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Amid debate about “fake news,” peer-review papers offer vital, objective insight.

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The term “fake news” has been a hot-button issue recently. By definition, “fake” news is news that is false; this content may be disseminated as “true” to influence opinion or to deceive. In the current political environment, some identify any information that runs counter to their opinions as “fake news.”

Thanks to special-interest online publications and blogs, cable news channels, and even traditional news outlets, finding news that is “real” to you is only a click away. Your “real” news, most likely, is “fake” to those with opposing views. Facts, scientific consensus, and real-word evidence have become secondary to opinion and vested interests in the political arena. Fact-based information, however, is still the foundation for discussions and decisions in science-driven industries.

The editors of BioPharm International routinely screen pitches from representatives of supplier companies about the importance and capabilities of new products or the quality of the services they offer. Experts at biopharma companies look to promote their capabilities or receive recognition for their achievements. We embrace our role as gatekeepers, sorting through the sometimes overtly promotional pitches to compile objective information and insight about industry trends and developments. We also receive article contributions from industry experts whose primary objective is to share information to help advance the science and technology of biopharmaceutical development. We are grateful for their contributions.

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Readers report that they prefer articles that provide practical descriptions of technical topics, as well as peer-reviewed papers, formats that are the foundation of our coverage. While the editors interview and work with industry professionals for the technical features, we turn to experts to provide the peer-reviewed contributions.

While other publications offer pay-for-publish models or require authors to have their papers reviewed prior to submission, manuscripts submitted to BioPharm International for peer review are double-blind reviewed by the publication’s editorial advisory board using procedures standard for scholarly and technical journals. Thus, this peer-review process validates the credibility of the scientific or technical work described in the paper. The review period typically takes four to six weeks. Accepted papers are scheduled for publication in BioPharm International on a first-accepted basis and usually are published four to six months following acceptance.

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Since confirmation as FDA commissioner in May 2017, Scott Gottlieb has moved aggressively to deliver on promises to Congress and the public to promote innovation while also ensuring medical product safety and patient access to needed therapies. A top priority is to address the deadly opioid epidemic by reducing drug misuse and abuse and encouraging development of safer painkillers. Although FDA officials usually avoid involvement in drug pricing, Gottlieb has addressed the issue head-on, mapping out strategies to accelerate development of competitive generic drugs and biosimilars, as well as initiatives to bring more life-saving therapies to patients.

In addition to these high-profile initiatives, FDA faces the considerable task of implementing the 21st Century Cures legislation. The new law sets tight deadlines for issuing guidance documents, reports, and regulations and for holding public meetings with stakeholders to devise and revise key programs for product development. Cures Act provisions call on FDA to better coordinate the review of combination products and establish a framework for approving regenerative therapies and accelerating the development and approval of critical medicines.

In his first months on the job, Gottlieb also had to overcome administrative hurdles, such as negotiating an exemption for FDA from the administration’s federal hiring freeze. And it appears that Congress will provide a reasonable budget for FDA by ignoring the White House’s proposal to slash agency appropriations and greatly increase user fees. FDA will need considerable resources to accomplish all the goals within the timeframes set by Gottlieb’s multiple action plans.

**COMBATING OPIOIDS**

First on the agenda is the imperative to curb excess prescribing and distribution of powerful opioids and to produce safer alternative pain therapies. To this end, FDA is collaborating with the National Institutes of Health and industry to speed the development of non-addictive analgesics, effective overdose reversal interventions, and better diagnostics that can prevent death from overdose.

At the same time, FDA is examining more closely whether opioids with abuse-deterrent features (ADFs), which FDA has encouraged manufacturers to devise, are effective in resisting misuse or actually aggravate overdosing. In June 2017, FDA urged Endo Pharmaceuticals to pull Opana ER from the market (which the company did in July 2017) due to evidence that abusers merely shifted from snorting the reformulated product to injecting it, igniting outbreaks of HIV, hepatitis C, and a serious blood disorder.

Agency officials are weighing the removal of additional ADFs and examining strategies for evaluating their safety. At a public workshop in July 2017, experts discussed what data and analytical methods could best assess these products, as outlined in an FDA issues paper (1). A new Opioid Policy Steering Committee, headed by FDA Deputy Commissioner Rachel Sherman, is examining whether FDA reviewers sufficiently consider risk of abuse during the evaluation of new pain medicines, along with the adequacy of current dosing recommendations and label information, and whether mandatory education of healthcare professionals is needed to ensure appropriate opioid prescribing (2).

**PROMOTING COMPETITION**

Instead of permitting broader import of cheaper medicines from abroad to expand access to less costly therapies, Gottlieb looks to speed more competitive generic drugs and biosimilars to market. For example, FDA officials say they expect interchangeable biosimilars to appear within two years, following publication of guidance on how to test and develop such products (3). FDA had approved...
five biosimilars as of June 1, 2017, and manufacturers were developing 66 additional therapies.

Furthermore, a Drug Competition Action Plan outlines strategies for accelerating the review and approval of “high-need,” priority generics and for addressing concerns about brands using Risk Evaluation and Mitigation Strategies (REMS) to block the development of new generic competitors (4). Gottlieb scheduled a public meeting in July 2017 to examine whether innovator firms were gaming FDA rules to block generic-drug sponsors from access to samples needed for bioequivalence testing of abbreviated new drug applications (ANDAs). A related issue is the struggle to negotiate single shared REMS programs for marketing high-risk brand and generic therapies. At the recent meeting, FDA experts heard recommendations from manufacturers and other parties on how to revise the REMS program to halt anticompetitive behavior without undermining new drug development. FDA is accepting comments on these issues through mid-September, and Commissioner Gottlieb is exploring with members of Congress what legislative change is needed in this area and what issues FDA can address with existing authorities.

FDA also seeks to quickly approve additional competitive generics by extending priority review of ANDAs to products with less than three approved alternatives to a reference drug (5). FDA outlined in a June 2017 update to its internal procedures that accelerated review (8 vs. 10 months) applies to drugs with limited competition, as well as to first generics and to products related to drug shortages, public health emergencies, and other special situations (6).

To encourage development of these high-need therapies, the agency also issued a “hit list” of more than 250 branded drugs that are off-patent, have no exclusivities, and thus qualify for priority review. The list includes some notorious, high-cost products that are eligible for immediate agency evaluation, and more complex therapies that raise scientific and legal issues and warrant prior consultation with FDA staff (7).

The importance of manufacturer readiness for accelerated approval of priority generics is highlighted in a draft guidance that maps out a new process for identifying and vetting production facilities for priority generics. To enable faster ANDA reviews, FDA wants manufacturers to provide a pre-submission facility correspondence (PFC) two or three months before filing an application for a priority generic product (8). FDA says it needs the extra time to determine if testing facilities and planned manufacturing sites require inspection in order to schedule such visits within the abbreviated review timeframe.

INNOVATION KEY

Ultimately, Gottlieb regards the development of more innovative and effective therapies as key to lowering healthcare costs over the long run. Such gains involve streamlining development of treatments for chronic diseases and clarifying R&D requirements to limit unnecessary regulatory costs. The commissioner told the Senate Appropriations subcommittee in June 2017 that a new Medical Innovation Development Plan will address these issues and when FDA may approve a targeted drug based on genetically-defined factors and define more clearly a range of clinical trial enrichment strategies (9).

An initial priority under the Innovation Plan is to spur development of treatments for rare diseases by improving the process for evaluating orphan drug designation requests, which have more than doubled in recent years. By mid-September, according to a new Orphan Drug Modernization Plan, FDA will eliminate its backlog of 200 pending designation requests and establish procedures for vetting such requests within 90 days (10).

Despite these efforts, medical product costs will remain on the table at FDA. “It’s not debatable,” Gottlieb told the Senate Appropriations subcommittee in June 2017, that the United States is subsidizing lower drug prices in other countries through “the high prices we pay here to support research and development.” But whether market competition and more innovation will alter that truism remains to be seen.

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8. FDA, ANDAs: Pre-Submission Facility Correspondence Associated with Priority Submissions, Guidance for Industry, Draft Guidance (CDER, June 2017).
Uncertainty about the White House’s agenda has impacted pharma and the contract manufacturing organization (CMO)/contract development and manufacturing organization (CDMO) sector. There’s been some progress in the past few months, including the appointment of FDA Commissioner Scott Gottlieb, MD, but there remains a great deal to be worked out.

In the near term, one of the most critical areas to be resolved is the FDA Reauthorization Act (FDARA), which reauthorizes four major FDA user fee packages: the Prescription Drug User Fee Act (PDUFA), the Generic Drug User Fee Amendments (GDUFA), the Biosimilar User Fee Act (BsUFA), and the Medical Device User Fee Amendments (MDUFA). The new packages will kick in on Oct. 1, 2017 (when the federal government’s FY2018 begins), and without timely approval of FDARA, FDA will be compelled to issue intent-to-furlough notices this summer to staff who are paid with user-fee funds. That would cause drug approval timelines to skyrocket for both innovator and generic drugs, and potentially hazardous facilities to go uninspected.

On July 12, 2017, the House of Representatives approved FDARA (HR 2430) by voice vote; the bill was finalized in pre-conference with the relevant Senate committee, so this version should be the same one that the Senate will vote on. It retains the negotiated agreements between FDA and industry, while adding amendments intended to prioritize the review of generic drugs where they can help drive down drug prices. FDARA also includes stricter accountability and reporting of FDA’s use of user fee funds.

The Pharma & Biopharma Outsourcing Association (PBOA) was deeply involved in the negotiations with industry and FDA for GDUFA II, and subsequently worked with Congress to explain some of the changes to the program, how they’re intended to increase patient access to affordable generic medicines, and how the new fee structure is fairer to various industry stakeholders. PBOA also worked through the amendment process to ensure that additions to FDARA wouldn’t harm the CMO/CDMO sector.

Drug pricing, immigration, tax reform, and other topics could impact CMO/CDMOs.

That doesn’t mean everyone’s happy with FDARA. The White House has issued statements and an FY18 budget that runs counter to the negotiated agreements, zeroing out the federal funding that is part of each UFA program and increasing the user fee totals to offset that cut. The day HR 2430 was passed, the White House’s Office of Management and Budget (OMB) issued a statement that included the following: “The Administration urges the Congress to provide for 100% user fee funding within the reauthorized programs. In an era of renewed fiscal restraint, industries that benefit directly from FDA’s work should pay for it” (1).

Congress has turned down the White House’s request, retaining the existing UFA budget models. This should provide a degree of predictability for the user fee-paying sector.

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it’ll be a bad sign for FDA, the pharma sector, and patients.

