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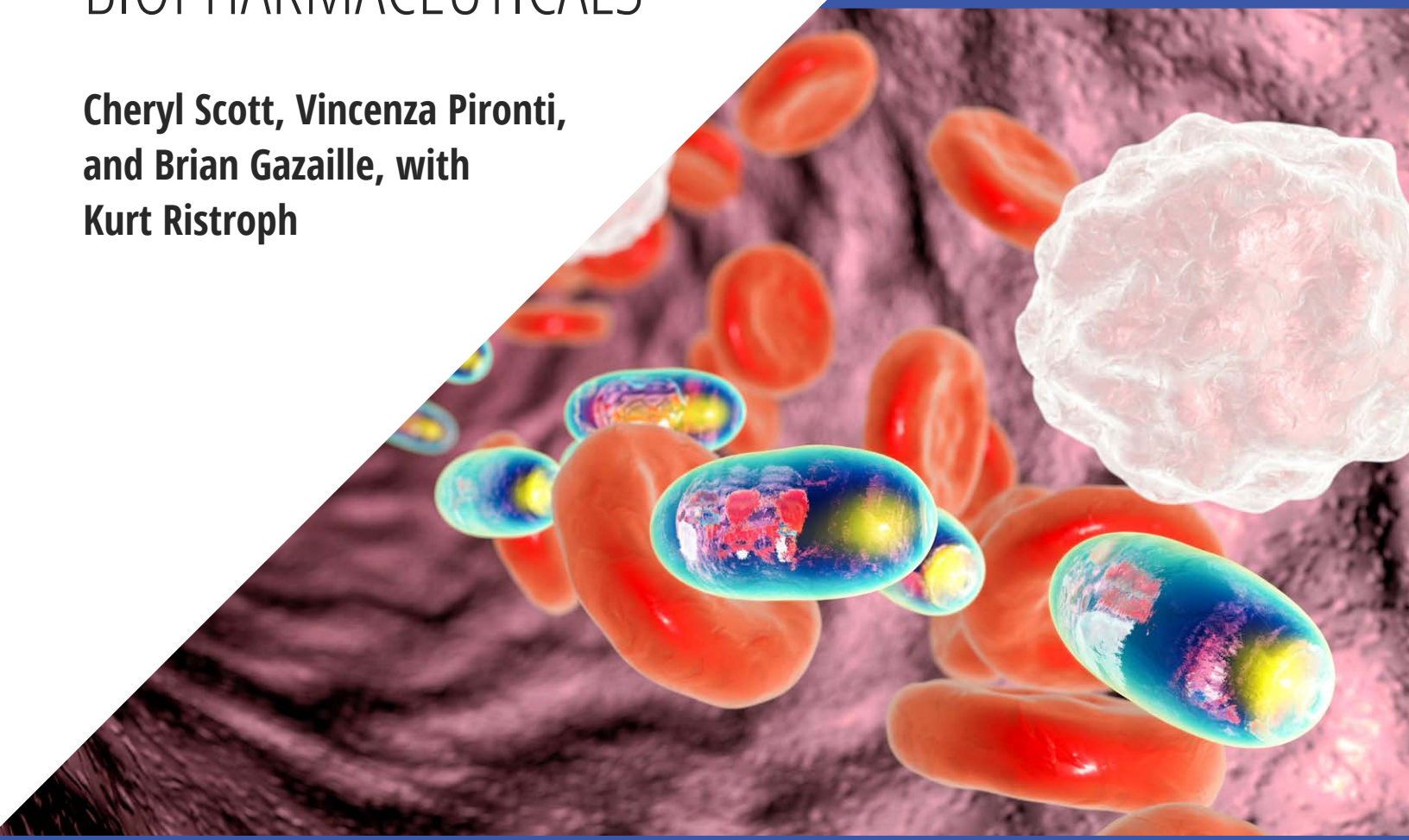
DRUG DELIVERY

