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FEATURES

SPECIAL REPORT: EXCIPIENT QUALITY

Mitigating the Risk from Excipient Variability

This article reviews sources of excipient variability, including raw materials and processing, both of which may vary from supplier to supplier and from plant to plant for a single manufacturer.

Progress in the Development of CAR NK-Cell Therapies

Further advances in construct design and manufacturing scalability are still needed.

Overcoming Engineering Challenges to Enable Commercial Scale mRNA Vaccine Manufacturing

This article provides an overview of solutions to address key challenges facing vaccine developers or CDMOs wishing to build their own commercial-scale mRNA production facilities.

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ANALYTICS

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What Goes Wrong When API Quality is Compromised?

The nitrosamines contamination in “sartan” drugs emphasized the importance of risk assessment in the manufacture of active pharmaceutical ingredients.

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The gold standard for topical development and manufacturing.
n the pharmaceutical industry, the Venn diagram intersection between shortages and quality manufacturing issues occupies more space than anyone wants. There has long been discussion about the best way to reduce this. Recently, FDA formed a “multidisciplinary, multi-Center working group to facilitate the development of a Quality Management Maturity (QMM) rating program. This working group is developing a framework to objectively assess and rate the QMM of manufacturing sites through the integration of interactive site assessments and relevant intelligence pertaining to the site’s state of quality.” To some degree this framework has been borrowed and modified from similar manufacturing industries. In discussions, FDA is “…considering standardized assessment tools, policy approaches, industry incentives, transparency, and communications” (1).

In a recent Pharmaceutical Technology Drug Solutions podcast, Cindy Buhse, Deputy Director of Operations, Office of Pharmaceutical Quality at FDA, made a relevant insightful business observation saying, “I think the key words here are business processes and continual improvement. It’s not about the product. We already know the product is of high quality because its being made under current good manufacturing practices (CGMPs) with inspections and assessments and everything FDA does, so QMM is really about the business processes, and rewarding those manufacturers who have robust processes … because if you have robust business processes and really understand your supply chain, you have risk management plans, you have continuous improvement plans in place, … you end up making sure your particular product does not end up in shortage” (2).

A paper by the Brookings Institution discusses the ongoing baby formula shortage, but also calls out excipients such as Microcrystalline cellulose and Magnesium Stearate, as of special consideration given the number of drug products affected were there to be a disruption in supply (3). It continues, saying the baby formula shortage falls into the National Academy of Science “A Framework for Resilient Medical Product Supply Chains” recommendations (4), and so should include those excipients also. Given what we now know, it’s hard to disagree.

References
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Navigating the Future

In August it feels odd to be speculating what people will find important on the final days of December 2023, but that’s the job when creating next year’s editorial calendar. Sensible navigation typically means maintaining a series of fixed compass headings. But to optimize any journey one must make calculations regarding prevailing weather patterns, current good health, and the indisputability of certain predestined ports of call. As part of a large team, this engenders loads of discussion. Ultimately, however, we end up in the same spot and in the same boat.

Writing about the calendar allows me to illustrate how inputs arrive from diverse sources. This includes feedback from articles, discussion at conferences, and mining our own podcasts, video interviews, and symposia presentations. An upcoming September Drug Solutions podcast is a conversation with Dr. Uwe Schoenbeck, chief scientific officer, External Research and Development Innovation (ERDI); and senior vice president, Worldwide Research and Development (WRD), Pfizer. Quite a job title and quite the challenging job. He is also navigating the industries future, albeit with a broader mission than our editorial team. In this dialogue, Uwe covers what’s important to Pfizer when it comes to partnering, by emphasizing areas such as repeat expansion disorders, senescence, DNA damage response and nucleic acid sensing, deubiquitinase pathways, and neuroinflammation. Pfizer feels these hold special promise for next year’s editorial calendar.

And I was struck by a Parthian shot. “An area everyone, including us, is looking into for further improvements,” he fired at me, “is targeted delivery. True not just for RNA but true for small molecules, large molecules, and also oligo-based therapies … there’s a lot of activity for lipid nanoparticles for local delivery, there’s a lot of activity in the capsid space, so any kind of tropism that you can introduce that would really be selective, and could be dialed in and out as desired, would be a very promising platform that we would be very interested in, obviously.”

I can look into the future and it is manifestly obvious targeted delivery will be on next year’s calendar.
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FDA Joins Effort to Curb Drug Patent Abuses

Jill Wechsler

FDA and the USPTO hope to promote competition by challenging strategies designed to block timely approval of generics and biosimilars.

As part of the Biden administration’s ongoing campaign to reduce outlays for prescription drugs, FDA is collaborating with the US Patent & Trademark Office (USPTO) to limit patent extensions for minor changes to a medicine. The initiative aims to promote competition in the biopharmaceutical market by challenging strategies designed to block timely approval of less costly generics and biosimilars to innovator products.

The joint effort was outlined by FDA Commissioner Robert Califf and USPTO Director Kathi Vidal in a statement issued July 6, 2022 on the need to prevent the patenting of “incremental, obvious changes to existing drugs.” The aim is to prevent delays in generic drug competition “based on trivial changes” to a drug product (1). While the “issuance of robust and reliable patents to incentivize pharmaceutical innovators is critical, our patent systems must not be used to unjustifiably delay generic drugs and biosimilar competition beyond that reasonably contemplated by law,” President Biden noted in an Executive Order last year.

The two agencies aim to “leverage our collective expertise” in advancing innovation, competition, and the approval of safe and effective drugs.

To ensure that patent examiners have the time and resources needed for thorough review of pharma patent applications, FDA will assist the USPTO by providing patent examiners with training on pharmaceutical and biologics state-of-the-art development to help determine whether similar innovations already exist. FDA will ensure USPTO access to sources maintained by the agency for this purpose, and the two agencies will collaborate on developing policies aimed at protecting and promoting US innovation, a process that will solicit public input in upcoming events and listening sessions.

FDA’s role in addressing patent practices likely to delay competition were described earlier in a September 2021 letter from then-Acting FDA Commissioner Janet Woodcock to her PTO counterpart, urging greater collaboration and engagement between the two agencies to prevent “possible misuse of the patent system” (2). Woodcock outlined how FDA’s Orange Book aims to ensure transparency in patent listings, as does additional similar information on certain licensed biological products. She further described concerns about companies creating “patent thickets” by obtaining multiple patents on different aspects of the same products, as well as patent “evergreening” and “product-hopping” practices designed to forestall competition without advancing innovation.

Woodcock suggested that FDA assist USPTO by offering patent examiners training on its public information and databases that can help identify what is “prior art” to a claimed invention. Joint efforts could cover product eligibility for patent term extensions and information to help USPTO “accurately and fairly” grant patent term extensions where they are appropriate.

Curbing high prices

The FDA-USPTO collaboration comes as Congress appears to be moving forward with drug pricing legislation, which Democrats hope to include in key budget reconciliation legislation before Congress. The Senate leadership released the text of a bill that updates previous price reform efforts and claims to have support from all 50 Senate Democrats (3). The main proposal requires the Department of Health and Human Services (HHS) to negotiate lower prices paid by Medicare for certain prescription drugs, with the exception of insulin, where separate legislation has been proposed to address the need for significant cost controls. Additional provisions in the Senate bill would cap out-of-pocket spending on drugs by Medicare patients at $2000 a year, and ensure free vaccines to patients. The measure also revises manufacturer rebates on Medical Part B and D and repeals the controversial Part D rebate rule proposed by the Trump administration.

Biotech and pharma innovators have strongly attacked the legislation as a threat to innovation and global competitiveness of US-based...
firms, predicting big drops in developing new cancer drugs and therapies for rare diseases. At the same time, marketers of generic drugs and biosimilars claim that the revised Medicare negotiating process would undermine the development of lower-cost therapies.

FDA officials acknowledge that the agency plays only an indirect role in drug pricing debates, largely by instituting added efficiencies to drug development and ensuring a transparent and responsive product review process. At the same time, Woodcock noted earlier that the agency is “committed to identifying abuses of the system that can impede competition” and to recognizing how pharmaceutical patents impact access to approved medicines.

References

FDA Continues to Grapple with Accelerated Approval Issues

The approval of certain critical medicines based on preliminary clinical evidence continues to raise questions about reliance on too-weak efficacy data and delays in sponsors submitting confirmatory evidence from postapproval trials. FDA has approved dozens of valuable therapies under the program, many of them able to confirm efficacy through subsequent clinical studies. However, the controversial approval in 2021 of Biogen’s Aduhelm to treat Alzheimer’s Disease by the Center for Drug Evaluation and Research (CDER) continues to haunt the program, triggering widespread scrutiny of accelerated approvals and generating a range of reform proposals to accelerate the conduct of confirmatory studies. FDA’s Oncologic Drugs Advisory Committee (ODAC) added to the debate with its April 2021 meeting that challenged several “dangling approvals” for cancer therapies. That prompted some manufacturers to withdraw earlier approvals for certain indications and others to renew confirmatory study efforts. The agency recently announced another ODAC meeting on Sept. 22 and 23, 2022 to review additional medicines that raise questions about efforts to confirm indications in later studies. The expert panel will assess a multiple-myeloma treatment from Oncopeptides, where a confirmatory trial demonstrated “worse overall survival and failed to verify clinical benefit,” the FDA announcement stated (1). Also on the agenda is Secura Bio’s lymphoma therapy, which has raised questions about the benefits and risks of overall survival information.

Reference
1. FDA, Oncologic Drugs Advisory Committee; Notice of Meeting; Establishment of a Public, Federal Register, July 21, 2022.

—Jill Wechsler

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What Goes Wrong When API Quality is Compromised?

Susan Haigney

The nitrosamines contamination in “sartan” drugs emphasized the importance of risk assessment in the manufacture of active pharmaceutical ingredients.

Effective and safe APIs provide the necessary element of drugs to treat patients with a variety of health conditions. It is, therefore, crucial that the quality of APIs, along with other ingredients that make up a medicine, is of primary importance to drug developers and manufacturers.

“It is the API, that attributes the efficacy of a drug,” says Aloka Srinivasan, principal and managing partner at RAAHA LLC. “In other words, a drug is a drug because of the API, otherwise it is just a placebo. Thus, it is of utmost importance that the pharmaceutical manufacturers assure the safety of the API.”

According to Meera Khullar, senior vice president of Quality and Compliance, Curia Global, API quality is an essential requirement. “Current regulations worldwide provide zero flexibility when it comes to the quality of APIs released from [manufacturing] sites and reaching the market,” Khullar states. “Without global standards of quality, production of much-needed treatments can be derailed just when patients need them the most.”

“The clue is in the name; the API has an ‘active’ impact on the patient. The pharma manufacturer is ultimately responsible for the safety of the drug product and therewith of the API. In the worst case, an unsafe API can do harm to a patient, which can result in recalls, damage to reputation, financial loss, and potentially punitive actions by regulatory agencies,” warns Siegfried Schmitt, vice president, Technical at Parexel.

Best practices for API manufacturing

So how do API manufacturers and sponsor companies ensure the quality of their product? It’s all about assessing risk. Performing robust risk assessments of the manufacturing process is crucial.

“Robust risk assessments help manufacturers understand their processes, including degradation pathways and impurity formation, and optimize them to meet quality targets. In this way, manufacturers can ensure quality by design—developing a process that detects and minimizes impurities and guarantees quality,” says Ankur Deshpande, director, Process Development Quality at BIOVECTRA.

“One of the key considerations for API and resulting [drug product] safety is assessing potential impurities that may be introduced from the interaction of the manufacturing process stream to manufacturing component materials (e.g., filter membranes, polymeric or elastomeric tubing, polymeric storage vessels and closures, etc.),” says Xiao Chun Yu, senior principal scientist, PPD. “The approach for evaluating and controlling this risk is well described in United States Pharmacopeia <1665>.”

A quality system that employs good manufacturing practices (GMPs) is also...
necessary, adds Schmitt, and the personnel involved in the quality system are key. “A system is only as good as the persons who apply it and adhere to it. Thus, having suitably trained and qualified staff and of course, also suitably designed and equipped facilities are a prerequisite. It sounds simple and logical, but it requires a quality mindset to implement and follow.”

Documentation is also key, continues Mike Johnson, senior director, Quality, Cambrex High Point. “A sponsor might develop an API that is a miracle to the patient, but if the quality system has not been properly built or continuously managed, missing parts or inaccuracies within the development package may arise. This can cause delays in approvals and rework, as auditors and regulatory reviewers may question the quality and validity of the API being developed,” he says.

A crucial area in API quality is cleaning with contamination controls, specifies Johnson. He also points to facility design, equipment, and personnel training as part of a robust cleaning program. “When an API is produced in a reactor or isolated for final pack out, we must confirm that only the one API is present and there are no concerns for contamination from other materials,” says Johnson.

Testing effectiveness. Performing testing on APIs, whether produced in house or obtained from a supplier, is essential to establishing the quality of the API used in the finished drug product. High-performance liquid chromatography (HPLC) is a key testing method for ensuring API purity, asserts Johnson, and it is a common and critical testing method. Mass spectrometry (MS) and gas chromatography (GC) are also effective testing methods, but numerous methods should be used to meet regulatory guidelines, stresses Johnson. “A series of different methods and analytical techniques must be used to ensure a full battery of tests is performed to support that the API is effective and safe for patients,” he says.

Other testing methods include charged aerosol detectors and multi-angle light scattering (MALS) detectors, according to Peter Phillips, vice president of Quality at BIOVECTRA. “But the use of these methods depends on the areas of risk identified by the sponsor through their clinical trials. This information drives the manufacturer towards the appropriate technology to detect and eliminate that risk,” he says.

“Continual advances in the sensitivity, specificity, and accuracy of analytical technologies contribute to increasing confidence in the ability of API manufacturers to ensure the quality of final API products,” says James Lawler, general manager at C² PHARMA. “For small-molecule APIs, common techniques include Raman, ultraviolet, infrared, and UV spectroscopy; HPLC; LC–MS; LC–tandem MS (MS/MS); GC; GC–MS and GC–MS/MS; and particle analysis, among others. These methods are used during product and process development and for final API release. GMP drug substance release testing requirements are extensive and include testing materials against specification for identity, purity, the level of impurities, certain physical properties, among other attributes. The specific requirements depend on the modality.”

Sourcing and the supply chain. Sponsor companies often source APIs from third-party suppliers. Sourcing of these materials often requires a commitment to quality above costs to ensure that the final product is effective and safe.

