the 1x fast-thaw samples). Interestingly, the negative impact of slow thawing appeared to be exacerbated by fast freezing, as this condition generated the highest levels of aggregates. Conversely, slow freezing followed by fast thawing had the lowest levels of aggregate formation.

The aggregation of mAb-A was impacted by the number of freeze-thaw cycles under all test conditions (3x samples had higher aggregate levels compared with corresponding 1x samples). Among all the 3x samples, 30mL fast freeze/slow thaw sample had highest level of aggregates. After 3x fast freeze/slow thaw cycles, the aggregate level in different size samples was found to be in the following order: 30mL sample size >100mL sample size >6.2L. Moreover, the trends observed with respect to freezing and thawing conditions were the same regardless of the scale.

Representative (3x fast-freeze/fast-thaw, fast-freeze/slow-thaw, medium-freeze/medium-thaw, slow-freeze/fast-thaw, and slow-freeze/slow-thaw samples) SEC chromatograms from the study are shown in **Figure 3**. These overlays show that the soluble aggregates are predominantly comprised of dimers. Due to limitations in the analytical capability of the SEC method used, and the relatively low aggregate level, it was not possible to quantitatively assign the percent of dimer versus higher-order multimers.

To assess whether subvisible particles were also impacted by freeze-thaw conditions, the 3x freeze-thaw samples were evaluated by MFI (**Figure 4**). The freezing rate had a minor impact on subvisible particle counts. The thawing rate had more pronounced impact on subvisible particle counts (subvisible particle counts of 3x fast freeze/slow thaw samples were found to

be 28–175 times higher than 3x fast freeze/fast thaw samples). The particle counts were found to be impacted by the sample size (the particle counts of 3x fast freeze-fast thaw, fast freeze-slow thaw, and slow freeze-slow thaw samples were found to be in the following order: 30 mL > 100 mL > 6.2L). Therefore, the trends in sub-visible particle counts were the same as that for aggregation (by SEC) discussed previously.

Aggregation of proteins during freeze-thaw cycling has generally been attributed to partial unfolding of protein molecules caused by the perturbing conditions such as pH variation, low temperature, freeze concentration of solutes, exposure of proteins to ice-liquid interface or surfaces induced by excipient crystallization, and/or adsorption to materials of contact (8, 10, 13, 16, 28, 33, 35). The formulation buffer used in the current study contains glycine as a bulking agent. It is known that glycine may crystallize under frozen storage conditions and form a new surface, thereby potentially causing protein denaturation (27, 31). During thawing, recrystallization can cause additional protein perturbations at the ice-liquid interface (38). The cause for higher protein unfolding under slow-thawing conditions has been linked to prolonged exposure of protein to low temperature and high solute concentration medium (39). The findings of the current study were in good agreement with those reported by Cao et al. (38), wherein slow thawing conditions caused higher protein degradation and loss of activity (38).

One key finding from this work is that fast freezing followed by slow thawing resulted in greater degradation of the mAb than did slow freezing followed by slow thawing. If glycine crystallization is the primary cause of aggregation, it would suggest that the crystallization of glycine during thawing is more detrimental than the crystallization of glycine during the freezing step. This finding is assumed because rapid freezing would be more likely to leave glycine in the amorphous phase after the freeze compared with slow freezing. It would then follow that rapid freezing followed by slow thawing would be more likely to lead to higher levels of glycine crystallization during the thaw. The authors sought to study this phenomenon by mDSC. The findings of mDSC were found to be in good agreement with this hypothesis. In the DSC experiment, where fast freezing was followed by slow thawing, an exothermic peak at approximately -25 °C onset, likely corresponding to glycine (eutectic) crystallization, followed by a likely eutectic melt around 6.6 °C onset, were readily observed during the slow thawing step (**Figure 5**). Neither the exothermic thermal event nor the endothermic melt were observed after fast freezing and fast thawing conditions (data not shown).

Figure 4: The effect of freezing and thawing stress on sub-visible particle formation of mAb-A. Sub-visible (1–100 µm) particle counts of mAb-A samples (measured by micro-flow imaging [MFI]) exposed to different freezing and thawing conditions. FF: fast freeze; MF: medium freeze; SF: slow freeze; FT: fast thaw; MT: medium thaw; ST: slow thaw. *The frozen control was exposed to one MF/MT cycle with extended storage at -80 ˚C.