SUPPLY CHAIN WORRIES
FDARA isn’t the only worrisome deadline the industry faces. Under the Drug Supply Chain Security Act (DSCSA), the federal law mandating FDA secure the drug supply chain, drug manufacturers and packagers had a deadline of Nov. 27, 2017 to make sure that all packages are marked with a product identifier, serial number, lot number, and expiration date.

One of the most critical areas to be resolved is the FDA Reauthorization Act.

This has proved to be an immensely complex project (actually, a series of immensely complex projects). PBOA’s Serialization Working Group held numerous meetings to discuss the CMO sector’s readiness for that deadline, exchanging best practices and sharing other aspects of implementation. The lack of standardization, especially for data exchange, can put CMOs in an especially tough position, where they must accommodate the platforms of their various customers.

In recent months, PBOA has met with FDA to discuss the CMO/CDMO sector’s readiness for that deadline, both in concert with other industry groups and in a one-on-one setting. In previous years, the agency had heard from a few individual CMOs, but most of their perspective came from other trade groups that represent the customer-client side of the equation. This necessarily led to an incomplete picture of the pharma manufacturing supply chain, and FDA seemed glad to talk directly with CMOs about the pending DSCSA deadline.

At the end of June 2017, FDA announced that it will use enforcement discretion for 12 months following that November 27 date. Effectively, the agency will not cite manufacturers that are not ready to introduce serialized products into the marketplace. This gives CMOs and their customers some breathing room, but CMOs should be reminded that 12 months is not a lot of time when it comes to implementing a serialization solution.

QUALITY METRICS AND CMOs
Nothing has brought PBOA and its member companies to the table with its industry peers as FDA’s Quality Metrics initiative has. Since the agency’s first draft guidance for quality metrics was introduced, PBOA’s Quality Technical Group has worked with a cross-industry consortium—including Pharmaceutical Research and Manufacturers of America (PhRMA), Biotechnology Innovation Organization (BIO), Association for Accessible Medicines (AAM), Bulk Pharma Task Force (BPTF), International Society for Pharmaceutical Engineering (ISPE), and the Parenteral Drug Association (PDA)—to push FDA to clarify the many ambiguities (and occasional contradictions) contained in that document and its successor.

Both iterations of FDA’s draft guidance would have placed CMO/CDMOs in a position where they would be responsible for reporting data that are either confidential or outside of their purview (such as data from their customers’ other contract service providers). Reporting timelines that differ from those of Annual Product Reviews (APRs) would create new workflow and staffing requirements for CMOs. Other aspects of the program could also penalize CMOs, or incentivize them to avoid certain types of products for fear of getting a “bad score”.

PBOA worked to convey to FDA that the quality metrics compliance burden would be immense for both in-house and outsourced manufacturing processes, and that the agency had yet to make a strong case for the benefits that would accrue from the program. Docket submissions and industry presentations have carried that message.

The White House issued an Executive Order calling for federal agencies to repeal two regulations for every one that they introduce (2). That order also called for the incremental cost of new regulations to be zero (when offset by repeal of other regulations). While those are somewhat basic goals, it’s clear that quality metrics would carry a massive cost to industry to implement, and needs to be put on the back-burner.

Drug pricing, immigration, tax reform, and other topics could impact CMO/CDMOs. In some respects, the industry is still in a wait-and-see mode regarding the administration, Congress, and FDA, but as an industry, we’re more involved now than we’ve ever been.

REFERENCES
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SEVERAL INTERACTION MODES

There are four major chromatographic techniques employed to purify biologic drug substances: affinity, ion-exchange (IEX), mixed- or multi-mode, and hydrophobic-interaction chromatography (HIC). Metal chelate, size-exclusion, and hydroxyapatite resins are also used on a limited basis, according to Andrew Bulpin, head of process solutions strategic marketing and innovation with MilliporeSigma.
Monoclonal antibodies (mAbs), which today account for approximately 50% of the total revenues of all biopharmaceuticals according to David Westman, global product marketing manager for GE Healthcare Life Sciences, are typically purified using a platform approach in which protein A chromatography resins are used in the first capture step. Polishing is then achieved using some combination of anion exchange (AEX), cation exchange (CEX), mixed mode, and HIC resins.

“Protein-A based affinity chromatography is very popular and more widely used because it typically affords purities above 95% in one step,” observes Nandu Deorkar, vice-president of research and development for Avantor. IEX chromatography is widely used due to its versatility, while HIC is an orthogonal technique to IEX and, therefore, the two are often used in series, according to Westman. Generally for mAbs, both strong CEX and AEX resins are preferred, according to Shelly Parra, global field applications senior manager with Thermo Fisher Scientific.

Mixed-mode and multi-mode resins are considered second-generation IEX chemistries. “Traditionally with IEX chromatography, one type of ionic interaction occurs. In multi-mode and mixed-mode chromatography, two interactions take place simultaneously, or one right after the other. This approach is becoming more popular because it allows for better separations of very closely related impurities, with the opportunity to avoid a step in the process,” Deorkar explains.

**MANY SEPARATION MECHANISMS**

The different interaction modes separate biologic drug substances from impurities through different binding mechanisms. “In all cases, resins are selected because of their ability to separate the target molecule from process- and product-related impurities,” Westman notes.

IEX resins, according to Bulpin, have ionic groups on their surfaces comprising weak or strong acids (cation exchangers) or bases (anion exchangers). These groups discriminate between different macromolecules (e.g., proteins, antibodies, etc.) due to the net charges of the target compounds. Salt, or a pH shift, is then used to disrupt the interactions and, if used for capture, to free the biologic drug substance from the resin. IEX resins are noted for their versatility and robustness and thus are applicable for most biologic separations.

IEX resins can be used for capture of biomolecules or polish, according to Parra, with these different chemistries used depending on the application. For example, she notes that strong CEX resins like sulfopropyl are suitable for mAb aggregate removal and charge variant separation, while strong AEX resins are suitable for mAb polish due to their ability to bind low pH impurities such as DNA, endotoxin, leached protein A, and viruses. “Different ion exchange chemistries can offer unique selectivity depending on the purification needs, so a variety of chemistries are tested based on the application,” Parra says.

Affinity resins such as protein A, on the other hand, separate biomolecules based on highly specific biological interactions, according to Westman. “More specifically, protein A resins have affinity ligands on their surfaces that have the appropriate shape and chemical substituents to allow for a three-dimensional steric fit with the Fc portion of antibodies,” says Bulpin. Within the main site of interaction, according to Deorkar, ionic charges exist for other interacting sites. “Similar reverse charges are present on the ligand as well, so they fit together like two pieces of a puzzle,” he explains.

The result, according to Westman, is high-purity recovery under generic conditions in just one separation step and enables a plug-and-play approach that leads to reduced process development times. In fact, according to Parra, highly selective affinity resins with antibody-like specificity can significantly improve a purification process making it simpler and more economical for a variety of molecules from mAbs, to recombinant proteins, to viruses.

Hydrophobic interaction resins separate molecules based on differences in hydrophobicity and are primarily used in high-salt conditions and for challenging resolutions, according to Westman. “HIC can be an orthogonal and powerful chromatography tool used in both bind/elute and flow-through modes of operation,” asserts Orjana Terova, product manager for purification with Thermo Fisher Scientific. In cases where the molecule is highly hydrophobic or contains a very hydrophobic conjugate, it can be difficult to elute even in low salt conditions. “Recently developed HIC resins from Thermo Fisher Scientific offer a differentiating range of selectivities by altering the hydrophobicity of the resin surface. These resins are appropriate for a wide variety of hydrophobic molecules while also addressing mAb aggregate removal in flow-through mode in low salt conditions,” she comments.

With mixed-mode resins, two different interactions occur. They can both be ionic interactions, or they may be ionic and hydrophobic.

**OPTIMIZING RESIN CHEMISTRIES**

“Resin development is basically driven by what is required at the end of a process—whether it is the ability to bind more protein, more separation in less
time, high selectivity, or low pressure,” says Deorkar. For high selectivity, the ligand interaction is changed, which is where engineered mixed-mode chemistry comes in. Increased binding capacity can be achieved by changing the linker chemistry on the surface of the polymer so the ligands can be spaced equally in an optimized way, allowing more molecules to be attached. The resin particles can also be optimized with respect to shape, size, and mechanical properties (i.e., compressible, non-compressible, can be crushed under pressure, etc.) to, for instance, achieve particles with high surface areas but that do not afford high pressures, according to Deorkar.

Process development scientists must also balance multiple factors when developing new processes to address current challenges and industry demands. “Capacity, resolution, and the speed at which the process can be run must all be simultaneously optimized,” asserts Parra. Process development scientists also need to consider the ability to achieve good capacity and separation under higher salt concentrations for added flexibility and process simplicity, according to Parra. “Using tangential flow filtration (TFF) between steps reduces product yield and lengthens process times. A salt-tolerant resin can be very beneficial to a purification process by decreasing the need for dilution or eliminating TFF steps all together,” she notes.

“Next-generation IEX resins that offer high capacity, high resolution, and salt tolerance at faster flow rates have significantly improved process flexibility and allow for easy adaptation to integrated processing,” Parra continues. “By increasing the throughput, process flexibility, and process performance, the costs associated with purification can be decreased and more productive processes can be realized,” she states.

Ion exchange resins that offer high capacities at high linear flow rates help to scale-up processes for the economic production of large-volume mAb drugs, says Bulpin. “The base beads of these materials exhibit good mechanical strength while maintaining high porosity,” he says. In addition, Bulpin notes that the three-dimensional presentation of the ligands in the open pore spaces of ion exchange ligands is important. “This effect can be achieved through the use of so-called tentacles, grafted linear chains presenting the ion exchange functions in a more flexible way that allows multi-point interactions with the proteins of interest,” he observes.

The past decade has, in fact, seen a steady increase in usage of multimodal chromatography resins, according to Westman. Because these resins separate molecules based on more than one mode of action, they are being used when other chromatography techniques do not give the desired selectivity. He adds that they are also commonly used in polishing steps during mAb purification.

Process development scientists must also balance multiple factors when developing new processes to address current challenges and industry demands.

Multimodal resins most often separate molecules based on ligand properties, but there are examples of multimodal resins for which the base matrix design contributes to the separation. “This resin chemistry is beneficial for the separation of large molecules, such as viruses, vaccines, and immunoglobulin M,” says Westman.

BIOSIMILARS AND OTHER CHALLENGES

The increased trend toward the development of two-step chromatography processes and higher protein doses is requiring more performance from chromatography products, according to Parra. “Process development scientists need new tools that will clear even more impurities in a single step and achieve even cleaner biologic drug substance,” she says.