The selection of low-quality APIs to reduce manufacturing costs can put the whole supply chain at risk, notes Lawler. “The lowest commonly acceptable quality API manufacturer typically put everyone else out of business for that API while also being the one with the highest risk of failure. Once they fail, like a domino effect, they wipe out the whole supply chain leading into shortages which are extremely detrimental to patient health,” says Lawler.

Raw material selection is an important aspect of API quality, especially combined with robust process validation, according to Adriana Malizia, director of Quality, Aceto. “Raw material selection and qualification involve the evaluation of the supplier QMS [quality management system] and lay the foundation for the processing of the material to manufacture the API,” says Malizia. “This is further supported by a thorough validation of the manufacturing process which should identify critical process parameters that will act as the control mechanisms to establishing consistent process and product quality.”

A holistic approach to qualifying suppliers is also key, emphasizes Khullar. This approach should consider the quality aspects of the supplier at the beginning of the relationship and include the supplier’s technical capabilities, equipment, technology, and personnel. “The assessment also should evaluate a supplier’s track record of reliability for producing consistent products in line with emerging requirements, including its history of regulatory compliance. Furthermore, it should address whether the supplier’s ongoing quality performance is in line with the sponsor’s standards across the supply chain. Sponsors should gauge and confirm an environment and culture of continuous improvement. Ultimately, the strongest sponsor and supplier partnerships require alignment between each organization’s quality systems,” says Khullar.

Site audits of API suppliers must be performed as part of a sourcing program, asserts Johnson. “Keep in mind that audits are a snapshot in time, so it’s important to incorporate additional technical and business oversight throughout the relationship. Sponsors can ensure a second confirmation of quality checks at periodic time-points through a use test in the production environment, followed by quality control testing,” Johnson says.

Suppliers should have a robust quality system to detect and correct problems with their product, confirms Phillips, and API manufacturers should have the technical background and expertise to plan for problems. “It is essential that manufacturers can walk sponsors through the technical process, both from a manufacturing and an analytical perspective,” says Phillips.

An additional consideration for sourcing of APIs is ensuring critical quality attributes (CQAs) of the API. “For example, shipping an ingredient at the wrong temperature can cause issues severely impacting quality. Depending on the particular product and its unique [CQAs], some factors might be more important than others, but it is always case-dependent, and there are always multiple areas that can impact API quality,” says Phillips.
The nitrosamines problem
An example of what can go wrong with APIs when impurities are present is the nitrosamine contamination in sartan drugs (1). Individuals are exposed to nitrosamines, which are common in water and foods, to some level in their environment. However, these impurities may increase the risk of developing cancer in some people, according to FDA, and in 2020, industry began issuing recalls of drug products contaminated with nitrosamines (2).

“The nitrosamine saga is ongoing, and industry is learning new things every day related to nitrosamines,” says Srinivasan. “Currently, the agencies have moved beyond low molecular weight nitrosamines, which arise from reagents and solvents to larger API like nitrosamines, which FDA has termed as NDSRIs (nitroso drug substance related impurities). These nitrosamines are formed when the API itself is an amine (secondary, tertiary, or quaternary) and is capable of forming a nitrosamine in presence of a nitrosating agent like nitrite, which are ubiquitous. This has made the industry aware that they need to make sure that the water or the reagents they use are nitrite free. It has also made the industry more careful regarding the cleaning of shared equipment to avoid any contamination with a nitrosating agent. Industry is rethinking the route of synthesis for many APIs, including “sartans” to see if nitrosating agents can be avoided. Last but not least, industry has started paying serious attention the quality of recycled solvents, which could be a player in the formation of nitrosamines.”

Root cause: the process. The presence of impurities may not be obvious, according to Duu-Gong Wu, Regulatory Affairs, PPD, especially when an interaction with non-toxic excipients results in impurities in the API. With the nitrosamine contamination in sartan medications, the manufacturing process itself was the culprit and dimethylformamide combined with sodium nitrite in the presence of an acid led to the formation of N-nitrosamines.

According to Lawler, the N-nitrosodiethylamine (NDMA) and N-nitrosodiethylamine (NDEA) contamination in these drugs occurred because recycled solvents were used to reduce costs, and formulations of some APIs with specific excipients resulted in nitrosamine formation. “When the problem first arose (due to poorly considered process changes), awareness of the need for better risk analysis of the synthetic routes used to manufacture APIs with respect to the potential for production of both known and unknown impurities and how to prevent/control them increased substantially across the industry. The importance of effective process-change control processes and the need for careful oversight of API suppliers was also highlighted,” Lawler says. “Furthermore, the critical need for scientific competence and the negative impact that continuous cost-cutting measures can have on quality was clearly demonstrated from the nitrosamine crisis.”

The nitrosamine situation stressed the importance of a robust process validation, which can identify by-products and/or impurities created during manufacturing, explains Malizia. “The nitrosamine contamination … has underscored the importance of these initial process analyses and risk assessments which should be supported by analytical testing where doubt exists. It further demonstrated that the collaboration of raw material and excipient suppliers in identifying potential risk factors is necessary for early detection and ultimately patient safety,” says Malizia.

Because contaminants can be introduced early in the manufacturing process, which can then impact the entire process, the need for “strict control” over the pharmaceutical supply chain was also emphasized by the nitrosamine situation, emphasizes Deshpande.

Srinivasan believes the scope of the nitrosamines problem has expanded from small nitrosamines created from reagents and solvents to NDSRIs, and the problem may take years to resolve. “Considering that more than 40% of drugs approved by agencies all over the world are secondary and tertiary amines, there are potentially hundreds or thousands of drugs that need to be evaluated for these larger nitrosamines, which have very little to no data in the public domain. Agencies have recommended limits which in most cases are unachievable based on currently available analytical capabilities,” she says. “The API industry should take a holistic look into the route of synthesis of an API and the reagents, solvents used to make sure that they are not missing anything related to mutagenic impurities. The other aspect, which cannot be stressed enough, is the need to follow appropriate GMP during API manufacturing and avoid contamination.”

The nitrosamines situation has shown that an analytical assessment of the manufacturing process, in addition to a paper assessment, is essential, according to Niamh Lynch, Executive Director, Global Head of Quality, Lonza Small Molecules, Basel. “It also became apparent that if a change is made to a process, whether to solvents, catalysts, or a new supplier of starting materials, it can result in the formation of additional impurities,” Lynch says.

Industry and regulators react. Industry and regulatory bodies jumped quickly to tackle the nitrosamine problem. Regulators across the globe have collaborated to assess the risk and develop recommendations, according to Khullar. “For example, the US guidance [issued by FDA] goes beyond products of known concern and applies to all chemically synthesized APIs and drug products containing chemically synthesized APIs. Recommendations for both API and drug product manufacturers cover approved and marketed drugs as well as those in development. This is an ongoing effort, which underscores the need for continuous improvement and the need to adjust to meet evolving standards,” says Khullar.
Regulators are using tools to track potential contamination trends, asserts Phillips, but manufacturers should remain diligent and use risk assessment tools to anticipate potential issues before their products reach patients. “That being said, we are dealing with complex molecules and products, so it can be very difficult to predict everything that can happen. Therefore, it’s important that CDMOs [contract development and manufacturing organizations] and Pharma manufacturers work hard to foster a culture where quality is highly valued,” says Phillips.

“In general, awareness of the nitrosamine contamination issue is high within the pharmaceutical industry across the entire value chain,” says Lawler. “Collaboration between international regulatory agencies and API and drug product manufacturers continues with respect to identifying any additional sources of nitrosamine impurities not yet uncovered. As more information is obtained, greater understanding and tightening regulations will likely lead to a reduction of such occurrences in the future. Unfortunately, the root cause of the nitrosamine crisis, namely poor scientific knowledge at the API manufacturer and continuous cost cutting programs for APIs is not addressed,” confirms Lawler.

Malizia stresses that while the nitrosamine problem may not be fully resolved, better control strategies have been implemented across the world that may help the industry avoid similar situations. “The fact that risk assessments and testing are being adopted by less regulated suppliers (raw material and excipient manufacturers) demonstrates that there is a better understanding of the importance of fully comprehending a product’s impurity profile and that collaboration across the industry is key in avoiding similar recurrences for other potential contaminants,” Malizia says.

“Manufacturers are under pressure to better understand the nitrosamine problem, and regulators require manufacturers to perform specific assessments for nitrosamines under M7 ICH [International Council for Harmonisation],” concurs Phillips. “There is always the potential of a new nitrosamine-type issue arising, but the agencies and the companies themselves are continuing to monitor processes and products to ensure this does not happen again.”

Conclusion
Quality control at each stage of API manufacturing is crucial to ensure the quality, safety, and effectiveness of drug products. “The compliance of all stages of manufacturing to CGMP also plays a critical role in maintaining the quality and safety of API, especially after the regulatory approval,” says Derek Wood, senior principal scientist, PPD.

“The moment an API leaves our site, we must have full confidence that we have ensured the API is the correct quality and has been fully tested to meet current product specifications. Strong quality oversight ensures the patient can confidently use their medicine without hesitation. Low-quality and ineffective APIs simply break the trust of the patient, and more importantly, could cause harm to them,” concludes Johnson.

References

Ensuring API Quality—A Pharmacopeia Perspective

Pharmaceutical Technology spoke with Ed Gump, VP Small Molecules at the US Pharmacopeia (USP) about API quality from the standards perspective.

PharmTech: How crucial is it for Pharma manufacturers to ensure the safety of APIs?

Gump (USP): To ensure the quality (and by extension, the safety) of their products, it is crucial that Pharma manufacturers ensure the quality of their drug product ingredients—the API as well as excipients. In the [United States] (and in many other countries), the legal burden to ensure the quality of each ingredient, including excipients, is on drug manufacturers.

Unlike APIs, excipients are often not pure and tend to be complex in nature and can be sourced from natural (animal, plant, mineral), synthetic, and semisynthetic origins. And many (if not most) excipients have uses beyond pharma, so it is especially important for manufacturers to test the quality of their excipients and ensure they are fit for pharmaceutical use.

PharmTech: What are the steps to ensure quality throughout the development and manufacture of an API?

Gump (USP): Manufacturers should manufacture their products under CGMPs [current good manufacturing practices], adhering to all regulatory requirements at the federal, state, and local level.

When ordering APIs from suppliers, manufacturers should qualify their suppliers, perform regular quality audits of their suppliers’ facilities, develop specifications to ensure that API is fit for use in their product formulations and that API conforms to USP standards, ensure the quality of the API they receive such as by testing the API against the USP monograph and reference standard.

PharmTech: What has the industry learned from the nitrosamine problem?

Gump (USP): We have learned that it is not just blood pressure medications that are at risk of nitrosamine contamination. Many recent drug recalls are due to a specific sub-category of nitrosamine impurities known as drug substance-related nitrosamine impurities (DSRNIs), a class of nitrosamines sharing structural similarity to the API. DSRNIs (and nitrosamines in general) can form in several different ways. One of the most common reactions is when an API molecule has what’s known as secondary amine group in its structure, and when nitrite and acidic conditions are present. Under these conditions, the API molecule with a secondary amine can react to form a nitrosamine.

Nitrites from excipients could react with amines in APIs, or low molecular weight amine impurities. Because excipients are generally the greatest component of medicinal products, sufficient amounts of nitrite could be present as a reaction partner.

However, this is not the only chemical route that can potentially lead to formation of a nitrosamine. Given that there are a variety of possible routes, it’s critical for manufacturers to understand what medicines and associated manufacturing processes are at risk to form nitrosamines, confirm if they are forming, at what levels, and how they can establish controls to ensure they are not present at levels that could be harmful.

—Susan Haigney

Editor’s Note: Visit PharmTech.com to read the full interview.
Excipients are substances other than the API, which have been appropriately evaluated for safety and are intentionally included in drug delivery systems. Excipients are typically more complex than APIs, because they are frequently multi-component and less well-defined. In addition, excipient variability can present itself in various forms, compositionally, physically, or functionally.

Most excipients are not pure substances. Other residual and trace components present in excipients can impact key excipient functional characteristics when used in pharmaceutical formulations (1). The International Pharmaceutical Excipients Council (IPEC) refers to these other unavoidable substances in excipients as concomitant components (2). Performance of many excipients in a drug formulation may rely on the presence of such substances in the excipient. These excipient components are not considered impurities and could vary due to changes in raw materials, process variability, and from supplier to supplier. Excipients are not controlled at an individual parameter level. There are many degrees of freedom associated with polydispersity of particle size, molecular weight, and chemical composition, all of which may be process or supplier dependent. Particle size is usually controlled as an average or distribution, and dilute solution viscosities are often used for routine analysis to monitor batch-to-batch consistency of polymer molecular weight. The composition profile of a complex mixture may not be well defined, and the performance impact of the many components may not be well understood.

Silverstein contrasted the control of API quality versus that of excipients (3):

“API quality is improved by reducing the presence of all materials other than the desired chemical. […] Extraneous substances may be harmful to the patient in that they may lead to side effects, or they are inert, thus reducing API purity and thereby compromising efficacy.”

“Excipient quality is described quite differently. While one would again refer to compliance with the compendial monograph (if there is one) or the manufacturer’s specification, a higher assay is not always better. While this may seem counterintuitive, excipients are often complex mixtures that include constituents arising from raw materials, catalyst, solvent, initiator residue, or side reactions” (3).

Excipients are often mixtures of materials with major and minor components, and the minor components may contribute to the overall excipient performance in a particular application (4). For example, the compactibility of coarse grade dibasic calcium phosphate dihydrate is influenced by the presence of “impurities” in the crystal lattice, which cause dislocations and weakness, allowing brittle fracture to occur. Very pure coarse-grade dibasic calcium phosphate dihydrate does not compact as well. However, in general, the exact relationship between excipient composition and excipient performance is not often well understood and will vary with each application (5).

Mitigating the Risk from Excipient Variability

This article reviews sources of excipient variability, including raw materials and processing, both of which may vary from supplier to supplier and from plant to plant for a single manufacturer.

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Pharmaceutical Technology

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Excipient attributes can arise from many sources such as, but not limited to those in Table I (6).

Excipient variability for some excipients can also be influenced by degradation, which also can be impacted by packaging and/or environmental conditions during storage and distribution.

