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Spray Drying and Particle Engineering in Dosage Form Design for Global Vaccines

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Abstract

Vaccines are a very important tool in the effort to reduce the global burden of infectious diseases. Modern vaccines can be formulated in several ways to induce specific immunity, including through the use of live bacteria, subunit antigens, and even genetic material. However, vaccines typically need to be transported and stored under controlled refrigerated or frozen conditions to maintain potency. This strict temperature control is incompatible with the available infrastructure in many developing countries. One method of improving the thermostability of a vaccine is through drying of a liquid presentation into a dry dosage form. In addition to enhancing the capability for distribution in resource-poor settings, these dry vaccine forms are more suitable for long-term stockpiling. Spray drying is a drying method that has been successfully used to stabilize many experimental vaccines into a dry form for storage above refrigerated temperatures.

Additionally, the use of spray drying allows for the production of engineered particles suitable for respiratory administration. These particles can be further designed for increased out-of-package robustness against high humidity. Furthermore, there are already commercial dry powder delivery devices available that can be used to safely deliver vaccines to the respiratory system. The research in this field demonstrates that the resources to develop highly stable vaccines in flexible dosage forms are available and that these presentations offer many advantages for global vaccination campaigns.

Keywords: global health, spray drying, stabilization, vaccine

Introduction

The development of vaccines is one of humanity's greatest health care achievements. Through vaccination, the human immune system is able to develop the required specific immunity to fight off the pathogen of concern upon infection. Extensive vaccination campaigns have been very successful in reducing disease progression. A coordinated global vaccination effort eradicated smallpox in 1979. Before eradication, this contagious disease plagued the world for thousands of years, with the more common strain having a mortality rate of 30%.[1] Prevention of infectious diseases has become more crucial in light of increasing antibiotic-resistant strains. Widespread antibiotic resistance in common bacterial infections has rendered previously effective and simple drug regimens unsuccessful. For instance, the current recommended treatment for tuberculosis (TB) consists of a relatively inexpensive treatment schedule that is only 6 months long and has been reported to have an 85% success rate.[2] However, the treatment of drug-resistant and multidrug-resistant strains of *Mycobacterium tuberculosis* requires the use of second-line drugs that are more toxic and much more costly. Moreover, the treatment of multidrug-resistant strains of TB is significantly less successful, reportedly only 56% globally.[2]

A list of vaccines fully licensed for use and vaccines approved for emergency use in the United States by the Food and Drug Administration (FDA) is given in Table 1. The primary types of vaccines currently in use are live attenuated, inactivated, subunit, and toxoid. Live vaccines induce immunity with a weakened form of the pathogen that is still capable of replication. Inactivated vaccines are produced using the killed pathogen. Subunit vaccines use only components of the pathogen rather than the entire microbe to induce immunity. These vaccines are typically considered safer; however, they are often unable to elicit sufficient

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Table 1. List of Storage Conditions for Licensed Vaccines and Vaccines Approved for Emergency Use by the Food and Drug Administration

Trade name	*Disease*	*Vaccine type*	*Dosage form*	*Administration Route*	*Storage temperature*
ACAM2000	Smallpox	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	−15°C to −25°C
ActHIB	Hib	Conjugate vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
Adacel	Diphtheria, tetanus, and pertussis	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
AFLURIA quadrivalent	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Agriflu	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
AUDENZ	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
BCG vaccine	Tuberculosis	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
BEXSERO	Meningitis B	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Biothrax	Anthrax	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Boostrix	Diphtheria, tetanus, and pertussis	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Cervarix	HPV	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
DAPTACEL	Diphtheria, tetanus, and pertussis	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
DENGVAXIA	Dengue	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
Engerix-B	Hepatitis B	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
ERVEBO	Ebola virus disease	Live vaccine	Suspension	Parenteral injection	−60°C to −80°C
FLUAD quadrivalent	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Fluarix quadrivalent	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Flublok quadrivalent	Influenza	Recombinant vaccine	Solution	Parenteral injection	2°C to 8°C, do not freeze
Flucelvax quadrivalent	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
FLULAVAL quadrivalent	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
FluMist quadrivalent	Influenza	Live vaccine	Suspension	Intranasal delivery	2°C to 8°C, do not freeze
Fluvirin	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Fluzone quadrivalent/ Fluzone high-dose	Influenza	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Gardasil/Gardasil 9	HPV	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze. Stable at temperatures up to 25°C for 72 hours
HAVRIX	Hepatitis A	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
HEPLISAV-B	Hepatitis B	Recombinant vaccine	Solution	Parenteral injection	2°C to 8°C, do not freeze
HIBERIX	Hib	Conjugate vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C
Imovax	Rabies	Inactivated vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
Infanrix	Diphtheria, tetanus, and pertussis	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
IPOL	Polio	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
IXIARO	Japanese encephalitis	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze

(*continued*)

Table 1. (Continued)

Trade name	*Disease*	*Vaccine type*	*Dosage form*	*Administration Route*	*Storage temperature*
Janssen COVID-19 Vaccine	COVID-19	Viral vector vaccine	Suspension	Intramuscular injection	2°C to 8°C, do not freeze. Unopened vials can be stored between 9°C and 25°C for up to 12 hours
JYNNEOS	Smallpox and monkeypox	Live vaccine	Suspension	Parenteral injection	−15°C to −25°C
KINRIX	Diphtheria, tetanus, and pertussis	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Menactra	Meningococcal disease	Conjugate vaccine	Solution	Parenteral injection	2°C to 8°C, do not freeze
Menomune-A/C/Y/W-135	Meningococcal disease	Recombinant vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
MenQuadfi	Meningococcal disease	Recombinant vaccine	Solution	Parenteral injection	2°C to 8°C, do not freeze
Menveo	Meningococcal disease	Conjugate vaccine	Lyophilized powder	Parenteral injection upon reconstitution	2°C to 8°C, do not freeze
M-M-R II	Measles, mumps, and rubella	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	8°C to −50°C
Moderna COVID-19 Vaccine	COVID-19	mRNA vaccine	Suspension	Parenteral injection	−15°C to −25°C
Pediarix	Diphtheria, tetanus, pertussis, hepatitis b, and polio	Toxoid, inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
PedvaxHIB	Hib	Conjugate vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Pentacel	Diphtheria, tetanus, pertussis, polio, hib	Toxoid, inactivated and conjugated vaccine	Suspension	Parenteral injection upon reconstitution and mixing	2°C to 8°C, do not freeze
Pfizer-BioNTech COVID-19 Vaccine	COVID-19	mRNA vaccine	Suspension	Parenteral injection	−60°C to −80°C
Pneumovax 23	Pneumococcal disease	Inactivated vaccine	Solution	Parenteral injection	2°C to 8°C
Prevnar 13	Pneumococcal disease	Conjugate vaccine	Suspension	Parenteral injection	2°C to 8°C. Stable at temperatures up to 25°C for 96 hours
ProQuad	Measles, mumps, rubella, and varicella virus	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution and mixing	−50°C to 8°C
Quadracel	Diphtheria, tetanus, pertussis, and polio	Toxoid, inactivated vaccine	Suspension	Parenteral Injection	2°C to 8°C, do not freeze
RabAvert	Rabies	Inactivated vaccine	Lyophilized powder	Oral administration on reconstitution	2°C to 8°C
Recombivax HB	Hepatitis B	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze. Stable at temperatures up to 25°C for 72 hours
ROTARIX	Rotavirus	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution and mixing	2°C to 8°C
RotaTeq	Rotavirus	Live vaccine	Solution for oral	Oral administration	2°C to 8°C
SHINGRIX	Shingles	Recombinant vaccine	Suspension	Parenteral injection upon reconstitution and mixing	2°C to 8°C, do not freeze
TDVAX	Diphtheria and tetanus	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
TENIVAC	Diphtheria and tetanus	Toxoid vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
TRUMENBA	Meningococcal disease	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze

