



Special Topic Commentary

Addressing the Cold Reality of mRNA Vaccine Stability



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ARTICLE INFO

Article history:

Received 7 December 2020

Accepted 8 December 2020

Available online 13 December 2020

Keywords:

Cold chain

COVID-19

Formulation

Lipid nanoparticles

mRNA

Shelf life

Stability

Vaccine

ABSTRACT

As mRNA vaccines became the frontrunners in late-stage clinical trials to fight the COVID-19 pandemic, challenges surrounding their formulation and stability became readily apparent. In this commentary, we first describe company proposals, based on available public information, for the (frozen) storage of mRNA vaccine drug products across the vaccine supply chain. We then review the literature on the pharmaceutical stability of mRNA vaccine candidates, including attempts to improve their stability, analytical techniques to monitor their stability, and regulatory guidelines covering product characterization and storage stability. We conclude that systematic approaches to identify the key physicochemical degradation mechanism(s) of formulated mRNA vaccine candidates are currently lacking. Rational design of optimally stabilized mRNA vaccine formulations during storage, transport, and administration at refrigerated or ambient temperatures should thus have top priority in the pharmaceutical development community. In addition to evidence of human immunogenicity against multiple viral pathogens, including compelling efficacy results against COVID-19, another key strength of the mRNA vaccine approach is that it is readily adaptable to rapidly address future outbreaks of new emerging infectious diseases. Consequently, we should not wait for the next pandemic to address and solve the challenges associated with the stability and storage of formulated mRNA vaccines.

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Introduction

Only a few early-stage clinical trials with several different mRNA vaccine candidates, mostly focused on treatment or protection of small groups of recipients, were in progress in January 2020 at the time of the outbreak of the COVID-19 pandemic. This situation dramatically changed in the first months of 2020, when mRNA vaccines 'overnight' became COVID-19 vaccine candidates and a global pandemic had to be tackled as fast as possible. Currently, three non-replicating mRNA vaccine candidates against COVID-19 are being tested in human clinical trials, i.e., sponsored by Moderna, Pfizer-BioNTech, and CureVac.¹ Many more mRNA vaccine candidates are being evaluated in preclinical studies.² The first results from large-scale, Phase 3 clinical trials for the Moderna and

Pfizer-BioNTech vaccines have been reported as very promising with high efficacy rates (~95%). Moreover, no clinical holds due to unacceptable adverse effects have been encountered so far.

As of the writing of this commentary, one mRNA-based COVID-19 vaccine (from Pfizer-BioNTech) has received conditional approval by the British MHRA (Medicines and Healthcare products Regulatory Agency) and more regulatory bodies are expected to follow soon.³ Considering the potential of the mRNA vaccine approach to control or even stop the pandemic, a rolling review approach has been adopted by regulatory authorities such as FDA (US Food and Drug Administration) and EMA (European Medicines Agency) to ensure both a rigorous and rapid review and approval process.⁴ When approved, an enormous number of mRNA vaccine doses will need to be manufactured, shipped across the globe, stored at end-user sites, and then administered in large-scale vaccination campaigns. Before the COVID-19 pandemic, the storage temperature of mRNA vaccine candidates in development had not been given much attention. Typically, small batches were kept frozen at −80 °C, and then thawed and administered as needed. Along with the growing clinical promise of mRNA-based COVID-19

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vaccines, however, there arose a growing perception that storage, transport and delivery under these conditions would create quite a challenge when hundreds of millions (eventually billions) of doses were to be administered all around the world.^{5,6}

In this commentary, we describe the current proposals (based on publicly available company information) for the storage of mRNA COVID-19 vaccines across different stages of the supply chain including in-use stability conditions. This is followed by an overview of the literature of what is known about the stability of mRNA vaccines, especially the final drug product. We then discuss attempts made to improve their stability during storage, analytical methods to monitor their stability, and international regulatory guidelines for stability testing. Finally, we summarize our current knowledge base and identify outstanding challenges and opportunities with regard to improving the stability profile (and assessments) of formulated mRNA vaccines.