NEW FORMATS FOR NOVEL BIOPHARMACEUTICALS

Cheryl Scott, Vincenza Pironti, and Brian Gazaille, with Kurt Ristroph

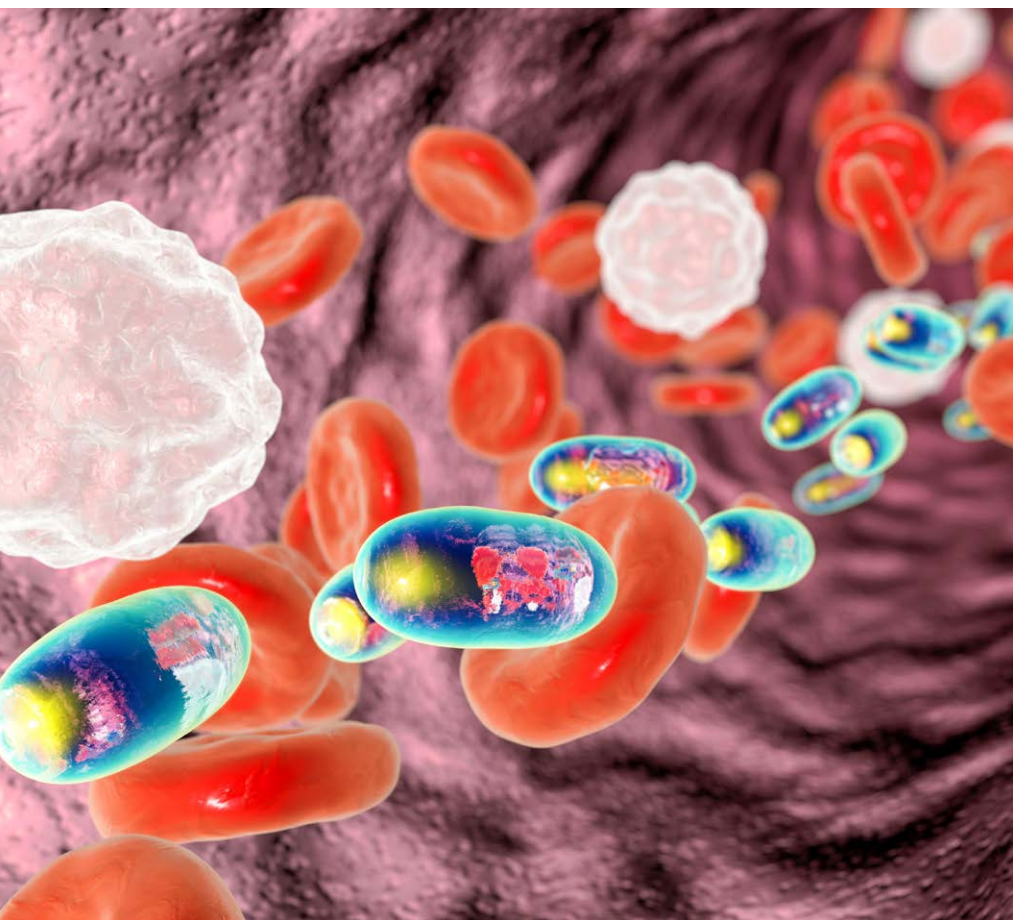


June 2024

Drug Delivery

New Formats for Novel Biopharmaceuticals

by Cheryl Scott, Vincenza Pironti, and Brian Gazaille with Kurt Ristroph



Advances related to drug delivery, formulation, and fill–finish have paralleled drug-modality research in recent years. Perhaps inevitably, the newest formulations and devices bring forth a number of technical and operating challenges in areas such as contamination control, standardization (especially for prefilled syringes), lyophilization, serialization, and management of outsourcing/partnering relationships. New product modalities such as oligonucleotides and advanced therapies are intensifying the traditionally sizable demands on formulation, fill and finish groups in the biopharmaceutical industry. The authors in this eBook address some such concerns. First, a CDMO executive highlights the latest options in container technology. Then, BPI's managing editor discusses key aspects of LNP technology with a leading researcher.

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Introduction

Cheryl Scott

New product modalities such as oligonucleotides and advanced therapies are intensifying the already sizable demands on formulation, fill and finish groups in the biopharmaceutical industry. Historically, delivery of biologics has been a matter of injection — typically intravenous (IV) and intramuscular (IM) — or IV infusion. These sensitive parenteral products must by-pass the metabolic activities of the gastrointestinal tract, making oral delivery thus far an unrealized dream.

A recent survey report from CPHI (part of Informa Markets) highlights the rise of both partnerships and drug-delivery/device innovation, often involving relatively small technology companies (**1**). Survey respondents noted continued expansion in development of autoinjectors and prefilled syringes as well as rising interest in needle-free devices and wearables for parenteral drugs in the patient-centric future. Dual-chambered autoinjectors were predicted to be the most successful technology over the next five years, with pen injectors and prefilled syringes tied for second place.

In that report, consultants connect mRNA and personalized medicines through lipid-nanoparticle (LNP) delivery technology and highlight progress toward inhalation technologies for biologics (**1**): "There is now a new challenge for drug-delivery devices to be able to handle . . . often-delicate formulations. This has led to a shift in the design landscape for inhaled pulmonary drug-delivery devices."

Advances related to drug delivery, formulation, and fill–finish have paralleled drug-modality research. And perhaps inevitably, along with the newest formulations and devices comes a number of technical and operating challenges related to, e.g., contamination control, standardization (especially for prefilled syringes), lyophilization, serialization, and management of outsourcing/partnering relationships (**2**). The authors in this eBook address some such concerns. First, a contract development and manufacturing organization (CDMO) executive highlights the latest options in container technology. Then, BPI's managing editor discusses key aspects of LNP technology with a leading researcher.