(*continued*)

TABLE 1. (CONTINUED)

Trade name	*Disease*	*Vaccine type*	*Dosage form*	*Administration Route*	*Storage temperature*
Twinrix	Hepatitis A and B	Recombinant vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
TYPHIM Vi	Typhoid disease	Inactivated vaccine	Solution	Parenteral injection	2°C to 8°C, do not freeze
VAQTA	Hepatitis A	Inactivated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Varivax	Varicella	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	–50°C to 8°C
Vaxchora	Cholera	Live vaccine	Lyophilized powder	Oral administration on reconstitution	2°C to 8°C, do not freeze. Stable at temperatures up to 25°C for 72 hours
VAXELIS	Diphtheria, tetanus, pertussis, polio, hepatitis B, and Hib	Toxoid, inactivated vaccine, conjugated vaccine	Suspension	Parenteral injection	2°C to 8°C, do not freeze
Vivotif	Typhoid disease	Live vaccine	Lyophilized capsules	Oral administration of capsules	2°C to 8°C
YF-Vax	Yellow fever	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution and mixing	2°C to 8°C, do not freeze
Zostavax	Shingles	Live vaccine	Lyophilized powder	Parenteral injection upon reconstitution	–50°C to 8°C

From Food and Drug Administration.[9]
BCG, Bacillus Calmette–Guérin; hib, Hemophilus influenzae type b; HPV, human papillomavirus; mRNA, messenger RNA.

immune responses on their own and are typically administered with an adjuvant. Finally, toxoid vaccines are based on toxins produced by the representative microbe to induce immunity. Additionally, messenger RNA vaccines, a type of nucleic acid vaccine, have been successfully produced in response to the COVID-19 pandemic and approved for emergency distribution. Nucleic acid vaccines act through the introduction of genetic material into the body that allows the body's cells to manufacture the antigens required to induce immunity.

As can be seen in Figure 1, the bulk of FDA-approved vaccines require storage under refrigerated conditions, and several need to be kept frozen at even colder temperatures to maintain potency. Only four of the listed vaccines can be kept at room temperature for a few days, and none is stable long-term above refrigerated temperatures. Also, nearly all vaccines are given through injection, which requires trained health care personnel, sterility, and proper sharps disposal. These attributes make the vaccines ill-suited for distribution in resource-poor settings.

Modelling of Niger's vaccine supply chain has shown that introducing a more thermostable vaccine would improve the availability of temperature-sensitive vaccines by reducing bottlenecks and increasing availability of temperature-controlled storage space.[3] Even short-term improvement of stability can greatly enhance the distribution capability of a vaccine. The MenAfriVac vaccine is a lyophilized meningitis vaccine that can be kept at temperatures up to 40°C for up to 4 days.[4] This vaccine is part of the World Health Organization-controlled temperature chain (CTC) program, in which a vaccine must exhibit some potency after at least 3 days of storage at 40°C.[5] Analysis of a MenAfriVac campaign in Benin found that using the thermostable presentation reduced the logistical burden and increased vaccination reach due to fewer wasted doses.[6] In addition to improved distribution, the use of the CTC-approved vaccine as compared with a vaccine requiring cold storage was estimated to reduce the cost of a vaccine campaign by 50%.[7] Lyophilization, the method used to stabilize MenAfriVac, is thus far the most common drying method to improve stability of vaccines.[8] However, there are other drying methods that offer advantages over lyophilization.

Processing Methods Used to Stabilize Vaccines

Drying methods that have been used to stabilize vaccines in a dry presentation include lyophilization, spray drying, spray freeze drying,[10] and foam drying.[11] Lyophilization is performed by adding stabilizing excipients to the formulation and subsequently freezing the liquid formulation. The ice is then sublimed out of the frozen concentrate, leaving a dry, mostly amorphous ''cake.'' Lyophilization has frequently been used to stabilize vaccine formulations for eventual reconstitution and subsequent parenteral administration (cf. Table 1). However, lyophilization is a fairly slow batch process that is difficult to scale up economically. Spray drying consists of atomizing the liquid formulation by means of a drying gas medium that dries the droplets into a dry powder composed of many particles. These particles are then separated from the flow for collection. Spray freeze drying is a method that involves aspects of both the spray drying and lyophilization processes.

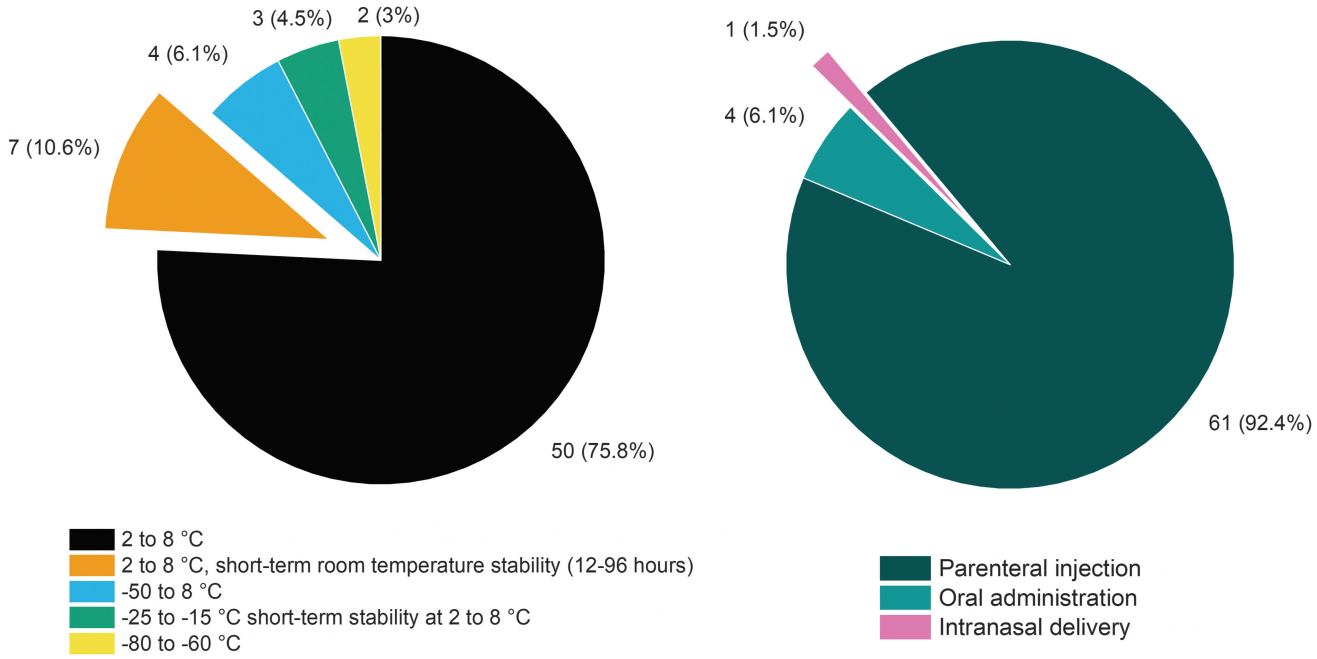

FIG. 1. Storage conditions (left panel) and route of administration (right panel) for 62 Food and Drug Administration-licensed vaccines and 3 vaccines approved for emergency use.

The spray freeze drying process begins with the atomization of a liquid formulation into a cryogenic medium, forming frozen droplets. The ice in the collected frozen droplets is then removed through sublimation, leaving behind highly porous particles. This method appears to be useful for forming inhalable powders from heat-sensitive formulations but has not been fully commercialized. Foam drying consists of foaming up the liquid formulation under reduced pressure before subjecting it to a lyophilization cycle to produce a solid foam structure. This article will focus on spray drying as a method of stabilizing vaccines because of its benefits over the other processing methods. For instance, spray drying can be scaled up to very large batch sizes or semicontinuous production and therefore potentially carries lower processing costs.