Description of the mRNA COVID-19 Vaccines Being Tested in the Clinic

Historical Perspective

The (short) history of the development of the concept of using mRNA vaccines for prophylactic or therapeutic indications has been expertly summarized.^{7–9} In these reviews, the special character of mRNA macromolecules consisting of approximately 1000–5000 nucleotides is discussed, and various strategies to modify 'natural' mRNA molecules to make them more resistant to enzymatic and non-enzymatic degradation are explained along with different approaches to decrease potential innate immune reactions. Moreover, Pogocki and Schöneich provide comprehensive insights into the chemical degradation reactions that mRNA molecules can undergo, including hydrolysis of N-glycosidic bonds (depurination), hydrolysis of phospho-diester bonds, deamination of cytosine derivatives and oxidation of nucleobases or sugar moieties.¹⁰ Reaction kinetics not only depend on the solution environment (e.g., pH, buffers, presence of oxygen) but also on secondary structure formation of the mRNA molecule in solution as well as in the presence of cationic lipids (see below).^{11,12} It is important to recognize that the complete, intact mRNA molecule is essential to its potency as a vaccine. Even a minor degradation reaction, anywhere along a mRNA strand, can severely slow or stop proper translation performance of that strand and thus result in the incomplete expression of the target antigen. In contrast, therapeutic proteins and protein antigens may undergo multiple chemical degradation reactions (e.g., Asn deamidation, Met oxidation) which, if they occur outside the active site or an important epitope, do not necessarily affect potency.

Particular attention must be paid to the critical issue of delivering mRNA inside cells and the essential contribution of formulation using various delivery vehicles.^{13–15} For example, lipids (as in lipid nanoparticles, LNP) and proteins (e.g., protamine, a naturally-occurring basic/cationic polymer) are used to enhance intracellular mRNA delivery. These critical components of a formulated mRNA

vaccine modulate mRNA distribution in the body, help mRNA molecules enter cells, and affect protein antigen expression and immunogenicity as well as the safety profile.^{14,16} Moreover, they likely impact mRNA stability during storage. For example, in a theoretical study, Wayment-Steele et al. predict a hundred-fold increase in cleavage rate of mRNA when it is incorporated in a cationic lipid formulation.¹² Based on these considerations, the nature, quality and supplier of these excipients, along with the design of the formulation manufacturing processes, may affect the pharmaceutical stability of formulated mRNA vaccine candidates in terms of chemical stability of the mRNA/excipients as well as the colloidal stability of their complexes. A reliable supply-chain of the raw materials is therefore of great importance to assure consistent quality of the vaccine product, which can be challenging in times of a pandemic as was recently experienced by Pfizer-BioNTech.¹⁷

Storage Conditions for COVID-19 mRNA Vaccines: Current Situation

Available information on the stability profiles of the mRNA COVID-19 vaccine candidates in development is regularly updated. The current shelf-life and temperature storage conditions published by three vaccine manufacturers (i.e., Moderna, Pfizer-BioNTech and CureVac) are listed in Table 1. At this time (December 5, 2020), such information has been provided by the vaccine manufacturers only, with no confirmation from regulatory authorities. It is now clear, however, that the required storage conditions during manufacturing, shipping and at the end-user site are considered important characteristics of the mRNA vaccine drug product, as they might offer a competitive (dis)advantage.

Stability Data of mRNA Vaccines in the Literature: A Rare Commodity

There is a wealth of information on stabilizing mRNA molecules themselves, as expertly reviewed.^{8,9,18} In contrast, when searching the literature for stability and storage of formulated mRNA drug product stability (i.e., LNP-mRNA and protein-mRNA complexes), little information can be retrieved as of the time of this writing. In a review by Muradlihar et al., on nucleic-acid based macromolecules (NAM), including but not restricted to mRNA vaccines, the authors draw a similar conclusion: 'there is a need for a comprehensive overview of all the challenges and mitigation strategies for naked NAM stability and formulation in nonviral systems.'¹⁹ That need is even more urgent now, considering the emerging importance of mRNA vaccines in fighting the global COVID-19 pandemic.

