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LYOPHILISED AND THERMOSTABLE MRNA-LIPID NANOPARTICLE VACCINES: EXCIPIENT SELECTION, PROCESS DESIGN AND COLD-CHAIN-INDEPENDENT DISTRIBUTION FOR LOW- AND MIDDLE-INCOME COUNTRIES

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ABSTRACT

The messenger RNA lipid nanoparticle (mRNA-LNP) platform reached the clinic at extraordinary speed, yet it carried an inconvenient inheritance: a liquid drug product that survives only at deep sub-zero temperatures. The frozen chain that this demands, running from roughly -80 to -20 degrees Celsius, is precisely the infrastructure that low- and middle-income countries (LMICs) most often lack, and it converts a scientific triumph into a distribution failure at the last mile. This review argues that the instability is a formulation problem with a formulation solution, and that lyophilisation is the most mature route to a refrigerator-stable or ambient-stable mRNA vaccine. It examines why the aqueous particle degrades, working through the chemistry of RNA backbone hydrolysis and lipid ester cleavage that the resident water in the particle core sustains. It then treats the two levers that a formulator actually controls. The first is excipient selection, where disaccharides such as sucrose and trehalose vitrify the matrix and replace hydrogen bonds at the lipid headgroups, while bulking agents and a matched buffer set the collapse behaviour of the cake. The second is process design, where the freezing, primary drying and secondary drying stages must each be held below the critical temperature of the freeze concentrate to avoid collapse, aggregation and residual moisture that would defeat the exercise. Evidence that an optimised cycle can compress a multi-day process to under a day, hold particle size and encapsulation within narrow bounds, and confer refrigerated shelf lives measured in months is now consistent across several independent groups. The review closes on the distribution question, quantifying what a warm-stable presentation would mean for LMIC immunisation and setting out the manufacturing and regulatory work that still stands between the bench result and a licensed product.

Keywords: mRNA vaccines; lipid nanoparticles; lyophilisation; freeze-drying; thermostability; cryoprotectants; cold chain; low- and middle-income countries.

1. Introduction

The clinical arrival of mRNA-LNP vaccines against severe acute respiratory syndrome coronavirus 2 compressed a decade of expectation into a year of deployment. Two products, tozinameran and elasomeran, reached billions of recipients and demonstrated that a synthetic, cell-free, sequence-programmable vaccine could be designed, manufactured and distributed at global scale. The platform's promise extends well beyond a single pathogen, because the drug substance is defined by its nucleotide sequence rather than by a bespoke biological process, and a new antigen is in principle a new sequence in an unchanged manufacturing train.

That promise is constrained by a single physical fact. The approved products are liquid dispersions of a fragile nucleic acid inside an equally fragile lipid assembly, and they retain potency over useful periods only when frozen. Long-term storage is specified at roughly -90 to -60 degrees Celsius for tozinameran and -25 to -15 degrees Celsius for elasomeran, and once thawed the labelled shelf life collapses to weeks under refrigeration and to hours or a small number of days at room temperature [1,2]. This is an anomaly rather than a norm. Most marketed human vaccines are distributed at 2 to 8 degrees Celsius with shelf lives of two years or more, and the introduction of a frozen presentation reversed decades of accumulated logistical assumption.

The consequence falls hardest where it can least be absorbed. A cold chain is a continuous, monitored, temperature-controlled path from the fill-finish line to the injection site, and each link is a point of failure. In many LMICs the ordinary 2 to 8 degree chain is already unreliable, and an ultracold requirement is simply out of reach for most peripheral facilities. A national assessment cited widely in the access literature found that as recently as 2014 around one fifth of health facilities in LMICs had no cold storage at all, and that only about one in seven met the World Health Organization's temperature-control criteria; the same body of work attributes wastage of up to half of some vaccine doses to supply-chain and cold-chain failures [3]. An ultracold vaccine layered on top of that reality does not reach the populations with the highest disease burden.

The instability, however, is not an immovable property of the modality. It is a property of a specific presentation, the aqueous dispersion, and presentation is something a pharmaceutical scientist can change. Removing water is the oldest and most direct way to arrest the hydrolytic chemistry that drives the decay, and lyophilisation, or freeze-drying, is the drying method best suited to a heat-sensitive, colloidally delicate material. This review is organised around that thesis. It first sets out why the aqueous mRNA-LNP degrades, because the degradation chemistry dictates what a successful dried product must prevent. It then examines excipient selection and process design as the two coupled variables that decide whether a freeze-dried mRNA-LNP survives drying, storage and reconstitution with its identity intact. It considers

the alternative drying routes that are competing for the same role. Finally it returns to the distribution question that motivates the entire enterprise, and asks what a warm-stable mRNA vaccine would actually change for the countries that need it most, and what still stands in the way.