Spray Drying

Spray drying is an established method of encapsulating and stabilizing pharmaceuticals in a dry form.[12] A simplified schematic of the spray drying process is given in Figure 2. This figure shows a cocurrent configuration, wherein the feed and the drying gas are flowing in the same direction. Another possible configuration is the counter-current configuration,

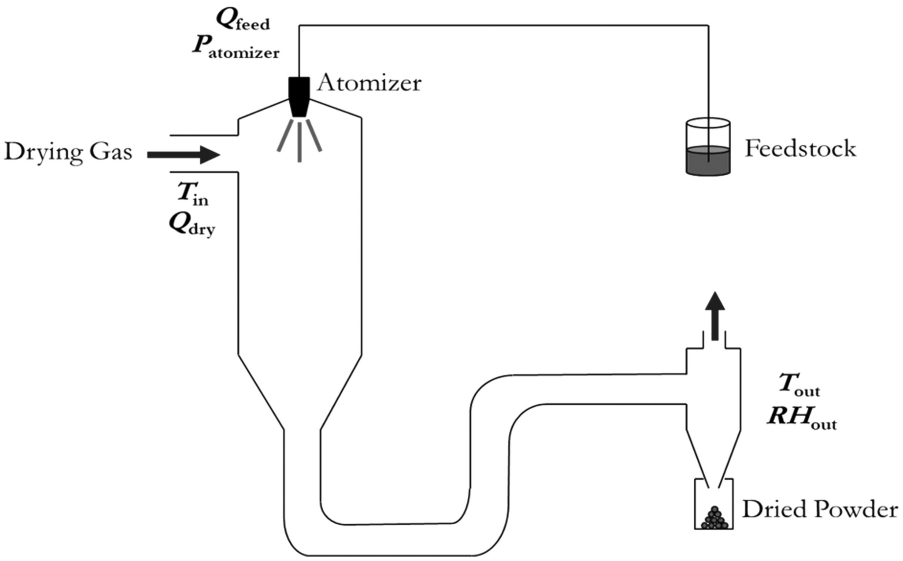

FIG. 2. Simple schematic of the spray drying process, wherein a liquid feedstock is atomized and dried into a powder. RH, relative humidity.

wherein the feed and the drying gas flow in opposite directions. The collecting device used to separate the powder from the flow illustrated in this figure is a cyclone; however, alternative collection methods such as bag filters or electrostatic precipitators can be used.

The primary input processing conditions are the atomizer settings, liquid feed flow rate, drying gas flow rate, and drying gas temperature. These parameters will affect the throughput, evaporation rate, resulting outlet temperature, and outlet humidity. Typical atomizers include the rotary atomizer, vibrating mesh atomizer, and twin-fluid atomizer, the latter being the most popular for inhalation products. Clean dry air or inert nitrogen gas are typically used as the drying gas. The available time for drying, which is the droplet residence time, is dependent on the size of the spray drying chamber and the drying gas flow rate. High inlet and outlet temperatures are often desired because they correlate with high throughput in spray drying. During evaporation, the drying droplets experience an evaporative cooling effect such that the droplet temperature remains much lower than the drying gas temperature. However, the dried particles are subjected to the outlet temperature and any elevated temperature in the collector, where this thermal stress can lead to degradation of labile pharmaceuticals if the process is not properly designed.

Spray Drying Vaccines

Many studies have established that spray drying is a feasible method of stabilizing different types of vaccines. Several of these studies are listed in Table 2 and summarized in Figure 3. These spray-dried dosage forms have shown clear improvement in thermostability over their liquid counterparts under the same storage conditions.[13–15] For example, Gomez et al.[16] spray dried a complex TB vaccine candidate using relatively mild processing conditions. This TB vaccine candidate, ID93+glucopyranosyl lipid adjuvant (GLA)-squalene nanoemulsion (SE), is a subunit vaccine consisting of an antigen and an adjuvant system. The adjuvant system was formulated as a SE with GLA, a toll-like receptor 4 (TLR4) agonist, situated at the nanoemulsion droplet interface. The antigen and agonist components were especially susceptible to heat stress. The antigen band of the liquid presentation, as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), was no longer present after only 1 month of storage at 37°C.

Similarly, the GLA product was completely degraded after 3 months of storage at 37°C, and only 50% of this agonist was retained at 25°C. Furthermore, the vaccine contained squalene, which is liquid at room temperature, making this a particularly challenging format for high-temperature storage. A low inlet temperature of 65°C, atomizer settings geared toward large droplet sizes, and a low feed flow rate of 0.6 mL/min were used to achieve mild outlet conditions of ~36°C and 7% relative humidity (RH), which led to a low moisture content in the powder. Analysis of the spray-dried formulation showed low processing losses compared with those of the liquid feed, with the measured properties all well within target values.

In addition to low processing losses, the spray-dried formulation also demonstrated greatly improved thermostability. Antigen band intensity was comparable to a control after 2 months of storage at 40°C and was still present at a reduced intensity after 26 months of storage. The agonist was stable, with >80% retention after 1 month at 40°C and 26 months at 25°C. Additionally, the resulting powder exhibited excellent physical stability, with moisture content and amorphous solid state retained after 26 months at 40°C. Particle morphology was maintained after 26 months of storage at 25°C, with only minor fusing exhibited at 40°C after 26 months.

The most common primary stresses exerted on biologics during spray drying are exposure to the air–liquid interface through atomization, and drying and heat stress during the drying and collection phases. These losses can be mitigated through appropriate choice of equipment, processing parameters, and stabilizing excipients. Atomizer configuration and atomizing conditions have been shown to affect the stresses imposed on the feed solution. Spray drying of L-lactic dehydrogenase, a protein sensitive to shear and temperature stresses, led to 78% loss of the enzymatic activity when the formulation was atomized with a vibrating mesh configuration.[17] By comparison, atomization using a twin-fluid configuration greatly reduced the loss of enzymatic activity to only 23%. Similarly, spray drying of a recombinant adenovirus under a high shear rate induced high activity losses.[18] However, the use of a moderate shear rate resulted in reduced activity losses as compared with a control sample. The authors of the study reporting these findings suggested that atomizing the formulation under the moderate conditions deaggregated the adenoviruses that had aggregated during preparation of the feedstock.

Several studies have specifically investigated the effect of spray drying conditions on vaccine processing loss. LeClair et al.[19] assessed the effect of the inlet temperature and spray gas flow rate on the processing loss and loss during storage when spray drying an inactivated herpes simplex virus type 2 (HSV-2) vaccine candidate with either trehalose or sucrose as a stabilizer. Their study found that when HSV-2 was spray dried with trehalose, processing losses increased with increasing inlet temperature. However, the processing loss with increasing spray gas flow rate was parabolic, with the lowest losses exhibited under moderate gas flow. A different relationship between inlet temperature and spray gas flow rate was found for the formulations using sucrose as a stabilizer. Increasing losses with increased inlet temperature were also exhibited; however, high gas flow rates were more beneficial for retention at moderate inlet temperature than were low gas flow rates. In addition to affecting the initial processing loss, spray drying conditions will affect the storage potential of the resulting powder.