**Process variability**

As described in the IPEC quality-by-design (QbD) guide (6), “excipient manufacturers can control excipient variability only within the limits of their process capability and for which the processing equipment was designed and constructed." Process capability is a general statistical concept based on the ratio of the specification limits to the process variability. If the variability is the same as the specification range the process capability is one, with no room for error. The higher the process capability the lower the risk of failure. Less than one means that not all batches will meet specification. Therefore, arbitrary narrowing of a specification by users may adversely impact sustainable excipient supply. Robust formulations are not critically impacted by typical excipient variabilities, not all of which may be reflected in the specification.

“In addition, the equipment train used in the process and the type of processing and unit operations included may all influence variability. Various excipients are continuously manufactured in many metric tons per annum. Results reported on a certificate of analysis (CoA) will often be a composite or average, potentially underestimating variability. Not all excipients will have the crystallization or precipitation steps typically used to purify fine chemicals. Users should discuss inherent variability with the supplier and their capability to supply product as it relates to the user’s needs (7). It is the responsibility of the user to identify potential critical material attributes (CMAs) for the excipients used in their formulations but the suppliers may be able to assist. A risk-based approach will probably be appropriate” (6).

### Table I. Potential variability due to excipient source.

<table>
<thead>
<tr>
<th>Plants or animal derivatives</th>
<th>Mineral excipients</th>
<th>Synthetic materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>• weather conditions during</td>
<td>• geographic source</td>
<td>• composition of products derived from</td>
</tr>
<tr>
<td>• growing</td>
<td>• of the excipient or</td>
<td>crude oil or gas feedstock varies with</td>
</tr>
<tr>
<td>• harvesting</td>
<td>• crude ore</td>
<td>• well location</td>
</tr>
<tr>
<td>• transporting</td>
<td>• processing conditions</td>
<td>• sulfur content</td>
</tr>
<tr>
<td>• storage</td>
<td>• calcining</td>
<td>• elemental impurities</td>
</tr>
<tr>
<td>• processing</td>
<td>• temperature</td>
<td>• organic components</td>
</tr>
<tr>
<td>• packaging, etc.</td>
<td>• type of milling</td>
<td>• multiple phase vs</td>
</tr>
<tr>
<td>• environmental conditions in</td>
<td></td>
<td>• single-phase reactions.</td>
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<tr>
<td>• different geographic regions</td>
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<tr>
<td>• nutrient levels</td>
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<tr>
<td>• elemental impurities</td>
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<tr>
<td>• pollution</td>
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<tr>
<td>• etc.</td>
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</table>

**Variability due to excipient source**

Different excipient manufacturers are likely to use different raw material sources as well as different manufacturing processes. Therefore, variability introduced by alternate sources encompasses variability of both raw materials and processing. Excipients from alternate sources (additional sites within a manufacturer and/or alternate supplier(s)) may not be compositionally, physically, or functionally identical, but may still demonstrate equivalent performance within the drug product depending on application. Excipient users should define functional performance requirements for alternate excipient sources (8).

When evaluating alternate excipient sources, users should not assume that all sources conforming with the same compendial requirements are interchangeable in all drug product formulations. Variability in excipient composition and other attributes among excipient sources could impact performance. To gain an understanding of the potential origin for various components that may be found in excipients from different sources, see Figure 1.

Confirmation of equivalent performance is necessary to support interchangeability. The critical quality attributes (CQAs), including stability, of the drug product manufactured with the alternate source must be demonstrated. Distributors should not substitute an alternate excipient source without prior notification to the excipient user to allow evaluation of the interchangeability of the alternate source in their specific drug applications.

In a 2008 article on “Comparative Tableting Properties of Sixteen Microcrystalline Celluloses” (9), Doelker et al. noted that, “Great differences in packing and tableting properties and in sensitivity to the addition of a lubricant were generally observed between products from the various manufacturers. In contrast, lot-to-lot variability was quite acceptable.”

**Mitigating the impact of excipient variability**

Moreton defined a robust formulation as being “able to accommodate the typical variability seen in API, excipients, and processes without compromising the manufacture, stability, performance, or any other attribute of the product critical to the patient’s care or well-being” (5). Qualifying multiple sources should be considered when establishing the design space to reduce the time required to switch to an alternative supply after launch (10). If the impact of an excipient variability on finished product CQAs is unacceptable, then additional controls (CMAs) should be considered. Both limits and methods for CMAs must be agreed between user and excipient manufacturer.

It may be necessary for excipient manufacturers to make changes to their processes for various reasons that have the potential to impact excipient performance and thus finished product performance. Excipient manufacturers should consult the IPEC Significant Change Guide for Pharmaceutical Excipients (11) whenever a change is to be made to excipient raw materials, manufacturing process, and/or finished excipient testing...
and assess the need for customer notification. Based on a risk assessment, the excipient manufacturer should apply the same reasoning (as far as is practicable) to their own suppliers of starting materials, reagents, solvents, processing aids, and additives used in the manufacture of the excipient.

When investigating potential impacts of notified changes, it is strongly recommended that a risk assessment be undertaken by the excipient user. Based on the results of the risk assessment, the excipient user should determine their need for further action.

It may be necessary for the excipient user to build product inventory, thus requiring pre-change excipient supply, because regulatory filings may be subject to extended review times. This may, in turn, require coordination between excipient manufacturer and user.

It should also be remembered that not all changes to an excipient will be detrimental to the performance of the excipient or pharmaceutical finished product. All changes should be considered as significant unless it can be scientifically justified as not a significant change. Significant changes should be notified to the excipient user (11).

Factoring excipient variability into design of experiments, control strategy, and life cycle management will increase finished product robustness (12), which will improve the overall product quality and performance, leading to better patient outcomes.

**Conclusion**

Excipient variability should always be a concern unless proven otherwise. Relying only on the excipient sales specification or the compendial monograph is not enough to account for potential variation(s) because many CMAs may not be part of these requirements. Drug product manufacturers should have a robust dialogue with their excipient suppliers to understand the variability of material attributes.

**References**

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Further advances in construct design and manufacturing scalability are still needed.

Autologous chimeric antigen receptor (CAR) T-cell therapies have been shown to be effective for the treatment of many types of cancers. Challenges with scalability and manufacturing associated with autologous cell therapies have led to rapid advancement of allogeneic off-the-shelf cell therapies. However, allogeneic cell therapy products have their own challenges including problems with excessive immune responses and concerns about graft-versus-host disease (GVHD).

T cells are not the only immune cells, however, and there is significant interest in leveraging natural killer (NK) cells in a similar manner. NK cells are lymphocytes that have the ability to target tumor cells, releasing chemokines and cytokines that activate the innate and adaptive immune systems. They can be isolated from a variety of sources and do not have to be patient-specific. Progress is being made in the development of scalable production processes. Clinical trial data from engineered allogeneic NK cells is already in the public domain, additional clinical data continues to emerge from early-generation products, and data read-outs will soon begin for next-generation engineered products.

Several advantages over CAR-T cells

NK cells are important lines of defense the body has against infectious agents and tumors. They target any cells lacking a major histocompatibility complex (MHC), a group of genes that code for proteins found on the surfaces of cells that are unique to each individual and part of the human leukocyte antigen (HLA) system, according to Heather Stefanski, vice president of medical services at the National Marrow Donor Program (NMDP)/Be The Match. “Each T cell has a unique T cell receptor that recognizes a cognate antigen presented in the MHC complex; the repertoire of human T cells includes millions of unique T cells. Tumor cells can evade the immune system as tumors often do not have antigens that T cells can recognize, and therefore, the T cells do not attack,” Stefanski says.

NK cells have the intrinsic ability to mediate tumor killing through innate recognition pathways without prior sensitization, comments Ryan Larson, vice president and head of translational science at Umoja Biopharma. “These intrinsic anti-tumor properties can be augmented by engineering NK cells to express synthetic tumor targeting proteins, such as CARs or adapters compatible with tumor-specific monoclonal antibodies that can be administered in combination,” he notes.

Next-generation CAR cell therapies, contends William Rosellini, co-founder, president, and director of CytoImmune, are focused on NK cells because they are one of nature’s best killing machines. “The NK cell has unbelievable tumor-killing activity with significantly reduced safety issues like on-target/off-tumor effects, GVHD, and cytokine release syndrome (CRS),” he explains.

NK cells do not have endogenous T cell receptors, nor do they cause the secretion of interleukin 6 and other cytokines that can cause undesired immune responses, Stefanski notes. As a result, NK cells typically have shown reduced cytokine production and expansion, and thus reduced rates of some of the serious side effects of CAR T-cell therapies, such as CRS and immune effector cell-associated neurotoxicity syndrome (ICANS), according to Larson. In addition, NK cells do not express antigen-targeting receptors that are tuned specifically to their host, thus eliminating the graft-versus-host risk that exists with allogeneic T-cell therapies.

That latter attribute also makes the development of NK-cell therapies less complex than the development of allogeneic CAR T-cell therapies, Larson adds. “For any allogeneic T-cell product, a genetic manipulation must be made to eliminate T-cell receptor expression. This step is not required for NK cells, and thus simplifies their engineering and manufacturing processes, as well as removing a potential safety issue,” he observes.
The ability to produce allogenic products from healthy starting cells is another advantage. "Autologous, or patient-specific, CAR T-cell therapies are produced using cells collected from patients who are very sick. Those T cells are potentially not ideal for a cell therapy product as some may not be good at expressing CAR proteins. In addition, the types of T cells present in each person will vary, and those differences can have a direct influence on the ability to modify those cells and generate an effective therapy," Stefanski explains.

Overall, concludes Chris Nowers, CEO of ONK Therapeutics, allogeneic NK-cell therapies have the potential to deliver treatments with compelling clinical profiles that are better tolerated and more easily administered, logistically simpler to manufacture, and produced at lower cost. "We believe that through the reduction of costs and a growing accumulation of clinical data demonstrating the positive longer-term benefits, we will be better able to demonstrate measurable value," he states.

Several sources
NK cells are present throughout the body and can be harvested from a number of sources. They can be separated from peripheral blood or isolated from umbilical cord blood or the placenta for the development and production of allogeneic therapies. Another option involves differentiation of human pluripotent stem cells (iPSCs). NK cells may also be generated from CD34+ stem cells present in bone marrow.

According to Rosellini, NK cells isolated from umbilical cord blood samples have been shown to have a higher cytotoxic capacity and span as much as 30% of white blood cells recovered, while NK cells isolated from peripheral blood tend to be a more mature population exhibiting more refined cytotoxic capabilities with a lower 15% of white blood cells recovered.

The lymphoma-derived NK-92 cell line is one established cell line designed to be easy to maintain and expand, as well as easily genetically modified using both viral and non-viral methods. It does require irradiation prior to clinical use, however, which can impact activity.

All of these different sources have been used to produce CAR NK-cell therapies undergoing clinical development. ONK Therapeutics, for instance, uses umbilical cord blood provided by Anthony Nolan Cell & Gene Therapy Services for development of its engineered NK-cell therapies. "We believe that cord blood offers a widely available source of NK cells that has been clinically validated and provides cells with a potential attractive phenotypic makeup," Nowers observes.

Larson’s perspective is that we don’t know yet whether the source will affect the application. "It is likely that some combination of source, CMC [chemistry, manufacturing, and controls] processes, engineering/synthetic biology, and tumor-targeting approach (e.g., CAR) will determine the best therapeutic applications," he states.

Similar production methods
As with CAR T-cell therapies, once the NK cells for development of the therapy have been isolated, they must be expanded and genetically modified to express the CAR protein. The methods for cell expansion and cell engineering are similar for NK cells and T-cells, with some specialization accruing to each cell type, according to Larson. In most cases, large-scale expansion using cytokines and feeder cells is required to produce sufficient NK cells for clinical applications, notes Nowers. This approach facilitates efficient engineering.

Rosellini adds that retroviral, lentiviral, or non-viral delivery of genetic material is used, with viral-vector gene delivery systems currently considered the most effective. ONK Therapeutics is an exception. The company uses a combination of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) editing and transposition of genes employing a non-viral TcBuster transposon approach (Bio-Technne) to produce large numbers of edited CAR-NK cells, according to Nowers.

Larson believes that the method that gives the largest scale possible will be the best to commercialize, as long as it can accommodate some engineering to make sure that the cells will perform well, including in the tumor micro-environment (TME) for solid tumors. He does note, however, that there are schools of thought that think it best to use minimally expanded cells.

While the theoretical unlimited expansion capacity of iPSCs makes them an attractive option for the production of CAR NK-cell therapies, Larson also points out there have been challenges to translating this feature to downstream yield of relevant immune cell progenitor populations in an efficient and scalable format. "Solutions for scaling a directed differentiation manufacturing process will realize the full value of iPSC as a starting material from a CMC perspective in terms of simplicity, reducing cost, and patient access," he remarks.

In this vein, Umoja recently entered a collaboration with TreeFrog Therapeutics to develop CAR NK-cell therapies (1). Umoja’s iPSCs are engineered with a synthetic rapamycin-activated cytokine receptor (RACR) to drive differentiation to, and expansion of innate cytotoxic lymphoid cells, including but not limited to natural killer (NK) cells in the absence of exogenous cytokines and feeder cells. TreeFrog’s proprietary C-Stem technology relies on the high-throughput encapsulation (>1000 capsules/second) of iPSCs within biomimetic alginate shells that promote in vivo-like exponential growth and protect cells from external stress.

Scalable manufacturing essential
One of the less-desirable attributes of CAR NK-cell therapies when compared to CAR T-cell therapies is their lack of persistence in the body. NK cells have a much shorter half-life on the order of weeks. Administration of cytokines such as interleukin 2 to patients can prolong NK cell lifetimes, but Stefanski notes this approach often comes with harmful side effects.

The need for lymphodepletion is another important issue associated with allogeneic CAR NK-cell therapies. Prior to administration, patients receive a short course of chemotherapy to kill their existing T cells as a means for increasing persistence. “Lymphodepletion adds a veneer of immune system damage that provides short-term benefits in enabling engraftment of therapeutic cells but is detrimental to immune system function and may contribute to recurrences or other problems in the long term,” Larson says.
It is important to employ strategies to improve drug exposure through engineering strategies and be able to dose more than once at high dose levels. “Scalable manufacturing solves a lot of problems,” Larson states. Developing NK cells that secrete their own cytokines, such as interleukin 15, is also an approach to improving NK persistence, according to Nowers. He notes that this strategy can be synergistic with the knockout of the cytokine inducible SH2-containing (CISH) protein.