2. The origin of the cold chain: architecture and degradation of the mRNA-LNP

2.1 A four-component particle built around water

The lipid nanoparticle that carries mRNA is not a simple capsule. It is a self-assembled colloid of four lipid classes, each with a defined role: an ionizable lipid that is protonated at the acidic pH of manufacture to condense the polyanionic mRNA and is near-neutral at physiological pH to limit toxicity; a helper phospholipid such as distearoylphosphatidylcholine that supports the bilayer geometry; cholesterol that fills packing defects and lends rigidity; and a PEGylated lipid that occupies the particle surface, controls size during assembly and confers colloidal stability [1]. These components are combined at defined molar ratios, and small changes in that ratio or in the identity of any component alter size, encapsulation and stability.

The critical feature for shelf life is that the particle is not anhydrous. Small-angle scattering of representative formulations has shown that the isotropic core holds a substantial volume fraction of water, on the order of a quarter of the core, and that the encapsulated mRNA sits at least in part within water-filled compartments in contact with the ionizable lipid [6,11]. The mRNA is therefore never fully protected from the solvent that degrades it. Encapsulation shields the strand from external nucleases, but it does not remove the intramolecular chemistry that water sustains from within.

2.2 Why the aqueous particle decays

Two coupled degradation processes limit the shelf life of a liquid mRNA-LNP. The first is chemical scission of the RNA itself. Ribonucleic acid carries a 2'-hydroxyl group on every ribose, and that hydroxyl is a built-in nucleophile: under the influence of heat, extremes of pH or trace divalent metal ions, it attacks the adjacent phosphodiester bond in an in-line transesterification that cleaves the backbone [4]. Because the reaction is intramolecular and available at every internucleotide linkage, a long transcript is statistically fragile; the probability that at least one cut has occurred rises with length, and a single cut in the coding region abolishes the biological function of the whole molecule. This length dependence is not a minor detail for a platform whose transcripts run to thousands of nucleotides, and it means that the very size that makes an mRNA a versatile antigen template also makes it a large target for hydrolytic scission [4]. Backbone hydrolysis of this kind proceeds measurably at storage-relevant temperatures and accelerates with warming, which is the molecular reason the liquid product must be kept cold [4,5].

Two features of the chemistry sharpen the case for water removal. First, the cleavage is catalysed by species that are difficult to exclude entirely from a formulation. Trace divalent metal ions, magnesium in particular, promote transesterification, and the reaction is sensitive to pH, so that both acidic and basic excursions raise the rate; chelation of adventitious metals is one reason buffer and excipient purity are treated as stability-critical [4]. Second, secondary and tertiary structure modulate the local rate, so that the same nucleotide is more or less exposed depending on how the strand is folded, which is why sequence and structure design has become a parallel stabilisation strategy to formulation [4]. Neither of these levers, however, removes the underlying requirement, because the reactive 2'-hydroxyl and the water that enables its attack are intrinsic to RNA in an aqueous environment. Taking the water away is the one intervention that addresses the root cause rather than the catalysts.

The second process is degradation of the carrier. The ester linkages present in the helper phospholipid and in many ionizable lipids are themselves susceptible to temperature- and pH-dependent hydrolysis, and the products of that hydrolysis change the surface chemistry and the fusogenicity of the particle [1]. In parallel, the colloid can destabilise physically: particles aggregate, fuse or leak their cargo, and the encapsulation efficiency that protects the mRNA falls. Particle size is not a cosmetic attribute in this system, because the size distribution influences potency, and drift in size on storage is a marker of the very reorganisation that erodes activity [7]. The removal of water addresses both processes at once, since it is water that both feeds RNA hydrolysis from within the core and mediates the hydrolytic and mobility-dependent decay of the lipids.

2.3 What the cold chain buys, and at what cost

Freezing slows this chemistry but does not stop it, and it introduces problems of its own. The consequence in practice is the storage envelope already described: deep-frozen for long-term stability, refrigerated for weeks, ambient for hours [1,2]. The gap between that envelope and the two-year, 2 to 8 degree envelope of a classical vaccine is the entire difficulty. It is a gap that no amount of logistics investment fully closes, because the physics of the liquid product sets the ceiling. The rest of this review concerns the alternative: to change the product from a metastable liquid into a kinetically arrested solid, in which molecular mobility is so low that the degradation reactions effectively cease at temperatures a refrigerator, or even a shaded shelf, can hold.