In terms of mRNA drug product stability studies, a few reports with lyophilized mRNA formulations have appeared. For example, a study on the effect of freeze-drying on the integrity of naked mRNA showed that a trehalose solution performed better than distilled water.²⁰ In a later publication, a mRNA-protamine vaccine candidate against rabies was freeze-dried and tested in mice. The dried formulation was stored at temperatures ranging from –80 to +70 °C. Under the chosen read-out conditions (1 vaccine dose

Table 1
Current Stability Profile, Dose and Dosing Schedule of mRNA COVID-19 Vaccine Candidates in Development (Status December 05, 2020).

| Manufacturer | Stability in Frozen State | Stability at 2–8 °C | Stability at Room Temperature | Dose (Injection Volume); Dosing Schedule | References |
|-----------------|----------------------------------|---------------------|--|--|------------|
| Moderna | –20 °C, up to 6 months | 30 days | Up to 12 h | 100 µg (0.5 mL); day 1, day 29 | 36,37 |
| Pfizer-BioNTech | –80 °C to –60 °C, up to 6 months | up to 5 days | Up to 2 h (up to 6 h after dilution ^a) | 30 µg (0.3 mL); day 1, day 21 | 5,38,39 |
| CureVac | ≤ –60 °C, at least 3 months | At least 3 months | Up to 24 h | 12 µg (no information); day 1, day 29 | 40–42 |

^a The thawed vaccine must be diluted in its original multidose vial with unpreserved 1.8 mL sodium chloride 9 mg/mL (0.9%) solution for injection (not provided with the vaccine).³⁹

level), the vaccines stored for 12 months at 5, 25, 37 and 45 °C gave adequate neutralizing antibody levels and protected mice from a rabies infection. Even after stressed storage at +70 °C for 3 months, protection of the mice was observed. Unfortunately, the study does not give details about the formulation or about physicochemical quality attributes before and after lyophilization and storage.²¹ In a series of patents from CureVac, claims are made to maintenance of the activity of mRNA formulations (with and without protamine) upon freeze-drying and storage by using lyoprotectants such as sugars.^{22–24} For example, Ketterer et al. used trehalose as a lyoprotectant to stabilize mRNA-protamine complex formulations during freeze drying and storage at –80, 5, 25 and 40 °C.²⁴ Stability analysis included appearance, RNA integrity, RNA content, pH value, and osmolality, and the text states: ‘The results show that all quality attributes analyzed during the experimental period (up to 36 months) meet the stability specifications of a stable and safe RNA medicament.’ However, no justification for such stability specifications were given. Other patent publications describe spray-drying of mRNA²⁵ or the generation of lysospheres, i.e., freeze-dried droplets with mRNA.²⁶ In 2020, Zhao et al. published their findings on the performance of freeze-dried LNP-mRNA (encoding for luciferase) with different lyoprotectants compared to fresh LNPs.²⁷ They examined both *in vitro* and *in vivo* mRNA expression (single-dose, i.v. injection) in mice. These reports, scattered throughout the (patent) literature, lack a mechanistic basis. Clearly, the speed at which mRNA vaccine candidates have recently been developed has prioritized optimization of *in vivo* delivery, *in vivo* expression, and animal immunogenicity and safety results over storage stability, which now places critical limitations on future, large-scale mRNA vaccine deployment.

Regulatory Guidelines

In June of 2020, the FDA published a ‘Guidance for the development and licensure of vaccines to prevent COVID-19’ (thus, not only mRNA vaccines), which contains paragraphs on ‘Chemistry, Manufacturing and Controls’ (CMC).²⁸ The following quotes are taken from this guidance document: ‘Data on the qualification/validation for all quality indicating assays should be submitted for a BLA’ (biologics license application) and ‘For vaccine licensure, the stability and expiry date of the vaccine in its final container, when maintained at the recommended storage temperature, should be demonstrated using final containers from at least three final lots made from different vaccine bulks.’ Naik and Pesen, from the FDA Office of Vaccines Research, provide more detailed insights into the current thinking within the FDA regarding CMC issues of mRNA vaccines.²⁹ Specific ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human

Use) or FDA guidances for mRNA-based vaccines have yet to be developed, and no examples of specific release and/or stability acceptance criteria for quality attributes are in the public domain.