The development of stealthy NK cells that are edited to evade the immune system is another strategy being pursued by several companies, according to Nowers. ONK Therapeutics is also looking to increase the ability of its NK cells to target specific tumors by using cells that express high levels of favorable chemokine receptors such as CXCR4 and CXCR3 and E-selectin ligands. “These features ensure a higher likelihood that the NK cells will traffic to the site of the tumor within their natural lifetimes,” says Nowers.

Other challenges for CAR NK-cell therapies, according to Stefanski, are similar to those faced by CAR T-cell therapies: difficulty infiltrating the TME of solid tumors, the use of lentiviral vectors for delivery of genetic material, and the need to know the target antigen. In addition, although CRS is avoided by using NK cells, other off-targets are possible that must be avoided. There are also complexities associated with garnering regulatory approval for cell therapies, Rosellini adds.

Cancer targets predominate
As next-generation treatments, the development of CAR NK-cell therapies is not as far along as that of CAR T-cell therapies. Most programs are preclinical to early-phase clinical, including both Phase I and II trials. Cancer is the focus, with the greatest number of candidates targeting blood cancers including various leukemias, lymphomas, and multiple myeloma, but there are trials underway in solid tumors such as glioblastoma, non-small cell lung cancer, head and neck squamous cell carcinoma, and prostate cancer.

Rosellini points out that academic research groups are hard at work understanding how CAR-NK cells could be used on other diseases too. “Similar to cancer cells, we could train NK cells to target other malfunctioning cells in inflammatory diseases,” he notes. Stefanski suggests there is a potential for genetically modified NK cells to fight viruses that infect patients following stem-cell transplants and to treat autoimmune diseases driven by malfunctioning B cells. “Theoretically, CAR NK-cell therapies could be used to treat any disease that involves proliferation of abnormal cells,” she concludes.

Early days yet
The CAR NK-cell therapy field is still quite new. Additional clinical data from Affimed and Fate and readouts for some of the hypo-immune cells from Century are expected during the next 6–12 months, according to Nowers.

Stefanski anticipates seeing the number of Phase II trials continuing to grow. Within the next year, she believes at least one hematological malignancy candidate may advance to Phase III. Five years from now, she hopes there will be at least one CAR NK-cell therapy on the market.

CAR NK-cell therapies, Stefanski also suggests given their limited persistence, may have the potential to get patients into remission and in a position to undergo stem-cell transplants they wouldn’t be eligible for otherwise. The limited persistence of NK cells may also be an advantage from a safety point of view, according to Nowers, because it could lead to reduced risk of prolonged on-target, off-tumor toxicity.

Improvements in manufacturing technologies are also anticipated, whether using NK cells isolated from umbilical cord blood or differentiated from iPSCs. “Enhanced manufacturing procedures and the increasing use of automated closed platforms both have the potential to significantly reduce cost of goods,” Nowers notes.

More advanced cell modifications are also being explored. Approaches that include multiple receptors and different ligands on the NK cells have shown potential. “Researchers are beginning to figure out what is best for a given application, because solutions that work well for one type of cancer aren't necessarily optimal for others,” Stefanski says. As information is gained about the current targets and how existing CAR NK-cell therapy candidates behave clinically, Rosellini believes that much will also be learned about how to improve their safety and potency.

For instance, Larson observes that organizations including Umoja will introduce second-generation approaches that avoid lymphodepletion, include engineering strategies to enhance engraftment and pharmacokinetic profiles, and enable the production of larger numbers of doses with higher cell numbers per dose. “As these advances are layered on, I expect we will see better and better clinical results,” he states.

Similarly, companies that are exploring gene editing to benefit the cytotoxic potential, metabolic health, and durability of engineered cells in-vivo will demonstrate enhanced persistence and metabolism, improved tumor homing, reduced exhaustion and immune invasion, and maximization of antigen-independent killing, according to Nowers.

Longer follow up and more mature data from ongoing studies should give greater confidence in the durability of CAR-NK approaches as well as in their safety, says Nowers. “The breadth of ongoing research and an increased move to better tolerated, logistically simpler allogeneic NK cell-therapy platforms will allow evolution toward a more traditional treatment model for advanced cell therapies. Simultaneously, the use of big data and artificial intelligence to direct selection of the best patient candidates will lead to better efficacy, greater patient benefits, and increased understanding,” he contends.

The end result will potentially be significantly reduced list price costs combined with a growing clinical database demonstrating the positive longer-term benefits of CAR NK-cell therapies, according to Nowers. “Stronger demonstration of value will help support the willingness to pay of payors and subsequently drive broader patient access and adoption,” he reiterates.

Reference
A 24-hour streaming program
For Health Care Professionals, By Health Care Professionals
Season 6 is streaming now!
www.medicalworldnews.com
The option of using prefilled syringes for vaccinations is not (yet) available for all types of vaccines. Stakeholders are working to expand that option while also pursuing other non-traditional routes of administration.

Vaccines have been in the news and on the minds of people everywhere in recent years, thanks to the aggressive effort and timetable that was required to discover, develop, and disseminate the COVID-19 vaccines. Meanwhile, developers of other types of pediatric and adult vaccines have continued to advance the manufacturing, drug-administration, and packaging options that are used to support their products.

Vaccines are traditionally packaged and distributed in vials that contain either single or multiple doses. This approach requires the healthcare professional to prepare the vaccine for injection, withdrawing the precise dosage from the vial using a syringe, administering it into the patient, and safeguarding the remaining doses until the vial is empty.

A newer alternative—prefilled syringes (PFS)—has continued to gain favor among vaccine manufacturers and healthcare providers, thanks to several demonstrable advantages. During manufacturing, PFS are prefilled with a single dose representing one vaccination, in accordance with good manufacturing practices (GMPs).

“Today there is very strong preference among physicians and patients that if a therapy or vaccine can be supplied via PFS, it should, and the tangible results the PFS brings—where applicable—can create strategic marketing opportunities for drug makers,” says Cornell Stamoran, PhD, vice-president of corporate strategy and government affairs for Catalent. He is also a co-founder and co-chair of Catalent’s Applied Drug Delivery Institute, a research and advocacy arm founded in 2012 that explores how advanced drug delivery options can improve patient outcomes.

“Globally, PFS currently account for approximately half of the total market for prescription-based vaccine volume in the Americas and Europe, according to IQVIA’s MIDAS database,” notes Stamoran. (Note: The MIDAS database reflects vaccine doses that are dispensed with a prescription in retail or hospital settings, so the COVID-19 vaccines and other public-health-administered vaccines are not fully reflected in that data set.) Stamoran adds, “However, across the rest of the world, vial-based vaccines still make up the majority of the volume dispensed.” Thus, there is plenty of room for growth for this newer administration device.

The benefits of PFS for vaccines

The use of PFS has been shown to streamline and simplify vaccine administration, reduce dosing errors and wasted product, and minimize the potential for microbial cross-contamination and pathogen transmission, which can happen when multiple doses are withdrawn from a single vial. As a result, PFS are now widely used for annual flu shots, heparin injections, and a growing list of injectable therapies across multiple disease states.

“When vaccine is filled in a PFS, no further manipulation is required to safely administer it to the patient,” notes Vincenza Pironti, PhD, senior staff scientist, research and development, pharma services for Thermo Fisher Scientific. “For this reason, the risk of dose failure or injuries during manipulation is reduced to almost zero.”

Similarly, the use of PFS can help to streamline mass-vaccination efforts, especially in situations where the availability of trained staff may be limited.
By not having to prepare traditional syringe-and-vial vaccines for injection at the point of care, frontline workers do not need the same level of training. This, along with the inherent sterility of vaccines in PFS form, is a key differentiating factor supporting broader use of PFS in developing nations and remote regions where the number of skilled medical professionals may be limited.

According to a 2021 article authored by Guillaume Lehée, R&D Innovation Leader for BD Medical—Pharmaceutical Systems, the use of PFS to vaccinate 300 million individuals in the United States in the event of a future pandemic could save more than three million hours of healthcare practitioners’ time (1).

Traditional vaccines (including the flu vaccines) that are based on proteins must be maintained at 2–8 °C (refrigerated conditions). "One potential downside to PFS is that in some retail or hospital pharmacies, vaccines stored in PFS take up more refrigerator shelf space (compared to traditional vials), so this may limit uptake by smaller pharmacies or smaller physician offices,” notes Stamoran of Catalent. "Similarly, if you're trying to vaccinate in a developing country, having multidose vials may make more sense due to limited shipping capacity and refrigerator space; in more developed regions, those limitations may not exist.”

PFS provide advantages for vaccine manufacturers, as well. “There is no need to overfill the single-dose syringes,” Pironti says. “This increases the overall yield of the manufacturing process and preserves valuable vaccines and drug substances from losses.” By comparison, multidose vials are routinely overfilled and excess vaccine material is left behind when the multidose glass vials are discarded.

Meanwhile, “if the vaccine manufacturer is considering converting its product from a traditional vial-delivery system to a PFS, it may need to revise the formulation and carry out additional testing (and in some cases seek additional regulatory approval),” notes Stamoran, adding: “The developer will also need to explore other sources for packaging and materials, and all of this must be factored into the commercialization timeline and supply chain planning.”

**Frozen and ultrafrozen vaccines — not candidates for PFS (yet)**

Despite their advantages, PFS are not suitable for all vaccine types. Notably, the COVID-19 vaccines that are based on the messenger RNA (mRNA) technology platform (from Pfizer/BioNTech and Moderna) require frozen and ultrafrozen temperatures. They cannot, therefore at this time, be packaged and delivered in single-dose PFS form. “Generally speaking, today’s PFS are not yet proven to be compatible with ultrafrozen temperatures as the existing glass materials and other components may not stand up to the extremely low required temperatures,” explains Stamoran.

**Traditional vaccines that are based on proteins must be maintained at 2–8 °C.**

When PFS technology is able to advance sufficiently to support frozen and ultrafrozen vaccines, the breakthrough will be met with open arms, as the pursuit of more mRNA-based vaccines is already underway.

Every year, the seasonal results in roughly five million cases of severe illness and 290,000 to up to 650,000 deaths worldwide, according to the World Health Organization (2). And the currently available seasonal flu vaccines prevent just 40–60% of the disease in the best-matched seasons, according to the US Centers for Disease Control and Prevention (CDC) (3). In September 2021, Pfizer launched a Phase I study to evaluate a single-dose quadrivalent mRNA vaccine against influenza (4). Unlike conventional seasonal flu vaccines that are based on inactivated virus, the investigational process of designing vaccines based on mRNA “requires only the genetic sequences of the viruses, enabling more flexible, rapid manufacturing and the potential opportunities to improve upon the efficacy of current flu vaccines,” said the company at the time of the announcement. Hoping to capitalize on the “immense scientific opportunity of mRNA,” Beyond the seasonal flu vaccine, Pfizer is also exploring mRNA-based vaccines targeting other viral diseases, including HIV, Zika, and rabies, and is pursuing mRNA-based therapies in oncology and other genetic diseases.

Moderna is also pursuing flu vaccines based on mRNA, as well as mRNA-based vaccines against the mosquito-borne viral disease chikungunya, malaria, respiratory syncytial virus (RSV), endemic human coronavirus, Zika, Epstein-Barr virus, HIV, and others (5). Sanofi is also developing mRNA-based flu vaccines, while GSK is partnering with CureVac on both flu and second-generation COVID-19 mRNA vaccines.

**Efforts to make PFS cold-compatible**

With the advent of the ultrafrozen COVID-19 vaccines, “rubber materials and glass were challenged to demonstrate that the sterility barrier will be preserved even when ultra-frozen conditions are required,” says Pironti. “Potential mechanical solutions have been introduced and are in a preliminary phase, such as design alterations that allow the plunger to be rod blocked in order to avoid any unwanted movement, and customized combinations of plungers and syringes that could prevent vaccine degradation at extremely low temperatures.”

Stakeholders are also working to address this limitation by evaluating alternative materials, such as plastics or resins and glass-coated resin syringes that remain stable at extreme temperatures. "Ongoing studies and efforts to develop new materials are underway to address this challenge, with the hope of expanding the use of PFS for frozen and ultrafrozen vaccines in the next few years,” says Stamoran.
Becton, Dickinson and Co. (BD) completed a study in 2021 to assess the stability of drugs contained in glass PFS at frozen and deep-frozen temperatures (-20 °C to -40°C), and to evaluate how the syringes themselves cope at these extremely low temperatures (6). The company’s analysis involved syringes with different glass barrel coatings, different volumes, and other characteristics that could influence the response to deep cold storage. Tests were conducted on several combinations of glass barrel coatings, different formats (from 0.5 mL to 3 mL), different tip and flange designs, and multiple elastomeric closures with various state-of-the-art methods. “We’re encouraged by these results and look forward to partnering with pharmaceutical companies to further advance PFS in deep cold conditions,” said Bruno Baney, vice president of R&D for Pharmaceutical Systems at BD, at the time of the September 2021 announcement.

In December 2020, BD announced plans to invest roughly $1.2 billion over a four-year period to expand and upgrade manufacturing capacity and technology for PFS and other advanced drug-delivery systems across its six global manufacturing sites, and the company is adding a new manufacturing facility in Europe (to be online by late 2023) (7).

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**There is still plenty of room for growth in the use of PFS.**

Meanwhile, in May 2022, BD and Mitsubishi Gas Chemical (MGC) signed a letter of intent to explore a partnership agreement to explore new ways to advance biologic drug development. The companies will investigate ongoing development of OXYCAPT—an innovation from MGC that “integrates the best of plastic and glass for plastic syringes”—to support the next generation of PFS for advanced biological pharmaceuticals. OXYCAPT is a multilayer structure applied on silicon-free plastic syringe barrels, to provide high breakage resistance, oxygen and vapor barrier, lower protein adsorption, very low extractables, high UV barrier, and pH stability, according to the company (8).

### Over the horizon for vaccines: Oral and inhaled dosing and transdermal patches

Prescribers and patients alike appreciate safe and easy vaccine administration. Today, of the 900 or so vaccine-development programs that are reported to be in development in Informa’s PharmaProjects database with known route of administration, one quarter are being evaluated in non-injectable formats, including oral, inhaled, and nasal routes, notes Cornell Stamoran, PhD, vice-president of corporate strategy and government affairs for Catalent, and about one third of these non-injectable programs are already in the clinical phases.