Ohtake et al.[20] assessed the influence of spray drying parameters on the stability of a live measles vaccine. The study assessed different outlet temperatures, atomization pressures, and solution feed rates and compared the stability of the formulations after 1 week of storage at 37°C. The authors reported that the lowest titer loss was achieved with the mildest conditions tested, that is, an outlet temperature of 40°C, an atomizing pressure of 103.4 kPa, and a feed flow rate of 0.5 mL/min. However, selection of excipients will also affect the storage stability and the effect of processing conditions on powder stability. For instance, spray drying of the HSV-2 vaccine with sucrose as a stabilizer showed a large range in loss after 10 days of storage at 45°C across the different spray drying conditions used.[19] By contrast,

Table 2. List of Recent Experimental Spray-Dried Vaccine Studies and Stability Achieved With the Lead Formulation

Vaccine	*Vaccine type*	*Excipient system*	*Intended route of administration*	*Stability*	*Reference*
Adenovirus vaccine platform	Recombinant (viral vector)	Trehalose, mannitol, dextran, lactose, and mixtures of them	Parenteral injection of reconstitution vaccine	Greatest retention of ~30% in the mannitol/dextran formulation after 30 days at 37°C	(21)
Anthrax	Recombinant	Trehalose, hydrolyzed gelatin, and additional tested additives (Tween 80, Alhydrogel)	Parenteral injection of reconstitution vaccine	Immunogenicity maintained in mouse model after 3 months at 40°C/75% RH	(22)
Cholera	Inactivated	Eurdragit and triethylcitrate with aliginate or carbopol as mucoadhesive agents	Oral administration of dry powder	Antigenicity maintained after 12 months of storage at 25°C/60% RH and 6 months at 40°C 75% RH	(23)
Herpes	Live	Trehalose or sucrose	Parenteral injection of reconstitution vaccine	<1.5 log loss in activity after 10 days at 45°C storage exhibited in trehalose formulation	(19)
HPV	Recombinant (bacteriophage)	Combinations of leucine, trehalose, mannitol, and dextran	Oral administration of dry powder or parenteral injection of reconstitution vaccine	Immunogenicity maintained in mouse model after 14 months of storage at 37°C storage	(24)
HPV	Recombinant (bacteriophage)	Mannitol, trehalose, dextran, leucine, and inositol	Parenteral injection of reconstitution vaccine	Protection conferred in mouse model after 34 months of storage at room temperature	(25)
HPV	Recombinant	Mannitol, dextran, l-leucine, PVP, trehalose, myo-inositol	Parenteral injection of reconstitution vaccine	Protected from challenge in mouse model after 3 months of storage at 40°C/75% RH	(14)
Influenza	Live (viral vector)	Combinations of trehalose, mannitol, dextran, and lactose	Parenteral injection of reconstitution vaccine	Greatest retention of ~15% in the trehalose formulation after 30 days at 37°C	(21)
Influenza	Inactivated	Trehalose	Inhalation of dry powder or parenteral injection of reconstitution vaccine	Stable for 3 months of storage at 60°C based on bioassay	(26)
Influenza	Inactivated	Trehalose and leucine	Pulmonary route through dry powder inhalation	Stable for 2 months of storage at 40°C based on bioassay	(27)
Influenza	Recombinant	Trehalose and hydrolyzed gelatin	Parenteral injection of reconstitution vaccine	Physically stable for 6 months of storage at −20°C to 50°C. Immunogenicity maintained in mouse model after 3 months of storage from −20°C to 50°C	(28)
Measles	Live	Trehalose, sucrose, myo-inositol, potassium phosphate, l-arginine, glycerol, pluronic F68	Parenteral injection of reconstitution vaccine	<1 log loss exhibited in optimized formulation after 8 weeks of storage at 37°C	(20)
Tuberculosis	Live	Mixture of leucine, mannitol, BSA, trehalose, PVP	Pulmonary route through dry powder inhalation or parenteral injection of reconstitution vaccine	Immunogenicity maintained in mouse model after 2 years of storage at room temperature	(29)

(*continued*)

TABLE 2. (CONTINUED)

Vaccine	*Vaccine type*	*Excipient system*	*Intended route of administration*	*Stability*	*Reference*
Tuberculosis	Live	Mannitol and dextran	Parenteral injection of reconstitution vaccine	Immunogenicity maintained in mouse model after 90 days of storage at 20°C/<10% RH	(30)
Tuberculosis	Recombinant	Mannitol, cyclodextran, trehalose, and dextran	Pulmonary route through dry powder inhalation	Insignificant change in virus titer after 1 year of storage at 25°C and 5 weeks of storage at 37°C	(31)
Tuberculosis	Recombinant	Trehalose	Parenteral injection of reconstitution vaccine	Adjuvant system is stable after 26 months at 25°C and antigen present after 26 months at 40°C based on bioassay	(16)
Tuberculosis	Recombinant	Trehalose, trileucine	Pulmonary route via dry powder inhalation or parenteral injection of reconstitution vaccine	Adjuvant system stable after 1 year at 25°C, 45% of antigen retained after 1 year at 50°C	(32)
Tularemia	Live	Mannitol, trehalose, dextran, leucine, and inositol	Pulmonary route via dry powder inhalation	Stable for 180 days at 40°C/75% RH when stored with protective packaging, based on bioassay	(33)
Vesicular stomatitis virus	Live (viral vector)	Combinations of leucine, trehalose, mannitol, and dextran	Parenteral injection of reconstitution vaccine	Immunogenicity maintained in mouse model after 15 days of storage at 37°C for the formulation containing only trehalose	(13)
Vesicular stomatitis virus	Live (viral vector)	Combinations of leucine, trehalose, mannitol, and dextran	Parenteral injection of reconstitution vaccine	Greatest retention of ~50% measured in trehalose and trehalose/dextran 3:1 mixture after 30 days of storage at 37°C	(21)
Whooping cough	Recombinant	Trehalose	Inhalable or injectable reconstitution	Stable for 30 days of storage at 40°C and improved stability for 30 days of storage at 65°C as compared with a liquid control	(15)

BSA, bovine serum albumin; PVP, polyvinylpyrrolidine; RH, relative humidity.

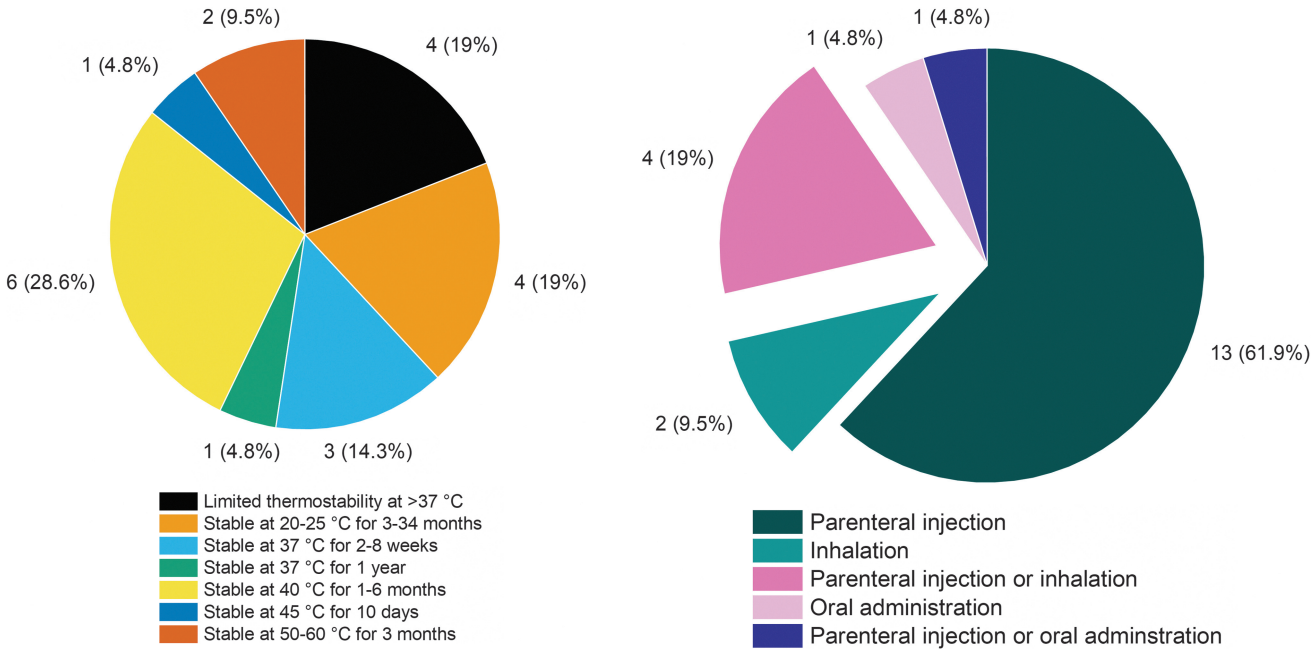

FIG. 3. Thermostability (left panel) and administration route (right panel) for spray-dried experimental vaccines.

losses on storage of the trehalose-based formulations appeared to be relatively consistent among the different spray drying conditions. Clearly, then, appropriate choice of excipients can enhance powder stability.