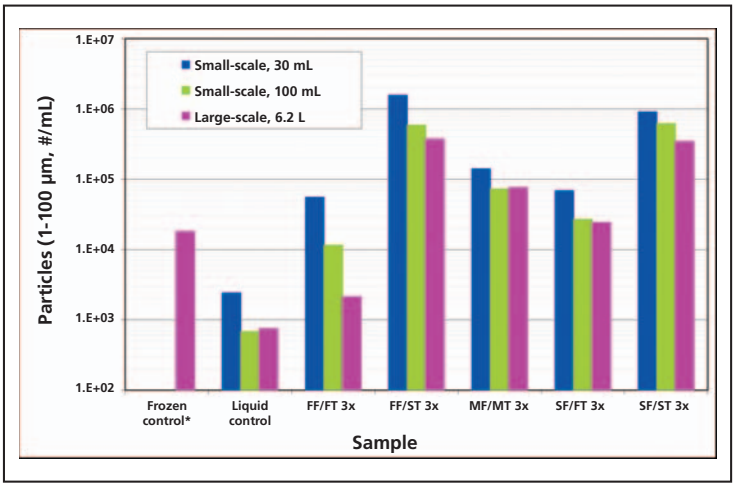
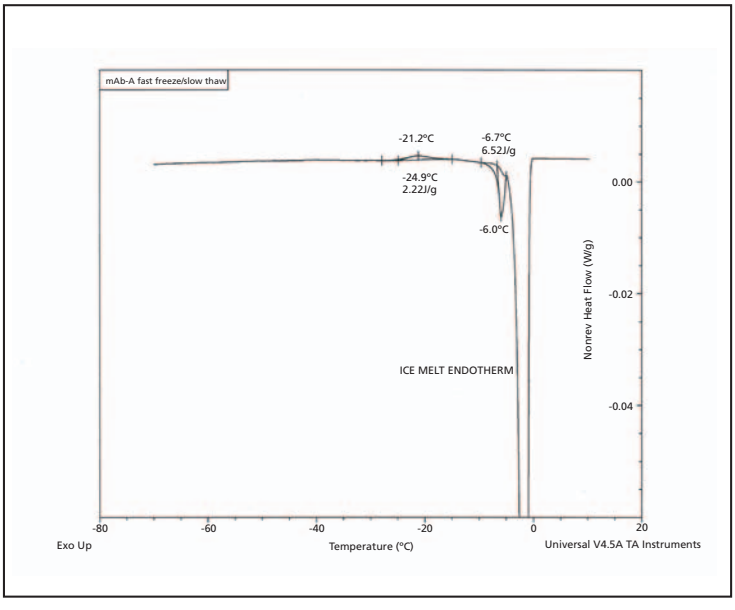


Figure 5: Modulated differential scanning calorimetry scan (heating leg) of fast-freeze/slow-thaw sample of mAb-A.



It can be summarized that fast freezing and fast thawing conditions are optimal for mAb-A to prevent potential effects such as protein aggregation and subvisible particle formation, but that the fast thawing rate is the more important parameter. The small-scale model successfully identified the impact of manufacturing-scale freezing and thawing rates on aggregation and subvisible particle formation of a mAb-A, thereby suggesting its feasibility and benefits in biopharmaceutical manufacturing. The simulation at small-scale amplified the affects observed at large-scale possibly due to difference in interfacial contact effects (e.g., ice crystal, air, and container surface).

CONCLUSION

A robust manufacturing-scale, freeze-thaw process suitable for mAb-A was developed through large-scale and small-scale assessment models. A small-scale process simulation model evaluated in the current study successfully identified risk at manufacturing-scale conditions on aggregation and subvisible particle formation of mAb-A. The study also demonstrated the importance of thawing parameters. Slow thawing was shown to have the most negative impact on the product (highest levels of aggregate formation). Fast freeze-fast thaw conditions were found to be optimal for mAb-A. Multiple freeze-thaw cycles are sometimes inevitable during manufacturing due to unforeseeable issues. Multiple cycles under fast-freeze/fast-thaw conditions are also considered to be feasible for mAb-A.

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Evaluating E&L Studies for Single-Use Systems

Irene Cecchini, Daniele Mastroianni, Silvia Rocchi, and Gabriella Angiuoni

Photo courtesy of Merck Serono Aubonne

Extraction studies demonstrate approaches for evaluating single-use biopharmaceutical manufacturing materials.

Biopharmaceutical companies have increased the adoption of single-use systems (SUS) and components (i.e., polyethylene bags, filters, tubing, connectors, etc.) for manufacturing processes, in addition to multi-use (MU) materials (i.e., glass bottles, stainless steel tanks, etc.) (1). Regulatory guidelines require that the product contact items "shall not be reactive, additive, or absorptive" to assure drug product quality and safety (2). The manufacturer is, therefore, responsible to examine various materials used in manufacture of both drug substances and drug products to ensure that the materials are appropriate in terms of efficacy (process performance and product quality) and safety for the final drug product.

Suppliers are advised by the BioPhorum Operations Group (BPOG) and BioProcess Systems Alliance (BPSA) to provide comprehensive extractables test data. Many companies have now adopted the BPOG extractables protocol (3) as their user requirement.

EXTRACTABLES AND LEACHABLES EVALUATION

An extractables and leachables evaluation should begin with a risk assessment of the materials to determine whether there is a significant risk that may require additional studies on all or part of the product contact materials (4).

A risk rating is determined by applying three main factors: the severity of the harm caused by substances leaching from the manufacturing component; the

Irene Cecchini is lab manager; **Daniele Mastroianni** and **Silvia Rocchi** are junior researchers, all in the E&L and Process Troubleshooting Lab, Protein Chemistry Dept. PADBP, Merck Serono. **Gabriella Angiuoni** is QC Market & LCM Unit Head–Global Manufacturing & Supply, Merck Serono.

probability that leaching will occur; and the probability of detecting the leached substances through in-process manufacturing controls.

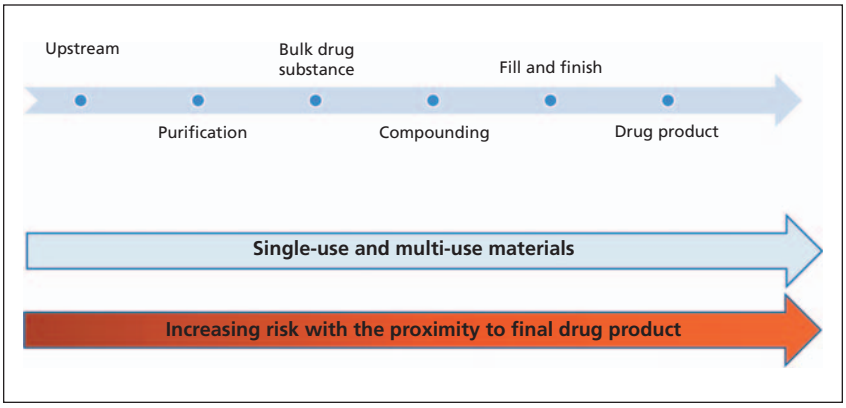
The risk analysis of each product contact material used in the process takes into account variables including:

- Proximity to the API/drug substance and final drug product
- Extraction capability of the solvent/solution
- Contact time
- Temperature
- Product contact surface area
- Material pre-treatment
- Material compatibility/resistance.