Biosimilars also present chromatographic challenges. “For biosimilars, it is necessary to produce a comparable pattern of protein variants. That requires removal of closely related byproducts in proteins, such as charge or glycosylation variants,” Bulpin says. He adds that the drive away from bind/elute chromatography steps to more efficient flow-through steps is impacting resin development. Single-use chromatography is another challenging area. “To date it has been difficult to provide cost-effective devices that can also be scaled up,” notes Bulpin.

Purification processes for mAbs have improved significantly over the past 20 years, but Westman notes that shortcomings remain to be addressed. In particular, the binding capacity of Protein A resins is still lagging behind other chromatography techniques, such as IEX chromatography. “An increase in binding
capacity will be beneficial for process economy reasons when purifying steadily increasing upstream titers,” he states. Current protein A resins are also exposed to feeds with high concentrations of cell-culture nutrients yet are more sensitive to the high concentrations of sodium hydroxide needed for cleaning and sanitation, which increases the risk for bioburden issues, according to Westman. “We believe that both binding capacities and chemical resistance can be further improved for future protein A resins,” he observes.

GROWING DIVERSITY

The increased diversity of pipeline therapeutic molecules is a major challenge for chromatographic separation. “The development of novel scaffolds for therapeutic molecules (e.g., bispecific antibodies, antibody fragments, smaller protein drugs, and cell-based therapies) will have an impact on the development of new resins. There is a need for more economic downstream processes to ensure that modern therapeutic regimes can be offered to more patients worldwide,” observes Bulpin.

Adds Terova: “The landscape in molecules is changing with more difficult and unique modalities and therapies leading to new challenges for purification. As more and more challenging molecules are introduced into the industry pipeline, new purification tools are needed with unique functionality.”

The platform approach, which has been so successful for the development and manufacture of mAbs, cannot easily be applied for such diverse drug substances, according to Westman. As a result, each separate biomolecule may need extensive process development, which will impact both time and cost. “When a targeted, specific affinity purification solution does not exist for a biomolecule, the protein purification scheme can be very complex. As the number of required purification unit operations increases, the product yield decreases. Yield drives cost of goods (COG), so not having an affinity purification solution can greatly impact the COG for biotherapeutic manufacturing,” Terova explains.

With the increase in more challenging molecules and the desire to make processes more efficient, she adds that there has been a significant increase in interest for custom resins on high-performing beads. Most chromatography vendors have developed new affinity resins designed for use with the next-generation biologic products being developed today, including antibody fragments, bispecific antibodies, and antibody drug conjugates (ADC), as well as cell and gene therapies and vaccines.

To address the need for different selectivities, new modalities have to be developed that offer more than one mode of interaction, according to Bulpin. He adds that these interaction principles will not only be attached to chromatographic beads, but to a variety of different base matrices, such as adsorptive membranes, monolith, or fiber-based materials. The main driver for new matrices is the wish for pre-sterilized single-use materials that offer both process economy and performance.

As suppliers expand their portfolios of resins to enable efficient separations of newer biomolecules, they must also consider the potential to increase the column capacity, binding/separation capacity, resin lifecycle, and/or column cleaning efficiency, according to Deorkar. “In addition to these opportunities to optimize performance, it is also important to look at improving the overall ecosystem around the chromatography process, by considering the buffers, cleaning agents, etc., and how they all work together in tandem,” he comments. “Chromatography doesn’t work in isolation.”

CONTINUOUS IMPACT

Continuous manufacturing is an important topic for cost-efficient and fast production of protein drugs. “Continuous chromatography enables shorter bed heights with lower pressure flow constraints, enabling the use of smaller beads with higher binding capacities and better resolution,” notes Westman. By going to continuous chromatography, resin utilization and capacity and separation efficiency are increased by using smaller particle size resins and operating at slightly higher pressures in smaller columns, according to Deorkar. The higher particle surface areas result in greater efficiency.

The ultimate goal for continuous manufacturing, according to Bulpin, would be to purify the target protein in an all flow-through mode, binding only the impurities and eliminating the need to store the target protein in an intermediate buffer tank. “Given the interest in continuous processing, planning for use in a continuous chromatography process must be taken into account when developing purification tools,” Parra states. She does add, however, that many in industry feel there are enough good tools in the industry now to make continuous processing a reality.

Switching chromatography processes from batch to continuous isn’t a simple exercise, however. Deorkar believes that the best way to make improvements in chromatography processes is to first reduce the number of steps. A process with three or four chromatography steps will not be easy to move to a continuous process. “Multi-mode, mixed-mode resin chemistries that offer the potential to reduce the number of steps will, therefore, play an important role in facilitating the adoption of continuous chromatography,” he concludes.
Increased competition, the diversification of biologic drug substances, and globalization of manufacturing have accentuated the need for processes that are not only compatible with modular, flexible, smaller plants but also carry reduced total costs. Continuous and intensified processing is an attractive alternative that fits the needs of current and future manufacturing requirements.

Cell-culture media directly impact the performance of upstream biomanufacturing processes; they dictate the potential productivity and reproducibility of specific processes while also influencing product quality attributes, according to Andrew Bulpin, head of process solutions for MilliporeSigma. “Cell-culture media must be optimized for each process, particularly perfusion processes and fed-batch systems that support higher profile fed-batch processes designed to minimize cost and speed development,” he observes.

MEASURABLE MEDIA IMPACT

In addition to the cell line and cell-culture process parameters, cell-culture media is a key driver of bioproduction processes. Cell-culture media can have significant impacts on cell growth and viability and also drive protein production and protein quality, according to James Brooks, manager of worldwide media services and media development for BD.

While significant time and effort are invested in the generation of an optimal cell line—from transfection to isolation and cloning, to full characterization—it
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is often the case that little thought is given to selection of the optimal medium for the clone, according to Brooks. He also notes that suboptimal formulations have been shown to have deleterious effects on bioproduction processes and limit the potential of cell lines.

Cell-culture media provide not only nutrients to the cell, but stimulatory and regulatory effects. “Media can impact cellular signaling pathways and be involved in the regulation of enzymatic activity within the cell,” Brooks explains. For example, metals contained within media formulations, such as manganese, copper, and iron, can serve as co-factors for enzyme activation and play a key role in driving protein titer and protein quality. In addition, sufficient levels of amino acids are required for normal cell growth and function, but also for the production of the correct target protein,” Brooks says. “Limitations in the amino acid compositions in some chemically defined (CD) media formulations have been shown to result in amino acid substitutions and the generation of sequence variants of the target proteins,” he says.

**CUSTOMIZED MEDIA IS IMPORTANT**

The inherent diversity and complexity of animal cells limit the applicability of a universal cell culture media that can be used across all different production processes with optimal performance. Each cell line is unique, and to achieve its full potential, it is imperative to match an individual cell line with its full potential, it is imperative to achieve the optimal cell-culture medium that specifically meets its metabolic requirements.

For example, notes Brooks, from the isolation of Chinese hamster ovary (CHO) cells into culture in the 1950s, the cell line has diverged significantly, giving rise to a multitude of CHO subtypes and lines used for bioproduction today. “Each CHO subtype and each individual clone has its own specific nutritional and metabolic requirements. Through a medium optimization process, these requirements can be met, allowing the cell line to reach its full potential,” he explains.

“Customization of a cell-culture medium requires the right tools and a specialized team of experts that can guide clients in the right direction and/or modify an existing formulation or design a new one if necessary in a minimum amount of time,” states Bulpin.

**FACING THE CHALLENGES OF CHEMICALLY DEFINED MEDIA**

Companies have been moving toward the use of chemically defined media to avoid the potential contamination issues associated with animal-derived ingredients. They have been challenged, however, to achieve the increasingly higher volumetric productivity levels now expected for fed-batch systems, according to Bulpin. “Chemically defined media are not necessarily the optimal process solution,” Brooks agrees.

Nutritional limitations in chemically defined media formulations have been shown to impact protein titer and protein quality. Additionally, chemically defined does not mean chemically pure, according to Brooks. “Contaminants in CD media, such as trace elements present in vitamin stocks and some salts, have been repeatedly shown to impact cell-culture processes, particularly protein quality,” he says. Multiple companies have, in fact, published information where trace element contaminants in their CD formulations have impacted their monoclonal antibody (mAb) titers or glycosylation profiles, according to Brooks. “This issue is particularly pertinent in the development of biosimilar mAbs, where it is critical to match the quality parameters of the originator molecule,” he adds.

Bulpin notes that nutrients that were traditionally supplied with complex solutions such as hydrolysates need to be now supplied as individual chemically defined chemicals. Some of these nutrients, such as cysteine and tyrosine, have a maximum solubility and stability level below the concentration requirements for optimal performance. “In order to overcome this issue, companies choose different strategies; most commonly, feed solutions are divided in multiple parts, often with extreme pHs, and clients need to add them separately to the bioreactor, leading to potential unwanted pH shifts,” says Bulpin.

MilliporeSigma developed a single-feed concept in which the nutrients are concentrated to more than 130g/L, allowing a reduction of the volume of feed added to the medium and thereby increasing the volumetric productivity. The feed is added at neutral pH using a simple reconstitution protocol and high solubility, according to Bulpin. It also contains cysteine and tyrosine derivatives that have been shown to release free cysteine and free tyrosine slowly throughout the culture. “This time-dependent release avoids tyrosine depletion, which can lead to sequence variants. It also results in free cysteine release while maintaining a reduced redox environment, which has been related to higher cell growth and productivity,” Bulpin says.

Poloxamer-188, which has traditionally been available from only a single supplier, is a specific cell-culture ingredient that has caused difficulties in biopharmaceutical manufacturing in recent years. “Significant numbers of lots have resulted in toxic or low shear stress-protectant effects for cells,” Bulpin explains. MilliporeSigma
developed an analytical test that allows the identification of the desire variety of poloxamer-188 and is employed by the company to validate each lot of poloxamer-188 to be used in cell-culture media. In addition, MilliporeSigma worked intimately with an alternative manufacturer and has validated this producer as a second supplier for poloxamer-188, according to Bulpin.

**GROWING INTEREST IN PEPTONES**

The issues with chemically defined formulations are creating a renewed interest in peptone supplementation for cell-culture media across the biopharma industry, according to Brooks. “While chemically defined remains the ultimate desire, peptones continue to be used as cell-culture media supplements to enhance production and protein quality,” he says. “Peptones are well-characterized and long-established cell-culture supplements and feeds that can enhance cell growth and/or protein titer while helping maintain or achieve desired protein quality. The rich composition of peptones enables them to serve as an optimal nutritional source for cell culture. Peptones have also been shown to inhibit apoptosis and positively affect cell cycles,” Brooks adds.