The regulatory pathway in the EU for mRNA vaccines against infectious diseases is described by Hinz et al.³⁰ The EMA considers mRNA vaccines for infectious diseases as a class of medicines known as biological medicinal products. These products fall under the responsibility of the Committee for Human Medicinal Products (CHMP) and follow the centralized procedure to obtain marketing approval through EMA. Schmid summarizes the quality data needed for an EU Investigational Medicinal Product Dossier (IMPD), which is part of the clinical trial application (CTA).³¹ The latter is similar to the USA Investigational New Drug (IND) Application. The IMPD contains information on the mRNA molecule itself as well as degradation products (i.e., product impurities) and process impurities such as residual DNA, proteins and solvents from the manufacturing process. The next section of the IMPD deals with the control of the drug substance with information on analytical procedures and their acceptance criteria (i.e., specifications). Finally, data on critical mRNA product stability parameters is requested where stability-indicating parameters for the mRNA component are: mRNA integrity, content and potency as well as pharmaceutical properties including pH, appearance, and the microbiological status of the drug product. Details on specifications and stability testing for a biological bulk drug substance and final drug product can be found in the ICH Q6B and ICH Q5C guidelines, respectively. At the end of their chapter, Hinz et al. provide an exemplary list with analytical tests for a liquid mRNA-based drug substance and a freeze-dried drug product without giving corresponding acceptance criteria.³⁰ In the next section, we briefly discuss key analytical techniques for mRNA (vaccine drug product) testing and characterization.

The Analytical Toolbox

The qualitative composition of two mRNA COVID-19 vaccine candidates formulated as mRNA-LNPs is shown in Table 2. The analysis of the identity, purity, potency, safety and stability of the mRNA bioactive and the mRNA-lipid/protein complex formulations is obtained through a series of analytical methods. Poveda et al. reviewed key techniques currently available to monitor the critical quality attributes of mRNA vaccine candidates (Table 3).³² We expanded the list by including additional assays to characterize attributes related to formulated (i.e., lipid- and/or protein-containing) mRNA drug products including general quality attributes for parenterally administered vaccines.^{19,33} Unfortunately, a critical review on analytical techniques to characterize mRNA-(cationic) lipid complexes, as has been published previously for plasmid DNA-lipid complexes,³⁴ is clearly missing.

Table 2
Qualitative Composition of mRNA COVID-19 Vaccine Candidates Formulated as mRNA-Lipid Nanoparticles (LNPs)^a.

| Category | Pfizer-BioNTech Vaccine Candidate | Moderna Vaccine Candidate |
|----------------------------------|---|---|
| Active pharmaceutical ingredient | BNT162b2 RNA | mRNA-1273 |
| Lipid nanoparticle components | ALC-0315 = (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis (2-hexyldecanoate) ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N ditetradecylacetamide 1,2-Distearoyl-sn-glycero-3-phosphocholine Cholesterol | SM-102 (proprietary ionizable lipid) PEG2000-DMG = 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 1,2-Distearoyl-sn-glycero-3 phosphocholine Cholesterol |
| Buffer | Phosphate (potassium dihydrogen phosphate, disodium hydrogen phosphate dihydrate) | Tris (tromethamine) |
| Other excipients | Potassium chloride, sodium chloride Sucrose Water for injection | Sodium acetate Sucrose Water for injection |

^a Source: Pfizer-BioNTech: Reg 174 information for UK healthcare professionals for COVID-19 mRNA vaccine BNT162b2 concentrate for solution for injection³⁹ and Moderna mRNA-1273-P301 Clinical Study Protocol.⁴³

Table 3Analytical Methods to Determine and Monitor Quality Attributes and Stability of mRNA Vaccine Bulk Drug Substance and Final Drug Product^a.

| Assay | Purpose |
|---|--|
| Characterizing DNA templates and RNA transcripts | |
| DNA template sequencing/mRNA sequencing | Identification of mRNA |
| UV spectroscopy (A260 nm, A260/A280, A260/A230) | Quantification – purity dependent |
| Fluorescence-based assays (e.g., residual DNA) | Quantification – purity assessment |
| Agarose/acrylamide electrophoresis | Molecular mass, RNA integrity and quantification |
| Reverse transcriptase qPCR | Identification and quantification of mRNA |
| Blot for dsRNA | Quality assessment |
| mRNA capping analysis | Quality assessment |
| mRNA polyadenylated tail analysis | Quality assessment |
| Chromatographic assays ^b | Quantity and quality assessment |
| Characterizing mRNA-encoded translation products | |
| In vitro translation – cell free medium | Translation into target protein |
| mRNA evaluation using various cell-based systems | Translation product analysis and potential toxicity assay |
| Characterizing mRNA-lipid/protein complexes | |
| Light scattering ^c | Particle size (distribution) |
| (Gel) electrophoresis | Assessing bound/unbound mRNA and surface charge |
| Laser Doppler electrophoresis | Zeta potential |
| Chromatographic assays ^b /mass spectrometry | Quantification and integrity of carrier lipids/protein |
| Fluorescent dyes | Encapsulation efficiency |
| General pharmaceutical tests | Appearance, pH, osmolality, endotoxin concentration, sterility |