3. Excipient selection for a dried mRNA-LNP

Drying an mRNA-LNP is not a matter of simply removing water. The freezing and dehydration steps impose mechanical and osmotic stresses that can rupture, fuse or fatally reorganise the particle, and the empirical record is unambiguous that an unprotected formulation dries badly: early work reported that lyophilisation of lipid-based

mRNA carriers could preserve particle metrics while nonetheless abolishing biological activity, a warning that survival of size and encapsulation is necessary but not sufficient [16]. The excipient system is what stands between the particle and that outcome. Its members do distinct jobs, and a workable formulation assembles them deliberately rather than reaching for a single additive.

3.1 Disaccharides as the core stabiliser

The central excipient in a freeze-dried nanoparticle is a stabilising sugar, and the disaccharides sucrose and trehalose are the reference choices [13,14]. Two mechanisms are usually invoked, and both matter here. The first is vitrification: on freezing and drying, the sugar forms a rigid amorphous glass of very high viscosity that immobilises the embedded particles, so that molecular motions required for aggregation and fusion are suppressed on any practical timescale. The second is water replacement: the sugar hydroxyls hydrogen-bond to the polar headgroups of the lipids in place of the departing water, which holds the spacing and geometry of the bilayer through the dehydration transition. Molecular dynamics simulation of a phospholipid membrane with and without sugar in an anhydrous environment makes the second mechanism concrete. In the hydrated system, water competes for the lipid surface and only a handful of sugar-lipid hydrogen bonds form, on the order of twenty to thirty; in the anhydrous system, the sugar coats the membrane and the count rises to roughly one hundred and forty [11]. That difference is the physical substance of the water-replacement hypothesis, and it explains why a sugar that is merely present is less effective than one present at a concentration sufficient to blanket the lipid surface.

The two reference disaccharides are not interchangeable, and the distinction is instructive. Trehalose has a higher glass transition temperature in the dry state than sucrose and is often favoured where the finished product must tolerate warm storage, because a higher transition temperature keeps a wider margin between the storage temperature and the point at which the glass softens and mobility returns. Sucrose, by contrast, tends to give a lower freeze-concentrate transition temperature but has repeatedly proved gentler to the particle through the freezing and rehydration transitions of this specific platform. The choice between them, and the case for using them together, therefore turns on whether the limiting stress is the drying process or the downstream storage climate, and a formulator who fixes on one sugar by reflex forgoes a genuine design variable [13,14].

Sucrose has emerged as the most consistently reliable single sugar for this platform. In a direct comparison of cryoprotectants for an mRNA-LNP, particle size and encapsulation were essentially unchanged through freezing when sucrose was used, whereas trehalose or maltose alone allowed a measurable increase in size and a small loss of encapsulation, an outcome attributed to sucrose's efficiency in replacing water at the lipid membrane and in forming a viscous glassy matrix that resists osmotic change [10]. Sucrose concentrations in the region of 10 to

20 percent weight by volume recur across independent studies as effective for both conventional and continuous freeze-drying [8,12]. The choice is not automatic, however, because the sugar also sets the thermal properties of the freeze concentrate, and those properties govern the process, as the next section develops.

3.2 Bulking agents, matrix formers and the case for mixtures

A single amorphous sugar produces an elegant but delicate cake, and it constrains the process because its glass transition temperature in the maximally freeze-concentrated state is low. This is the rationale for combining a protective sugar with a crystalline bulking agent. Mannitol is the archetype: it crystallises during freezing to give the cake mechanical rigidity and an elevated collapse temperature, which in turn permits a warmer, faster primary drying step without structural failure. A study that optimised a three-component lyoprotectant of sucrose, trehalose and mannitol for an mRNA-LNP found that the mixture raised the eutectic and collapse temperatures of the system and produced a rigid, highly porous cake described as ginger-root-like, which tolerated rapid temperature increases and gave up its water efficiently [11]. The same work showed the failure mode of the unsupported sugar directly: sucrose alone produced a cake riddled with holes and evidence of collapse, whereas the ternary mixture protected the structure. The lesson is that excipient selection and process design are not independent choices. The excipient sets the thermal ceiling, and the thermal ceiling sets how aggressive, and therefore how economical, the cycle can be.

3.3 The ionizable lipid, the buffer and the coupling between them

Two further variables belong in the excipient discussion because they change drying outcomes even though they are not conventionally thought of as protectants. The first is the ionizable lipid, which is the most chemically active component of the particle. Work on batch and continuous lyophilisation has shown that successful freeze-drying depends jointly on the cryoprotectant and on the identity of the ionizable lipid, so that a formulation that dries well with one lipid may not with another; the drying process cannot be optimised for the sugar in isolation from the lipid it is protecting [12]. The second is the buffer. Because RNA hydrolysis is acid-, base- and metal-catalysed, the buffer species and the pH of the pre-lyophilisation solution influence both the rate of chemical degradation and the freezing behaviour of the matrix, and a buffer that shifts pH on freezing can expose the cargo to a damaging local environment during the freeze concentration step [4]. A defensible formulation therefore fixes four things together: the protective sugar, the bulking agent, the buffer and its pH, and the identity and ratio of the lipids. Table 1 summarises the functional roles.