**THE NEED FOR PERFUSION SOLUTIONS**

The continued move toward perfusion-based production has highlighted the need for more optimally designed formulations that can support the perfusion process. “It is a challenge to achieve steady-state, high-density cultures while maintaining consistent product quality. Most media formulations today are not designed for this application,” states Brooks. The overall cost of the base medium for perfusion processes is also a concern. With higher perfusion rates and medium exchange rates as frequent as 1–2 times per day, significant volumes of media can be consumed during a perfusion process. “It is therefore essential to have a medium that can achieve the desired growth, titer, and quality parameters within a cost-effective and manageable process,” Brooks asserts.

In response to this need, MilliporeSigma developed and recently introduced a medium specifically for use in perfusion processes. It is designed to reduce the medium exchanges per day in a perfusion process while maintaining or even increasing specific productivity and product quality, according to Bulpin.

**ACHIEVING ACCEPTABLE PROTEIN QUALITY**

MilliporeSigma expects biopharmaceutical industry needs for intensified fed-batch and perfusion processes and for high control over protein quality attributes to continue to increase, according to Bulpin.

Brooks agrees that protein quality continues to be a major concern and focus in the industry—particularly given the increasing numbers of biosimilars and biobetters in development—and must be considered when developing cell-culture medium formulations. “The best way to make sure the formulation in use provides acceptable protein quality, such as N-glycan or charge-variant profiles, is to optimize the formulation specifically to match the cell line metabolic requirements,” he says.

Protein quality supplements designed to modulate quality parameters following addition to existing cell-culture base media, are also beginning to emerge in the market, according to Brooks. “The most efficient way to achieve the desired protein quality parameters, however, is through the development and customization of the cell-culture medium to the specific cell line in use,” he stresses. ◆
Keeping it Clean: Biopharmaceutical Cleaning Validation

Caroline Hroncich

Industry experts weigh in on best practices, challenges, and mutual recognition of cleaning validation standards.

FDA and the European Medicines Agency (EMA) will begin recognizing each other’s cGMP inspections as early as November 2017, the agencies revealed in March 2017 (1). Since the announcement, there has been some discussion about how this will impact the standardization of cGMP procedures for bio/pharmaceuticals (2). Cleaning validation is an important part of GMPs, and experts have mixed opinions on whether this will affect existing standards. While there are some known differences on how cleaning limits are calculated between the United States and Europe (3), biopharmaceutical manufactures around the world face similar challenges when it comes to cleaning validation. To discuss some of these challenges, BioPharm International posed some questions to Karen Ginsbury, CEO, PCI Pharmaceutical Consulting Israel Ltd; Richard Forsyth, principal consultant with Forsyth Pharmaceutical Consulting; Richard Yeaton, president, Atlantic Technical and Validation; and George Verghese, director, Technical Service at STERIS Corporation.

COMMON CHALLENGES

BioPharm: What are some common challenges you see biopharma manufacturers facing when it comes to cleaning validation processes?

Ginsbury (PCI): The challenges for cleaning validation in biopharma processes are somewhat different from traditional validation of small-molecule chemical residues. In biopharma, the main issue...
Increase viable CHO cell density by supplementation with recombinant Insulin Human AF

CHO cells are one of the most widely used platforms for the production of biopharmaceuticals. Increased demand for safety and reliability has moved the standard for CHO cell culture media from Serum to Serum free and further on to chemically defined media. UAB in collaboration with Novo Nordisk Pharmatech (world’s largest supplier of recombinant insulin) has shown that addition of animal origin free insulin to three leading commercially available off-the-shelf chemically defined media resulted in significant increases in viable cell density. In addition to this benefit insulin has been proven to aid in the expression of difficult to express proteins.

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is microbiological contamination, in particular viral residues, and almost all of those can be overcome by using sodium hydroxide in the cleaning process. Therefore, the challenges that remain are:

• Reproducibility of manual cleaning of small parts and non-clean-in-place (CIP) systems.
• Effective design of fully automated CIP systems to avoid dead legs and lack of drainability.
• Build-up of physical protein residue on the walls of equipment cleaned by CIP. This process happens over time, and if there is no periodic manual scrub down, this can result in a thin layer over the inner surface of a vessel, which can be scraped off with your fingernail.
• Failure of CIP systems over time due to blocked steam traps, poor maintenance practices, or even replacement of a steam trap with a new one installed upside down.

I’ve been on the job for 30 years and seen some weird things. A word of caution—because using sodium hydroxide denatures the protein and therefore inactivates the pharmacological residue, you do not need to calculate a health-based exposure limit as required for small molecules in EMA’s 2014 guidance (4). In fact, the guide has a specific exclusion clause in section 5.3.

‘Therapeutic macromolecules and peptides are known to degrade and denature when exposed to pH extremes and/or heat, and may become pharmacologically inactive. The cleaning of biopharmaceutical manufacturing equipment is typically performed under conditions which expose equipment surfaces to pH extremes and/or heat, which would lead to the degradation and inactivation of protein-based products. In view of this, the determination of health-based exposure limits using PDE limits of the active and intact product may not be required. Where other potential routes of cross-contamination exist, the risks posed should be considered on a case-by-case basis’ (4).

Therefore, provided that you can demonstrate that you have degraded and denatured the protein to a pharmacologically inactive substance, there would be no need to calculate a PDE.

You still have to be sure that you remove the denatured protein residue and restore pH to neutral before the next cycle of manufacture but you do not need to calculate permitted daily exposures (PDE). I was recently in an inspection where neither my client nor the inspector was familiar with the clause. A polite intervention on my part saved the client from spending a lot of unnecessary resources on trying to calculate a PDE for a biopharma product.

Vergheese (STERIS): Some of the common challenges faced in biopharma include:

• Developing acceptance criteria and validation strategies incorporating degradation or deactivation of proteins and their breakdown products
• Transitioning from traditional approaches to health based limits when applicable
• Harmonizing cleaning process risk-assessment methodologies, considering their implementation requirements and cost
• Addressing air-liquid interface issues in bioreactors from a cleaning and validation perspective; ensuring a high-level of cleanliness to prevent potential for microbial excursions and biofilms; and determining the impact of rogue formation on surface cleanliness, acceptance criteria, and validation

Yeaton (Atlantic Validation): Keep things clean. Once you clean a vessel, FDA is going to expect you to have some data that says you can hold that vessel clean, and have it still be usable for some period of time—you have to have data to support that claim. So usually a client will pick a time period that they want to hold, and they’ve got to hold the clean tank one day beyond that, and then do some sort of bioburden analysis to show that tank is still meeting criteria. It’s surprising how short that time can be sometimes, especially if you’ve got a large vessel, where you can’t quite get it as dry as you would like. If you’ve got any residual moisture that will eventually start growing bioburden, you’ll find that you’re failing your bioburden requirement. That tells you just how long you can hold that tank. You can dry a vessel out by blowing clean compressed through it. Dry nitrogen is more expensive but more effective.

I’ve also noticed a lot of biopharma manufacturers want to establish a ‘dirty hold time.’ This means you don’t have to clean your tank immediately after it’s used to produce. You can wait some period of time and then clean it. I understand why production managers say they want that flexibility, a lot of times they’ll say they want to be able to hold a dirty tank over a three-day weekend and still be able to clean it effectively when people come back from the weekend. But I have found that getting your operations people to let you have that tank, three times for the three-day period plus the one day safety margin can be challenging. I’ve had operations managers say there’s no way I can give you my tank for that long, I need it too much. And then we have to do a little bit of negotiation, and say if you need it that much, why do you want to be able to hold it over a three-day weekend? And then we have a little heart-to-heart about what’s realistic. However, once you let organic materials dry on a stainless-steel surface, you’ve made that cleaning challenge much more difficult.

Dry proteins are very tenacious to wash off. Especially if you can’t have any mechanical scrubbing or mechanical moving of soil. I’ve convinced a number of my clients to proceduralize in their production
batch records that once production is done, it’s followed by a 5- or 10-minute high-purity water flush, just to get the majority of your stuff off the surface of the stainless steel. You’re not going to get it all, but it’ll get a good 80% of your materials off your vessel until you start your official dirty hold time start. Now the downside to that is, if you do that, now you’re committed to do that every time. Even if you’re going to be cleaning your tank immediately after use you still have to do that 10-minute high purity water flush. The way I look at it, 10 minutes is okay if that will reduce your CIP cycle from eight hours down to two hours.

**Forsyth (Forsyth Pharmaceutical Consulting):** Some challenges include:

- Calculating cleaning limits based on PDE/accepted daily exposures (ADE)
- Ability to denature/degrade products as part of the cleaning process and how to apply that to the cleaning limits
- Well-controlled cleaning procedures.

**BEST PRACTICES FOR PROCESS CONSISTENCY**

**BioPharm:** What are some best practices to ensure cleaning process consistency for biologic drugs?

**Forsyth (Forsyth Pharmaceutical Consulting):** Make sure all cleaning procedures have sufficient detail and training to ensure consistent execution and periodic monitoring of cleaning execution. It is also important to assure personnel are following the cleaning procedures.

**Yeaton (Atlantic Validation):** I recommend that my clients use both a caustic and acid cleaning agent. The accepted conventional wisdom that is your caustic cleaning agent goes after your protein, and your acid cleaning agent goes after more polar things, salts. You usually don’t have that kind of a cleaning challenge for salts; they’re usually very water soluble. The reason why I like to use both caustic and acid cleaning agents is when you go with your caustic wash followed by some sort of intermediate rinse, then you do an acid flush, you’re bouncing your pHs around so much you’re effectively driving your bioburden down to a very low level. There are microbes that don’t like low pHs but they like high pHs, or vice versa, I’m not a micro person but I’m not aware of any microbes that can handle both ranges of pH.

**Ginsbury (PCI):** A well-designed, validated, and well-maintained CIP system is really the only way to ensure a reproducible cleaning process. I advise the use of disposables whenever possible, and today there are single-use systems available which can negate the need for cleaning at all. I have seen companies implement a serious risk-based approach to turning a facility from dedicated usage to multi-product using single-use systems so that no product contact parts are multi-use.

If using manual cleaning, you need to have a much more robust cleaning process to allow for variability in application of the cleaning between different operators on different days. Personally, I am not convinced that you can have a reproducible manual process because there is always the possibility that an operator will fail to clean an item at all, or skip over a step in the process, use less water or cleaning agent etc., and it is difficult to control.

**Verghese (STERIS):** Best practices include designing and qualifying an effective and robust cleaning process based on risk assessment, understanding the relationship between cleaning process parameters and quality attributes, reducing process variability/spread to help achieve optimization, maintaining continuous process verification, and establishing appropriate change control procedures to address changes in both the manufacturing and cleaning process.