Formulation development for long-term stability

The spray-dried experimental vaccines shown in Table 2 typically use complex multicomponent excipient systems to achieve high stabilization. Many of these excipient systems include at least one amorphous sugar, with mannitol, trehalose, and dextran as the most common stabilizers, and combinations of these sugars can improve stabilization more significantly than any one sugar alone.[34] The primary mechanisms by which saccharides provide protection against drying are hydrogen bond replacement and vitrification in a glassy matrix. More detailed reviews of these mechanisms in relation to the stabilization of proteins and lipids can be found elsewhere.[35–37]

Hydrogen bond replacement refers to the replacement of the hydrogen bonds between the biologic and water with hydrogen bonds between the biologic and the stabilizer. This mechanism requires the stabilizing excipient to be able to fit closely to the biologic to maintain its shape. The molecular flexibility and size of the stabilizing excipient affects its ability to effectively stabilize a pharmaceutic. The molecule should be small enough to closely match the structure. Tonnis et al.[10] demonstrated that small, flexible sugars were better able to stabilize several model proteins than were larger, rigid sugars. Additionally, excipients need to be in an amorphous solid phase to be able to effectively mix with and stabilize biologics. For instance, mannitol is able to form hydrogen bonds and has been successfully used as an excipient when spray dying vaccines. However, it may be unsuitable for certain systems as it readily crystallizes upon drying and may then induce destabilization due to its crystalline nature.[38] Vitrification is the rendering of a biologic immobile within a solid matrix, thus preventing unfolding of proteins. Hydrogen bond replacement acts primarily to limit local mobility, whereas vitrification acts to limit global mobility.

For amorphous stabilizing systems, another key consideration is the glass transition temperature, T_g. The glass transition temperature is the temperature at which an amorphous solid transitions from brittle, glass-like properties to more rubber-like ones. This increase in molecular mobility reduces the ability of the stabilizing system to prevent conformational changes. An often-quoted rule of thumb is that the T_g should be $\sim$50°C above the storage temperature for long-term stability.[39,40] Grasmeijer et al.[40] demonstrated with a spray-dried model protein that vitrification is the primary mechanism of stabilization when the storage temperature is close to T_g, whereas the water replacement mechanism determines stability at higher glass transition temperatures.

The T_g of amorphous systems decreases with increasing water content, a process called plasticization. Therefore, once the moisture sorption behavior of the material is known, a suitable T_g for spray-dried powders can be targeted through the processing conditions. The T_g can be predicted using the Gordon–Taylor equation,[41] where the subscript s refers to the stabilizing excipient, typically a sugar, the subscript w refers to water, T_g is the glass transition temperature, w refers to the weight fraction, and k is an empirically determined parameter:

$$T_g = \frac{w_s T_{g,s} + k w_w T_{g,w}}{w_s + k w_w} \quad (1)$$

Because high water content substantially depresses the T_g of sugars, high residual moisture content in powder or exposure to a high humidity environment is detrimental to long-term stability. The effect can be assessed using supplemented phase diagrams that have been developed for various sugars indicating the T_g based on moisture content.[42]

The moisture content of spray-dried powder, and with it the glass transition temperature, can be manipulated by controlling the RH at the collection point. However, a very low water content can also be detrimental to stability. Kunda et al.[33] found that storing a commercial desiccant sachet directly with a spray-dried live-attenuated tularemia vaccine powder led to immediate loss in viability of the encapsulated live bacteria.

Best strategies for stabilization of vaccines encapsulated in lipid structures, such as lipid-based adjuvant systems or viral vectors with lipid envelopes, are still being developed. Depending on the type of lipid used, it may be necessary to stabilize lipid membranes to avoid fusion of vesicles or leakage of components upon drying.[36,44] Solid phase change of phospholipids has also been found to lead to membrane destabilization upon rehydration.[44] Trehalose has been shown to be a suitable stabilizer for lipid systems as it can prevent phase transitions of the membrane during the dehydration and rehydration processes.[45]

Lastly, the overall solid content of the feeds can influence the processing loss and storage loss of a formulation. Spray drying of the herpes vaccine HSV-2 with trehalose as a stabilizer showed that an increase in the concentration of trehalose reduced activity loss.[19] Similarly, spray drying of bacteriophage CP30A with higher concentrations of trehalose significantly reduced processing losses.[46] Increased solids concentration promotes earlier shell formation and leads to the generation of larger particles. Larger particles have a lower surface-to-volume ratio, which mitigates possible surface-mediated degradation mechanisms.

This review has so far focused on general stabilizing mechanisms; however, the appropriate excipient system is dependent on the properties of the respective vaccine. For instance, Toniolo et al.[21] spray dried three viral vector vaccines, the enveloped vesicular stomatitis and influenza vaccines and one nonenveloped human adenovirus type 5 vaccine. Each was spray dried with excipient systems containing either trehalose, mannitol, dextran, lactose, or mixtures of these sugars, and then evaluated over 30 days of storage at 37°C. The results showed that the formulations composed primarily of trehalose best stabilized the enveloped viral vectors. However, the nonenveloped virus was better stabilized by the mannitol and dextran mixture. This example demonstrates why screening excipients is a necessary step in the development of dry dosage forms of vaccines. For optimal stabilization, more than one excipient may be needed.

Inhalable vaccines

As can be seen in Figure 1, most licensed vaccines are designed to be delivered by parenteral injections. Powder vaccines produced by spray drying are also suitable for injection after reconstitution. However, as can be seen in Figure 3, dry powders are a flexible dosage form that also allows for direct administration of the dry powder to the respiratory system through inhalation. Development of a presentation suitable for inhalation can be accomplished with engineered particles. Spray drying allows for engineering of particles without the need for secondary processing. Inhalation is a promising alternative route of administration, especially for global health purposes, as it mitigates drawbacks associated with needle delivery, such as the risk of transfer of blood-borne illnesses, difficulty safely disposing of the used needles, and low compliance due to the invasive nature of needles. Currently, the only licensed vaccine that is delivered to the respiratory system is the FluMist® Quadrivalent influenza vaccine (AstraZeneca, Gaithersburg, MD), which is administered as a liquid spray to the nasal passages.

There are two routes of vaccine administration for the respiratory system: pulmonary delivery and intranasal delivery. Further discussion on the biological mechanisms relevant to vaccination through inhalation can be found elsewhere.[47–51] Successful pulmonary drug delivery of aerosols requires deposition in the lung. To achieve consistent lung deposition, particles must not be retained in the delivery device, nor deposit to a large extent in the mouth–throat, nor be exhaled out. Particle size is a significant factor affecting deposition. Large particles are likely to deposit in the mouth, while very small hydrophobic particles are likely to be exhaled out. The target aerodynamic diameter range for liquid or dry vaccine particles intended for pulmonary delivery is between 1 and 5 μm,[52] with increasing peripheral deposition tied to decreasing particle size.[53] Such particles and associated delivery devices have been developed successfully for many commercial pulmonary drug delivery applications, and this know-how is applicable to vaccine delivery. However, intranasal delivery offers several advantages over pulmonary delivery of vaccines.