^a Adapted from Poveda et al., 2019³² and Muralidhara et al., 2016.¹⁹^b Size-exclusion chromatography, anion-exchange chromatography, affinity chromatography, reversed-phase chromatography.^c Dynamic light scattering, static light scattering, nanoparticle tracking analysis.

In summary, mRNA based product candidates for several therapeutic and prophylactic indications have been tested in the clinic and numerous publications have reported on the nature of the mRNA drug product involved. Nonetheless, key CMC information on the critical quality attributes and corresponding justification of acceptance criteria, especially as related to determining the stability profile of formulated mRNA drug products (i.e., shelf-life and storage temperature), has yet to reach the public domain.

Concluding Considerations

The mRNA vaccine candidates from Pfizer-BioNTech and Moderna are promising frontrunners for protection against the COVID-19 pandemic. Although detailed efficacy and safety information from the clinical trials as well as required storage conditions are shared with the public through publications in peer reviewed journals and company press releases, no background information on the quality attributes that control and limit stability is available. For example, what are the assay capabilities and acceptance criteria for the relevant quality attributes for the proposed storage conditions for mRNA vaccines (Table 1)? In this context, the extended controlled temperature chain (ECTC) initiative of the World Health Organization (WHO) is worth mentioning.³⁵ In this case, it would potentially allow a mRNA vaccine to be kept at temperatures outside of the frozen cold chain (e.g., 2–8 °C for a limited period of time) under monitored and controlled conditions.

While the present mRNA vaccine formulations in late-stage COVID-19 clinical trials may already be finalized in terms of composition, there is presumably room for subsequent, “second generation” formulations with stability improvements. For example, alternative excipient choices to develop improved, refrigerated stable mRNA vaccine formulations, e.g., as either liquid or lyophilized products, could be pursued. From the above discussion, one can see that stability assessments of mRNA vaccine candidates are in their infancy with only scattered reports currently in the literature. A systematic, mechanism-based approach looking into chemical and physical degradation pathways is lacking. Taking a more rational approach to the design of new formulations to ensure stability of mRNA vaccine final drug products should

therefore have top priority in the pharmaceutical community. Such formulation development studies including the selection of excipients (e.g., stabilizers and/or the inclusion of preservatives), formulation milieu (e.g., pH and tonifying agents), and manufacturing processes (e.g., liquid or lyophilized dosage forms) should be part of an integrated effort to move away from freezing conditions for long-term storage. To this end, the inherent stability of the active mRNA molecule should be optimized without compromising its potency. The LNP (consisting of mRNA and lipids; cf. Table 2) composition and structure are critical to *in vivo* delivery, protein antigen generation, and subsequent immunogenicity and vaccine safety. Therefore, *in vivo* testing should be part of this development program for second generation mRNA vaccine formulations, as optimization of shelf-life and storage temperatures should not interfere with presently established *in vivo* performance.

Finally, as mRNA vaccines take a prominent place in global strategies to successfully fight the COVID-19 pandemic, we believe it is short-sighted and unwise to wait for another pandemic before solving the storage stability issues of this versatile, rapidly-deployable vaccine platform technology. Instead, a better understanding of the causes and mechanisms of the instability of mRNA vaccine formulations, combined with rational selection of appropriate stabilization technologies, will undoubtedly lead to improvements in mRNA vaccine stability. Such second generation mRNA vaccine formulations with optimized stability, i.e., enabling shipping and storage at refrigerated or ambient temperatures across the entire vaccine supply chain, should be developed now to facilitate more rapid worldwide distribution of mRNA vaccines in the future.

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