**BioPharm:** Are there any new technological advancements available to improve cleaning validation processes for biologics? What kinds of improvements can be made in the future?

**Verghese (STERIS):** Improvements in the future include advanced inline and online analytical methods for quick and direct surface residue detection and quantification.

**Ginsbury (PCI):** I think the design of CIP systems are constantly improving. There is continuous monitoring of critical process parameters in CIP: temperature, contact time, water pressure, cleaning agent concentration etc., which ensure the cycle was completed as planned. I am not aware of major breakthroughs in new technologies in this area, and I suspect that we will see increasing use of disposables/single-use systems and reduction in cleaning, which is ecologically sound because of the amounts of water and environmentally unfriendly cleaning agents. Of course, you can counter that the single-use systems are not good for the environment either, but overall my impression is that they are the lesser evil.

**Yeaton (Atlantic Validation):** Well there are some technologies that are still pretty new. There are some technologies getting close to being able to complete real-time endotoxin tests and bioburden tests that use fluorescents with DNA to take bioburden, things like that. I don't know if they’re 100% ready for prime time yet, but they’re getting close. I think anything that can be done to minimize the wait time for either doing bioburden testing or endotoxin testing, is worth it. The closer you can get to a real-time release of a clean vessel and say I have data that I can show to an inspector that shows that I’m confident my vessel is clean—instead of waiting a week for your incubation to come back to say my bioburden is good, or a couple days for someone to run an
Cleaning Validation

endo test back in the lab—the better. The closer you can get to real-time results, or close to near to real-time results, the smoother things go and the more dependable that is an inspection. Because you can say that if for some reason something upsets my cleaning process, I will know just in time to run an extra cycle instead of having to go back and quarantine whatever was produced with equipment that might not have been clean enough. The closer you get to a real-time release of clean equipment, the happier I think you’re going to be. I think it’s well worth the capital expense to purchase this equipment. Each company is going to have to evaluate that equipment to see if they’re ready for it. It’s evolving, every year I go to these product shows and I see more stuff out there that’s getting more and more robust and rugged. It’s definitely worth investigating. Part of the problem is that most GMP companies don’t want to be the first one to convince the FDA that their new technology works. Most companies want to be the second or third ones.

FDA does not seem to have adopted it or at least has been quiet as to whether they do or don’t agree with the approach. And as mentioned above, EMA has excluded therapeutic proteins and macromolecules from their approach anyway. I suspect that, as has been the case to-date, cleaning validation in biopharma where multi-use equipment is in place will continue to focus on microbiological risks and validation of viruses ... as indeed it should.

**Verghese (STERIS):** Hopefully it will reduce the number of inspections and result in gained efficiencies for companies as well as the regulatory agencies. Further harmonization in regulations, guidance documents, inspector expectations, and industry practices may be helpful. For example, in the context of setting health based limits, various practices are followed such as the International Society for Pharmaceutical Engineers Risk Management approach of using ADE more common in the United States, EMA’s PDE, and the traditional approach. The recent draft Q&A on health-based exposure limits from the EMA is a step in the right direction (5).

** Yeaton (Atlantic Validation): I have heard an FDA inspector give a presentation on that [during] a [Parenteral Drug Association] chapter meeting a couple of months ago. They really are looking for a way that they can say, well if the EMA inspected these people, we can trust their results. So yes, they are serious about that. I would say the Europeans, the Americans, and the Japanese are pretty consistent with each other. Now human nature being what it is, yes we have harmonization, but you do get a certain amount of ‘we’re different, we’re better’ on the part of the inspectors. Whether it’s the Americans looking at the Europeans, or the Europeans looking at the Americans, but yeah, they do want to be able to trust each other. The catch is going to be some of the more third-world countries. And this inspector was very frank, that they’re concerned about corruption, they’re concerned about favoritism where some regulatory agent is going to look more favorably on a native company than a foreign company. They have a long way to go before they can really trust some of these other inspection agencies.

I think you’ll see that phased in gradually, where FDA may start saying if I can review the EMA results, we either may not have to go inspect or we may have an abbreviated inspection. The other side of that is EMA may come in and say we’ve looked at the inspection report from FDA and so we want to focus on the same issues FDA had. That cuts both ways. If you had a problem in an inspection with FDA don’t be surprised when EMA asks you about the very same thing, and you better show that you’ve been making progress with that.

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Investigations-focused regulatory standards that apply to life-sciences organizations, such as FDA 21 Code of Federal Regulations (CFR) 211.192, mandate that all deviations from written procedures must be thoroughly investigated and that the results of those investigations must be adequately documented. When life-sciences companies are unable to identify potential root causes and subsequently keep sufficient records of those events, their internal investigations are deemed incomplete by regulatory agencies.

A recent high-profile example of this occurred when FDA noted during a 2016 inspection that Busch & Lomb's “written records of investigations into the unexplained discrepancies do not include the conclusions and follow-up” regarding the company's investigations into its non-viable particle testing for aseptic filling lines (1). This investigational infraction, which resulted in the issuance of a Form 483 Investigational Observation from FDA, serves as an illustration that if a company does not perform its quality due diligence in the investigation of these types of events, such oversights could have long-lasting damage on a company's reputation and its branded products, not to mention potentially catastrophic results for consumers.

**THE ROOT OF THE PROBLEM**

The companies cited for these investigational failures are not alone. Historically, corrective actions and preventive actions (CAPAs) have been over-

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**Avoiding Investigational Failures and Discrepancies**

Walt Murray and James Jardine

Investigational failures and discrepancies can be avoided through the proper execution and documentation of investigations.

Walt Murray is a compliance and risk consultant with ARC Experts. James Jardine is a marketing content specialist for MasterControl.
used industry-wide as a part of the investigation process within the overall quality event management scheme. Even industry leaders have found themselves suffering from “death by CAPA” after cramming every quality event that occurs into their CAPA systems. It is becoming more evident that the key to effective and efficient investigations is to eliminate quality events as issues before escalating them from a correction to a corrective action. For this reason, the International Organization for Standardization (ISO) 9001 revised quality management standard has eliminated the term CAPA from its parlance and has now outlines separate processes for preventing action (PA) and nonconformance/corrective action (NCCA).

As referenced in FDA’s Quality Systems Approach to Pharmaceutical CGMP Regulations guidance, an “investigation” refers to the formal procedural process evaluation of a problem resolution (2). The Form 483 Investigational Observations issued most commonly for poor or ineffective resolutions are due to a poor or nonexistent investigation process by the offending organization. All the data in the world won’t do any good if the process is inherently bad. By very definition, an investigation is the formal gathering of the factual boundaries of a problem or issue with the intention of uncovering clues which, by experiential treatment, are the source of generating the root cause (see Figure 1).

It should be noted that this investigational process demands the validation of action(s) to eliminate recurrence or proof that a plan of action will effectively address the resolution of an event. An action is not corrective if it cannot be proven to work on a repeatable basis and resolve the issue.

Finding Out What You Don’t Know That You Don’t Know

When done correctly and according to an established, proven process, an investigation can be as straightforward as following a road map. But, just like following a map, if you don’t know where you are, you won’t be able to figure out where you’re going. Figure 2 below illustrates how a closed-loop quality event management system can act as a road map for investigations.

An investigation equation has three essential and interdependent components: clarification, decision making, and plan analysis.

Clarification. An investigation should start by thoroughly examining the input to clearly identify each respective issue of the event so that it may be completely addressed. These inputs come in the form of quality events such as customer complaints, deviations, nonconformances, out-of-specification incidents, and audits.

Decision making. The next potentially hazardous step where organizations can go wrong in their investigations is the decision-making phase where data are gathered and assessed. In this risk-based issue review phase an incident can be corrected or mitigated and closed, or a CAPA can be initiated, depending on what was discovered during the review. Each issue must be assessed for impact (negligible, minor, important, or critical) and frequency (rarely, frequently, or occasionally). Some issues may not be contained at the department level and must be
escalated into a formal CAPA process. Different situations require different actions and, that being the case, the impact and frequency of the quality event can dictate what actions should be taken. A correction, for example, is ostensibly an immediate fix that can be made to eliminate an existing undesirable situation. For issues that can’t be resolved quickly, however, the company may carry out a series of corrective steps to determine the root cause(s) and prevent the reoccurrence of the problem(s).

**Plan analysis.** At the completion of the issue review phase, a documented plan analysis of the implementation is required, which gets back to the concept of knowing where one is in order to determine where one needs to be. The initiation of a CAPA marks the beginning of the third stage of a closed-loop quality event management system. The process of establishing corrective actions and preventive actions and effectiveness checks continues through this phase. It is during this investigational phase where root causes are conclusively determined, actions are implemented, and the event achieves closure once appropriate effectiveness checks have been finalized. Tracking and documenting these actions during this analysis phase is crucial to maintaining regulatory compliance.

Be aware, however, of an investigational truism that many working in regulatory environments have learned the hard way: for every solution, there is a cost. As such, several questions need to be taken into consideration when selecting the best action for eliminating a root cause:

- Are multiple components needed to achieve complete removal of the problem?
- Will the solution completely remove the root cause?
- Is there a possibility that the solution will cause other problems?
  Once these questions have been answered, the best course for resolving the quality event comes into focus.

During the course of the investigation process, the guiding principle behind all efforts should be to find out exactly what you don’t know that you don’t know. Otherwise, the bulk of your efforts will just be guesswork.

**EQMS: THE KEY TO AVOIDING INVESTIGATIONAL FAILURES AND DISCREPANCIES**

An organization’s ability to integrate data and documentation from investigations with the rest of the company’s overall quality system is the foundational element of efficient quality management. Life-sciences companies can dramatically simplify their investigations and documentation of quality events by implementing an enterprise quality management system (EQMS) software solution that automates the handling of every phase of the quality event investigation process, from initiation, investigation, and all the way through to closure. An EQMS software solution that provides forms and workflow routes optimized according to industry best practices can provide a repeatable methodology for guiding quality teams through every step of quality event evaluations and CAPA implementation (i.e., identification of the problem, investigation of root cause, correction of problem, and prevention of recurrence). Such systems can be invaluable tools for implementing and sustaining dependable, repeatable, and compliant closed-loop investigation management protocols. Some robust customizable EQMS solutions even include functionality that automatically handles quality events in accordance with scalable preconfigurations and triggers CAPAs whenever established thresholds are met.

Other investigation-centered benefits of EQMS software solutions include:

- Automated routing, notification, delivery, escalation, and approval of CAPAs and all related documentation
- A central, secure repository for all documents pertaining to investigations
- Connections to all departments, product lifecycles, and quality processes
- Reduces duplicated efforts and data entry errors
- Simplified tracking, trending, and report creation
- Optimized quality processes and streamlined implementation of preventive measures.

