How Lyophilisation Can Overcome mRNA Stability Challenges

In this article, Vincenza Pironti, Head of Business Development at Recipharm, explores the latest trends in messenger RNA (mRNA) drug development, dives into the development challenges this modality poses, and explains how lyophilisation can hold the key to optimising stability in storage and transit.

The mRNA therapeutics market was estimated to be worth $18.7 billion in 2023, and is forecast to grow to $40 billion by 2033, expanding at a compound annual growth rate (CAGR) of 8.2% throughout the forecast period.[1]

Stability is a Hurdle

While mRNA therapeutics offer a number of benefits for pharmaceutical companies seeking novel solutions for currently untreatable diseases, they do present challenges in development and manufacturing. These need to be addressed to deliver an effective and commercially viable end product.

A particular problem for mRNA-based therapeutics is their inherent instability. The mRNA molecule is naturally both physically and chemically unstable:

- Physical instability concerns include the loss of secondary or tertiary structure, as well as aggregation and precipitation, which affect the translation of mRNA molecules.
- Chemical instability issues include potential degradation due to hydrolysis and oxidation.[2]

Failure to address these issues in development can lead to a product with a short shelf life, or one that requires frozen or ultra-frozen storage and transport – as we saw with COVID-19 vaccines, some of which required storage at -70°C.[3] These storage and shelf-life challenges can have implications for global accessibility of novel therapeutics – it may be difficult to transport doses long distances overseas, and emerging markets, where there is limited cold-chain infrastructure, may end up being underserved.

Lyophilisation Presents a Potential Solution

One possible answer to this issue for future mRNA-based vaccines, cancer treatments and other therapies is the use of lyophilisation in the formulation process. Lyophilisation or freeze-drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the sublimation of water. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption).

The advantages of lyophilisation include:

- Enhancing the stability of highly sensitive products
- Gentle removal of water from the formulated mRNA
- Rapid and easy dissolution of reconstituted product
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Lyophilisation Challenges to be Overcome

To harness the benefits of lyophilisation in overcoming mRNA instability, pharmaceutical companies do need to answer a number of questions.

1. Does the Lyophilisation Process Effectively Optimise Stability of the Product in Question?

For successful drug administration, the vial must contain a high-quality final cake that can be easily reconstituted when it is time to inject the dose without compromising the formulation's integrity. This is a particular issue for low-dosage products like mRNA-based therapies due to the disproportionate ratio of ingredients-to-dose-volume. In such cases, the incorporation of suitable cryo-protectants or bulking agents is crucial. These additives must be compatible with the formulation and the vial and ensure the stability of the final drug product. Extensive analytical testing of the formulation well before commercialisation can help determine the appropriate lyophilisation methods to preserve product stability and extend shelf-life once the therapy enters commercial use.

2. How to Maintain the Integrity of Lipid Nanoparticle Formulations (LNPs)?

Many mRNA-based therapies use LNP technology, where the active substance is encapsulated within lipids to enhance its bioavailability and stabilise it. In such cases, the lyophilisation process must be carefully designed to minimise potential damage to the LNP envelope and preserve the viability of the drug product (DP) upon reconstitution. It is essential to employ prolonged freeze-drying cycles to prevent harm to the LNPs within the formulation, which could compromise their performance and stability.

Strategies to Harness Lyophilisation

To address potential challenges with mRNA

therapeutics lyophilisation, alternative approaches can be explored.

One approach is to optimise the process by ensuring it is as gentle as possible. A thorough study of each freeze-drying phase is required, rather than opting for an accelerated method that may save time but risk damaging the formulated mRNA.

Another approach is to modify the formulation. Factors such as different ionic strengths within the buffer can significantly impact the resulting cake post-lyophilisation, potentially improving stability and facilitating reconstitution. Adjusting the mRNA concentration pre-lyophilisation can also influence the freeze-drying process and should be addressed to ensure effectiveness. For LNPs, selecting an appropriate surfactant is crucial to address aggregation challenges.

A number of technological advancements in lyophilisation in recent years have the potential to further overcome challenges when harnessing the process for mRNA products. Controlled temperature automated loading and unloading of freeze-dried products, for instance, have significantly improved the overall manufacturing and filling processes of mRNA products, ensuring optimal product stability. mRNA formulations remain sensitive to temperature even after lyophilisation, so automating and accelerating unloading minimises the risk of exposure to impactful temperatures on final stability.

The Value of Seeking Lyophilisation Guidance Early

The lyophilisation process is time-consuming, especially for mRNA candidates. For pharmaceutical companies with a limited timeframe to move mRNA candidates from early development to clinical trials, bypassing lyophilisation during clinical manufacturing may be necessary to expedite the process. Nonetheless, the earlier lyophilisation is incorporated into the formulation development journey, the more straightforward it is to establish an effective process. This ensures the final mRNA treatment benefits from enhanced stability and simplified storage capabilities provided by lyophilisation.

The mRNA technology is frequently approached as a platform. Developing an effective process for lyophilisation - also known as a lyocycle - and selecting an appropriate formulation require a comprehensive understanding of the technology.

With this in mind, more and more pharmaceutical companies are seeking expert external advice on lyophilisation feasibility from contract development and manufacturing organisations (CDMOs). The rapid increase in the number of mRNA treatments under development in recent years has seen such partners gain significant experience in this field, so CDMOs are well placed to assist pharmaceutical companies in accessing lyophilisation advances. This enables pharmaceutical companies to effectively leverage these advancements in a structured manner, supporting the efficient commercialisation of their mRNA products when they reach the commercialisation stage.

Learning Lessons from the Past

Due to the speed with which companies needed to develop and commercialise the vaccines, lyophilisation was not taken into account during the first COVID-19 pandemic. This meant it was necessary to have frozen and ultra-frozen transport and storage in place for the first wave of vaccines, which hindered the global vaccination process, especially in emerging markets, which lacked the necessary infrastructure. This presents clear lessons for the industry to learn from for the next outbreak.

Lyophilisation – and the stability at ambient temperatures it confers – presents significant advantages for mRNA vaccines developed to combat future pandemics, and the logistical requirements for any vaccination programme. A lyophilised product can greatly simplify logistics when vast quantities of vaccine doses need to be manufactured and distributed worldwide. This eliminates the requirement for specialised cold-chain transportation and storage, making the process more manageable and cost-effective. Lyophilisation also offers plenty of potential for mRNA therapeutics targeting cancers and other conditions, simplifying handling during manufacturing, and supply chain needs, making it easier for treatments to reach patients all over the world.

To harness the technology, it is important for pharmaceutical companies to work with a CDMO with specialist experience and infrastructure for lyophilisation and subsequent sterile fill and finish of mRNA treatments. By leveraging existing knowledge of successful strategies, it is possible to

streamline the development process. This eliminates the need for redundant efforts, as companies can build upon proven methodologies rather than starting from scratch.

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