“There are also examples of promising R&D activities involving microneedle patches (which deliver the vaccine subcutaneously via a reservoir or coating on the needles), and, potentially, certain vaccines could be dried and stabilized in the microneedles to overcome issues related to logistics and ultracold storage conditions,” notes Vincenza Pironti, PhD, senior staff scientist, research and development, pharma services for Thermo Fisher Scientific. However, Pironti continues, “the regulatory landscape is still unclear about the acceptance criteria of these newer forms, as methods for release are still unclear, but these new modalities can represent potential solutions in the future.”

“Initial theoretical work and early R&D efforts suggest that the transdermal route may work, but more study and clinical trials are required to establish the safety and efficacy profiles and demonstrate benefits of this alternative approach, and then move toward the manufacturing considerations to allow these novel products to reach the market,” adds Stamoran.

In June 2022, Catalent announced it is carrying out a feasibility study with Israeli biopharmaceutical company MigVax, to investigate the possibility of delivering the COVID-19 vaccine to the oral mucosa (for absorption through the mouth and intestines) using Catalent’s proprietary Zydus Bio orally disintegrating tablet (ODT) technology, which creates a freeze-dried tablet that disperses in the mouth without water (1).

MigVax’s lead oral vaccine program for COVID-19, MigVax-101, which would be transported using the Zydus Bio ODT in unrefrigerated vehicles and stored in standard warehouses, “has shown positive results in preclinical tests,” said the companies at the time of the announcement. MigVax is also working with the Coalition for Epidemic Preparedness Innovations (CEPI) to develop oral tablet vaccines that are broadly protective against both SARS-CoV-2 variants and other coronaviruses, such as severe acute respiratory syndrome and Middle East respiratory syndrome.

Early work is also underway to pursue intranasal vaccines for COVID-19. Today, several dozen companies are at work, exploring not just aerosolized sprays but also nasally administered drops, powder, and gels (2).

### References


—Suzanne Shelley
such projects, teams with expertise across a wide range of specialisms are needed to design, build, and operate this new class of therapeutics facility.

Manufacturing process
Production of mRNA-based vaccines differ from the approach used in conventional protein or viral vector-based vaccines as they are produced in cell-free systems. mRNA manufacturing involves the production of a DNA template, typically using linearized plasmids, followed by an in vitro transcription (IVT) enzymatic capping reaction, which produces the mRNA (See Figure 1).

Following purification using chromatography techniques, the mRNA drug substance is encapsulated with a selection of nanolipids to form lipid nanoparticles (LNP), before further purification and buffer exchange using chromatography coupled with tangential flow filtration (TFF) to make the bulk drug product (BDP) (5) (See Figure 2). Low-temperature storage is needed until such time the BDP can be formulated, sterile filtered, filled or lyophilized in vials, labeled, and packed to make the vaccine. At present, multidose vials plus diluents comprise the pandemic vaccine product, but this is likely to change in the future (6).

The different process needs necessitate different facility design requirements. Examples are given in Table I.

From a manufacturing perspective, mRNA’s potential lies in the capability of smaller-scale facilities to produce clinical trial materials as well as larger volumes of patient doses with the same equipment. The latter can be achieved via more intensive production schedules, or semi-continuous processing techniques.

Challenges and solutions
As a rapidly evolving field using disruptive approaches and technology, mRNA requires a reset in terms of the thinking around facility design. In contrast to the conventional approach, each mRNA project needs solutions providers to provide a ‘bottom up’ strategy, which should emanate from teams on the ground who have the necessary insight into the spe-

**Manufacturing**

**Overcoming Engineering Challenges to Enable Commercial Scale mRNA Vaccine Manufacturing**

Julie Vickers, Peter Cramer, Ranna Eardley-Patel, and Oliver Excell

This article provides an overview of solutions to address key challenges facing vaccine developers or CDMOs wishing to build their own commercial-scale mRNA production facilities.

Messenger RNA (mRNA) is an ingenious natural molecule involved in the creation of proteins, which are essential to biological processes. This same molecule is the basis of a new breed of mRNA vaccines, which were adapted in a matter of months to combat the SARS-CoV-2 pandemic (1).

Rapidly evolving mRNA technology will be key to addressing many unmet needs in vaccines and oncology, as well as supporting a more rapid, global response to future pandemic threats. The mRNA process offers the potential to rapidly produce more vaccine doses compared to conventional vaccines that use cell-culture based production routes, which are often more prone to failure (2).

Advances in mRNA, coupled with the need to ramp up production capacity to meet global demand (3), provide an opportunity to build flexible manufacturing capacity (4). However, larger-scale mRNA vaccine manufacturing poses challenges relating to facility design. As each project will have a unique set of requirements, it is important for solutions providers to address each consideration.

New mRNA good manufacturing practice (GMP) facility design must comply with strict regulatory and sustainability codes of practice, which vary globally. When it comes to delivering

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**Table I**

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<th>Challenge</th>
<th>Solution</th>
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<td>Manufacturing process aspects</td>
<td>Enhanced regulatory and sustainability codes of practice (5)</td>
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<tr>
<td>Low-temperature storage</td>
<td>Low-temperature storage is needed until such time the BDP can be formulated, sterile filtered, filled or lyophilized in vials, labeled, and packed to make the vaccine. At present, multidose vials plus diluents comprise the pandemic vaccine product, but this is likely to change in the future (6).</td>
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<tr>
<td>Diverse project requirements</td>
<td>Different facility design requirements are needed for each project.</td>
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<tr>
<td>Equipment utilization in GMP facilities</td>
<td>Flexible manufacturing capacity can be achieved via more intensive production schedules, or semi-continuous processing techniques.</td>
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**Figure 1**

Schematic representation of mRNA manufacturing process highlighting key steps.

**Figure 2**

Schematic representation of mRNA vaccine formulation highlighting key steps.
**specific design, equipment, and materials handling considerations.**

Biopharma companies should provide their best forecasting estimates for future capacity, number of products, and various formats that mRNA vaccines can be formulated into (e.g., liquid vs. lyophilized, multi-dose vs. single dose vials, transdermal patches, or inhalants).

Where mRNA vaccines currently fall short versus their cell culture-based product competitors is their dependency on steady supplies from others of costly and scarce starting materials (plasmid DNA, enzymes, nanolipids), plus the need for lower temperature storage of intermediates and products.

The latter point provides a geographic constraint for manufacturing and distribution sites. Both have contributed to a higher cost of
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goods, which may limit market penetration during non-pandemic times and affect the facility’s operational and environmental sustainability.

In the absence of such a capacity estimate, mRNA projects could be restricted when it comes to scale, and manufacturers may also face limitations in relation to handling and containment measures for the raw materials used to produce mRNA.

While each project has a unique set of manufacturing and facility design challenges, the key challenges facing mRNA facility projects are scalability, use of flammable materials, and equipment and manufacturing support.

**Process scale.** Process scalability provides the potential to manufacture product in greater volumes. However, scaling out an mRNA process requires careful considerations and can introduce specific design requirements, which mean that it may not be possible to renovate a brownfield site or an existing facility.

The mRNA process can produce more doses than conventional biologics projects, so best practice involves estimating in advance the number of doses per batch that a project or facility will manufacture, establishing how many doses are required in what timeframe, and to evaluate the use of continuous processing strategies. This process will act as the starting point to guide each mRNA project and will determine the parameters for the design of a commercial facility.

To address this challenge, prefabricated modular facilities offer a solution for phased capacity expansion/scale out, while reducing interruption to existing operations.

**Use of flammable materials.** The use of significant quantities of flammable materials that are central to the mRNA production process requires tailored containment, handling, and disposal measures. These measures vary from project to project and effectively necessitate solutions providers to deliver one-off, bespoke solutions. Wider regulatory and sustainability issues also need to be considered.

Increasing production capacity may necessitate the facility to cater for higher hazardous area category (e.g., H-Occupancy) design features, such as specialized building construction and potential blast zones. These are typically undesirable as they add to the cost and complexity to the project, especially if the project is a retrofit.

Once the scale of operations is defined, it is necessary to determine flammable quantities and assess whether there is a need for hazardous area compliant construction. It is recommended to assess if the process can be scaled back or run differently (more batches per year) to avoid this specialized aspect by operating below the relevant maximum allowable quantities (MAQs).

Use of semi-continuous processing strategies can minimize solvent use. Such an assessment should cover:

- Quantification of flammable material use for production steps, including buffer preparation and LNP storage
- Equipment and facility cleaning strategies that contribute to the facility flammable materials inventory
- Impact of HVAC design to avoid hazardous atmospheres (e.g., full fresh air), use of local exhaust ventilation (LEV) or fume hoods

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Currently, there is limited commercial times are not on the critical path to mRNA purification and LNP assembly for each mRNA project, consideration of process analytical technologies (PAT) to automate selected process steps (8).

Operational capacity will be mainly dictated by the availability of a skilled workforce for GMP manufacturing. Thought should be given to staff welfare facilities, which are key to retain skilled workers. Onsite canteens, adequate spaces for parking, lockers, and shift handover meetings need to be considered as well as the manufacturing suites.

From an operational perspective, process and facility design should consider continuous processing and implementation of process analytical technologies (PAT) to automate selected process steps (8). Low-temperature storage capacity should be included to allow for processing breaks (e.g., freezing of intermediates) to enable day-shift production.

Finally, the limited capacity for outsourcing of supporting functions, such as facility environmental monitoring or product sterility testing, should be considered during concept design. Just because the process is cell-free, the analytical in-process testing and release methods needed may not be. Inclusion of fully-segregated microbiological testing laboratory suites in the scope of the project should be considered if outsourcing is not an option. Basing designs on the use of recent advances in microbiological testing such as enzymatic indicators and rapid sterility testing can reduce the footprint of these labs (9, 10). An example of an LNP manufacturing area is shown in Figure 3.

**Figure 3. Example of a lipid nanoparticles (LNP) manufacturing area.**

**Equipment and manufacturing support.** Currently, there is limited commercial offering of dedicated, single-use, and/or explosion-proof equipment both for mRNA purification and LNP assembly at commercial scales (7).

Prompt equipment specification and procurement is needed to fix the facility design and ensure that their lead times are not on the critical path to plant start-up. At present, the process cannot be fully single-use, so thought needs to be put into the cleaning and sterilization processes, plus the analytical support infrastructure needed for reusable product-contact surfaces.

Therefore, it is recommended that for each mRNA project, consideration is given to the following aspects to determine the link between the equipment available and the facility design:

- Need for custom/proprietary equipment
- Independent production rooms with “through-wall” buffer transfer through iris ports in from logistics corridor (Buffer Prep/Hold)
- Room electrical classification needs versus process step
- Equipment selection versus electrical and fire code requirements
- Benefits and limitations of implementing single-use technologies, given that the process will be hybrid (with stainless steel).

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**Conclusion**

Linking process engineering knowledge to facility design is critical for compliant and competitive manufacturing in the life sciences sectors.

Novel mRNA-based therapeutics are on the cusp of transforming product and project delivery and has the potential to revolutionize modern medicine. By overcoming challenges related to manufacturing and facility design, solutions providers can play an important role in helping mRNA to face down future pandemics and, more broadly, make these life-changing medicines accessible to a greater number of patients, faster.

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A wound on the skin triggers a whole series of events that prompt the healing of the wound. The specific purpose of the wound healing is to restore the skin’s barrier function. If these mechanisms fail, often due to pathological conditions, recovery can be prolonged, and an acute wound can morph into a chronic condition. Under these circumstances, the wound remains in an inflammatory stage, where the presence of pro-inflammatory cells and cytokines promote a proteolytic environment that generates the degradation of growth factors (GFs), which are necessary for the proliferation and migration of cells—thereby, delaying wound healing (1–3).

Currently, there are numerous treatments for wound healing, most of them focused on preventing microbial contamination at the site, maintaining a certain degree of hydration, or promoting regeneration by including GFs. These systems generally include only one or two GFs that may be affected by the proteolytic environment (4–6). In such situations, the external administration of a mixture of these GFs can help to reduce the healing process time. Platelet lysate (PL) is a product derived from platelet GFs-rich granules that result from the disruption of platelets when they are subjected to various processes, such as sonication or freeze-thaw cycles (7–8). However, despite being a mixture rich in GFs, the conditions of a damaged skin site can negatively impact the activity and efficacy of this PL if it is administered externally due to its polypeptide nature requiring frequent administrations. Therefore, the inclusion of PL in a nanocarrier can provide the necessary protection, ensuring the preservation of its biological activity. In addition, if the carrier offers a controlled release for a suitable period, this will prevent the PL from being completely exposed to the unfavorable conditions of the damaged site (9). Various topical delivery systems have been designed to enhance the efficacy of skin regeneration by maintaining local high concentrations of GFs for extended periods (4). Elastic liposomes—biocompatible bilayer vesicular systems—consist of phospholipids, surfactants as edge activators,
and an inner aqueous compartment enclosed within a lipid bilayer capable of encapsulating hydrophilic and lipophilic molecules. The deformation capacity of the vesicles allows them to squeeze and thus penetrate intact through spaces much smaller than their diameter (10). Certainly, a disadvantage of liposomal dispersions is their low viscosity, complicating their application and retention on the wound. That is why its inclusion in dressings or gels that avoid these drawbacks has been proposed. In this sense, aqueous solutions of poloxamers offer a good alternative based on their reverse thermal gelation properties and the fact that they can be fluid at room temperature—which facilitates their administration—and can form a hydrogel when the temperature increases as a result of contact with the biological environment (11–12).

The aim of this research was to develop, characterize, and evaluate a PL-loaded elastic liposome dispersion included in a thermogelling matrix based on Pluronic F-127 and with potential application in treating damaged skin conditions.

**Materials**

L-α-phosphatidylcholine (PC) from soybean, Pluronic F127 (Sigma-Aldrich, St. Louis, Mo.), polysorbate 80 (Tween 80 [T80], Merck KGaA, Darmstadt, Germany), sodium hydroxide, potassium phosphate monobasic, and ethanol (Ferment, Mexico City, Mexico) were all obtained. All solutions were prepared using water from a Milli-Q (MQ) filtration system (MilliporeSigma, Darmstadt, Germany).

**Biological material.** Blood was donated by Banco de Sangre, Hospital Juárez de México, and platelets from the blood were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum, 400 U/ml penicillin, 400 μg/ml streptomycin, 2 mM sodium pyruvate, 10 mM sodium bicarbonate, and 1 mM sodium pyruvate in a humid environment (11–12).