It is presumed that any impurity entering the production stream can have a negative impact on the product quality and the severity of such a failure mode is considered to be high. A process-specific assessment takes into account the production stream in which the component is used in, and accordingly, it assigns a risk rating. As the production process advances towards the final drug product state, the risk to the patient, represented by any leachable, increases (**Figure 1**). Therefore, polymeric components used in process steps closer to the drug substance or the drug product will carry a higher risk rating compared to the earlier steps of the process. For example, a bag or a filter used for the final filtration of bulk drug substance will have a significantly higher rating compared with the components used in the upstream process steps.

The BPOG *Best Practices for Mitigating Risk from Leachables in Single-Use Systems* suggests a risk assessment rating (5) that companies can use to develop their own risk assessments (see **Table I**) (5). The risk ratings of each factor will be weighted as in the BPOG example and summed to obtain the final risk score that is then categorized as low, medium, or high. If the risk is classified as low, it may be sufficient that the material meets the compendial requirements. If the risk is medium, in addition to the low-risk requirements, the company should evaluate available extractables data from the supplier, or generate in-house study data for a toxicological risk assessment. If the risk is classified as high, in addition to the medium risk requirements, a process-specific leachables risk assessment must be carried out; if data are not available from the supplier, or the data do not correspond to the

Figure 1. Distance along the production stream.



ALL FIGURES ARE COURTESY OF THE AUTHORS

Table I. Example of risk assessment from BioPhorum Operations Group (5).

Consideration	Ratings[a]		Weight[b]
Distance along production stream (DAS)	1	Synthesis: Vial thaw, inoculum, expansion, production, harvest, plasma	0.40
	3	Purification: Affinity chromatography, viral inactivation, ion exchange chromatography, viral filtration, ultra/diafiltration	
	5	Bulk Drug Substance: Formulation, filtration, storage	
	9	Final formulation, fill/finish Potency adjustment, sterile filtration, filling	
Exposure Temperature (ET)	1	Frozen	0.15
	3	0 °C to < 8 °C	
	5	> 8 °C to <30 °C	
	9	> 30 °C	
Exposure duration (ED)	1	Transient (≤ 60 minutes)	0.15
	3	Short (≤ 24 hours)	
	5	Medium (≤ 7 days)	
	9	Long (>1 week)	
Process Fluid Interaction (PFI)	1	Limited penetration into polymeric component (i.e., Water)	0.15
	3	Low solvation power or low penetration of polymeric component (e.g., neutral pH without organics, surfactants, etc.)	
	5	Medium solvation power or medium penetration of polymeric component (e.g., surfactant low concentration organics, high/low pH solutions without organics/detergents)	
	9	High solvation power or high penetration of polymeric component	
Dilution ratio (DR)	1	$<1 \times 10^{-03}$ m²/L	0.15
	3	1×10^{-02} to 1×10^{-03} m²/L	
	5	1×10^{-01} to 1×10^{-02} m²/L	
	9	$>1 \times 10^{-01}$ m²/L	

Abbreviations: DAS = distance along production stream; DR = dilution ratio; ED = exposure duration; ET = exposure temperature; PFI = process fluid interaction.
[a] Parameter range definitions in this table represent examples only. Individual companies should develop their specific range definitions according to their internal policies/standard operating procedures.
[b] Weighting levels used in the table represent examples only. In this table, 0.40 is used for DAS rating, and 0.15 is used for all other considerations. Individual companies may use equal weight distribution or may assign weighting levels according to their internal policies.

processing conditions, a dedicated extraction study and/or leachables study will be necessary.

A dedicated extraction study representative of the processing conditions should define the worst case in-use parameters in terms of type of solvents, duration, and temperature of contact, and surface-to volume ratio.

Finally, the definition of the study design is a very important and crucial step. Different extraction study design options are available for the manufacturing process contact materials:

Table II. Advantages and difficulties of the different extraction study designs.

	Advantages	Difficulties
Option 1: On-site assembly study	• Easier to perform: the solvents re-circulate in the system loop. • Fewer samples to test.	• The samples may be contaminated from several materials and buffers. • Data interpretation may be difficult. • Big volumes in use. • Correlation between extractable compound and the source of material may be needed.
Option 2: Singular material study	• Extraction data for each single part of the process may useful for future material changes.	• Management of the extraction of small parts, O-rings, or filters is difficult. • Increased number of samples to analyze. • Smaller volume used. • Increased number of extractables species and more complicated data interpretation. • Need for scaling the results to the real in-use conditions.
Option 3: Small-scale assembly	• Easier to perform • Can be adapted in the laboratory to the real needs; possibility to exclude some parts to avoid sample contamination and to simplify data interpretation. • Less volume in use. • Less number of samples to analyze. • More practical and representative of the real case, though can simplify the analytics and the extraction preparation.	• No direct correlation between extractable compound and the source of material, though Option 3 can be easier than Option 1. • It may be necessary to repeat the study or at least the simulation study for a different product.