**CONCLUSION**

Investigational failures and discrepancies can be avoided through the proper execution and documentation of investigations conducted by organizations that have the capability to securely maintain written records of their investigations within a central, audit-ready document management system. An EQMS can be integral to a life-science company’s ability to conduct and document investigations that meet even the stringent quality standards of global regulatory agencies.

**REFERENCES**


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Successful pharmaceutical manufacturing strategies depend on raw materials that are of good quality. While much effort is spent to develop active ingredient specifications, excipients and other non-active raw materials are often taken for granted. This is especially true in biological manufacturing, in which a variety of raw materials may be used in a single process. Some materials can increase the risk of introducing adventitious agents into the process and final product. Thus, raw material qualification is extremely important in ensuring process control and final product quality. Qualification programs rely on risk assessment strategies that may include testing raw materials before they are introduced into manufacturing.

This article describes some of the challenges involved in qualifying raw materials for use in biologics production, and how quality standards may evolve to support biologics manufacturing. The International Council for Harmonization’s (ICH) Q7 guidelines define raw materials as “starting materials, reagents, and solvents intended for use in the production of intermediates or APIs.” With biopharmaceuticals, however, this definition can be expanded to include other materials that are added to the manufacturing process, or that may come in contact with the active ingredient, including process materials such as buffer and media, selection devices, ancillary materials, packaging materials, and excipients. Each of these types of materials...
must be qualified before manufac-
turing can safely begin.

The risk-based approach will depend on the potential for raw materials to remain present, at trace levels, in finished product.

Quality by design (QbD) principles call for the use of systematic approaches to development, starting with predefined objectives and an emphasis on product and process understanding and process control. This approach underscores the importance of raw materials and all the components of the manufacturing processes.

In addition to regulations and guidances, standards developed by the United States Pharmacopeia and other pharmacopeias can help biopharmaceutical manufacturers meet requirements.

The use of raw materials that have been manufactured in a GMP-compliant environment provides assurance that the processes used to make the materials are reproducible and that the quality of final products is controlled. In such cases, changes to the process cannot be implemented without assessing their impact on the finished product.

When raw materials are procured from commercial, non-GMP compliant sources, however, the risks of manufacturing failure increase. In this type of situation, raw material specifications may change without an assessment of their impact on finished product quality. In addition, specifications for non-GMP-compliant raw materials are often wider than would be acceptable in a GMP-compliant environment.

QUALIFICATION PROGRAMS

It is important to use formal programs to qualify the raw materials used in biopharmaceutical manufacturing to ensure the safety of finished products. For biologics, raw material qualification can be a lengthy, multi-step process. When choosing a supplier, finished drug manufacturers should consider the type of quality systems in place at the supplier's manufacturing facility, the possibility of referencing a drug master file (DMF), and the supplier's financial stability.

The technical information (e.g., certificate of analysis [CoA]) obtained from the supplier provides insight into specifications for these materials, which may need to be considered when evaluating their potential impact on the quality attributes of finished products. But the CoA alone may not be sufficient.

RISK-BASED ASSESSMENT

Risk-based assessment can be used to determine the criticality of the raw material. The level of risk will determine the level of testing required, beyond the information found in the CoA.

Generally, a raw material that is used in large quantities in the downstream process will pose a higher level of risk than one that is used in small amounts in an upstream process. Similarly, a material that is used in steps of the process with long holding times will pose a greater risk than one used where there are shorter holding times.

The use of animal- or human-derived materials presents the additional risk of introducing adventitious agents or communicable diseases into the process. When these materials are used in a process, screening for potential contaminants is a crucial element of the control strategy. The risk-based approach will depend on the potential for raw materials to remain present, at trace levels, in finished product. It is also important to study the impact of the raw material on the development and manufacture of biological drug substances, as per ICH Q11 (2).

ANCILLARY MATERIALS

USP <1043> Ancillary Materials for Cell, Gene and Tissue-Engineered Products, provides guidance for assessing the risk, and qualifying the use of materials used to manufacture cell, gene, and tissue-engineered products.

However, the principles described in <1043> may be extended to raw materials used in biomanufacturing in general. In <1043>, raw materials are grouped in four different tiers, depending on the level of risk they present, and guidance is offered on how to reduce risk so that these materials can safely be used in biological manufacturing.

The risk-based categorization takes into account the initial intended use of these materials (e.g., pharmaceutical grade material is considered low risk, while research-grade material would be high risk). Also considered are:

- The amount of material
- The stage at which it is used
- Its potential to remain in the finished product.

Table I shows the criteria that define the level of risk for these four tiers of raw materials used in the manufacturing of cell therapy products and biological manufacturing in general.

Building on USP <1043>, USP began to work with expert com-
mittees on documentary standards, including test procedures and acceptance criteria, for some of the critical materials that are used in the manufacturing of biological products, such as cell therapies. These efforts also resulted in reference standards that support the test. An example of this work, and the hierarchy of approach, is presented in Figure 1.

These reference standards may be used as calibrators as well as critical materials to develop residual testing for these materials and control the level of their removal from finished therapeutic products. Standards for the raw and ancillary materials used in pharmaceutical and biopharmaceutical manufacturing have always been included in United States Pharmacopeia-National Formulary (USP-NF), in the form of monographs and supported by reference standards. Examples of these standards include some for inorganic salts, vitamins, amino acids, carbohydrates, and other buffers and components of raw materials.

The test procedures and associated reference standards for these materials may address their quality as excipients and when they are used as raw materials in a manufacturing process. If they are used as other components in manufacturing, however, additional work may be required to demonstrate that these tests adequately address potential questions about the material’s quality, in its new use.

### RAW MATERIALS: COMPENDIAL USE AND BEYOND

Risk-assessment strategies are key to successful manufacturing processes; they help set proper specifications for raw materials and ensure that adequate testing approaches are in place. Developing test methods to verify the identity and quality of materials used in biopharmaceutical manufacturing can help prevent the use of unsuitable raw materials, providing a solid foundation for a successful process.

Pharmacopeial standards provide valuable tools for users of these materials. Standards can save them the time and expense that would be required if they were to develop and validate test methods themselves.

The reference standards, when they are available, serve as calibrators or comparators to ensure users that the material can pass the required tests. Suppliers may

---

**Table 1. Levels of risk.**

<table>
<thead>
<tr>
<th>Level of Risk</th>
<th>Definitions and criteria that define the level of risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1: Low risk</td>
<td>Intended for use as licensed drugs, biologics, or medical devices. Suitability for use as a manufacturing component is required because the formulation, stability profile, and other quality aspects of these materials may change once the material has been introduced in the manufacturing process.</td>
</tr>
<tr>
<td>Tier 2: Low risk</td>
<td>Intended to be used as ancillary materials. These materials are well-characterized and produced under quality systems well-suited for biological manufacturing, but the materials are not licensed medical products. Many are produced specifically for use in the manufacture of biological products.</td>
</tr>
<tr>
<td>Tier 3: Moderate risk</td>
<td>Research-grade materials not intended for use in biological manufacturing. Sometimes, these products are approved by regulatory agencies as part of an in vitro diagnostic device. Tier 3 requires more qualification than Tier 1 or Tier 2 materials.</td>
</tr>
<tr>
<td>Tier 4: High risk</td>
<td>Materials produced as industrial or research-grade materials and may contain harmful impurities. They may also contain animal- or human-derived components with potential contaminants. This tier requires extensive qualification before use as component in biological product manufacturing.</td>
</tr>
</tbody>
</table>

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**Figure 1. Standards for raw and ancillary materials: from general to specific.**
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Raw Material Quality

declare their raw materials to be compliant with USP standards when they meet compendial analytical specifications.

Raw materials that have been processed under GMP conditions and meet compendial monographs have established some base level of quality. However, their suitability for use in the process must still be proven. For example, pharmaceutical-grade human serum albumin is available for use as a cell culture supplement, and its quality can be tested against existing monographs. However, in order to establish its quality, the impact of the albumin’s lot-to-lot variability on cell growth, its stability in the process, and possible interactions with other processing components must also be assessed.

Numerous regulatory guidelines spell out the need for raw materials to be included in manufacturing strategies. However, these guidelines don’t describe specifically how to do this.

Pharmacopeial standards can be used to fill this gap as they can provide tools for compliance. Reference standards can be used as calibrators to ensure that the materials are used consistently, meet the same specifications and, subsequently, will be translated into consistent manufacturing.

Additionally, the standards can be used to measure residual amounts of materials, especially with the increased risk of raw material components remaining in the finished dosage form. In USP-NF, the procedures that support the use of such standards are prescribed either in general chapters or in monographs.

The use of raw materials in multiple sites adds complexity to risk and quality assessment. Common quality attributes must be used for materials that are used by the same organization to manufacture the same products in multiple sites.

Some of the issues that manufacturers need to address relate to the transfer of processes from site to site. It is important to determine the best type of comparability testing to use, since testing will be required to ensure that the quality of the raw materials does not have an impact on the quality of finished products. The use of pharmacopeial standards can help mitigate the risks associated with manufacturing products in multiple sites.

Use of raw materials at multiple sites adds complexity to risk and quality assessment.

FUTURE DIRECTIONS

USP’s approach to setting standards for biologics is evolving, as it focuses on developing standards for some of the critical raw materials used in biomanufacturing, in addition to developing system suitability and performance standards that can be used to demonstrate method and process performance. As it evaluates the type of raw materials to be included in this strategy, USP is considering different types of standards that can help industry establish consistency in bioprocesses. For example, it is developing reference standards, without documentary standards, when a well-characterized material can be used as a standard against which raw materials suppliers or end users can calibrate their batches. Situations may arise in which the reference standard has a value only when procedures and acceptance criteria are available; in this case, USP will develop a documentary standard into a monograph or a chapter.

USP’s Biologics program is committed to using the information and data gathered during the evaluation of these materials to share best practices and lessons learned, either in white papers, journal publications, or USP informational chapters. Collectively, these standards will provide measurement tools to address the chemical identity of raw materials, characterization of some impurities, and the assessment of trace levels of chemical or elemental impurities that should not be present.

Critical materials that will be assessed as part of this new standard-setting initiative will include:

- Cell culture media
- Enzymes used in bioprocesses
- Complex extracts used as cell-culture supplements (e.g. protein hydrolysates)
- Polymers used in therapeutic protein-polymer conjugates

Additional market research and analysis will be conducted to develop a comprehensive list of raw materials to include in this strategy. USP welcomes feedback and recommendations on the types of standards that are most needed for these materials.

REFERENCES

Analytical Strategy in the Development of Biosimilars

Mario DiPaola

The author outlines an analytical strategy for establishing similarity in biosimilar development and approval.