**Methods: preparation and characterization of EL+PL**

**Preparation of elastic liposomes.** Both placebo elastic liposomes (EL) and elastic liposomes loaded with platelet lysate (EL+PL) were prepared according to a previously optimized process based on the thin film hydration technique (10), using PC as the principal component of the vesicle and T80 as the surface modifier. Briefly, 110 mg of PC and 15 mg of T80 were dissolved in 10 mL of ethanol, and this solution was poured into a ball flask where the solvent was evaporated under reduced pressure at 40°C with a rotary evaporator (R-300, BUCHI Labortechnik, Brazil). Then, the lipid film was hydrated with 10 mL of an aqueous solution of PL (in the case of EL+PL) or just MQ water for the placebo (i.e., EL). The solution was stirred with a vortex stirrer. The resulting suspension was left to stand for 30 min at 4°C, then sonicated for five minutes and extruded through a 0.1 μm polycarbonate membrane (MilliporeSigma, Darmstadt, Germany).

**Vesicle size, polydispersity index, and Z potential measurements.** Both EL and EL+PL were characterized by measuring vesicle size (VS), polydispersity index (PDI), and Z potential (zP) using a Zetasizer Nano ZS90 (Malvern Panalytical, Worcs, UK).

**Determination of entrapment efficiency and loading capacity.** Encapsulation efficiency (%EE) and loading capacity (LC) were calculated using Equations 1 and 2, respectively:

\[
\text{%EE} = \frac{\text{Initial amount of PL} - \text{Amount of PL in the supernatant}}{\text{Initial amount of PL}} \times 100\% \\
\text{(Eq. 1)}
\]

\[
\text{LC} = \frac{\text{Initial amount of PL} - \text{Amount of PL in the supernatant}}{\text{Total weight of EL}} \\
\text{(Eq. 2)}
\]

Free PL (in the supernatant of the liposome suspension) was separated by centrifuging EL+PL at 45,000 rpm/2 h, at 4°C (Optima L-90K centrifuge, Beckman Coulter, Indianapolis, Ind.). Three washes were carried out with distilled water, under the centrifugation conditions previously described. The aqueous extracts were combined, quantifying the PL by ultraviolet (UV) spectrophotometry at 280 nm using a previously validated method for the quantification of total proteins (14).

**Morphology.** Elastic liposomes were visualized by scanning electron microscopy (SEM) using a JSM-7600F electron microscope.

**CD spectroscopy.** Circular dichroism (CD) spectroscopy was performed in J-815 Perkin-Elmer equipment (Jasco, Easton, Md.) by placing the sample in a 1-mm cell. Conditions of the analysis included: standard sensitivity, a bandwidth of 1.00 nm, a scan speed of 10 nm/min, 25°C, a wavelength of 250–195 nm, and registering at 110 points for reading. The study was carried out for the PL solution, EL, and EL+PL. All systems had the same concentration of PL.

**Effect of temperature on VS.** The VS for PL solution, EL, and EL+PL was measured by dynamic light scattering (DLS) with an increase of temperature from 20 to 65°C.

**In-vitro release study of PL from the EL+PL suspension.** One mL of EL+PL suspension was placed inside a dialysis bag (Biotech Grade Cellulose Ester, molecular weight cut-off [MWCO] 1000 kDa, Spectrum Labs, San Francisco Calif.). The bag was immersed in 5 mL of phosphate-buffered saline (PBS) solution, pH 7.2 at 33°C, under constant magnetic stirring. The total volume of the receptor medium was withdrawn at predetermined time.
Intervals, and the PL content was determined by UV spectroscopy at 280 nm.

**Effect of PL on a fibroblast culture.** The biological activity of PL was evaluated by a scratch assay using a culture of dermal fibroblasts (15). Briefly, the fibroblast culture was diametrically scratched using a plastic tip. Once the formulation was placed on the culture, the progressive coating of the space generated by the scratching was followed by sequential photographs. For comparison purposes, fibroblasts were treated with a PBS solution, PL solution, EL suspension, and EL+PL separately.

**Methods: inclusion of EL and EL+PL in a thermogelling matrix**

**Preparation of the thermogelling matrix.** Both EL and EL+PL were included in a thermogelling polymeric matrix (HG) based on Pluronic F-127. The HG was prepared by the cold method (11–12), which consisted of dissolving a quantity of polymer equivalent to 20% (w/v) in deionized water at 4 °C under magnetic stirring. HG containing EL (EL HG) or EL+PL (EL+PL HG) were obtained by the same procedure.

**Characterization of the thermogelling matrix.** The viscosity was measured using a Brookfield CAP2000+ viscometer (Brookfield Engineering Labs., Stoughton, Mass.). A viscosity versus temperature profile (spindle No. 3, 50 rpm, 5–40 °C) was performed for HG, EL HG, and EL+PL HG.

In addition to viscosity measurements as a function of temperature, the sol–gel transition temperature, as well as the time needed to gel, were determined by the following methods (11):

- Determination of the non-flow time: once at 33 °C, the elapsed time for the gel to stop flowing was recorded by inverting the tube in which it was located.
- Loss of movement of a magnetic bar: magnetic stir bars were introduced into flasks containing the systems. The temperature was gradually increased, and the moment when the bars stopped completely was recorded.
- Brownian motion loss temperature: the VS was measured with a Zetasizer Nano ZS90 (Malvern Panalytical, Worcs, UK) as the temperature increased, and the temperature at which the VS dropped dramatically was recorded.
- Micellization temperature: thermograms were performed by differential scanning calorimetry (DSC) using a Q20 calorimeter (TA Instruments, New Castle, Del.) under the following conditions: sample equilibrium at 0 °C, isotherm for 1 min, and a heating ramp of 1 °C/min until 70 °C.

The occlusive capacity of the systems was determined by measuring the transepidermal water loss (TEWL) (12). This change in size is attributed to protein unfolding and aggregate formation (22). The VS of EL and EL+PL remained

**In-vivo evaluation of the EL+PL HG system.** A group of seven CD1 mice (seven to nine weeks of age) was used. The mice were anesthetized with a ketamine (90 mg/kg)-xylazine (7 mg/kg) cocktail, and the hair on their backs was shaved. Four punch biopsies were performed, generating a full-thickness cutaneous wound (3 mm size in diameter). Then, 5 µL of EL HG, EL+PL HG, or PL solution were deposited on three of the wounds, using the fourth wound as a control. The formulations were applied daily, taking photographs to monitor the evolution of the wound. Simultaneously, the wound area was determined as a function of time using the imageJ software (version 1.52q, National Institutes of Health, United States). Animal experimentation was approved by the Ethics Committee of Escuela Nacional de Medicina y Homeopatía-IPN, Mexico, (CBE/01/2018), in accordance with the Guide for the Care and Use of Laboratory Animals of the official Mexican standard NOM-062-ZOO-1999 (16).

**Results and discussion**

**Preparation and characterization of elastic liposomes.** Table I shows VS, PDI, and zP values for EL+PL as well as for placebo liposomes (i.e., EL). Size-reduction was achieved through the processes of sonication and extrusion (17). As can be seen, EL had a size of approximately 105 nm, which increased to about 135 nm with the addition of PL. In relation to zP, a value of -9.39 ± 2.27 mV, which was attributed to the phosphate group present in the PC structure, was obtained for EL+PL (17). Nevertheless, even if the zP value was low, the steric effect imparted by T80 provided stability to the vesicles because the bulky polar head of T80 prevented aggregation of the liposomes via a hindrance effect. This supposition is supported by the fact that EL suspensions were resuspendable and showed no evidence of aggregation or increase in VS.

Table I also shows %EE and LC when incorporating two different amounts of PL in the mix. Although %EE is higher with a 1:63 PL:PC-T80 ratio, a higher LC is achieved by increasing the amount of PL, as shown for the 1:16 ratio (18). Therefore, the 1:16 PL:PC-T80 ratio was used for the subsequent tests.

SEM images showed a semispherical shape for elastic liposomes (Figure 1a). The size observed in the micrographs was smaller than that obtained by DLS (Table I). The differences can be attributed to the preparation process of the samples and the effect of the electronic source on liposomes (19–20).

Figure 1b shows the circular dichroism spectra obtained for EL+PL and for the PL solution with similar ellipticity values for both, indicating the presence of secondary structures in a native and functional state within the nanocarrier. Considering that PL in liposomes is dissolved in the aqueous core, the similarity between these values can be explained (21).

Changes in the size of PL in solution, EL, and EL+PL were monitored as the temperature increased, without any significant changes observed until the temperature reached 40 °C (Figure 1c). The size of PL in solution increased after 40 °C (e.g., from 34 to 47 nm at 44 °C and then increasing dramatically from 45 °C upward). This change in size is attributed to protein unfolding and aggregate formation (22). The VS of EL and EL+PL remained
practically unchanged as the temperature increased due to the incorporation of PL into elastic liposomes.

Figure 1d presents the release of PL from EL+PL. As shown, the release occurs in a constant and controlled manner over time, which suggests the advantages of this system for the purpose sought in this work. On the other hand, to corroborate that PL does not lose its biological activity due to its incorporation in elastic liposomes, or during the manufacturing process, the effect of the systems on a fibroblast cell culture was evaluated. Figure 2 shows that the space generated in the fibroblast culture by the scratching technique was practically covered after 24 hours of contact, both with the PL solution and with EL+PL. This was not observed with the PBS solution used as control or with EL. This test demonstrated that PL maintained its biological activity when incorporated into elastic liposomes.

**Table I.** Section A shows values of vesicle size (VS), polydispersity index (PDI), and Z potential (zP) for plain elastic liposomes (EL) and platelet lysate-loaded elastic liposomes (EL+PL) before and after the sonication and extrusion processes. Section B shows values of encapsulation efficiency (%EE) and loading capacity (LC). PC-T80 is L-α-phosphatidylcholine with polysorbate 80.

<table>
<thead>
<tr>
<th>SECTION A.</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS (nm)</td>
<td>EL</td>
<td>EL+PL</td>
</tr>
<tr>
<td></td>
<td>1257.7 ± 718.6</td>
<td>2005.7 ± 833.6</td>
</tr>
<tr>
<td>PDI</td>
<td>0.790 ± 0.441</td>
<td>0.660 ± 0.155</td>
</tr>
<tr>
<td>zP (mV)</td>
<td>-9.47 ± 0.31</td>
<td>-8.94 ± 0.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SECTION B.</th>
<th>PL added (mg)</th>
<th>PL:PC-T80 ratio</th>
<th>% EE</th>
<th>LC (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>1:63</td>
<td>97.4 ± 1.3</td>
<td>15.5 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1:16</td>
<td>72.6 ± 2.4*</td>
<td>46.5 ± 4.2*</td>
</tr>
</tbody>
</table>

*p < 0.05 after addition of PL (n = 6). *p < 0.05 after increasing the amount of PL (n = 6)

**Figure 1.** Results of the tests performed to characterize and evaluate the platelet lysate (PL) in a phosphate buffered saline (PBS) solution, placebo elastic liposomes (EL), and elastic liposomes loaded with platelet lysate (EL+PL). a) Scanning electron microscope micrographs at different magnifications for EL+PL (the red arrows show some of the vesicles). b) The circular dichroism spectra. c) Vesicle size vs temperature. d) Release profile for EL+PL (PL:PC-T80, 1:16). PC-T80 is L-α-phosphatidylcholine with polysorbate 80.
liposomes into a hydrogel. This way, it could be applied fluidly at room temperature in the form of a spray. Subsequently, at the temperature of the skin, it will gel, allowing the formulation to be retained on the skin. The gelation temperature was determined by different techniques, including the measurement of viscosity as a function of temperature. As can be seen in Figure 3a, the viscosity increased with the temperature, with the highest viscosity value at above 32 °C; thus, it is expected that, at the moment of its administration, the matrix will gel completely, achieving permanence on the skin. Although the viscosity began to decrease after 36 to 37 °C, this decrease was not relevant, as the HG did not spill out of the skin site at those temperatures.

The thermograms shown in Figure 3b identify the point where the systems undergo a change from a fluid state with “free” polymer chains into a micellar state (23–24). The wide signal observed in the graphs indicated by a black solid arrow represents the micellization process. For HG, this occurs at approximately 32 °C (starting at approximately 10 °C), whereas for EL HG and EL+PL HG, this point was found to be below 15 °C. This observation agrees with reports that have indicated that the presence of structures, such as nanocarriers, decreases the micellization point of the hydrogel to a lower value than that of the system without EL (24). The signal that appears at approximately 55 °C for all systems represents a characteristic thermal event of the polymer (black dotted arrow).

In the case of determining the sol-gel transition temperature by the non-flow time method, it was found that the systems completely lost their fluidity after 10 minutes. However, it must be considered that a volume of 500 μl of gel was used; so, it should be expected that, for the thin layers deposited on the skin, the non-flow time will be much less. To determine the loss-of-movement of a magnetic bar and the Brownian motion loss temperature, sol-gel transition temperature values between 27 and 28 °C were obtained.

The occlusion capacity of the systems was determined by measuring the TEWL. The results showed a reduction in TEWL for all formulations in relation to untreated skin. As shown in Figure 3c, the presence of HG generated a reduction in TEWL of around 20 %, while for EL HG and EL+PL HG, a reduction of around 37 % was obtained. Significant differences were found between these two values (p < 0.05), which implies that the presence of liposomes contributes to the reduction in TEWL and, therefore, to obtaining a system with semi-occlusive properties.

It is desirable for a formulation or a wound dressing to generate a semi-occlusive environment to promote the autocatalytic activity that degrades dead tissue. This is only possible if a certain level of humidity is maintained in the damaged area without reaching total occlusion, which, on the other hand, would favor the growth of bacteria, exacerbate the proteolytic activity, and degrade endogenous growth factors (25). In this case, a partial occlusion was achieved with all systems.

Comparing the results of this study with those obtained in a previous work where PL was included in polymeric nanoparticles (11), the following points are relevant:

- The method of obtaining nanoparticles involved a longer process (e.g., the formation of a double emulsion, the use of a high-shear homogenizer, and the need to centrifuge to remove excess stabilizer), compared to the preparation process of the liposomes presented in this article.
- %EE and LC were higher with liposomes than with nanoparticles (an LC = 46.5 µg/mg with liposomes and 32 µg/mg with nanoparticles). The circular dichroism study showed that in no case (liposomes or nanoparticles) did the preparation method affect the protein structure of the platelet lysate.
- The zP had a higher value for nanoparticles (approximately -17.6 mV) than for liposomes (-9.39 mV); although, in both cases, it is below an absolute value of 30 mV, which is generally accepted as a minimum to consider that the system...
Both liposomes and nanoparticles allow a gradual release of PL for at least 12 hours.