Table 1. Functional roles of the excipient classes in a freeze-dried mRNA-LNP formulation. The classes are not independent: the protective sugar and the bulking agent together set the thermal properties that the drying cycle must respect, and the ionizable lipid conditions the choice of both.

Excipient class	Example(s)	Primary function in the dried product	Ref.
Protective disaccharide	Sucrose, trehalose	Forms an amorphous glass that immobilises the particles and hydrogen-bonds to lipid headgroups in place of the departing water	10,11,13,14
Crystalline bulking agent	Mannitol	Crystallises to give the cake mechanical rigidity and to raise the collapse temperature, permitting a warmer, faster primary drying step	11
Buffer	Low-drift buffers (e.g. Tris)	Sets and holds pH; a buffer that resists freeze-induced pH shift limits acid- and base-catalysed mRNA hydrolysis	4
Ionizable lipid	Formulation-specific	The most chemically active component; its identity co-determines whether a given cryoprotectant dries the particle successfully	12
Solvent / vehicle	Water for injection	Removed by sublimation then desorption; the residual moisture left behind is a critical stability attribute	11,13,15

4. Process design: turning a liquid into a stable glass

If the excipients decide what can be achieved, the cycle decides whether it is achieved. Lyophilisation removes water in three stages, freezing, primary drying and secondary drying, and each stage has a governing constraint that the excipient system has already set [13,15]. The overarching rule is that the product must be held below the critical temperature of its matrix, the glass transition temperature of the maximally freeze-concentrated solute or the closely related collapse temperature, at every point where ice is still present. Cross that threshold and the amorphous matrix flows, the cake collapses, and the residual moisture, reconstitution behaviour and stability of the product all deteriorate.

4.1 Freezing sets the template

Freezing is more than a preparatory step. It fixes the microstructure that primary drying must then work through. The rate of cooling determines the size of the ice crystals, which in turn determines the size of the pores left behind when that ice sublimes, and therefore the resistance the drying vapour must overcome [15]. Slow, controlled freezing tends to produce larger ice crystals and a more open, faster-drying cake but exposes the particle to a longer period of freeze concentration; fast freezing produces a finer, more resistant structure. Annealing, a deliberate hold above the glass transition of the freeze concentrate, is often used to grow and homogenise the ice crystals, which lowers the resistance to vapour flow and shortens primary drying, at the cost of an added step [15]. For a colloidal particle, the freezing stress is significant in its own right, and controlled cooling in the presence of an adequate protectant is what keeps the particle intact through this stage.

Freezing also carries a subtler chemical hazard. As ice forms, everything that is not water is concentrated into a shrinking liquid fraction, and in that freeze concentrate the local concentration of buffer salts, and with it the local pH, can shift substantially from the nominal value of the starting solution. A buffer whose two species freeze out at different rates can drive the pH

several units away from neutrality precisely when the mRNA is most concentrated and least protected, feeding the acid- and base-catalysed hydrolysis described earlier [4]. This is why buffer selection is a freezing variable as much as a storage variable, and why buffers known to resist pH drift on freezing are preferred for nucleic acid formulations. The interplay illustrates the general theme of the section: no stage of the cycle can be optimised in isolation from the formulation, and the freezing step in particular couples the mechanical fate of the particle to the chemical fate of its cargo.

4.2 Primary drying is the expensive step

Primary drying removes the frozen ice by sublimation under vacuum, and it is normally the longest and most energy-intensive part of the cycle. The product temperature must be kept a few degrees below the collapse temperature throughout, because sublimation is slow and the safety margin is narrow [15]. This is exactly where the bulking agent earns its place: by raising the collapse temperature, a crystallising excipient such as mannitol allows a higher shelf temperature and a shorter primary drying time without risking collapse. The practical stakes are large. Reports of empirical mRNA-LNP lyophilisation using a single protectant have described cycles of forty to one hundred hours, whereas a rationally optimised ternary system with an elevated collapse temperature compressed the full cycle to between eight and eighteen hours, with a demonstrated eight-hour cycle divided into four hours of freezing, two of primary drying and two of secondary drying [11]. A cycle that is five to ten times shorter is not a laboratory nicety; it is the difference between a process that can supply a region and one that cannot, and it bears directly on cost of goods.