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Sterile Drug Products

Key Considerations for Selecting a Final Container Format

Vincenza Pironti

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The biological drug market is growing rapidly, with more products being commercialized and launched annually than ever before. The sector was valued globally at US$322.8 billion in 2021 and is projected to be worth $689.59 billion by 2030, growing at a compound annual growth rate (CAGR) of 8.8% throughout the forecast period (**1**). The US Food and Drug Administration (FDA) approved 22 biologics in 2023, compared with 13 the year before and 12 in 2021 (**2**).

Most new biotherapies are administered by injection or infusion for several reasons. First, the size of active biopharmaceutical molecules creates inherent bioavailability issues that hamper efforts to formulate them for oral/inhaled administration. Second, the sensitivity of large-molecule biological active ingredients makes them vulnerable to degradation in the gastrointestinal tract when received orally. The need to formulate biological drug products for injection requires them to be manufactured, filled, and finished in a sterile environment following regulatory guidelines such as Annex 1 of the EU good manufacturing practice (GMP) guidelines (**3**).

As the biologics sector has grown, it has diversified to include a number of alternative container formats for injectable products beyond the standard glass vial. Each format has features that make it appropriate for different therapies and use cases. Product sponsors must select the most suitable format(s) for the needs of their drug products, patients and clinicians, and intended markets. Decision-makers must consider a number of factors to achieve that goal and ensure that chosen containers play a positive role in optimizing the performance and effectiveness of a finished biologic.

Selecting a Fill Format

Choosing a final fill form depends on a number of important considerations, such as a biologic's modality and route of administration. Biophysical compatibility of a container with the active pharmaceutical ingredient (API) and supporting excipients in formulation is as vital to bear in mind as is user preference.

Biologics pose additional challenges during container selection due to their inherent instability. Container compatibility becomes even more important for biopharmaceuticals than for synthetic chemical APIs, as does the need to consider additional manufacturing process steps (e.g., lyophilization) to eliminate the requirement for cryogenic storage and transport.

Advances in container technology have offered a number of benefits to the biologics sector. Some key developments include prefilled syringes (PFSs), blow–fill–seal (BFS) technology, and alternative delivery routes.

Prefilled Syringes: For users, PFSs come prepared with a single dose of a given biotherapeutic. That streamlines drug administration, minimizes the risk of over- or under-dosing, and helps reduce waste from unused medications left behind in multidose containers. PFS technology also has opened up the possibility of self-administration for a breadth of treatments to benefit patients.

Blow–Fill–Seal: BFS is a manufacturing process that produces lightweight plastic containers by blow-forming, filling, and sealing them in a sterile environment. This approach is ideal for offering single doses, further enhancing the potential for convenient self administration while reducing the risk of product waste and streamlining production and transport costs.

Alternative Administration Routes: Many alternative product presentations and delivery formats are in development, from transdermic microneedles to lipid nanoparticles. Inhalation offers particular advantages in ease of administration and enhanced patient comfort. It is highly attractive for localized treatment of chronic pulmonary diseases, with which patient compliance is crucial to successful disease management.

For most biologic drug-development projects, however, injection/infusion remains the most appropriate mode of delivery. In all cases, the choice of fill form depends on several priorities that change based on a project's development stage. For instance, the needs of a therapy in phase 1 clinical trials will differ greatly from those of a treatment that is entering commercialization. The right moment to choose a final fill form is not during phase 3 clinical testing, however. Rather, it should be considered and agreed upon during the early stages of development to ensure that measures will be in place to streamline movement of a program through clinical trials to commercialization.

The right moment to choose a final fill form is not during phase 3 clinical testing. It should be considered and agreed upon during the **EARLY STAGES** of development to ensure that measures will be in place to streamline movement of a program through clinical trials to commercialization.

Features and Benefits of Different Containers

Each container format has distinct features and benefits, whether it is a new technology or has been a fixture in the market for some time. No single option works for every product. The choice of format depends on the specifics of each biotherapeutic, its target product profile, and patient needs. Some benefits and key considerations of the three most important formats to the injectables market are described below.

Vials: Glass vials are well established on the market. Broadly available and appropriate production infrastructure makes them easy and cost-effective to fill, offering speed-to-market advantages. Vials are suitable for many biologic formulations and are ideal for phase 1–3 clinical-trial materials. These containers can carry multiple doses of a given treatment, which limits both filling and transport costs. When considering vials for a program, think about

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what stage the product is at in its development journey and whether a multidose container might be more appropriate than a single-dose container. If that is the case, then vials may be ideal.