- Perform an extraction study on the process parts as an on-site assembly (i.e., for each process step, combining all the materials included in each skid).
- Perform an extraction study singularly on each product contact material part, eventually bracketing materials of the same type/composition.
- Reproduce a small-scale assembly of the manufacturing process, which includes the most at risk materials (based on preliminary risk assessment).

These approaches have been applied in three different case studies and are summarized here, highlighting the advantages and the difficulties of each extraction study design option (**Table II**).

It is important to note that the case studies described in this paper are not to be intended in contrast with BPOG *Standardized Extractables Testing Protocol for SUS in Biomanufacturing*, which Merck adopted as single-use requirements. The extraction and leachables simulation study conditions presented here were designed to compensate for the insufficient/inadequate extractables data supplied by vendors and were intended to mimic the worst-case scenarios of use of the materials for their intended application within the company's specific manufacturing processes.

ANALYTICAL METHODS

In the three case studies, the following analytics were applied:

- Headspace sampling/gas chromatography/mass spectrometry (HS/GC/MS) and gas chromatography/mass spectrometry for volatiles and semi-volatiles
- Ultra-high performance liquid chromatography/MS/MS for semi-volatiles/non-volatiles
- Graphite furnace atomic absorption spectroscopy (GF/AAS) for silicone
- Inductively coupled plasma–mass spectrometry (ICP–MS) for elemental impurities
- Total organic carbon (TOC) on aqueous extracts only.

ON-SITE ASSEMBLY EXTRACTION STUDY

The on-site assembly option was applied to a downstream manufacturing process. Due to the complexity of the equipment and the single-use and multi-use materials involved, the extraction study was carried out by bracketing the different skids separately, according to the worst-case in-use conditions of each specific purification step, following the risk assessment. Extractables studies on the chromatographic resins were already available from the vendors so all resins involved in the purification process were disconnected and excluded from the extraction study, thus simplifying the study.

The worst-case organic and aqueous solutions selected as model solvents for the process skids extraction were the following: ethanol 20% in water, acetonitrile 50% in water, acetonitrile 50% with 0.1% trifluoroacetic acid (TFA), sodium hydroxide (NaOH) 0.1M and 0.5M, and a solution of NaOH 0.5M and sodium hypochlorite (NaClO) that was used to sanitize the line (**Table III**). These solutions were recirculated in each skid for a few hours up to seven days at normal process temperature, then

Table III. Extraction study design for option 1, on-site assembly for downstream processing. BPOG is BioPhorum Operations Group.

Process steps	Solutions	Volumes	Conditions
• Chromatography capture 1 • Ultrafiltration 1 • Chromatography capture 2 • Chromatography capture 3 • Ultrafiltration 2 • Chromatography capture 4 • Membrane filtration	Worst-case material contact solutions selected (working conditions as well as the cleaning and storage considered) Different contact solutions per different steps: • Sodium hydroxide (NaOH) 0.1M • NaOH 0.5M • NaOH/Sodium Hypochlorite • Ethanol 20% • Acetonitrile • Acetonitrile and trifluoroacetic acid • Water	Surface: Volume ratio 6:1 was applied in accordance with BPOG protocol. Different volumes used: Min: 0.5 L Max: 5 L	Recirculation for 7 days at normal process temperature Chromatography resins excluded as data were available from suppliers.

Table IV. Extraction study design for option 2, extraction of the single parts for fill and finish. BPOG is BioPhorum Operations Group.

Items	Solutions	Volumes	Conditions	Total number of samples
Group 1: Glass bottle Group 2: Stainless steel tank Group 3: Polyvinylidene fluoride (PVDF) filter membrane Group 4: Ethylene propylene diene monomer (EDPM) valve Group 5: EPDM diaphragm Group 6: EPDM and polytetrafluoroethylene (PTFE) diaphragm Group 7: EPDM O-ring Group 8: Silicone gasket Group 9: PTFE silicone hose Group 10: Silicone hose Group 11: Polycarbonate and silicone hose Group 12: Polystyrene connectors	Four aqueous solutions selected: • Sodium hydroxide 0.1M • Hydrogen chloride 0.1M • Water • Sample buffer (simulation study)	S/vol. ratio 6:1 and 1:1 for filters was applied as per BPOG protocol. Volume for extraction: 200–400 mL	7 days at 40 ˚C in closed container under mild agitation	48 samples 4 blanks (per analytical technique)

sampled and analyzed by performing the different techniques. The reference solutions, before starting the recirculation process, were sampled and tested as well.

Analytical studies detected a variety of volatile and semi-volatile organic compounds; several plastic/rubber additives such as phenolic antioxidants; plasticizers (e.g., phthalates/adipates); amides, and other plastic/rubber formulation related components (e.g., residual solvents, silicon polymers, glycols, and alkanes).

Having extrapolated from what was detected in the study to the real-case conditions of use of the process materials (namely, by taking into account the dilution factor), a safety assessment was made considering the daily dose of the final product (<1 mL in this case). The result was that the potential daily exposure of the patient to the species detected in this extraction study was below 1.5 µg/day, which is the safety threshold for genotoxic impurities. Therefore, no safety concern was determined for the materials used within the process.

Critical aspects and lessons learned

This experiment raised some difficulties within the execution and the analytics, and revealed some lessons learned:

- Due to the impact of contamination from the on-site solution

Table V. Extraction study design for option 3, small-scale assembly for fill and finish.