It is likely that, in the near future, research and development will focus on nasally delivered vaccines because they raise fewer toxicity concerns and because their required product attributes are easier to achieve. Powders for nasal administration simply need to exit the delivery device and deposit in the nose with minimal lung deposition. For this reason, the FDA guidelines on nasal sprays suggest that the fraction of droplets under 10 μm should be minimized.[54] To achieve a polydisperse aerosol whose particles are mostly above 10 μm, that is a $d_{a,1} \geq 1\,\mu$m in aerodynamic diameter, the appropriate median aerodynamic diameter can be estimated.

Assuming that the polydisperse aerosol follows a log-normal distribution with a geometric standard deviation of 1.6–1.9,[39,55] the corresponding median aerodynamic diameter of the powder must be above 40–45 μm, respectively. However, this calculation does not consider the effect of inhalation rate and state of agglomeration on particle deposition efficiency. High deposition rates in the nose have been shown at standard breathing (4 L/min) for particles with an aerodynamic diameter of $\sim 50\,\mu$m.[56] High deposition can also be achieved with smaller particle sizes of 14–25 μm at moderately higher breathing rates of 10–16.5 L/min.[56–58]

As shown in Eq. (2), the aerodynamic diameter of spray-dried particles, d_a, can be designed by modifying both formulation and spray drying process parameters. ρ_P is the particle density, ρ^* is the reference density (1000 kg/m^3), c_F is the solid concentration of the feedstock, and d_D is the diameter of the atomized droplets.[59] From this equation, it is apparent that increasing the solid concentration and the initial diameter of the atomized droplets will increase the aerodynamic particle diameter, and vice versa. The droplet diameter size is a function of the atomizer design and the

atomizing conditions. Eq. (2) shows that, for the production of particles for nasal delivery with a diameter ($d_a \sim 50\,\mu$m) and typical feed concentrations of 100 mg/mL, the median atomized droplet diameter needs to be larger than 100 μm, thus requiring a large drying chamber. However, during the development process of a nasal vaccine, powders with a very small particle size may also need to be produced, for example, to conduct animal studies in rodents. This requirement underscores the need for careful selection of spray drying equipment and associated processing conditions.

$$d_a = \left(\frac{\rho_P}{\rho^*}\right)^{\frac{1}{6}} \left(\frac{c_F}{\rho^*}\right)^{\frac{1}{3}} d_D \qquad 2$$

An added complication arises from the fact that powders may not be free flowing, and, especially where fine powders are concerned, particles may aggregate during storage due to relatively strong cohesive forces. Therefore, proper dispersion of particle aggregates into primary particles is essential; otherwise, aggregates will behave aerodynamically as inappropriately large particles or may not even exit the inhaler. Large carrier particles that do not contain any active pharmaceutical ingredient can be introduced into the powder to improve dispersibility.[60] However, introduction of these components reduces the active dose in the powder. Instead, the intrinsic powder cohesiveness can be directly reduced through particle engineering to improve the dispersibility. To better understand the design targets for particles with increased dispersibility, it is instructive to describe powder cohesiveness in terms of the contact mechanics between particles.

Many models have been developed for interparticle forces, but the Li-Derjaguin, Muller, Toporov (Li-DMT) model[61] is particularly useful for illustrating the relationship between the force of cohesiveness, F_c, between two particles and the particles' properties. This model is given in Eq. (3), where γ is the surface energy, r the radius, E the Young's modulus, υ the Poisson's ratio of the particles, and A_{eff} the effective contact area between the particles. The Li-DMT model assumes elastic deformation, dry micron-sized particles, similarly sized particle asperities, asperities that are in top-to-top contact, and Van der Waals attraction as the most significant attractive force.[61] Based on this model, cohesiveness can be reduced by lowering the surface energy, decreasing the deformability of the surface, and decreasing the contact area. Increase in the primary particle size also improves dispersibility as larger particles will experience greater dispersion forces.

$$F_c = \left(\frac{32\gamma_{13}}{9r\left(\pi\frac{1-\upsilon^2}{E}\right)^2}\right)^{\frac{1}{3}} \cdot A_{eff} \qquad 3$$

A suitable modification of surface morphology can be achieved through the addition of excipients that form a shell with favorable characteristics on the particle surface during drying. As indicated by the Li-DMT model, these excipients can improve dispersibility through a variety of mechanisms, such as forming a hard crystalline shell, decreasing the surface energy, or changing the surface from smooth to more rugose to decrease the contact area. Improved dispersibility may also be achieved if the chosen excipient has a hydrophobic nature as particles with hydrophobic surfaces were shown to have greater flowability than hydrophilic or hydroscopic surfaces.[62] However, this improvement of dispersibility is dependent on the extent of particle surface coverage of the agent, as well as on sufficient crystal growth in the case of crystallizing components. The level of surface coverage and time for crystal growth is a function of the formulation and processing parameters. Particle formation models have been developed to aid in the design of such structured particles.

Li et al.[63] demonstrated that spray drying increasing amounts of the amino acid leucine with the hygroscopic drug disodium cromoglycate increased surface coverage of leucine. Their study also demonstrated that increased surface coverage of the particle with leucine, which contains a hydrophobic side chain, improved aerosolization of the powders. Similarly, Momin et al.[64] assessed the manipulation of formulation and processing parameters when cospray drying the hygroscopic drug kanamycin and the hydrophobic drug rifampicin to achieve particles with hydrophobic surfaces. Recently, Ordoubadi et al. have provided detailed particle formation models for the dispersibility enhancers leucine[65] and trileucine,[66] which can be used for the rational design of such powders.

The choice of an appropriate dispersibility-enhancing agent for a vaccine requires careful screening. Suitable excipients for an inhalable presentation of a spray-dried TB vaccine candidate were investigated by Gomez et al.[67] The authors studied the addition of the polysaccharide pullulan, the amino acid leucine, and the tripeptide trileucine as dispersibility-enhancing agents to make the powder suitable for pulmonary delivery. All formulations were spray dried with trehalose along with the nanoemulsion-based TB vaccine candidate and assessed for compatibility with the vaccine and for aerosol performance. The formulations were designed using particle formation models in such a way that the respective excipient would accumulate on the particle surface. The pullulan-containing formulations and trileucine-containing formulations produced fully amorphous particles, whereas the leucine-containing formulation produced particles with an amorphous trehalose core and crystalline leucine on the surface. A trehalose formulation without any dispersibility-enhancing agent had excellent vaccine stabilization properties but formed particles with a smooth surface.

The lung dose was only 18% when the powder was administered with the Seebri Breezhaler, a commercial dry powder inhaler (DPI), with a flow rate of 100 L/min for 2.4 seconds, simulating a sharp inhalation. The pullulan-containing formulations also formed fairly smooth particles with indentations and had a lung dose of 19%–25%. The leucine-containing particles had a crystalline surface layer with many surface asperities and showed a much-improved lung dose of 32%. The trileucine-containing formulations showed very rugose particles and a demonstrated lung dose of 33%–34%. The lead candidate was established to be a fully amorphous formulation that used trehalose as the primary stabilizing excipient with 3% trileucine by mass as a dispersibility-enhancing agent.[32] In addition to improved dispersibility, a stability study on this lead candidate demonstrated that the addition of trileucine further improved the stability of the powder compared with the trehalose-only formulation.