In 2016, FDA announced its first three approvals of biosimilar products: Zorxio (filgrastim) and Erelzi (etanercept), made by Sandoz; and Inflectra (infliximab), made by Celltrion/Hospira (Pfizer) (1). While three approvals may not sound impressive, these approvals establish a clear path by which biosimilars can reach the United States market.

Since May 2016, applications for approval of biosimilars have been filed by Samsung Bioepis/Merck for infliximab; Coherus Biosciences for pegfilgrastim; Merck/Samsung Bioepis for insulin glargine; and Mylan/Biocon for trastuzumab. Four other filings have either not received FDA action yet or not received FDA complete response letters (1).

According to one publication (2), there are more than 1200 biosimilars in development against 16 major targets for potential US and global commercialization. The foundation for establishing similarity between a reference product and a biosimilar is a robust and sound analytical strategy demonstrating that the two products have similar primary, secondary, and tertiary structures, and similar functional and biological properties. This paper outlines some current methodologies and technologies available to develop a solid analytical strategy for use toward biosimilar development and approval.

ESTABLISHING BIOSIMILARITY

In the very early stages of biosimilar development, it is important to carefully analyze a series of lots of the orig-

Mario DiPaola is senior scientific director at Charles River Laboratories in Woburn, MA.
instructor product to determine the protein sequence, identify and quantify enzymatic and non-enzymatic post-translational modification (PTM), analyze biological functionality, and establish variability in product quality attributes against which the biosimilar product in development will be measured. Once lots of the biosimilar product become available, these lots should be tested against the reference product for physical attributes; primary, secondary, and tertiary structural properties; purity and presence of impurities, including those related to the product and its manufacture; and biological activity, all using orthogonal analytical methods that have sufficient sensitivity. It is important to recognize that product quality attributes should be ranked in criticality from critical to less critical and noncritical. For the critical quality attributes, it is expected that statistical equivalence be demonstrated between originator and biosimilar products. For less critical attributes, comparability should be based on reference product variability, typically based on the average ± 2 to 3 standard deviations; for noncritical product attributes, a graphical comparison will suffice for comparability. A comprehensive analytical characterization package that should be considered for comparison of biosimilar and originator products is provided in Table 1.

From the series of analytical methods listed in Table 1, one of the least complex tests to perform is intact mass analysis using a high-resolution mass spectrometer, preferably with electrospray ionization. Results from this method can reveal key product attributes, including the integrity of the molecule, post-translational modifications, and other types of protein modifications that may be present.

An example of the output from this type of analysis, often referred to as top-down mass spectrometry, is provided in Figure 1. Results shown in this figure were obtained by electron-transfer dissociation (ETD) rather than collision-induced dissociation (CID) in MS/MS. From the observed molecular weights in comparison to the theoretical one, one can easily conclude that major glycan species associated with this antibody are G0F (148083.0 Da) and G1F (148243.6 Da). Furthermore, there are species, including one with a mass of 146637.7 Da, corresponding to antibody with a single glycan substitution. More detailed analysis of the data has also revealed that a large fraction of the detected species lack the C-terminal lysine on the heavy chain.

PEPTIDE MAPPING

While intact molecular mass data can be informative, more detailed chemical information is typically extracted from a well-resolved peptide map of the protein, especially when analyzed by liquid chromatography tandem–mass spectrometry (LC–MS/MS). The mirror-image peptide maps, shown in Figure 2, are derived from the tryptic digest of adalimumab, followed by analysis by reverse-phase high-performance liquid chromatography (RP–HPLC) with detection at 210 nm and tandem mass spectrometry (MS/MS).

Not only can the analysis by peptide mapping with LC–MS/MS confirm the protein sequence, it can also identify and locate within the primary structure of the molecule any post-translational modifications, including enzymatic ones such as N- and O-linked glycosylation, phosphorylation, and other common modifications; and non-enzymatic modifications including oxidation, deamidation, N-terminal cyclization, etc. In addition, by running maps with and without reduction, in most cases, it is possible to assign cysteines involved in the formation of disulfide linkages. Fragmentation by electron-transfer dissociation (ETD) rather than collision-induced dissociation (CID) in MS/MS can further facilitate the localization of post-translational modification and disulfide bonds.

Visual comparison of the two maps shown in Figure 2 indicates that the two molecules are quite similar overall in primary structure. Upon closer inspection, however, there is evidence of differences in height for some of the peptide peaks and the presence or absence of some of the low-intensity peaks. These differences typically should be closely scrutinized to better understand the types of molecular changes that would trigger changes in peptide maps, their significance, and if they could affect the efficacy and safety of the product.

GLYCAN CHARACTERIZATION

Glycosylation can potentially impact not only the biological activity of the glycoprotein, but also the product circulation half-life and can render the protein immunogenic. Beyond establishing the location of glycosylation onto the glycoprotein, it is important to fully characterize the glycans. To do this, N-linked glycans can be simply released enzymatically and then derivatized for analysis by chromatography with fluorescence detection and LC–MS/MS for structural and linkage confirmation. O-linked glycans are released chemically by beta elimination and then permethylated before analysis by LC–MS. The glycosylation comparability between an originator antibody and a biosimilar is shown in Figure 3, with fluorescence profiles displayed as mirror images. The profiles are quite comparable in that the major species are G0F and G1F, but there are minor differences with respect to the observed lower-abundance glycan species. Interestingly, in this case, the originator molecule
High-Resolution SPR Surrogate Potency Assay to Facilitate Comparability and Biosimilar Studies

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EVENT OVERVIEW:
Biotherapeutic drugs, such as monoclonal antibodies, are large, complex biomolecules whose structural features can impact their biological activity. Several molecular interactions contribute to antibody function and an array of potency assays is needed to measure and monitor critical quality attributes throughout the characterization process. Join Mr. Robert Karlsson as he uses Biacore systems to describe the combined use of dose response curves and direct comparison of sensorgrams to ensure unambiguous determination of relative potencies and to measure antigen and receptor binding in a single surface plasmon resonance (SPR) assay.

- Learn how SPR can measure not only antibody-target binding but also antibody interactions with Fcγ or FcRn receptors.

- Discover ways that Biacore assays can be simplified and used as surrogate potency assays to ensure comparability and product consistency.

- See how calibration-free concentration analysis can bring new perspectives to determining antibody potency.

The webcast will be followed by a live Q&A session.

Who Should Attend
- Scientists performing drug product characterization, process optimization, investigational studies, and release testing.

For questions, contact Ethan Castillo at ethan.castillo@ubm.com
### Table I. Suggested analytical approaches for establishing biosimilarity.

<table>
<thead>
<tr>
<th>Quality attribute</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary structure</strong></td>
<td>• Intact mass by electrospray ionization–mass spectrometry (ESI–MS)</td>
</tr>
<tr>
<td></td>
<td>• Peptide mapping by liquid chromatography–mass spectrometry (LC–MS) and MS/MS</td>
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<tr>
<td></td>
<td>• N-terminal sequence by automated Edman degradation</td>
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<tr>
<td></td>
<td>• Post-translational modifications</td>
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<td></td>
<td>o Glycosylation</td>
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<td></td>
<td>o Disulfides</td>
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<td></td>
<td>o Phosphorylation</td>
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<td></td>
<td>o Lipidation</td>
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<tr>
<td><strong>Bioactivity (potency)</strong></td>
<td>• Cell uptake</td>
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<td></td>
<td>• Cell proliferation</td>
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<tr>
<td></td>
<td>• Cytotoxicity</td>
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<td></td>
<td>o Antibody-dependent cell-mediated cytotoxicity (ADCC)</td>
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<td></td>
<td>o Antibody-dependent cellular phagocytosis (ADCP)</td>
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<td></td>
<td>o Complement-dependent cytotoxicity (CDC)</td>
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<tr>
<td><strong>Target binding</strong></td>
<td>• Radioligand binding</td>
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<tr>
<td></td>
<td>• Binding analysis by surface plasmon resonance</td>
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<tr>
<td></td>
<td>• Binding analysis by fluorescence anisotropy</td>
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<tr>
<td></td>
<td>• Immunoassays</td>
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<tr>
<td></td>
<td>• Radioimmunoassay (RIA)</td>
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<tr>
<td><strong>Strength (protein concentration/content</strong></td>
<td>• Absorbance at 280nm</td>
</tr>
<tr>
<td></td>
<td>• Colorimetric assays</td>
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<tr>
<td></td>
<td>• Chromatography (reverse-phase high-performance liquid chromatography [RP-HPLC], size-exclusion chromatography [SEC] etc…)</td>
</tr>
<tr>
<td><strong>High molecular weight species/aggregates</strong></td>
<td>• SEC–HPLC</td>
</tr>
<tr>
<td></td>
<td>• Size-exclusion chromatography–multi-angle light scattering (SEC–MALS)</td>
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<tr>
<td></td>
<td>• Native MS</td>
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<tr>
<td></td>
<td>• Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), (reduced and non-reduced)</td>
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<tr>
<td></td>
<td>• Capillary electrophoresis (CE)–SDS (reduced and non-reduced)</td>
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<tr>
<td></td>
<td>• Analytical ultracentrifugation</td>
</tr>
<tr>
<td></td>
<td>• Field-flow fractionation</td>
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<tr>
<td></td>
<td>• Dynamic light scattering</td>
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<tr>
<td><strong>High-order structures</strong></td>
<td>• Far and near ultraviolet (UV) circular dichroism</td>
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<tr>
<td></td>
<td>• Fourier-transform infrared spectroscopy</td>
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<td>• Antibody array</td>
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<td>• Intrinsic/extrinsic fluorescence</td>
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<td>• Differential scanning calorimetry</td>
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<td>• Hydrogen/deuterium exchange MS</td>
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<td></td>
<td>• Ion-mobility MS</td>
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<tr>
<td></td>
<td>• 1D and 2D nuclear magnetic resonance (NMR)</td>
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<td></td>
<td>• X-ray crystallography</td>
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<tr>
<td><strong>Charge distribution</strong></td>
<td>• IEF</td>
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<td></td>
<td>• cIEF</td>
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<tr>
<td></td>
<td>• Ion exchange chromatography</td>
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<tr>
<td><strong>Non-enzymatic post-translational modification (PTM): oxidation</strong></td>
<td>• RP-HPLC</td>
</tr>
<tr>
<td></td>
<td>• Peptide mapping with LC–MS</td>
</tr>
<tr>
<td><strong>Non-enzymatic PTM: deamidation</strong></td>
<td>• IsoQuant analysis</td>
</tr>
<tr>
<td></td>
<td>• Peptide mapping with LC–MS</td>
</tr>
<tr>
<td><strong>N- or C-terminal truncation</strong></td>
<td>• MS</td>
</tr>
<tr>
<td></td>
<td>• N-terminal sequencing</td>
</tr>
<tr>
<td><strong>Glycosylation</strong></td>
<td>• Oligosaccharide profiling</td>
</tr>
<tr>
<td></td>
<td>o HPLC (normal phase, hydrophilic interaction) with fluorescence detection</td>
</tr>
<tr>
<td></td>
<td>o HPLC with MS</td>
</tr>
<tr>
<td></td>
<td>• Sialic acid analysis</td>
</tr>
<tr>
<td></td>
<td>• Monosaccharide analysis</td>
</tr>
<tr>
<td><strong>Process-related impurities</strong></td>
<td>• Host cell proteins by enzyme-linked immunosorbent assay (ELISA) or LC–MS</td>
</tr>
<tr>
<td></td>
<td>• Residual deoxyribonucleic acid (DNA)</td>
</tr>
<tr>
<td></td>
<td>• Expression inducers by chromatography</td>
</tr>
<tr>
<td></td>
<td>• Anti-foam agents by chromatography</td>
</tr>
<tr>
<td></td>
<td>• Leachates by LC or gas chromatography</td>
</tr>
</tbody>
</table>
contains a higher abundance of high mannose species, which have been shown to lead to faster product clearance from circulation (3). Of course, significantly faster clearance of the originator product will translate into higher relative potency for the biosimilar if the two are dosed similarly.