Items	Solutions	Volumes	Conditions	Total number of samples
• Polyethylene bag • Silicone tubing • Pump head • Connectors	Four aqueous solutions selected: • Ethanol 50% • Hydrogen chloride 0.1M • Water • Sample placebo (simulation study)	Lowest scaled-down processing volume: 2.6 L for a 10 L bag	48 h recirculation at 35–40 °C	48 samples 4 blanks (per analytical technique)

preparation step (e.g., contact with plastic bags/bottles), there is a need to establish the right mindset within the manufacturing site.

- Many artifacts and analytical interferences were generated by strong/reactive solutions (as NaOH/NaClO), causing difficulties in the analyses and data interpretation; the use of strong saline/reactive solutions should be reconsidered and replaced with fit-for-purpose solutions.
- The impact of the already-used materials should be taken into consideration. Traces of residual compounds from previous manufacturing runs and from cleaning processes (e.g., ethanol) were also detected.

SINGLE-PARTS EXTRACTION STUDY

The single-parts option was applied to fill/finish manufacturing process parts. New parts (e.g., tubing, gaskets, valves, connectors, etc.) were used and extractions were done singularly within the laboratory, without involving the entire production area. Therefore, this option seemed simpler to perform. Furthermore, as most of the materials are used in different processes, a goal of the study was to use the extraction profile obtained from each material for all other processes in which the same material was involved, potentially reducing other extraction study efforts in the future. Therefore, for these fill/finish processes, a dedicated simulation study, with the drug product buffer/placebo as extracting solution, may be sufficient to complete the extractables and leachables data package. In addition, the data generated in this approach are useful for future changes of materials.

All the materials present in the process were evaluated and classified, grouping them by their main components (e.g., silicone, rubber, plastic type) resulting in 12 groups of materials. These materials were extracted with aqueous solutions only (NaOH, 0.1M; HCl, 0.1M; water; and drug product buffer as a simulation of the real-case conditions), because no organic solvent was foreseen to be used within this process (**Table IV**). The extraction was carried out by soaking the single pieces, with the selected model solutions, while the tubes were filled and closed at both ends. The volume of the extracting solutions reflected a surface-to-volume ratio ranging from 6:1 to 4:1. A light agitation was applied and the overall extraction was carried out in a climatic chamber under controlled temperature conditions.

A much higher number of samples were analyzed in this option, though the solutions chosen for the extractions were more compatible with the analytical techniques with reduced artifacts and contaminations. The main organic extractables identified were rubber and plastic materials formulation/manufacturing (polymer fragments, glycols, etc.) and additives (plasticizers, vulcanizing agents, lubricants, solvents, antioxidants, etc.). By using this extraction approach, it is worth noting that for some product contact items (i.e., ethylene-propylene diene monomer diaphragms), trace element results were affected by the presence of some metallic parts such as screws/external connections that were not supposed to come into contact with the product but were impossible to remove before the extraction, thus generating biased results.

Finally, the results were extrapolated to the normal operating volumes of solution in contact with the same materials, resulting in an overall dilution factor of 600 (surface-to-volume ratio <0.1). For this reason, the overall levels of organic/inorganic extractables/potential leachables identified in this study were negligible when referred to the final drug-product solution under normal operating conditions.

Critical aspects and lessons learned

This experiment raised the following difficulties within the execution and the analytics:

- It was difficult to extract small pieces or parts whose geometry was not completely compatible with the selected volume of the extracting solution.
- Higher numbers of samples had to be analyzed.
- Data evaluation and correlation with the process step volumes were difficult as the materials used in the different steps of the process.

- The possible contribution of external parts that do not normally come into contact with the product, but may affect the overall results, must be evaluated.

SMALL-SCALE ASSEMBLY EXTRACTION STUDY

The small-scale assembly option was applied to a fill/finish manufacturing process that involved the use of a single-use polyethylene mixing bag, sterile filter, pump, tubing, and connectors. These materials were considered most at risk as they are the closest to the final product and no further dilution is applied to obtain the final drug product. In this case, there was the possibility to use new items and to scale down the system to mimic the real conditions, dealing with less volume and a more friendly sample preparation.

The study was designed as a small-scale assembly within the manufacturing site, using materials that were representative of the real-case production. Based on the preliminary risk assessment and on existing information (from other extraction studies and from the supplier), some items were excluded from the small-scale assembly, simplifying the study and the data interpretation. In particular, the assembly was performed using a scaled-down 10-L bag (same type and supplier of the one used in production), silicone tubing, polypropylene connectors, and a pump with a Teflon head that contains rubber gaskets that come into contact with the product solution.

This small-scale assembly was extracted with three different model solutions (i.e., water, 0.01M hydrogen chloride, and 50% ethanol in water), covering the real worst-case conditions in terms of surface-to-volume ratio as well as contact time and temperature conditions. A scaled-down volume corresponding to the minimum batch size was used, and a dynamic extraction for 48 hours at 35–40 °C in a climatic chamber was performed. In addition, for a leachables simulation study, a simplified placebo solution was used as a model solvent. Separate extractions were carried out by using different equipment parts each time for each solution. The extraction experiment is shown in **Table V**.

The analytical results highlighted a few organic species (i.e., series of siloxanes and traces of some phenolic antioxidants related species) were detected as extractables from the plastic and rubber materials in contact with the product during the fill/finish operations. The quantity in the extracts was extremely low, however, especially in the simplified placebo (simulation study).

Based on these results, it was possible to conclude that no safety concern arises from the SU materials used during the fill/finish operations.