PFSs reach patients already charged with single doses ready for administration. These syringes improve dosing accuracy and minimize waste from leftover formulations. Easy for patients to self-administer, PFSs enhance both convenience and treatment-regimen compliance. As a result, these are ideal for high-value products with a crucial requirement of waste reduction. PFSs are also a good choice for projects that are ready for commercialization, providing enhanced useability of treatments for chronic conditions.

BFS plastics reduce risks for container breakage and bring cost benefits over glass. BFS vials can be filled with either multi- or single-dose volumes. The latter provide added benefits such as reduced microbial and particulate contamination risks in use because the vials are sealed immediately after filling — thus eliminating the need for preservatives in drug formulations. Prefilling each container with a precise dose also helps to improve dosing accuracy and minimize drug waste from what comes with multiple-dose containers. Process innovations have made BFS applicable for ophthalmic and injectable biologic projects, as well as any other high-value drug formulations requiring waste reduction and minimized container breakage risk. The efficiency of BFS technology brings advantages for high-volume filling of materials for commercialization and postlaunch market supplies.

Process innovations have made BFS applicable for ophthalmic and injectable biologic projects, as well as any other **HIGH-VALUE** drug formulations requiring waste reduction and minimized container breakage risk.

Other Considerations in Biologics Filling

In addition to fill form, other factors can affect the shelf life and stability of sensitive biotherapeutics. Leachables from primary containers and closure materials can cause premature expiration of formulations, as can oxidative stress during storage. Adequate extractables and leachables testing of containers and closures during early development is vital to ensuring that packaging will be compatible with the formulations they contain, thus maximizing shelf life as much as possible. For further reading, see the “Leachables and Extractables” box on the next page.

Temperature control is also often a key requirement — whether formulations are kept at fresh, frozen, or even ultrafrozen temperatures throughout transit and storage — to ensure that biologics remain stable when stored for defined periods and reach patients in good condition. However, temperature control poses logistical challenges when it creates the need for specialist storage and transport infrastructure. That can be a problem for products that are destined to travel long distances and across international borders — or for those being shipped to emerging markets and remote locations where cold-chain resources are less common.

Including lyophilization in a formulation manufacturing process can address some issues of formulation stability. Freeze-drying a liquid solution under a vacuum and drying the resulting product into a powder stabilizes it at moderate, noncryogenic temperatures.

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Lyophilization has to be built into a manufacturing process because it affects decisions made about other options (e.g., container selection) to maintain optimum manufacturing output and efficiency.

Working with Experts

Getting container selection right during the earliest possible phase of drug development is vital to ensuring the success and profitability of a biopharmaceutical program — and to delivering truly life-transforming treatments for patients. Working with a contract development and manufacturing organization (CDMO) partner that offers specialist experience in sterile fill and finish of an array of injectable container formats can give biopharmaceutical companies the guidance they need to make informed decisions that optimize the performance of their therapeutic candidates.

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Opportunities for Improving RNA Delivery

Brian Gazaille with Kurt Ristroph

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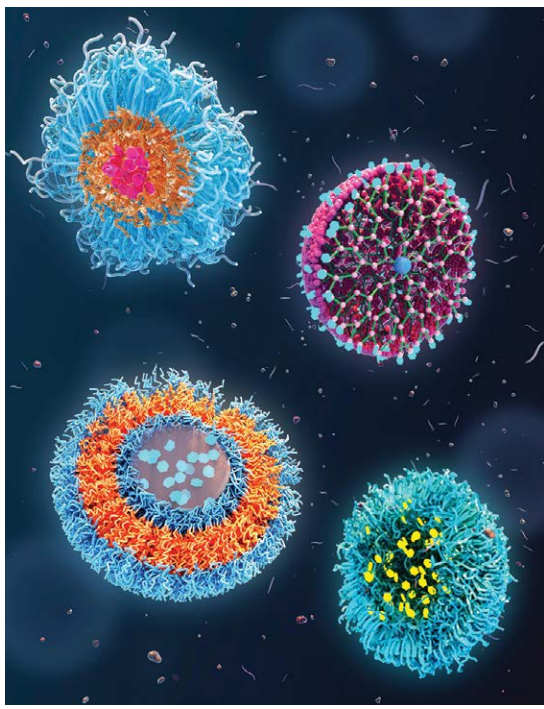
Pfizer's and Moderna's respective Comirnaty and Spikevax vaccines against COVID-19 embody decades of research into messenger RNA (mRNA). A key factor in bringing those products to millions of people around the world was the development of proprietary lipid nanoparticle (LNP) delivery systems. In October 2023, mRNA pioneer and Nobel-Prize winner Katalin Karikó told me,

> It is maybe ironic that I worked on a lipid team as an undergraduate [at the University of Szeged in Hungary] and then studied RNA [in graduate school]. From the beginning of my work with RNA, I was thinking about difficulties with its delivery. But I was naive about how RNA should be investigated as a therapeutic. Curiosity drove my science. (**1**)

Experience with lipids and liposomes ultimately proved to be formative for Karikó, whose invention of nucleoside-modified mRNA and work with LNPs would form the basis of the Comirnaty product.