Particle morphology was maintained even at very high temperature storage for 7 months, as shown in Figure 4. Conversely, the control formulation showed signs of small-particle fusing under long-term high-temperature storage. Additionally, the emitted dose and the lung dose did not change significantly over time, remaining at 98% and 38%, respectively, after 12 months of storage at 40°C, thus demonstrating the excellent physical stability of the spray-dried vaccine. This lung dose was significantly better than the performance of most commercial DPIs.[68] Both the trileucine-free and trileucine-containing formulations stabilized the adjuvant system for 1 year of storage at temperatures up to 25°C; however, the two formulations demonstrated differences in antigen stability. The trileucine-containing powder was able to retain ~45% of the antigen over 1 year of storage at 25°C, 40°C, and 50°C, whereas the trileucine powder showed no evidence of the antigen after 1 year of storage at 25°C and above. Clearly, trileucine exhibited a protective effect.

Trileucine was very promising for this particular vaccine; however, other vaccines may be made dispersible with lower-cost excipients such as leucine. For example, Kunda et al.[69] successfully spray dried a nanoparticle recombinant pneumonia vaccine for inhalable delivery using leucine as the sole excipient. The suitability for pulmonary delivery was assessed by actuating powder loaded in a commercial DPI into an impactor. The measured mass median aerodynamic diameter of the deposited powder was reported as 1.21 μm, suggesting it was suitable for delivery to the bronchioalveolar region.[69] Price et al.[29] successfully spray dried a live Bacillus Calmette–Guérin (BCG) vaccine for TB with an excipient system composed of leucine, mannitol, trehalose, polyvinylpyrrolidone, and bovine serum albumin. Their tested formulations were designed to consist primarily of leucine (71%–75% by mass) to achieve highly dispersible powders. The size distribution of aerosolized powder was also assessed using a commercial inhaler with an impactor. Here, the mass median aerodynamic diameter of the deposited powder was reported as 1.67 μm and thus within a suitable range for targeting the lung parenchyma.

Improvement of Out-of-Package Stability

When it comes to assessing the stability of dry powder vaccines, storage stability must be clearly distinguished from short-term robustness, which is necessary only during administration of the vaccine once it is removed from its protective packaging. Dry powder dosage forms generally need to be protected against moisture ingress during storage, so their long-term storage conditions are characterized by low RH. Upon administration, such dosage forms are typically taken out of their protective packaging configuration and then may be subject to extremely challenging conditions for a short while. Both scenarios need to be adequately addressed during dosage form design and testing.

Relatively few long-term stability studies on thermostable spray-dried vaccines have been published. Such studies must be conducted with consideration of the realistic environments to which powders may be exposed. Regulatory authorities (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use [ICH]) have issued general guidelines for the storage conditions of drug products for long-term, intermediate, and accelerated stability studies.[70] The storage conditions for powders in protective packaging are 25°C/60% RH or 30°C/65 RH for a minimum of 12 months for long-term studies, 30°C/65% RH for a minimum of 6 months for intermediate studies, and 40°C/75 RH for a minimum of 6 months for accelerated studies. Consideration of the effect of protective packaging is a necessary component of stability studies on vaccine powders.

Stability studies may simulate a protective packaging system for the final product, as shown by Gomez et al. in both their studies.[16,32] In this system, silica gel was used as a desiccant to regulate the RH of the package in which the powder was stored. First, the desiccant was left in an environmental chamber to equilibrate to 7% RH, the designed equilibrium humidity of the powder in the study. A vial containing the powder was then placed in an aluminum bag along with an equilibrated 7% RH desiccant pouch, and the bag double-heat sealed. This bag was then placed into another bag along with a desiccant pouch equilibrated to 0%

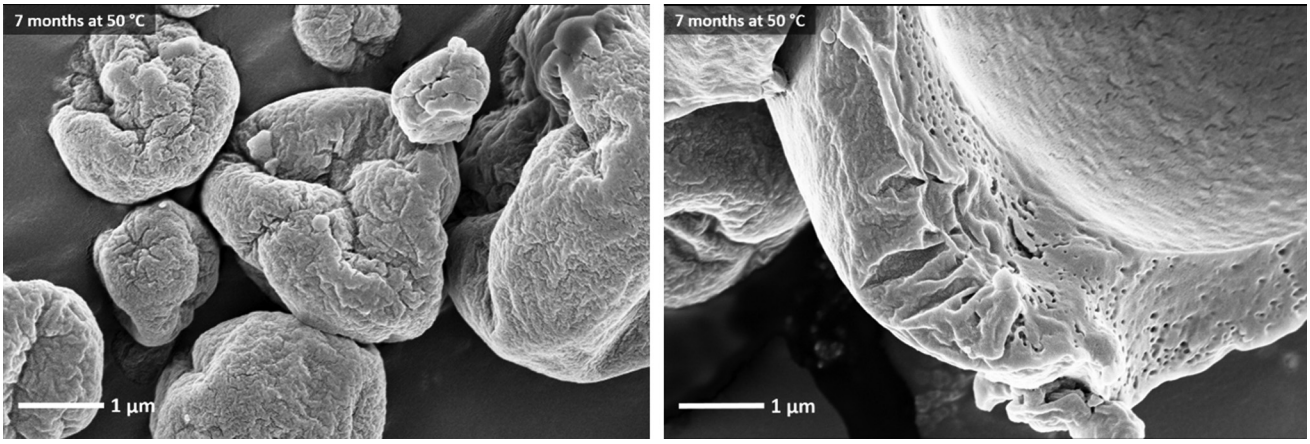

FIG. 4. Scanning electron microscopy (SEM) images of the lead inhalable spray-dried tuberculosis vaccine candidate after 7 months of dry storage at 50°C at low magnification (left) and at high magnification (right).[32] Interior and exterior particle morphology was preserved. Similar preservation of morphology was demonstrated for a sample held at 40°C for 12 months.

RH and the exterior bag also double-heat sealed. This secondary package was included to minimize the moisture difference between the innermost package where the powder was stored and the external environment. This system was capable of maintaining the initial moisture content of the powder for the duration of the stability study.

Other studies have demonstrated the necessity of protective packaging in assessing the stability of spray-dried vaccines. Price et al.[29] stored spray-dried BCG vaccine under conditions recommended by the ICH when the vaccine is packaged either in scintillation vials or in protective packaging. The protective packaging consisted of amber-colored vials that were previously purged with nitrogen gas and contained both a desiccant and a reactive oxygen species scavenger. Differences in packaging significantly affected the viability of the spray-dried vaccine. The powders stored in simple vials, which do not provide sufficient protection against moisture ingress, lost all viability after 2 weeks of storage at 40°C/75% RH. However, under the same environmental conditions, the powders with protective packaging experienced only 1 to 1.5 log loss after 12 months of storage.

Similarly, Kunda et al.[33] spray dried a live vaccine and assessed how changing the packaging would affect the stability of the dry powder for cases of no protection, storage with desiccant, storage with desiccant directly in the vial, initial purging of the vial with nitrogen gas, and addition of oxygen scavengers. Powders stored without any protection lost all viability after 50 days of storage at 37°C. Samples stored with nitrogen purging and inclusion of desiccant and oxygen scavengers in the packaging exhibited much greater stability, with $\sim$0.5 log loss after 180 days of storage at 40°C.

Improvement of out-of-package powder stability can be achieved through the addition of suitable excipients. The amino acid leucine has shown particular promise for the improvement of short-term robustness of powders, especially in the preservation of aerosol performance. Improvement of out-of-package stability has been demonstrated by Shetty et al.'s[71] work on spray drying the drug ciprofloxacin in a 1:1 ratio with either sucrose, lactose, trehalose, mannitol, or leucine. The spray-dried powder was exposed to 55% RH in open scintillation vials. Exposure of the powders to moisture showed that spray-dried ciprofloxacin began to crystallize within 1 hour under these conditions, whereas the sucrose, lactose, and trehalose formulations caked within the 10-day storage period.