It is important to highlight that in this type of extraction study design, if no dilution is applied from drug substance to drug product, the results in terms of concentration of the detected compounds are used as-is.

Critical aspects and lessons learned

The third option revealed the following considerations:

- The scaling down of the system had to be representative of the actual process and simulate worst-case conditions that mimicked the higher surface-to-volume ratio under normal operating conditions.
- As in the first assembly approach, there was no direct correlation between extractable compounds and the source of material, though in this experiment, the data interpretation was easier than in the first study. The small-scale option used less aggressive solutions and applied a simpler design assembled in the laboratory, with much lower risk of contamination.
- This last approach was considered to be the best way to conduct the extraction study on the manufacturing materials; it is friendlier to carry out and the conditions are more representative of the real situation.

CONSIDERATIONS AND CONCLUSION

The evaluation and practical study approaches of extractables and leachables for manufacturing process product contact materials concludes that each study should be carefully established case by case. Every extractables and leachables evaluation should begin with a risk assessment of the materials involved prior to the study design. Extraction studies should be as simple as possible. Model solvents should mimic the worst-case conditions, should be fit for the scope, and should not be too aggressive to avoid artifacts, sample contaminations, and difficult data interpretation. In addition, the authors recommend that special consideration should be given to the preparation of samples, especially if situated in the manufacturing areas instead of dedicated laboratories.

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Cold Chain: Zeroing in on the Last Mile

Agnes Shanley

Longer packouts are becoming the rule, as logistics service providers and sponsors gain experience planning logistics for clinical trials involving cell and gene therapies.

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Biopharmaceuticals are becoming more prominent in the overall pharmaceuticals markets. In 2016, according to projections by analysts at IMS' Institute for Healthcare Informatics, seven of the 10 top-selling drugs sold in the United States were large molecules (1). By the end of this year, IMS predicts that biologics will account for roughly 20% of the total pharmaceutical market.

As manufacturers move into new markets and launch new clinical research programs, including work in the development of cell, gene, and tissue therapies, ensuring the integrity of the cold chain has become more important, and more challenging, than ever.

Success involves careful partner selection and logistics planning, as well as proactive communication with shippers, freight forwarders, and logistics specialists. It also depends on new temperature-control technologies, a number of which have recently been commercialized. In 2016, UPS bought Marken Technologies, which specializes in clinical trial shipments, to strengthen its capabilities in this area.

In this overview, Ron Swistock, director of global healthcare strategy at UPS, discusses overall biopharm logistics trends, while he, Mike Sweeney, senior director of global service development, and James Klingelhoefer, regional sales director, both with World Courier, share insights into some of the challenges posed by personalized medicine, and innovative biological treatments such as gene and cell therapies.

MORE PRECISE CONFIGURATIONS

BioPharm: How are the biopharmaceutical industry's cold-chain packaging needs changing?

Swistock (UPS): Pharmaceutical companies are under increased pressure to understand carrier ambient environments in order to develop or justify their transportation methods and risk mitigation plans. Pharma companies are also finding a greater need to optimize the product carton and shipping carton, to minimize unused space, and select more precise packaging configurations.

You'll see more efforts placed on sustainable materials, and demand will grow for carriers to offer more temperature controls within their networks to minimize packout complexities, costs, and requirements. An increased number of pharmaceutical companies are also innovating and collaborating with reputable third-party logistics companies to create better efficiencies for shipping biopharmaceuticals and clinical trial specimens.

BioPharm: What specific changes are you seeing?

Swistock (UPS): There is more interest in reuseable shippers. These may be more expensive up front but they enable a 'just-in-time' business model. For the receiving lab, this approach not only offers environmental disposal advantages, but saves floor space because they no longer have to store used shippers.

BioPharm: What improvements are you seeing in packouts?

Swistock (UPS): Temperature-control technology is improving, and packouts can be much longer than they were in the past. Traditionally, the packout standard has been 24 hours up to 48 hours, but now efforts are being made to bring that up to 96 hours.

If shippers can provide this level of protection for a two-day trip, it will help ensure safety in case of transportation and weather delays.

Important capabilities include replenishment of dry ice and ensuring the integrity of shipment. UPS works closely with its business partner, Cryoport, and uses Cryoport's dewars, and shipments can be initiated from either Cryoport's or UPS' websites.

BioPharm: How is serialization affecting packaging equipment design and features?

Swistock (UPS): With the Drug Supply Chain Security Act (DSCSA), healthcare companies are getting in lockstep with establishing an interoperable track-and-trace system based on a unique numerical identifier. Complying with elements of the DSCSA is an ongoing process that must fully take shape sooner rather than later.

From altering highly regulated packaging to implementing a sophisticated system of issuing unique product identifiers, all of this aggressive maneuvering is aimed at better protecting the drug supply, consumers, and patients.

NEW BIOLOGICS POSE CHALLENGES

BioPharm: What role are new biological treatments (e.g., cell and gene therapies) playing in the clinical trial logistics business today, and how does planning for trials with these products differ from traditional clinical trial logistics?

Swistock (UPS): These treatments are still a small part of the clinical trials business, but they require a large amount of front-end planning. Over the past few years, we've seen that sponsors really want to know how integration can play a greater role. These studies require cryogenic shipping, so the ability to accommodate dewars seamlessly within our network will remain a priority.

Sweeney (World Courier): Cell- and gene-therapy products require extensive logistical planning. In the case of autologous cell therapies, the patient's cells are the active pharmaceutical ingredient used in the manufacturing of the end therapy. Therefore, the patient becomes a part of the supply chain, and can be directly impacted by delays or problems during the logistics process.