4.3 Secondary drying and the residual-moisture target

Once the ice has sublimed, water remains bound within the amorphous matrix, and at that point the cake may still hold several percent moisture. Secondary drying raises the shelf temperature, below the dry-state glass transition, to desorb that bound water toward a low residual level [13,15]. Residual moisture is a decisive stability attribute for two opposing reasons. Too much water and the glass is plasticised, its transition temperature falls toward the storage temperature, molecular mobility returns and the degradation reactions resume. Too little, and there is a theoretical risk of over-drying certain biologicals, though for this platform the dominant concern is excess moisture. Optimised mRNA-LNP cycles have reported residual moisture in the region of three percent while maintaining particle and cargo integrity, which appears to sit in a workable window for refrigerated storage [11]. The residual-moisture specification is thus a central quality attribute and a natural focus of process characterisation.

4.4 Reconstitution: the last chance to fail

A freeze-dried vaccine must return to a correct dispersion at the point of care, and reconstitution is a genuine stress rather than a formality, because the rehydrating particle can aggregate as readily as the freezing one. A well-designed cake

dissolves quickly and completely: optimised mRNA-LNP lyophilisates have been reported to reconstitute in under ten seconds on addition of water, to give a clear, opalescent dispersion indistinguishable to the eye from the freshly prepared material [9,11]. The quantitative outcome across several independent optimisations is consistent and encouraging. Particle size increases only modestly through the full freeze-dry and reconstitute sequence, for example from around 113 nanometres to around 121 nanometres with a polydispersity index held below 0.2, and encapsulation efficiency is largely retained [11]. Figure 1 sets out the overall transformation, and Figure 2 collects the physicochemical and molecular evidence that the change is tolerated.

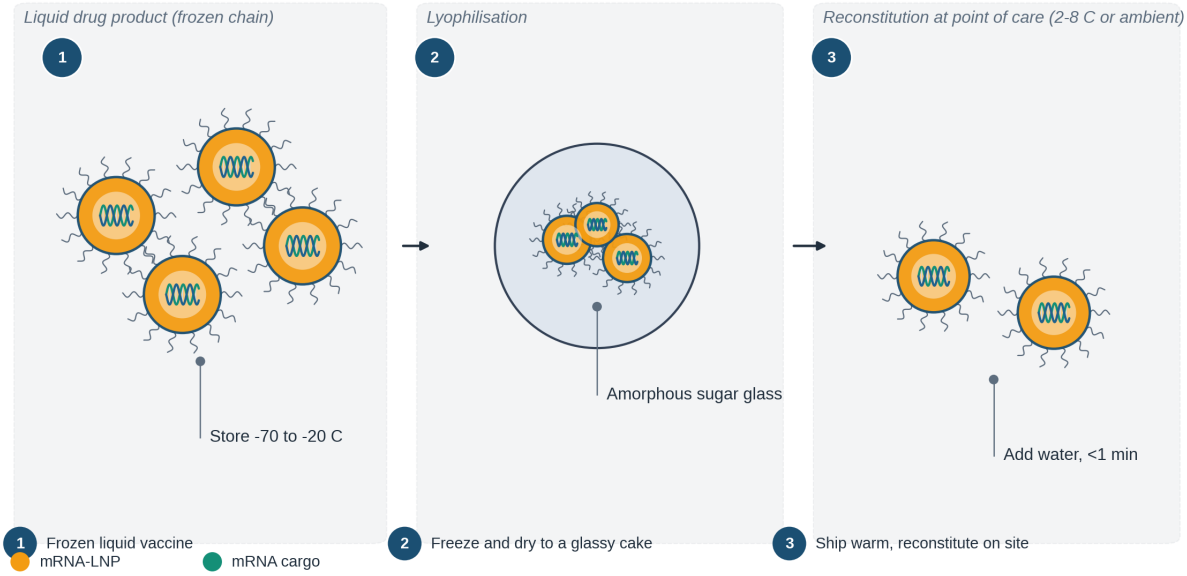

Figure 1. Lyophilisation converts a frozen-chain liquid mRNA-LNP into a refrigerator-stable glassy solid. (1) The approved products are aqueous dispersions of mRNA-loaded lipid nanoparticles that require storage at roughly -70 to -20 degrees Celsius. (2) Freezing and drying in the presence of a protective sugar system embed the particles in an amorphous glass, which arrests the water-dependent degradation of the mRNA and the lipids. (3) The dried cake is shipped and held at 2 to 8 degrees Celsius, or potentially at ambient temperature, and is reconstituted with water at the point of care in under one minute. Schematic, not drawn to scale.