When determining the degree of occlusion, the liposomes showed a reduction in TEWL of approximately 37%, while a reduction of more than 60% was achieved with nanoparticles, the latter of which is related to the hydrophobic and rigid structure of nanoparticles.

The effect of PL on wound closure, both in its free form (in solution) and included in EL+PL HG, was evaluated by a cutaneous excisional wound-healing model. To contrast the effects, a control (wound without treatment) and a placebo formulation (EL HG) were also monitored. A graph with the evolution of the wound area as a function of time as well as the digital images of representative wounds of the mice are presented in Figure 4. As shown, the treatment with EL+PL HG allowed for the complete recovery of the wound from day seven, while, for the rest of the systems until day 10, a trace of the wound was still present, even for the PL solution.

These results are attributed not only to the proliferative and migratory effects of PL, but also to the properties of the release system such as protection of PL, avoiding its degradation in the biological environment; controlled release of the PL; close contact, covering the area and acting as a wound dressing; and semi-occlusive effect. Similar results were obtained for PL formulated in nanoparticles (11). During the study, no weight loss or evidence of infection was seen in the mice.

**Conclusion**

A thermogelling matrix containing platelet lysate-loaded elastic liposomes was developed, representing a potential alternative for wound treatment based on the advantages the matrix offers, such as protection for the platelet lysate from the biological environment, gradual release, close contact with the affected site, semi-occlusive effect, and an adequate response in vitro and in vivo.

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Figure 4.  a) Representative images of the evolution of the four full-thickness wounds caused to mice, as a function of time, for each treatment. The arrowheads indicate the original wound places for platelet lysate in a PBS solution (PL sol.) and for HG containing platelet lysate-loaded elastic liposomes (EL+PL HG). b) Graph showing the excisional wound-closure area in the dorsal region of mice during different treatment days. c) Distribution of the wounds at the beginning of the evaluation. EL HG is HG containing unloaded elastic liposomes. The control is the wound without treatment. Data are presented as mean ± standard deviation (*p < 0.05)

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Automated finished product inspection has been widely adopted in the bio/pharmaceutical industry. The production of high-quality, defect-free finished products is a top priority in the bio/pharmaceutical industry, primarily to ensure the safety of the end-user—patients. The automation of finished product inspection is leading to gains in efficiency for companies and, as such, is being widely adopted.

To find out more about automated finished product inspection, some of the common tools used, potential pitfalls of approaches, the impact of increasing complexity of therapeutics in development, and regulatory changes, Pharmaceutical Technology spoke with Tyler Harris, applications engineer, and Oliver Stauffer, CEO, both from PTI—a specialist in packaging inspection technologies.

**Widely adopted**

**PharmTech:** Could you provide a little bit of background into automated finished product inspection, its importance, and where the industry currently sits on its use?

**Harris (PTI):** Automated finished product inspection has been widely adopted by the pharmaceutical industry with the use of various test methods. Additionally, not all these test methods are container closure integrity technology (CCIT) methods, non-deterministic, or likely to be accepted by regulatory agencies in the near future.

Visual inspection, torque testing, high-voltage leak detection (HVLD), headspace analysis, vacuum decay, and airborne ultrasound all inspect finished products in some form from label alignment to leak detection, and seal quality. No one test method is suitable for all applications, and more than one test method may be required.

Although methods such as visual inspection are widely adopted by the industry, CCIT methods have not yet become commonplace, although [they are] gaining traction. The newfound increase of CCIT in the automated finished product inspection space comes from the development of scalable test methods and regulatory pressure.

Benchtop, offline inspection methods validate automated sampling and 100% inline inspection systems. The practice of offline benchtop quality testing will never dissolve or be replaced by automated inspection; scalability decreases validation time, risk, and analysis time.

**Tools and shortcomings**

**PharmTech:** What sort of analytical tools are commonly used in automated finished product inspection?

**Stauffer (PTI):** The technologies used for automated inspection are application driven and depend heavily on the package format and container contents. Typically, headspace analysis, vacuum decay, or high-voltage leak detection (HVLD) are the go-to inspection technologies for parenterals.

**PharmTech:** Are there any pitfalls/analytical shortcomings that need to be addressed?

**Harris (PTI):** The most critical aspect of inspection is selecting the best test method for the application at hand. Some methods are suitable for a variety of applications while others have specific characteristics the applications must have to be effective. In regard to deterministic non-destructive CCIT methods, each have shortcomings that prevent robust, reliable, and repeatable results. Headspace analysis can only test transparent/translucent rigid applications before labeling and the package must have headspace.

One of the more common automated inspection technologies is traditional AC HVLD. Applications suitable for HVLD must be cylindrical, glass or plastic, and conductive liquid with low viscosity. High voltage can have negative effects on active drug product and produce large amounts of ozone.

Over the last half decade, MicroCurrent HVLD (HVLDmc™) has been proven to greatly reduce the voltage exposure to the product and reduce ozone to...
These two issues lead to false negatives. The increase in large-molecule, high-risk products has led to the need for automated handling mechanisms. Testing offline allows for increased attention to the package and lower machine costs. Additionally, many of these therapeutic products are manufactured in a manual process which does not warrant a need for automated inspection.

**Increasingly complex therapeutics**

**PharmTech:** How has automated finished product inspection adjusted to the increasingly complex therapeutic landscape?

**Harris (PTI):** Therapeutics require far more attention than standard pharmaceutical products. With small batch sizes and costly product, non-destructive methods are mandatory in this market segment. Therapeutics are typically large molecule and sometimes viscous liquids stored in vials, syringes, and intravenous (IV) bags. The increase in large-molecule, high-risk products has led to the need for HVLD, and HVLDmc in the automated inspection space has led to new innovations in handling, test cycle speed, and reliability.

Although superior to vacuum decay for large-molecule and viscous liquid products, HVLD struggles to effectively test the entirety of an IV bag. During an HVLD test, liquid must cover every interior surface of the package. IV bags are not easily rotated and typically contain a large air bubble. These two issues lead to false negatives and inconsistent results. This is where vacuum decay is highly effective. Although limit of detection (LOD) may be higher due to the molecule size, vacuum decay is able to detect leaks in IV bags in automated sampling inspection systems and offline systems.

With small batch sizes and high risk of therapeutic products, benchtop systems are capable of successfully testing product to ensure sterile barrier is intact and no leaks in the package are present. With small batch sizes and frequent changes to the production process/line, validation must be completed with every change. Automated inspection system validation can stretch across multiple weeks and hold up processing of time sensitive live product. Benchtop offline testing stretches across a few days and can be easily completed by a trained operator without the need for validation of the automated handling mechanisms. Testing offline allows for increased attention to the package and lower machine costs. Additionally, many of these therapeutic products are manufactured in a manual process which does not warrant a need for automated inspection.

**Regulatory updates**

**PharmTech:** What regulatory changes have impacted automated finished product inspection, in your expert opinion?

**Stauffer (PTI):** Foundational regulatory guidance documents have been updated in both the [United States] and European market space. *United States Pharmacopeia* (USP) <1207> was recently updated and reflects the shift away from probabilistic test methods to more reliable and traceable deterministic methods. USP <1207> is prescriptive about the technologies that should be considered as improved alternatives to traditional, less effective methods such as dye ingress.

In Europe, the European Medicines Agency (EMA) is updating its Annex 1 guidance, which is less prescriptive around the suggested technologies, but with guidance that frequency of testing and the method of testing should be scientifically appropriate for the commensurate level of risk associated with the application. The need to test fused containers still stands, and the proposed language further clarifies that IV bags also fall within that category. The final draft will most likely have some leniency related to that requirement. Regardless, in both the US and Europe, the regulatory guidance documents are driving towards increased testing frequency using deterministic test methods.

**PharmTech:** Are there further regulatory changes, particularly pertaining to analytical analysis, in the near future that you believe will impact automated finished product inspection?

**Stauffer (PTI):** The regulatory space is forever in a constant state of flux. The most recent proposed changes revolve around the European Union’s (EU’s) Annex 1 and its drafted revisions. The proposed changes call for 100% testing and deterministic test methods for package integrity on all fused containers. This refers to products such as ampoules, pre-filled syringes, and IV bags. The draft specifies that visual inspection alone is not suitable for these types of packages and must be used in conjunction with a deterministic method. At this moment, IV bags are the most difficult application to test whether automated or benchtop and there are two possible outcomes to the final revision of EU Annex 1. Regulatory demands can force innovation, or regulation must wait for further development of the aforementioned deterministic inspection methods.

**Future drivers**

**PharmTech:** Could you highlight any future trends that you deem to be important within automated finished product inspection in terms of analytics?

**Harris (PTI):** Inspection trends are driven by reliability, [regulations], and most commonly, cost. An uptick in automated sampling systems and scalable test methods is on the horizon. The ability to have the same technology in an automated format and in a laboratory setting is critical in being able to compare results, analyze data, validate, and ensure automated systems are functioning properly. Automated sampling systems allow for less human interaction, rapid inspection, and a decrease in inspection time. These benefits ultimately lead to a decrease in cost despite a larger capital expense. **PT**
The BioPharma industry is in a state of flux. As the industry modernizes to deliver on the promise of a new generation of drug modalities, such as cell and gene therapies, it is also seeking to take advantage of recent advances in digital technologies. Many companies have digital transformation initiatives to leverage the potential of big data, cloud computing, machine learning/artificial intelligence, and the Internet of Things (IoT). The ultimate goal of these initiatives is to improve operational efficiency, reduce costs and time to market, and stay ahead of the competition. To enable this transformation, the so-called Industry 4.0 movement must be built on a solid foundation of good data governance while accommodating the industry’s stringent regulatory requirements.

In the race to all things digital, data governance, data integrity, and regulatory compliance may not be at the top of the C-Suite’s mind. Yet, digital initiatives often provide an opportunity to modernize these legacy processes, replacing labor intensive and time-consuming manual methods with integrated systems that can reduce effort and improve overall data quality.

Recent years have seen the rise of “483s,” FDA regulatory warning letters, with data integrity violations accounting for most of the notices. In 2019, almost half (47%) of all warning letters issued by FDA concerned data integrity. By the end of 2021, that number had increased to 65% (1). This trend has prompted forward-thinking organizations to re-evaluate their infrastructure and ways of working in efforts to maintain compliance and avoid future risk through digitization of their business and operational processes. In many cases, these efforts should be seen as complementary to programs that modernize data governance infrastructure to meet Industry 4.0 aspirations.

The legacy systems headache
The industry has seen good adoption of tools to help manage operational data and improve data integrity, such as electronic lab notebooks (ELN), laboratory information management systems (LIMS), and manufacturing execution systems (MES). Larger organizations are also seeing a resurgence in investments in building centralized data repositories, such as data lakes, to help drive their digitization initiatives. A core business objective of many of these data lakes is to break down data silos to create centralized repository of data for end users that is easily accessible, coherent, and complete. However, automating the integration of such varied systems, whilst ensuring data integrity and regulatory compliance, remains a significant industry challenge (2).

Successful digital transformation in biopharma requires an integrated approach to the data lifecycle.

**An Integrated Approach to the Data Lifecycle in BioPharma**

Scott Weiss

*Scott Weiss* is vice-president of Business Development and Open Innovation at IDBS.
to provide adequate programmatic interfaces to enable automated system-to-system integration, creating silos of critical data.

A less appreciated and more nuanced data integrity issue is data contextualization. Even if an operator can extract data from a specific system (e.g., chromatography), the data may be of little use without combining it with data stored in other systems, such as the experimental conditions under which the sample was generated, or how the sample was stored or subsequently processed. Maintaining this information context or “chain of custody” is not only necessary for interpreting experimental observations (e.g., mechanism of action), but also critical when attempting to gain the higher level of business intelligence needed to optimize a drug candidate’s attributes or a process for drug manufacture.

**Digital initiatives can modernize legacy processes, replacing labor-intensive manual methods with integrated systems.**

Human operators are routinely required to manually transcribe and combine data from multiple systems, often using intermediary tools, such as spreadsheets. This human-centric process is time consuming and error prone, and the potential for errors escalates with each additional transcription required in data transfer. Additionally, manual data transcription workflows require extensive additional quality checks to ensure data integrity and meet regulatory requirements, such as 21 Code of Federal Regulations (CFR) Part 11 or good laboratory/manufacturing practices (GxP) (3).

Moreover, generating and accessing high-quality contextualized data is a major bottleneck to implementing machine learning and other advanced analytic techniques. Highly educated data scientists can spend an inordinate amount of time on a project simply searching for, combining, and cleaning data to generate datasets for training and validating models.

**Relieving the pain**

Improving data governance practices and systems integration is a critical pre-requisite to implementation of the automation and analytics aspirations of Industry 4.0.

FDA uses the acronym ALCOA to describe its expectations of data integrity to help industry technicians stay compliant with 21 CFR Part 11. Per ALCOA, data must be attributable, legible, contemporaneous (i.e., recorded in real-time when generated with a date and time stamp), original, and accurate. The concepts were expanded to ALCOA+, which incorporates additional features and specifies that data must be complete, consistent, enduring, and available (4).

The principles of ALCOA+ and requirements of 21 CFR Part 11 for maintaining data integrity are well established within the biopharmaceutical industry. More recently, similar concepts are being advocated to address broader data integration and system automation challenges by a movement known as the F.A.I.R Principles for scientific data management and stewardship (5). Established in 2014 by participants of a seminar called the Lorentz Workshop “Jointly Designing Data Fairport”, F.A.I.R ensures data are findable, accessible, interoperable, and reusable. More of a design principle than a standard, it recommends or relies on operators to subscribe to one system that enables crosstalk between two or more different systems in a machine-readable format. The output generates data that are comprehensible, reusable, and contextualizable. (6).

F.A.I.R. and ALCOA+ complement each other to help tie the data together. F.A.I.R. focuses on infrastructure, namely metadata, to increase the reliability of electronic data capture, while ALCOA+ addresses data integrity challenges to improve the trustworthiness of the data output in the process.