A variety of the methods listed in Table I are available to address charge variants, impurities, strength, and potency through binding and cell-based assay; for brevity, no further discussion on these attributes will be provided in this article.

**SPECTROSCOPIC METHODS**

When applied correctly, tools for addressing primary structural comparability and potency (Table I) will produce high-quality data that can be used to establish a detailed picture of the primary structure and post-translational modifications of the protein. Unfortunately, not nearly as many tools are available to address protein/glycoprotein secondary, tertiary, and as applicable, quaternary structures. While there are some high-resolution methods such as multi-dimensional nuclear magnetic resonance (NMR) and X-ray crystallography, the cost, complexity, and time investment to carry out these methods precludes their widespread and routine use. Instead, there is reliance on lower-resolution methods that do not provide information at an atomic coordinate level, but provide structural information that is spatially averaged over the protein.

These lower-resolution spectroscopic methods, including circular dichroism (CD), fluorescence, and infrared spectroscopy, require some form of reporter moiety that relates to a structural characteristic of the protein, and ultimately can be correlated to the non-covalent bonding pattern of

**Figure 1.** Molecular mass profile of adalimumab by electrospray ionization–mass spectrometry (ESI–MS).

**Figure 2.** Comparison of originator and biosimilar adalimumab lots by peptide mapping with detection at 210 nm.

**Figure 3.** Comparison of N-linked glycan profiles between originator and biosimilar antibodies.
### Table II. Comparison of analytical strategies used in the development of recently FDA-approved biosimilars. LC is liquid chromatography; MS is mass spectrometry; RP is reverse phase; HPLC is high-performance liquid chromatography; UV is ultraviolet; CD is circular dichroism; SEC is size exclusion chromatography; FTIR is Fourier transform infrared spectroscopy; DSC is differential scanning calorimetry; ITS is intrinsic tryptophan fluorescence; NMR is nuclear magnetic resonance; SDS–PAGE is sodium dodecyl sulfate polyacrylamide gel electrophoresis; CE is capillary electrophoresis; IEF is isoelectric focusing; cIEF is capillary isoelectric focusing; FFF is field flow fractionation; IEX is ion exchange chromatography; CZE is capillary zone electrophoresis; AUC is analytical ultracentrifugation.

<table>
<thead>
<tr>
<th>Product attribute</th>
<th>Method</th>
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<tr>
<td><strong>Primary structure</strong></td>
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<tr>
<td>Sequence confirmation</td>
<td>N-terminal sequencing</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td><strong>Intact mass</strong></td>
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</tr>
<tr>
<td></td>
<td>ESI-ToF MS</td>
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</tr>
<tr>
<td><strong>Post-translational modifications</strong></td>
<td>Peptide mapping with LC–MS/MS</td>
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</tr>
<tr>
<td><strong>Glycosylation</strong></td>
<td>DNA sequencing</td>
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<td>Monosaccharide</td>
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<td>FFF</td>
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the folded polypeptide chains. As the reporter moieties are not often associated with specific locations in the protein structure, the obtained information is a spatial average. By combining methods that rely on different reporters and different modes of measurement, it is possible to build an integrated picture of the protein structural motifs and establish biosimilarity. An example of CD results from side-by-side comparison of seven biosimilar lots with seven originator lots is provided in Figure 4.

CD spectroscopy of proteins is quite sensitive to the three-dimensional orientation of the peptide-bond group (far-UV CD; 190 to 250 nm), disulfide bonds (near-UV CD; 250 to 320 nm), and aromatic side chains (near-UV CD; 250 to 290 nm). Deconvolution of CD spectra in the far-UV can be used to quantitatively estimate the types of secondary structure, whereas near-UV CD spectra can provide useful information on the local folding environment surrounding aromatic residues. Separately, Fourier transform infrared spectroscopy (FTIR) should also be included in the study of protein secondary structure. The absorption bands from stretching vibrations of the C=O (amide I) and C–N (amide II) groups of the protein backbone are useful for quantifying different types of secondary structure; notably, FTIR is more sensitive than CD for beta-derived secondary structural conformations, which is useful in the characterization of beta-rich proteins like monoclonal antibodies.

**CALORIMETRIC TECHNIQUES**

Besides spectroscopic methods, calorimetric techniques such as differential scanning calorimetry (DSC) should also be applied for the characterization of biosimilars and comparability studies. DSC provides information on the structural stability of the folded polypeptide, given that the temperature (Tm) at which denaturation occurs is characteristic of the protein stability. Because the denaturation transitions should be the same for a protein drug product and its biosimilar, one can use the DSC thermograms to demonstrate that two products derived from different manufacturing processes are structurally comparable.

**ALTERNATIVE TECHNIQUES**

Other techniques that would complement optical spectroscopic methods include hydrogen/deuterium exchange mass spectrometry, antibody array mapping, and ion-mobility mass spectrometry. Unlike the spectroscopic methods, these alternatives require greater expertise, are likely to be more time consuming, and require significantly costlier instrumentation. However, they are expected to be much more informative than optical spectroscopy alone.

Protein aggregation is an undesired type of impurity that can result in enhanced product immunogenicity (4). Structural assays that have sufficient accuracy and precision to quantify such aggregates in protein drug products represent an important component in biosimilar comparability. Determination of the soluble aggregate levels in protein pharmaceuticals has historically relied on size-exclusion chromatography (SEC).

**Figure 4.** Comparison of N-linked glycan profiles between originator and biosimilar antibodies.
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ON-DEMAND WEBCAST  Aired July 20, 2017

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EVENT OVERVIEW:
Extensive characterization of therapeutic proteins such as monoclonal antibodies requires an arsenal of analytical techniques. In this webcast, experts will describe simple workflows that can reduce the complexity of the analytical process and provide an easy way to complete the determination of the many critical attributes with confidence.

Learn about methods and chemistries to improve protein characterization workflows including:

• Reproducible peptide mapping to determine critical attributes
• Charged variant analysis using a simple, robust workflow from method development to quality control
• Aggregate analysis using long-life column technology
• The benefits of native and denatured intact protein measurements with new workflows that provide information on multiple attributes

Who Should Attend:
■ Scientists who are developing protein biotherapeutics, including scientists in early discovery R&D, process analytical development, and QA/QC departments

Key Learning Objectives:
■ Gain understanding of various therapeutic protein characterization workflows
■ Learn how to simplify the measurement of critical attributes
■ Uncover chromatographic separation insights to increase throughput

Presenter
Ken Cook
EU Bio-Separations Expert
Thermo Fisher Scientific

Moderator
Rita Peters
Editorial Director
BioPharm International

For questions, contact Kristen Moore at kristen.moore@ubm.com

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In recent years, however, there has been an increased awareness that SEC can yield erroneous aggregation results (5), due to several factors: possible adsorption of the aggregates to the SEC stationary phase; analysis under non-native conditions due to specific mobile-phase requirements; physical filtration of large aggregates; and dilution effects resulting in disruption of weak aggregates (6, 7). To avoid these SEC shortfalls, two alternate orthogonal methods—analytical ultracentrifugation (AUC) and field-flow fractionation (FFF)—have been more widely used. Both methods employ instrumentation, and more importantly, separation mechanisms that differ from SEC. Indeed, in many cases, AUC can provide evidence of large soluble aggregates present in a drug product that can go undetected by SEC, for reasons described in the previous paragraphs. Furthermore, AUC, by contrast to SEC, does not result in analyte dilution or capture on a stationary phase, and can often be done directly in the formulation matrix. With recent improvements in AUC and FFF resulting in better precision and accuracy, either of these methods can be used to accurately quantify the aggregate content in support of biosimilar comparability studies.

COMPARABILITY STRATEGIES

To validate the analytical approaches discussed, a review of the comparability strategies related to FDA-approved biosimilar products has been undertaken. A summary of the comparability approaches used by Sandoz for filgrastim and etanercept and Celltrion for infliximab is provided in Table II.

Briefly, the developers placed significant emphasis on the confirmation by LC–MS/MS of the primary structure and post-translational modifications. Additionally, extensive effort has gone into the demonstration of biosimilarity by a combination of binding and cell-based assays. For aggregation and other high molecular weight species, the developers have relied on SEC but also with confirmation by either AUC or FFF. Interestingly, a less uniform strategy is evident among these three products for determining higher-order structures. The similarity in secondary/tertiary structures for filgrastim was conducted mainly through 2D NMR analysis, while crystallography and hydrogen/deuterium (H/D) exchange MS were used for etanercept, and a combination of CD, FTIR, and antibody array mapping was used for infliximab.

CONCLUSION

To date, three biosimilar products have been approved by FDA. Many other products are currently in review and are expected to receive approval in 2017. The regulatory path for getting to market in the US is becoming better defined; indeed, it is anticipated that there will be an acceleration in biosimilar approvals by FDA in the next five years.

As described in this paper, a variety of analytical tools are available to support the development of these biosimilars, especially with respect to chemical and biological/functional confirmation and comparability. While the three-dimensional structure of these molecules does present a significant challenge, there are several low-resolution methods that can be readily applied. However, more powerful methods are becoming available, including H/D exchange MS and ion-mobility MS, that can help with elucidating the spatial features of these molecules, and thus establishing comparability.

REFERENCES

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