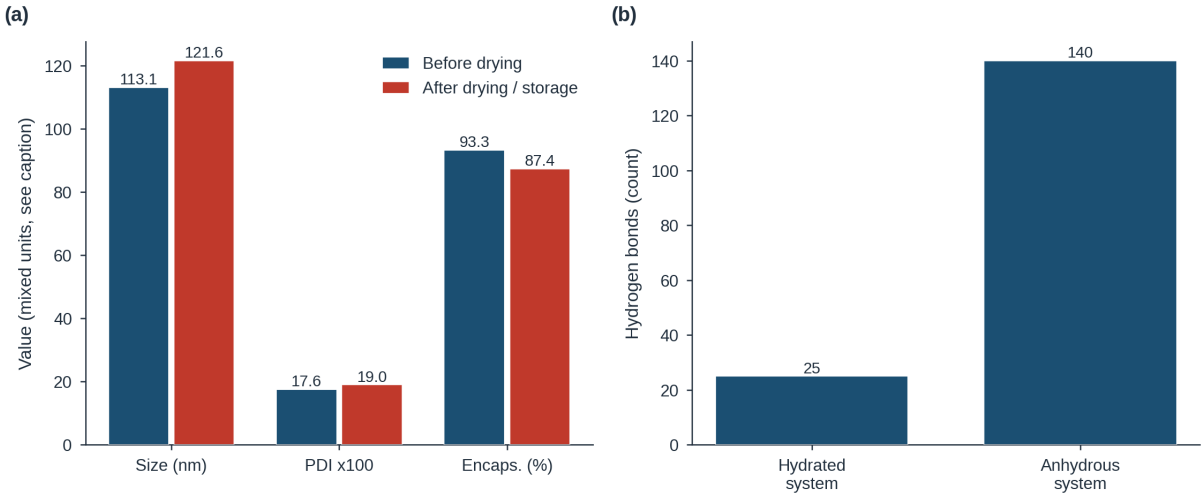

Figure 2. Physicochemical and molecular evidence that an optimised freeze-dry cycle is tolerated by the mRNA-LNP. (a) Selected quality attributes before drying and after drying with storage, for a representative optimised formulation: hydrodynamic diameter (nm), polydispersity index (plotted as PDI multiplied by 100) and mRNA encapsulation efficiency (%). Size rises modestly from 113.1 to 121.6 nm, the polydispersity index stays below 0.2, and encapsulation is largely retained. (b) Sugar-to-phospholipid hydrogen-bond counts from a 10 ns molecular dynamics simulation of a model lipid membrane in a hydrated versus an anhydrous system; removing water raises the count from approximately 25 to approximately 140, which is the molecular basis of the water-replacement mechanism. Values from Li et al. [11].

The stability payoff is the point of the exercise. Independent groups have reported that lyophilised mRNA-LNPs retain their physicochemical attributes and biological identity over months under refrigeration, with one nucleoside-modified product showing a preliminary shelf life of at least twenty-four weeks at 4 degrees Celsius, well beyond the liquid comparators [8]. Others have pushed into ambient and elevated-temperature challenges, reporting retention of particle metrics and a high fraction of intact mRNA after storage at 25 degrees Celsius for months and after an aggressive 40 degree challenge, with relative mRNA integrity still above seventy percent after two months at that temperature and no detectable lipid degradation by chromatography [9]. These are physicochemical and analytical endpoints, and the present review deliberately confines its claims to them rather than to biological potency data; the consistency of the physical result across laboratories is nonetheless the strongest available signal that dried mRNA-LNPs can escape the frozen chain.

5. Alternative drying routes

Lyophilisation is the most developed route to a dry mRNA-LNP, but it is not the only one, and the alternatives are worth situating because each trades a different set of properties. Spray drying atomises the formulation into a warm gas stream to produce a powder in a single continuous operation. Its appeal is throughput and cost, since a continuous powder process can in principle be cheaper and more scalable than vial-based freeze-drying, and its output is a free-flowing powder suited to routes such as inhalation. Its difficulty is that atomisation and the thermal excursion impose shear and heat stresses on a shear- and heat-sensitive particle, and mitigating those stresses through formulation and process control is an active area of development. Spray freeze-drying is a hybrid that atomises into a cryogenic medium and then sublimes the frozen droplets, an approach that seeks to combine the mild thermal profile of freeze-drying with the scalability of a spray process, and it has been proposed specifically as a route to the very high volumes a pandemic would demand [18].