**Emerging informatics trends**

The current state of biopharmaceutical informatics is heavily dictated by larger organizations which leverage considerable purchasing power. A high proportion of these organizations have made substantial investments in a trove of disparate systems stitched together with in-house integration code, bespoke data lakes/warehouses, and a variety of analytics tools. They are understandably hesitant to undertake the cost and risk associated with wholesale change, especially if the systems in question are validated to a GxP standard. Progress is often incremental, and this can stymie innovation by incentivizing incumbent vendors to maintain the status quo and creating a barrier to entry for newer, more disruptive technologies. There are, however, several emerging technologies to help automate the capture, integration, and contextualization of data from these legacy systems that can both improve data integrity and drive broader Industry 4.0 initiatives, such as implementation of IoT, robotics, and the creation of digital twins for predictive modeling.

Systems integration remains a perennial problem. Some organizations have the necessary IT/software development skills in-house to integrate their digital topography by working directly with individual vendors to create custom solutions. This approach is not ideal for many companies, however, because it is resource-intensive, time consuming, and creates custom software code that must be maintained in perpetuity, creating a resource overhead debt. Its viability is also dependent on support from hardware/software vendors for system integration, which varies considerably from vendor to vendor and should be a decisive factor when
procuring a new system or platform. When procuring new informatic systems or instruments intended to be integrated with your digital topography, key questions that should be asked include:

- How good are the vendors’ APIs and documentation?
- Do they offer services or have certified partners that support integration?
- Can they provide references to examples of successful integration projects?

Increasingly, procurement teams are placing an emphasis on integration support, putting pressure on hardware/software vendors to ascribe to F.A.I.R. principles when designing their products. For many small to mid-sized organizations, building an integrated ecosystem of hardware and software to drive automation is beyond their technical or budgetary reach. To address this need, a number of companies have developed softwares designed to simplify the integration of popular laboratory hardware and software platforms, by creating libraries of connectors to common laboratory equipment and key informatics applications, and providing the mechanism to automate the exchange of data between systems (7).

Cleaning, contextualizing, and aligning the data pulled from different systems presents a challenge as daunting as accessing the source data itself. Recreating the information “chain of custody” typically requires combining partial data sets from different systems, harmonizing terms and identifiers, and ensuring data are aligned correctly. This is one of the most tedious and error-prone steps, creating significant data integrity risks, particularly when done manually with a human operator.

Automating this process may be accomplished often in conjunction with some form of centrally-managed metadata/ontology library to automatically annotate data or create knowledge graphs that map terms from different systems and join together the data. The availability of ontology management, semantic enrichment, and knowledge graph tools continues to expand apace, led by open-source initiatives as well as a number of commercial vendors that specialize in this market.

Systems integration and automated data contextualization focus on integration of hardware, their control systems, and other operational tools, such as ELN, LIMS, or MES. However, the dynamic of wet-lab work does not always provide access to software interfaces for capturing all observations. Some data are still manually documented in hand-written notes, which can be critical to properly qualify an experiment or analytical run. This information may or may not become part of the electronic information chain of custody; it can be lost, erroneously transcribed, or intentionally omitted from the record.

**Improving data governance practices is critical to implementation of Industry 4.0.**

To address this issue, some companies have introduced scientifically intelligent digital voice assistants to provide a more efficient, hands-free user experience for lab workflow and data capture (8). For example, the assistant can prompt the user through a complex protocol with voice instruction, import data at critical steps directly from lab equipment, and capture key observations and ancillary notes by transcribing the operator’s voice dictation. These tools can operate both independently as well as integrate directly with other systems such as ELN.

**Looking ahead**

As small-molecule drug development gives way to newer drug modalities, the suitability of legacy informatics and hardware systems comes into question. Combined with a growing purchasing power of mid-market biotechnology companies, which are often greenfield sites with little or no legacy infrastructure, the opportunity for a more innovative and disruptive change to the traditional informatics landscape will emerge.

Many of these newer technologies seek to embrace a holistic integrated approach to BioPharma Lifecycle Management, by combining elements of out-of-the-box workflow execution, preconfigured system and hardware integrations, contextualized data stores built on F.A.I.R principles, and integrated analytics to drive business intelligence through an integrated digital platform. Their adoption will be driven on whether they can be implemented quickly, deliver business benefits immediately, reduce total cost of ownership, and provide a scalable foundation of data lifecycle management to accelerate Industry 4.0 initiatives.

Technology is finally maturing to address the challenge of integration and automation in the lab and manufacturing plant, whilst ensuring data integrity and regulatory compliance. Regardless of how organizations re-evaluate and modernize their current processes, the solution starts with a willingness to embrace new-world approaches to these old-world problems.

**References**

Automating Packaging of Bio/Pharmaceuticals

Susan Haigney

Automation of pharmaceutical packaging saves costs and time, say contract packagers.

A utomation plays a role in several pharmaceutical manufacturing operations, including packaging. Specifically, the automation of processes can save resources, time, and money. Outsourcing the packaging of bio/pharmaceutical products can offer sponsor companies and manufacturers a variety of options from clinical development to commercial production.

Pharmaceutical Technology spoke with Patrick Ferguson, managing director at Tjoapack, and Markus Hörburger, product and service manager at Vetter Pharma International GmbH, about the benefits of automating pharmaceutical packaging and the services contract organizations can provide.

Automating packaging

PharmTech: How can automation be used to optimize pharmaceutical packaging?

Hörburger (Vetter): Similar to other industries, the main benefit of automation in pharmaceutical packaging is replacing time-consuming manual assembly and packaging steps. Often, automation also has the advantage of being accompanied by digitalization. Digital processes allow for the gathering of various data around the automated packaging that are a significant basis for the identification of further process improvements (for example, predictive maintenance).

Ferguson (Tjoapack): The introduction of automation into pharmaceutical packaging processes has become the industry standard for any packaging company looking to positively impact the bottom line. Investment in automation has a direct and positive impact on quality, efficiency, productivity, and profitability. Additionally, it allows contract packaging organizations, such as Tjoapack, to be more competitive.

PharmTech: How is automation used for the different types of packaging, such as blister packs, prefilled syringes, vials, bottles, etc.?

Ferguson (Tjoapack): In the pharmaceutical packaging industry, equipment providers have continued to develop solutions to tackle nearly any task. Quality and robustness are being designed into the processes from the ground up. Inspections, verifications, integrity, and repeatability are all standard concepts in terms of the overall design of a particular solution.

Hörburger (Vetter): Automation adds the most value when various packaging process steps, such as labeling, assembling, and cartooning, can be processed within a fully automated line. Ideally, different packaging types can be processed on one line. For example, syringes...
can be automatically assembled in safety devices or autoinjectors respectively. Further down the line, syringes, assembled autoinjectors, or safety devices can then be packed in blisters and folding boxes or in more sustainable paper-based solutions depending on customer requirements.

**PharmTech:** What goes into formulating effective automation strategies for pharma packaging?

**Ferguson (Tjoapack):** In my opinion, the formulation of an effective automation strategy starts with an evaluation of the process needed to create the end product. Critical inputs include batch size, annual volume, margin, and the tasks required. Obviously, it doesn’t make sense to invest millions of dollars to fully automate a kitting process that runs only 500 units once a year. The investment into an automated solution must make good financial sense. Once the critical data has been collected and evaluated, a strategy can be developed with multiple automation options included. A cost-benefit analysis should then be developed for each option. Depending on the level of investment, proposals are then routed to the appropriate level with collaboration from multiple departments.

Working with equipment manufacturers and solution providers is another critical part of this process. Choosing the right vendor to partner with can make or break a project. Vendors often have standard solutions that work as is or can be customized to your need. When no solution currently exists, a good partner can take your concept and develop a custom, turn-key solution.

**Hörburger (Vetter):** The development of our packaging strategy starts by considering the needs of the end-user. As a CDMO [contract development and manufacturing organization] in high-quality filling, device assembly, and secondary packaging for injectables, developing an effective automation strategy involves the incorporation of many different assembly and packaging processes. As it pertains specifically to automation, the high complexity of the diverse processes plays a central role in the formulation of a suitable strategy. In addition, the lifecycle status of the products has a major influence on the degree of automation and, therefore, the automation strategy. In early clinical phases when batch sizes are small, highly automated processes can limit flexibility and efficiency. As the product matures, the batch size typically increases. When clinical Phase III is reached or at the launch and associated start of commercial production, highly-automated assembly and packaging processes are preferred.

“The development of our packaging strategy starts by considering the needs of the end-user.”

___ Markus Hörburger, Vetter

**PharmTech:** Which kind of automation services/equipment are available for pharma packaging?

**Hörburger (Vetter):** Common automation concepts include equipment that is being used for single-packaging processes; for example, assembly as well as the incorporation of a fully integrated line that combines several pieces of equipment for the entire packaging process. Collaborating robots (cobots) are another concept for the automation of pharma packaging. Through the use of cobots, the interaction between employer and machine is achieved, resulting in increased automation of manual processes. At Vetter, we have been using this collaborative approach for several years and are currently using cobots within our secondary packaging lines for feeding-in alcohol swabs. We have also gained experience in the assembly of piston rods and repacking syringes in trays in stand-alone systems.

**Ferguson (Tjoapack):** Automation comes in a variety of flavors. Many people not familiar with automation think of assembly lines with robots performing all the tasks. Automation in its entirety can range from lights-out operations with virtually no human interaction, to simple line-level, task-specific enhancements to a process. Sensors, gating mechanisms, and other mechanical inputs can easily be added to a line to improve a process and reduce labor and waste. Fully automated solutions can be implemented to perform a full sequence of tasks yielding a quality product with little human intervention. Naturally, the validation of these automated processes is critical.

**Outsourcing automation of packaging**

**PharmTech:** Why should manufacturers outsource their packaging needs?

**Ferguson (Tjoapack):** Contract packagers, such as Tjoapack, offer a wide range of services to drug manufacturers that they may not have been able to handle on their own. For example, a manufacturer may have a small-volume product or a unique pack-out that doesn’t fit into their existing capabilities or capacity. The contract packager is able to pool these together with other similar products from other manufacturers, making an investment into automation and technology more feasible (financially). In the end, the manufacturer is able to have their product packaged with a higher level of quality and with an overall reduced cost.

**Hörburger (Vetter):** Contract manufacturers typically run state-of-the-art production facilities that enable a wide range of services and packaging solutions. As a CDMO, we are a one-stop partner for our customers, offering services that range from clinical and commercial filling to final packaging solutions.

Having a proven track record demonstrates the ability of a CDMO to support customers’ special needs in secondary packaging. This includes production equipment for a broad range of order sizes and formats, broad expertise for country-specific needs, and extensive experience with international authorities. It also demonstrates leaner project coordination, streamlined fill-and-finish services, as well as reduced time to clinic and market. **PT**
reactants including master cell banks and fermentation reagents, equipment integrity, and performance. Further considerations for the downstream purification process variables and the effect of a final configuration (e.g., folding) also need to be considered.

The purpose of performing an investigation into a deviation is to determine why the deviation happened and what its impact was on the product quality. To determine the impact of the deviation on the product quality, it is important to determine the ‘root cause’ of the deviation. The process used in the industry to determine root cause is, of course, the investigation procedure. This procedure, regardless of whether the product you are investigating is biotech or traditional, should require the investigator to review various systems and determine whether they were the cause of the deviation being investigated.

The systems most often reviewed are equipment and machinery, the manufacturing process, the raw materials used in manufacturing, the specifications, the environment, and finally, the operators. This is not to imply that these systems are the only areas you should look at during the investigation but that these are the most probable areas where you will uncover the root cause of the deviation. Each investigation must address the following elements: root cause, impact to the material or product, the immediate correction taken, the corrective action to prevent re-occurrence for specific product/operation, and the preventive action taken to prevent re-occurrences for all products/operations. Once these elements have been investigated, results from the investigation must be documented.

The written narrative should clearly explain what happened, when it happened, and who was involved or observed what happened.

The written narrative should clearly explain what happened, when it happened, and who was involved or observed what happened. The narrative documents the solution and rationale for the root cause that was determined through the investigation process. If you follow your investigation procedure and thoroughly document your results, you should have an acceptable investigation regardless of whether you are manufacturing a traditional product or a biotech product.

Reference
1. US Public Health Service Act, Section 351, Part F—Licensing—Biological Products And Clinical Laboratories, Subpart 1—Biological Products Regulation Of Biological Products As Amended Through P.L. 117–159, Enacted June 25, 2022. PT
A biologics license application (BLA) is an application sent to FDA that provides information on introducing a biological product into interstate commerce. Similar to a new drug application (NDA), a BLA should include comprehensive product information generated during development, demonstrating safety, purity, and potency of the product including the proposed labeling information to be included in the packaging. All biological products are approved through the BLA pathway, are licensed under Section 351 of the Public Health Service (PHS) Act, and are regulated by the US Food, Drug, and Cosmetic Act (FD&C Act).

What is the difference between a BLA and an NDA?

BLAs and NDAs differ slightly in terms of their application content and submission requirements. The requirements for an NDA are that the drug is proven to be safe and effective, the labeling contains the necessary information, and the manufacturing process does not alter the identity, strength, quality, and purity of the drug. BLAs should also establish that the biological product is safe and potent, but because biologics are manufactured biological living material, the BLA must also provide information that demonstrates the final product does not contain potential extraneous material or viral contamination due to the nature of biologics and the complexities of manufacturing this type of product.

What types of products are filed as BLAs?

In Section 351 of the PHS Act, biological products are defined as a “virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, ... applicable to the prevention, treatment, or cure of a disease or condition of human beings” (1). FDA regulations and policies have established that biological products include blood-derived products, vaccines, in vivo diagnostic allergenic products, immunoglobulin products, products containing cells or microorganisms, and most protein products.

What FDA center has oversight of BLAs?

While traditional drug products (i.e., small molecules) are regulated by FDA’s Center for Drug Evaluation and Research (CDER), biological products can be regulated by either CDER or the Center for Biologics Evaluation (CBER), depending on the product’s classification.

How does the failure investigation process differ for biologics?

The short answer is there is no process difference when performing deviation investigations for traditional pharmaceutical products versus biological products. The differences lie in the complexity of the manufacturing processes and thus the variables that need to be considered regarding what could have impact on the deviation.

Chemical processes, although sometimes quite complex, often have fewer variables even though many of the categories are the same. For instance, when investigating an unknown impurity in a biological process from a simple oligopeptide fermentation process, the considerations may include fermentation conditions (e.g., temperature, oxygen uptake, byproduct production), potential contamination of...
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