Additionally, particularly with consideration to the severity of the patient's illness, the logistics timelines are always extremely tight and any deviation can have a far reaching impact on the clinical team, end-therapy manufacturer, and patient.

When contrasted with small-molecule or biologics products, we find that there is much less margin for error with the logistics of cell and gene-therapy products. Scoping suitable time, critical routings, and educating airlines on the special nature of these products are small parts of the overall process.

BioPharm: How should sponsors and contract partners approach collaboration for these projects? What questions are critical to ask logistics and shipping specialists, and what specific functions should each partner assume?

Swistock (UPS): Important questions should revolve around service partners' experience and safety record with dewars, as well as packouts and how long the shipments will remain viable without taking additional steps.

Klingelhoefer (World Courier): Sponsors and contract partners should collaborate in early planning stages for both early clinical and commercial development. Although early clinical-stage development requires careful planning, when the scale is increased to commercial application, it is absolutely imperative that sophisticated scheduling and tracking practices be put into place.

It is extremely beneficial for logistical plans for both early clinical-stage development and

commercial development to be discussed during the planning phases. Questions during these discussions should include the following:

- How many sites, countries and cities will be involved in the clinical trial?
- What will be the estimated number of patients?
- What is the nature of the contents—will the trial use kits? What type of packaging will be used, and will apheresis samples be included?
- Is temperature control management required?
- What's the nature of required chain of custody tracking?
- Is special shipment handling required (e.g., is x-ray permitted)?

All of these questions should be addressed at the very onset of project planning.

BioPharm: What issues must be taken into account, and how do they affect the choice of temperature-control and other technology, and overall planning?

Klingelhoefer (World Courier): Generally, it will help sponsors to have a basic understanding of the shipping lanes, transit time requirements, and temperature-control parameters in order to be able to anticipate potential issues and allow solutions to be pre-emptively created.

For example, if the end therapy is going to be transported in a liquid nitrogen dry shipper, then sponsors and partners should ask and decide upon the answers to such questions as:

- Who is going to provide the liquid nitrogen shipper?
- Who is going to make sure that the dry shipper unit has been properly charged with liquid nitrogen?

The answers to these questions will help determine responsibilities, and develop a plan of action and set project timeframes.

BioPharm: What are the unique challenges of moving autologous, as compared with non-autologous cell and gene therapies, and other personalized medicines?

Swistock (UPS): With autologous therapies, the biggest complexity is that, not one, but two really high-performance shipments are involved. There can be challenges, for instance, when the patient visits a facility where blood is collected. Often these trials are run in dispersed locations.

In some cases, there will be fewer labs available to send the blood. In general, with autologous treatments, special planning is particularly important for the journey's second leg.

Sweeney (World Courier): The obvious main difference is that an autologous cell-therapy scenario involves a circular supply chain, where the allogeneic model is more of a straight line between two points. With the circular supply chain, there is generally more of a need for careful coordination between apheresis and manufacturing slots.

With allogeneic, there is likely more room for maneuvering because it relates to the time between when the therapy is manufactured and the time it is needed for infusion into a patient. The more complex circular supply chain for autologous requires precision planning with no room for error or delays. The straight line supply chain for allogeneic relies on experienced specialists.

BioPharm: What special temperature-control technologies, storage, and transportation options are required for these types of advanced therapies?

Klingelhoefer (World Courier): This will vary on the product and pharmaceutical sponsor requirements. Sponsors often require temperature-controlled packaging for apheresis samples (2–8 °C packaging) and packaging for end-therapy product (typically, asking for liquid nitrogen dry shippers). In some cases, customers will ask for global positioning system (GPS) tracking devices to enhance the visibility of their shipments.

DIRECT-TO-PATIENT SHIPMENTS

BioPharm: Some clinical-trial materials are now delivered direct to patients' (DTP) homes. Do you see that approach being used in the future for these types of materials, and how would that complicate the overall process?

Swistock (UPS): There is great interest in DTP activity. DTP exists for rare diseases, but the logistics for clinical trials, involving ambient temperatures, are easier. We've had success with these studies. Investigational medicinal products must be delivered on a regular basis, typically in one or two doses.

In a clinical setting, they may be delivered less frequently. But if people have mobility problems, DTP studies speak to a patient-centric model we're seeing the industry lean toward more and more.

Sweeney (World Courier): At the moment, most shipments would be beyond the scope of home coordination. For many situations, the end-therapy product is sent at frozen temperatures, including some cryogenically frozen material, which then requires a sophisticated thawing process before infusion. Currently, it is unrealistic to expect a DTP scenario for most cell and gene-therapy products.

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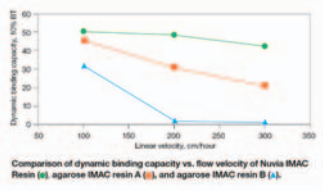

Comparison of dynamic binding capacity vs. flow velocity of Nuvia IMAC Resin (■), agarose IMAC resin A (□), and agarose IMAC resin B (▲).

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Ask The Expert—*Contin. from page 50*

manufacturing, so consideration must be given to how the materials and components will be introduced manually into the manufacturing area. This will require even greater emphasis on operator technique. In some instances, the manufacturing equipment used in small-scale batch production is portable, so this program should also encompass the introduction of the necessary manufacturing equipment into the production area.