A more radical departure abandons the lipid nanoparticle altogether for the drying step. One reported approach spray-dries mRNA into glassy polysaccharide microparticles and then encapsulates those microparticles in a thin protective inorganic shell by atomic layer deposition, to yield a lipid-free, thermally stable dry vaccine [17]. Approaches of this kind are early and mechanistically distinct from the LNP platform, and they raise their own questions about delivery and manufacture, but they

illustrate that the thermostability problem is drawing solutions from well outside conventional freeze-drying. For the near term, lyophilisation of the intact LNP remains the option closest to a licensable product, because it changes the presentation while leaving the validated particle and its established biology in place.

6. Cold-chain-independent distribution for low- and middle-income countries

The motivation for all of this formulation work is a distribution problem, and it is worth stating in concrete terms. A vaccine that is stable at 2 to 8 degrees Celsius, or better still at ambient temperature for a defined period, can travel on the same infrastructure that already delivers measles, diphtheria and hepatitis B vaccines to peripheral clinics. A vaccine that requires an ultracold freezer at every node cannot, because that infrastructure is thin or absent across much of the world. The gap is not marginal. Assessments of LMIC immunisation systems have repeatedly found unreliable refrigeration, intermittent electricity and cold-chain equipment that is outdated or non-functional at a substantial share of facilities, and the resulting temperature excursions and wastage fall on exactly the settings with the highest infectious-disease burden [3]. Layering an ultracold requirement onto that reality restricts an mRNA vaccine to well-resourced central facilities and undermines the equity argument that justified the global investment in the first place.

A refrigerator-stable dried presentation attacks the problem at its root. It removes the ultracold freezer, the dry-ice logistics and the tight post-thaw clock, and it replaces them with a familiar 2 to 8 degree chain and, potentially, an extended ambient window at the last mile. The evidence reviewed here indicates that this is achievable in physicochemical terms: optimised lyophilised mRNA-LNPs have retained their identity for months under refrigeration and have withstood ambient and elevated temperatures far better than their liquid parents [8,9,11]. A dried product also offers secondary logistical gains that matter in resource-limited settings, including reduced mass and volume for transport and, in principle, longer tolerance of the temperature excursions that are routine in field distribution.

The World Health Organization's concept of a controlled temperature chain gives this argument a concrete policy shape. Under that framework, a vaccine shown to tolerate a defined excursion outside the traditional 2 to 8 degree band, for example a period at ambient temperature immediately before administration, may be permitted to leave the cold chain for that window during a campaign, which transforms the practical reach of an outreach session in a setting with no field refrigeration. A dried mRNA-LNP with a demonstrated ambient tolerance is precisely the kind of product that could qualify for such a designation, and the physicochemical resilience reported at 25 degrees and above is the first evidence that the tolerance might be real for this platform [9,11]. The mass and volume reductions that accompany drying compound the

benefit, because they ease the cost and the carbon of transport and expand the number of doses a given cold box or vehicle can carry, a consideration that is far from trivial when the last leg of a supply chain is a motorcycle or a head-load.

The gains are not automatic, and three honest qualifications belong beside them. First, a lyophilised product must be reconstituted before use, which reintroduces a manipulation at the point of care that the liquid product does not require, along with the need for a diluent, a sterile procedure and trained hands; acceptability studies of dried presentations in some settings have found real resistance to added reconstitution steps, and that human factor cannot be waved away [3]. Second, the dried presentation must be manufactured, and vial-based freeze-drying is a slow, capital-intensive unit operation whose throughput has historically been a bottleneck, which is why cycle compression and continuous or spray-based routes matter so much for supply. Third, none of the physicochemical stability data reviewed here substitutes for the clinical and regulatory evidence a licensed product requires; a reformulated, freeze-dried version of an approved vaccine is a new product that must demonstrate comparability and stability under a defined program. Table 2 places the presentations side by side on the attributes that decide reach.

Table 2. Storage and handling attributes of the liquid and the lyophilised mRNA-LNP presentations, on the properties that decide reach in a low- and middle-income setting. Entries for the lyophilised column describe physicochemical findings from the reviewed literature, not licensed product specifications.

Attribute	Liquid (approved presentation)	Lyophilised (in development)
Long-term storage	Approx. -70 to -20 C	2-8 C, months reported [8,11]
Ambient tolerance	Hours to a few days	Retention of key attributes at 25 C and above [9,11]
Reconstitution	None	Required, under one minute [9,11]
LMIC last-mile fit	Limited by the ultracold chain	Compatible with the existing 2-8 C chain; potential controlled-temperature-chain candidate [3]
Transport mass and volume	Higher (frozen liquid, dry ice)	Lower (dry cake or powder)

7. Discussion

Taken together, the literature supports a clear and, by the standards of a young field, well-triangulated conclusion: the thermal fragility of mRNA-LNP vaccines is a solvable formulation problem, and lyophilisation is the most credible near-term solution. The convergence is notable. Independent groups, working with different mRNA sequences, different ionizable lipids and different equipment, have arrived at the same qualitative result, that a suitably formulated and processed mRNA-LNP can be dried and reconstituted with modest change in size, retained encapsulation and preserved mRNA integrity, and can then be stored under refrigeration for months rather than days [8,9,10,11]. When several laboratories reach the same physicochemical endpoint by different routes, the result is more likely to reflect the underlying material than any one group's technique.