Although global rollout of the currently available mRNA vaccines has established the viability of both the modality and LNPs, delivery of nucleic-acid therapeutics remains a critical research concern. Notably, those vaccines required ultracold storage until thawing and administration, a condition that hindered product distribution in regions without cryogenic capabilities. Thus, significant opportunities remain for scientists and drug developers to improve the thermal and chemical stability of mRNA therapies. Possibilities also abound for increasing the efficacy of LNP-mediated delivery and developing commercial-scale manufacturing methods and technologies.

Early in 2024, I spoke about such opportunities with Kurt Ristroph, an assistant professor of agricultural and biological engineering at Purdue University (West Lafayette, IN). Ristroph and his colleagues investigate a breadth of topics relating to drug delivery, including nanoformulation process integration and scale-up. The Ristroph laboratory's website explains, "There is a strong translational element to our research. We strive to develop processes that can be implemented at the industrial scale" and that can "improve product physical stability, reduce processing time, and promote dosage-form flexibility" (**2**). During our conversation, Ristroph described the chemistry underlying LNP encapsulation of mRNA, calling particular attention to the need for technologies that provide thorough, rapid mixing of lipid and nucleic-acid components in a fluid formulation. He also discussed



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research into lyophilization of mRNA-LNPs, noting physicochemical difficulties that will need to be addressed to create dry-powder formulations.

Ristroph holds a PhD in chemical engineering and materials science from Princeton University (Princeton, NJ) as well as bachelor's degrees in chemical engineering and classics from Louisiana State University (Baton Rouge, LA). He has worked in civil and environmental engineering at Carnegie Mellon University (Pittsburgh, PA) as a Schmidt Science Fellow and has spent time as a researcher at Moderna (Cambridge, MA) and the Monash Institute of Pharmaceutical Sciences (Parkville, VIC, Australia).

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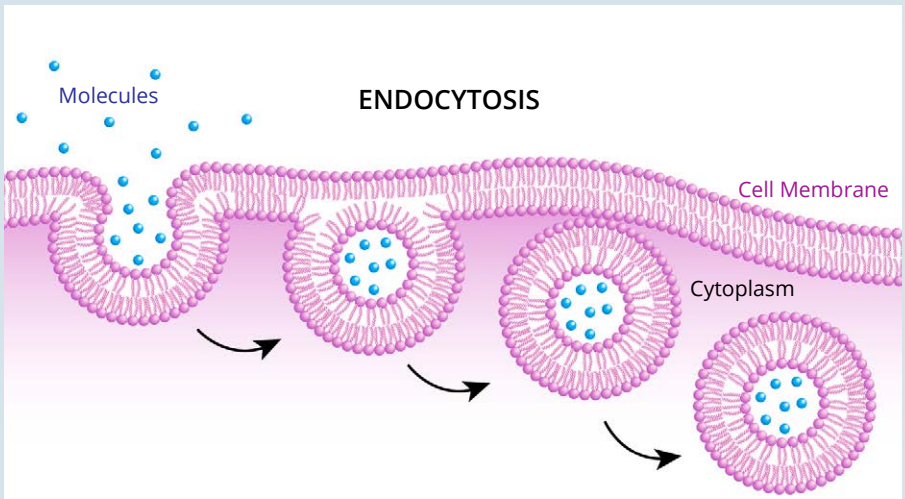
Chemistry Concerns

Why does mRNA require a delivery system? Researchers discovered mRNA in the 1960s, and initial work quickly revealed that it is a transient molecule (**3, 4**). Enzymatic processes break it down quite rapidly, and the immune system can recognize and clear it, so it requires packaging for protection.

Another consideration is that mRNA is like ticker tape. It bears a chain of instructions that must be pulled through, read, and translated by a ribosome so that a protein can be assembled. So an mRNA therapeutic molecule needs not only to reach a cell, but also to reach one of its ribosomes. Neither journey is straightforward because cells have many means of internalizing different molecules.

Solid LNPs such as those used in Moderna's and Pfizer's COVID-19 vaccines are large enough to undergo endocytosis (Figure 1). First, a cell pinches off a vesicle (called an endosome) from its membrane to subsume the LNP. Then, the cell decreases the endosomal pH level, at which point the vesicle begins to transform into a lysosome. When that happens, the nanoparticle is designed to become extremely positively charged on its surface, enabling endosomal escape of the mRNA: The positively charged nanoparticle fuses with the endosome membrane, releasing RNA on the other side. It is somewhat of an open question as to whether the nanoparticle remains fused with the endosome or punches through with the mRNA molecule (**5–9**). In either case, the nanoparticle breaks through the bilayer membrane of the lysosome to release its RNA into the cytosol. That is where you want the RNA to be.