There needs to be an established environmental monitoring program. The environmental monitoring program for small-scale batch production should provide the information on the quality of the aseptic processing environment including any ancillary areas such as the equipment/component processing area, gowning rooms, laminar flow hoods, floors, ceilings, walls, and equipment surfaces including those that come into contact with the product components as well as the product itself. Determining the appropriate monitoring locations should be determined through a comprehensive risk evaluation that should periodically be reviewed and updated to reflect the most current operating conditions.

Finally, there needs to be consideration to final product testing and the appropriate number of samples needed to ensure the product is sterile and safe for patients. The samples needed for testing should be taken from the beginning, middle, and end of the manufacturing run and the number taken should be reflective of the batch size.

Successfully manufacturing small-scale parenteral batches suitable for patients requires many of the same procedures and controls needed for large-scale parenteral manufacturing. If you keep in mind—regardless of batch size or manufacturing process—that the ultimate goal is to assure the sterility of the product throughout the manufacturing run and you use the data collected to determine the suitability of your processes, you should be able to produce any size batch in accordance with regulatory expectations.

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Analytical Advances —*Contin. from page 17*

Finally, DiPaola notes that the high cost of mass spectrometers presents another obstacle, especially when dealing with high-resolution, tandem systems. "Affordability is a sizable issue for small laboratories and companies with limited financial capacity. Additionally, these systems are rather complex and delicate, so that any repairs can only be performed by skilled engineers, adding additional costs," he explains.

Berger asserts that many of these limitations with mass spectrometry today do not involve the instrument itself, but other elements of the analytical workflow that interact with the system. "Faster and more robust sample preparation is needed to match the improvement in back-end data processing throughput for many applications of MS in the biopharmaceutical industry," he notes.

For instance, Berger points to clone screening and other early development activities, where reproducible microscale and nanoscale separations remain a challenge to many MS users. "Waters has developed a chip-based microfluidic platform, the ionKey/MS System, that simplifies the process of microscale LC–MS of proteins and peptides, but the industry is looking for further innovations that raise usability of systems at the microscale to that of analytical scale LC–MS," he observes.

Berger also notes that innovative software products such as those from Waters for automating biotherapeutic peptide mapping and intact mass analysis, intact mass, peptide mapping, and released glycan analysis characterization and monitoring have been key to advancing the productivity of labs using LC–MS instrumentation. He agrees, though, that "analysts are looking for smarter software, automated processing, custom calculations for critical results, and efficient reporting tools that simplify communication of the results, and they now want to be able to use this software in regulated environments."

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Ad Index

Ask the Expert

Ensuring Sterility in Small-Scale Production

Susan Schniepp, distinguished fellow at Regulatory Compliance Associates, discusses how to ensure sterility when manufacturing small-scale parenteral batches.

Susan Schniepp is distinguished fellow at Regulatory Compliance Associates.

Q: I am a small-scale parenteral manufacturer and have been approached to support a clinical program through Phases II and III. What considerations are important for the small-scale production lots?

A: This situation always raises some interesting discussions. The statistical assurances for sterility for normal commercial lots are not available because of the usually small lots during clinical production. At the same time, the need to minimize any unanticipated clinical risks remains high. The best advice is to consult the regulations and determine what requirements you must meet to help ensure the clinical material you are manufacturing is safe.

Clinical-trial material batches can range from as few as 200 to as many as 5000 units or more. It may not be feasible for these small-size batches to be produced on a traditional manufacturing line. In fact, some of the batches might be manufactured under a laminar flow hood by manual aseptic processing. It is important to remember that sterility assurance of the product must be maintained even when the clinical-trial materials are being prepared by hand because of the small lot sizes required. For these small-scale batches, manufacturers need to adhere to the recommended requirements for control of the environment as defined in FDA's aseptic processing guidance (1), the Parenteral Drug Association's *Technical Report* #62 (2) on manual aseptic processing, and in *EudraLex* Volume 4, Annex 1 (3). In other words, the environment must be maintained and monitored with the recommendations specified in the documentation governing aseptic processing.

Properly maintaining an environment suitable for the manufacture of aseptic drug products regardless of batch size is not as clear-cut as it seems. Quality personnel responsible for batch release need to take into consideration a number of factors before the lots in question can be released. These factors include assuring appropriate use of disinfectants before, during, and after manufacturing; proper gowning and aseptic technique of the operators; and making sure the environmental and qualification data support the operations.

CREATING STERILITY PROGRAMS

So, what does this all mean? It means the company must have a robust program in place to support the sterility of the batch regardless of the lot size. There needs to be a cleaning program defining the proper use of disinfectants/sporocides before, during, and after processing. It means there should be a gowning qualification/requalification program for personnel responsible for the product manufacturing. The gowning program should define how operators are initially qualified, how they are monitored for microbiological excursion during gowning, during manufacturing, and upon completion of manufacturing activities. It should also define how operators will be periodically requalified and how operators will be requalified in the event of an out-of-specification result. Operator aseptic technique should be tested through the conduction of media fills representative of the actual manufacturing runs. At a minimum, media fills should be performed annually.

There also needs to be a facility maintenance program that challenges the appropriateness of the air handling system. Establishing a periodic maintenance program for your high-efficiency particulate air (HEPA) air filtering system and performing periodic smoke studies to make sure the airflow is suitable for aseptic operations should provide the assurance that the airflow system is in control and is functioning appropriately.

In addition to the above recommendations, the company should have a program to control incoming components. Components used in the manufacture of aseptic processing on a small scale need to be rendered pyrogen free before being introduced into the manufacturing area. Small-scale batches will not be taking advantage of continuous processes associated with large-scale lot

Contin. on page 49

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