The mechanistic picture is also coherent, and that coherence is what gives the empirical results

their weight. The degradation chemistry identifies water as the common agent of both RNA scission and lipid decay [1,4]. The stabilisation strategy removes that water and replaces its stabilising hydrogen bonds with those of an amorphous sugar, a mechanism now supported at the level of molecular simulation as well as bulk measurement [11]. The process constraints follow directly from the thermal properties of the sugar-bulking-agent matrix, so that excipient selection and cycle design are two faces of one optimisation rather than separate exercises [11,12,15]. A reader can trace an unbroken line from why the particle fails to how the dried product is built to prevent that failure, and that line is the strongest feature of the current evidence base.

A related point concerns how stability is measured, because the credibility of a shelf-life claim rests on the assays behind it. The endpoints that recur across the dried-product literature are a coherent and demanding set: particle size and polydispersity by dynamic light scattering, encapsulation efficiency by a dye-exclusion assay, residual moisture by Karl Fischer titration, mRNA integrity by capillary or gel electrophoresis, and lipid integrity by chromatography [9,11]. Each interrogates a different failure mode, and a product that passes all of them has genuinely resisted the several routes by which a dried mRNA-LNP can decay. The field would nonetheless benefit from agreed acceptance criteria and stability-indicating methods applied consistently across formulations, so that a reported shelf life means the same thing from one paper to the next. Until that harmonisation arrives, cross-study synthesis of the kind attempted here must remain qualitative, and a numerical pooling of shelf lives would give a false precision that the underlying heterogeneity does not support.

The weaknesses are equally clear and should temper any triumphalism. The published stability datasets are still short relative to the two-year expectation of a routine vaccine, and much of the strongest evidence rests on physicochemical and analytical endpoints rather than on the biological and clinical outcomes that this review has deliberately excluded from its scope. Formulations, lipids and cycles differ enough between reports that cross-study comparison is qualitative rather than quantitative, and there is as yet no consensus formulation or standardised cycle for the platform. Manufacturing scale-up of freeze-drying, and the regulatory characterisation of residual moisture, reconstitution and comparability, remain substantial pieces of work rather than settled questions. The field has demonstrated feasibility convincingly; it has not yet delivered a licensed, refrigerator-stable mRNA vaccine, and the distance between those two states should not be understated.

8. Future directions

Several specific pieces of work would move the field from feasibility to product. The most valuable is long-duration, standardised stability testing of dried mRNA-LNPs under harmonised protocols, so that formulations and cycles can be compared quantitatively rather than described in

isolation, and so that a genuine refrigerated or ambient shelf life can be certified rather than projected. A second priority is the systematic mapping of the excipient design space, pairing ionizable lipids with sugar and bulking-agent systems, because the demonstrated coupling between the lipid and the cryoprotectant means that a universal excipient recipe is unlikely and a rational, lipid-specific framework is needed [12]. A third is the maturation of higher-throughput drying, whether through aggressive but controlled freeze-dry cycles, continuous lyophilisation, or spray and spray-freeze routes, since manufacturing capacity, not bench stability, is likely to be the binding constraint on supply [17,18]. Finally, the human and regulatory work deserves equal standing with the physical chemistry: field-oriented studies of reconstitution acceptability and error, and an early, explicit regulatory path for a reformulated presentation of an approved vaccine, would ensure that a technically stable product is also a usable and licensable one.

9. Conclusion

The mRNA-LNP platform's dependence on an ultracold chain is a consequence of its liquid presentation, not an irreducible property of the modality, and it is the single largest barrier between the technology and the low- and middle-income populations it could most benefit. The evidence reviewed here indicates that lyophilisation, built on a deliberately matched excipient system and a cycle designed around the thermal limits of that system, can convert the metastable liquid into a kinetically arrested glass that survives months under refrigeration and tolerates ambient excursions. The remaining work is real and largely non-scientific in character: longer stability datasets, manufacturing capacity and a clear regulatory route. The central scientific question, whether a thermostable mRNA vaccine is achievable, now has a defensible affirmative answer.

Declarations

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