Figure 1: Endocytosis (https://stock.adobe.com)



What have been the most popular options for RNA delivery? Currently, all of the commercialized options are solid LNPs, with variations on

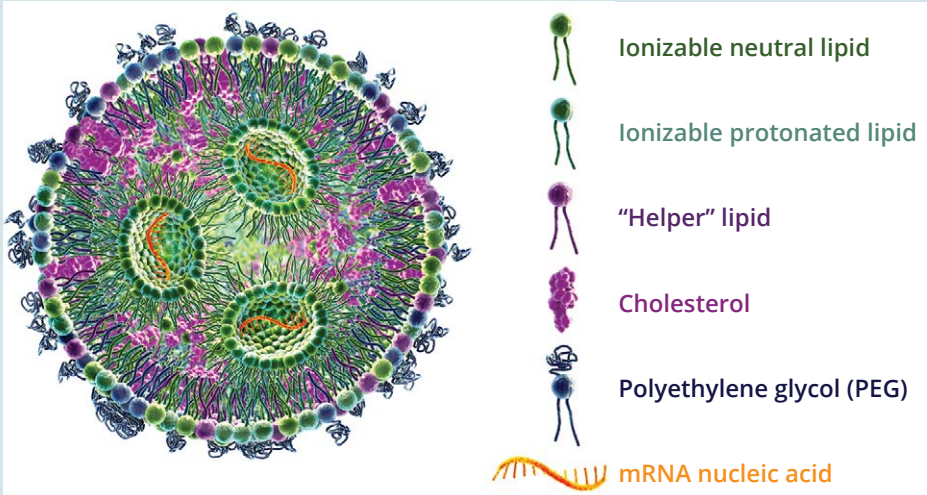
the same theme. The first such system to receive regulatory approval was for formulation of Onpattro (patisiran) from Alnylam Pharmaceuticals in 2018. Patisiran is a drug based on small interfering RNA (siRNA), which compared with mRNA is smaller and somewhat easier to work with. The Onpattro formulation comprises four types of lipids and the therapeutic siRNA molecule. With that product, Alnylam effectively demonstrated that it had developed a way to deliver nucleic acids. Companies have iterated on that initial design because it works. Moderna, Pfizer, and others are coming up with their own iterations of the initial nanoparticle design, particularly for the ionizable-lipid component.

An LNP contains four types of lipids (Figure 2): a cholesterol, a zwitterionic phospholipid (a helper lipid or bulking lipid) such as distearoylphosphatidylcholine (DSPC), a polyethylene glycol (PEG) lipid, and the key player, the ionizable lipid. All four types must be incorporated in a particular ratio. For instance, if the cholesterol mass fraction gets too high, then it will start to crystallize, and you do not want that to happen. The first two lipids provide the particle's structure. The zwitterionic phospholipid, for example, helps with particle curvature. It comes primarily toward the surface of the particle. PEG lipids keep an LNP colloidally stable by preventing aggregation. Without something to keep the lipids apart, those molecules would continue to aggregate. But the cationic ionizable lipid is where the magic of mRNA encapsulation happens.

Lipid delivery vehicles are like balls of fat (**10**). They are phase-separated for water: Water is a polar molecule, whereas lipids are nonpolar. Lipids want to minimize how much water they contact, so they precipitate into little balls. To load mRNA, which is polar and water-soluble, you need to do something to bring a molecule that wants to be in water into the ball of fats instead. RNA is a negatively charged polymer, so the answer is to introduce a positively charged lipid that has one or multiple positive groups and hydrophobic groups. Those hydrophobic groups want to be with the hydrophobic groups on other lipids, while the positively charged groups want to complex ionically through electrostatic interactions with mRNA. That ion pairing pulls the RNA into the particle.

The ionizable lipid is the star of the show. It is also the molecule that gains positive charge when an endosome becomes a lysosome to help the RNA escape the endosome into the cytosol.

Figure 2: Lipid nanoparticle mRNA vaccine (HTTPS://STOCK.ADOBE.COM)



Different companies have their own chemistries for ionizable lipids. Much of the intellectual property (IP) in the field of mRNA delivery lies in the chemical composition of the ionizable lipid.

Anticipating Industrial Application

How are LNPs assembled? What steps are involved in a typical workflow? All four lipid types are water-insoluble, but they are soluble in ethanol. So you dissolve your lipids in ethanol, dissolve your mRNA in an aqueous buffer, and mix those solutions together. The mixed lipids go from an environment that they like (ethanol) to one that they do not (a water–ethanol mixture). Thus, they find themselves way above their solubility limit and want to precipitate. In a mixed solvent, when a hydrophobic molecule encounters something else that is hydrophobic, those molecules stick together, and the combined structure diffuses in the solution. We call that assembly process *diffusion-limited aggregation* because the limiting factor is the speed of the molecules' diffusion. The mRNA will complex ionically with surrounding cationic lipids, which will diffuse and stick with other hydrophobic lipids.

A key feature of LNP assembly is the mixing process applied. Particularly for large-scale LNP manufacturing, you must have a reproducible mixing process, meaning that mixing must be consistent in the vessel over time. The mixing process should also be faster than the time required for a nanoparticle to assemble, which is tens of milliseconds. Ideally, the mixing process should be complete after a few milliseconds so that nanoparticle precipitation can occur in a homogeneous system (**8**). If a vessel has a high concentration of ethanol in one area and a high water concentration in another, then the lipids will precipitate unevenly. A poorly controlled process with precipitation occurring at different rates yields a polydisperse LNP population (**11**) — and pharmaceutical manufacturing necessitates extremely high reproducibility and control.

How difficult is that process to scale? I imagine that mixing is much more difficult to control in the large volumes needed for commercial manufacturing. The difficulty of scalability differs by mixing technology. It is public knowledge, for example, that Pfizer used an impinging-jet mixer to manufacture the LNP-encapsulated mRNA for its COVID-19 vaccine (**12–15**). That type of mixer was applied for pharmaceutical nanoparticle production by Robert K. Prud'homme at Princeton University, whose lab I worked in for my PhD.

The confined impinging-jet mixer that Pfizer uses is a turbulent-flow mixer. Remember that liquids flow in two regimes: laminar or turbulent. Laminar regimes occur at a low Reynolds number. Essentially, fluids slip past one another in sheets, and mixing takes place by diffusion between sheets. Such flow is achieved in extremely small vessels that are mixing at low velocities. Turbulent flow occurs when fluid elements are churning and mixing. Remember, too, that LNP assembly by diffusion-limited aggregation

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Particularly for large-scale LNP manufacturing, you must have a **REPRODUCIBLE** mixing process. Mixing must be **CONSISTENT** in the vessel over time. The process also should be **FASTER** than the time required for a nanoparticle to assemble (tens of milliseconds).

requires fast mixing. Mixing is much faster using turbulent rather than laminar flow. Turbulent flow is part of what gives Pfizer's impinging-jet mixer its scalability. Turbulent flow can be achieved in large vessels and with high flow rates, meaning high throughput. Other turbulent-mixer systems are available, too, but the Pfizer technology provides a well-known example.

Many scientists who research LNPs use microfluidic mixers that apply laminar flow or perform mixing by hand — e.g., by rapidly pipetting ethanol and lipids into a vial of mRNA. Both processes are hard to control at scale. One of my goals is to use processes in research that we know can be scaled up later. So if we want to have scalable, reproducible, and highly controllable mixing processes, then we want to use the kind of technology that Prud'homme developed and that Pfizer implemented for mRNA manufacturing.

Improving Stability and Storage

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Why does mRNA require ultracold storage, at least in current formulations? As I mentioned, mRNA is a transient molecule. It is susceptible to enzymatic breakdown, immune-system clearance, and even chemical decomposition (**16**). Ionizable lipids represent a double-edged sword in that respect. Interaction between negatively charged mRNA and a positively charged ionizable lipid drags the mRNA into a nanoparticle. That process provides the desired encapsulation, but the proximity of the positive charge and that particular ion-pairing interaction can help to accelerate mRNA degradation (**17**). If your mRNA ticker tape has even one rip in it, then it no longer works for protein translation.

Freezing a drug product slows down those degradation reactions considerably. We need that to happen to ensure mRNA stability.

We all remember the –80° C storage requirement for the initial Comirnaty COVID-19 vaccines. Does mRNA require such temperatures to maintain its stability, or was that parameter more of an "insurance policy" to ensure vaccine efficacy and safety upon administration? I can't say for certain, but I know that stability studies take a long time. The field did not have that kind of time during the COVID pandemic. Companies working with mRNA had internal data about temperature stability — enough that they could be confident about –80° C storage conditions providing sufficient chemical stability for the mRNA.

What are some of the obstacles to creating more thermostable mRNA drug products? On the face of it, you might want to try removing all of the water from the drug product to eliminate the possibility for hydrolysis reactions of the ionizable lipid (**17**). The result would be a powder formulation of your LNP. Some research teams are taking that approach — e.g., using lyophilization (freeze-drying), which is a popular and well-established method (**18–20**). Before lyophilization, the mixed ethanol–water–LNP solution would undergo diafiltration (DF) or

tangential-flow filtration (TFF) to remove the ethanol and replace the buffer with another buffer that is suitable for storage. Then, the formulation is frozen and the water is sublimated, leaving the LNPs as well as salts that were present in the storage buffer.

Aggregation represents an obstacle to LNP lyophilization. Remember that LNPs are essentially little balls of fat suspended in water. Given the chance, your nanoparticles will form into one big chunk to minimize their contact with water. In solution, they cannot do that because of the PEG lipids on their surfaces. Those molecules are amphiphilic, with both hydrophobic and hydrophilic regions. Such molecules keep LNPs colloidally stable while diffusing in water, preventing aggregation. But freeze-drying sublimates the ice that was separating the nanoparticles, pushing them together. The concern is that PEG lipids are unable to keep nanoparticles from aggregating as they get pushed together by the ice front.

So your formulations need a cryoprotectant (matrix form) to set up physical barriers between LNPs during lyophilization. Usually, a sugar is applied — e.g., sucrose, trehalose, or mannitol. Polymers such as PEG can be used, but small sugar molecules are more common in lyophilization processes. The difficulty lies in figuring out which sugars to use and how much sugar to add. Freezing needs to occur rapidly because the aggregation mechanisms that are at play during mixing can manifest during freezing, too. Solutions freeze from the outside in. If freezing happens too slowly, then the nanoparticles will concentrate in the center of the ice, where they can aggregate.

Several research challenges remain for lyophilization of mRNA LNPs. Scientists still need to optimize key parameters, including freezing rates, cryoprotectant concentrations, and lyophilization temperature and pressure.

Many laboratories, including mine, are exploring ways to process nanoparticle suspensions into dry-powder formulations. The “holy grail” would be to devise a dry powder that is stable at room temperature. Doing so entails a lot of characterization. After resuspension, the LNPs must be the same size as they were before drying. Even partial aggregation will be unacceptable. You need to ensure that the mRNA remains encapsulated. The mRNA must be intact to prompt a host cell to express a given protein, so you need to check its chemical and physical stability. Assuming that your lyophilization process has passed those tests, then you need to submit your powder to different storage conditions and then wait and see. That all takes time and effort.

How stable would a dry-powder formulation be? Would it still be susceptible to temperature, humidity, and so on? Yes, a dry powder’s stability depends on time, temperature, and humidity. Even a dry powder cannot be stable forever. It might be stable for a month, maybe for a year. Temperature potentially matters a lot. I worked on dry-powder susceptibility to humidity when I was in graduate school, for a project with the Bill and

CHALLENGES REMAIN for lyophilization of mRNA LNPs. Scientists still need to optimize key parameters, including freezing rates, cryoprotectant concentrations, and lyophilization temperature and pressure.

Melinda Gates Foundation on antimalarial formulations for use in Sub-Saharan Africa and Southeast Asia. For mRNA, you probably want to lyophilize your material in a glass vial, so that clinicians can add water to the drug product, let it resuspend, and then draw up the material into a needle for administration. With the right kind of packaging, you can keep humidity out of a dry-powder formulation — but temperature is still a factor that you need to account for.

LNP Prospects

What do researchers and drug developers need more of — in terms of technology or scientific understanding — to realize new options for mRNA and LNP formulation? The RNA-LNP is an extremely complex modality because it (currently) comprises a specific mix of five components (four lipid types and mRNA). Many other variables are at play, too: the solution pH, buffer composition, mixer type and geometry, type of sugar used as a cryoprotectant, and freezing or freeze-drying conditions.

Thus, mRNA LNP formulation is a multidimensional problem with a highly combinatorial experimental space. Some researchers are performing interesting work using "self-driving laboratories" that can analyze different iterations of the variable space rapidly (**21, 22**). That presents an opportunity to increase the throughput of mRNA-formulation experiments. Of course, one difficult aspect of increasing experimental throughput is the cost of lipids and mRNA. Both components are extremely expensive. Even when using a tiny amount in a high-throughput assay, the costs add up.

Another frontier to explore is LNP composition, figuring out whether we can achieve efficacy with structures beyond current four-lipid approaches. I heard a talk from Daniel Siegwart at the University of Texas Southwestern (Dallas, TX), whose laboratory is doing some excellent work in that regard. That team is experimenting with adding a fifth and even a sixth lipid, evaluating the resulting systems' efficacy for mRNA delivery (**23, 24**). The nanoparticles move to different organs in the body depending on their lipid compositions. Such research presents huge opportunities, but again, the experimental space is combinatorially large.

RNA is an exciting modality. The field has much more to do on the formulation side. We have a social responsibility to continue studying the long-term safety and efficacy of LNP-encapsulated mRNA. Safety and efficacy are two sides of the same coin, so we need to be conscientious about looking for off-target effects. I personally like to encourage researchers to use mixing technologies and processes that are representative of those used at industrial scales. All of those dimensions need to be studied carefully.

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