By contrast, the leucine-containing powder delayed crystallization of the drug by 3 days. Furthermore, when tested for *in vitro* aerosol performance with an RS01 monodose DPI, all spray-dried formulations, except the leucine-containing one experienced a significant decrease in measured fine particle fraction after 10 days of storage at 55% RH. Preservation of physical stability and aerosol performance for 10 days of storage was achieved in a formulation containing as little as 10% leucine by mass.

Wang et al.[72] recently assessed the out-of-package stability of spray-dried powders consisting of trehalose and the shell-forming excipients pullulan, trileucine, or leucine. Hydroxypropyl methylcellulose capsules were loaded with spray-dried powders and exposed to controlled humidity conditions in an environmental chamber set to 25°C and 90% RH. These powders were then actuated from a Seebri Breezhaler DPI and assessed for changes in emitted dose. The tested powders consisted of only trehalose, 70% trehalose and 30% pullulan, 97% trehalose and 3% trileucine, and 70% trehalose and 30% leucine. Tests were conducted at an inhalation flow rate of 60 L/min, with the results shown in Figure 5, where the error bars represent the percent difference between two experiments. Before exposure, all powders had a very high emitted dose of >90%. However, after 60 minutes of exposure, the emitted dose of the trehalose formulation was extremely low (<5%).

This result shows that, even though the powder was in a capsule, there was a drastic impact on performance, demonstrating how important it is that out-of-package robustness be considered during product development. The 70% trehalose and 30% pullulan particles also experienced a significantly reduced emitted dose of 8% under the same conditions, whereas the 97% trehalose and 3% trileucine powders' emitted dose was better at 43%. The 70% trehalose and 30% leucine formulation performed much better still, with the emitted dose showing no significant change.

This work also showed that a strong protective effect against moisture ingress can be achieved with lower mass fractions of leucine. Measurements using a lower inhalation flow rate of 15 L/min showed more pronounced effects of humidity on aerosol performance. After only 10 minutes of exposure to the environmental chamber, the emitted dose of the trehalose powders decreased from 66% to 43%. However, trehalose–leucine formulations containing as little as 10% leucine by mass maintained their emitted dose under the same conditions. These results show that successful particle engineering strategies for the improvement of out-of-package stability have been found. This improvement in short-term stability under harsh environmental conditions increases the chance of maintaining the inhaled dose, even when accounting for environmental exposure during administration.

Delivery Devices for Inhalable Vaccines

There are many benefits to using a DPI as an inhalation device to administer a vaccine to the respiratory system. For instance, if a vaccine is designed to be delivered as a dry powder, sterile water for reconstitution is no longer needed. Moreover, DPIs are minimally invasive, mostly simple to use, quick to administer, and highly portable.[68,73] Many commercially available devices have already been developed, and this field continues to expand. Several comprehensive reviews have been written regarding devices for nasal delivery,[74] pulmonary delivery,[75,76] and specifically DPIs.[68,77]

The majority of inexpensive pulmonary DPIs are passive devices, that is, they rely primarily on patient inspiration to aerosolize and disperse the powders. The question arises whether typical target patient groups for vaccination campaigns, for example, young children, can produce a sufficiently high inspiration rate to reliably disperse the powders for respiratory administration. However, a recent review of the capabilities of commercially available DPIs by Clark et al.[78] concludes that so long as a patient can produce a very modest 1 kPa pressure drop across the device—much lower than the often mentioned 4 kPa U.S. Pharmacopeia

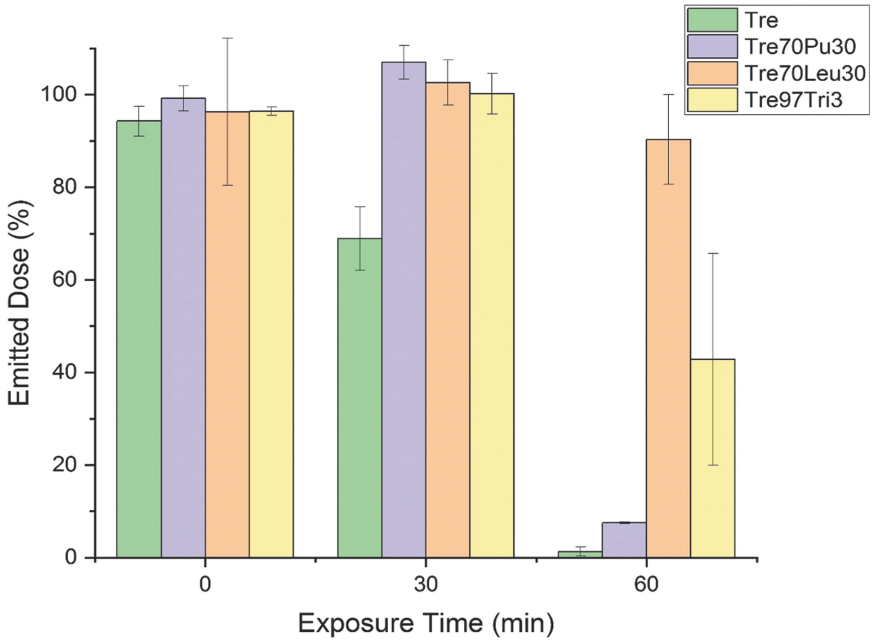

FIG. 5. Emitted dose from a dry powder inhaler for several spray-dried formulations at a simulated inhalation flow rate of 60 L/min. The formulations tested were trehalose (Tre), 70% trehalose and 30% pullulan (Tre70Pu30), 70% trehalose and 30% leucine (Tre70Leu30), and 97% trehalose and 3% trileucine (Tre97Tri3). Emitted dose was tested after exposure to an environment of 25°C and 90% RH for 0, 30, and 60 minutes. The error bars represent the percent difference between the two measurements. Reprinted by permission from Wang et al.[72]

test standard[79]—many commercial DPIs can still be considered viable devices. Lindert et al.[80] measured the emitted dose and pulmonary deposition of a lactose carrier-based test powder when administered with the simple DPIs Cyclohaler, Handihaler, and Spinhaler at different flow rates.

Pressure drops measured for the Cyclohaler at 30, 60, and 75 L/min were 0.22, 0.94, and 1.17 kPa, respectively. The Handihaler had pressure drops of 1.59 and 6.14 kPa, and the Spinhaler 0.14 and 1.04 kPa at 30 and 60 L/min, respectively. The study found that performance of the Cyclohaler was similar at the tested inhalation rates, with no increase in emitted dose found when the inhalation flow rate was increased from 30 L/min to 75 L/min. Pulmonary deposition increased by only 2%–7% across different throat models when the flow rate was increased from 30 to 75 L/min. Similarly, when the powder was administered with the Handihaler, the emitted dose and pulmonary deposition were not affected by the increase in flow rate from 30 to 60 L/min.

This study also demonstrated that the choice of inhalation device is important, especially for powders that are not very dispersible. Dispersion with the Spinhaler device showed poor performance, with only ~30%–50% of the dose emitted from the device when tested at 30 and 60 L/min. Administration of the same powder formulation under the same conditions with the Handihaler or Cyclohaler improved emitted dose to ~60%–70%. Similar results for the dependence of aerosol performance on DPI design were demonstrated by Sibum et al.'s[81] work on isoniazid powder spray dried with different dispersibility-enhancing agents. Their study found that using a Twincer DPI over a Cyclops DPI under the same inhalation condition of 4 kPa pressure drop improved the emitted dose by 10%–43%, depending on the formulation tested. It can be concluded that DPIs may be a useful option even for pediatric applications, provided that well-designed, simple-to-actuate devices are used in combination with dispersible powders.

Nasal dry powder delivery devices may be an even better option to induce mucosal immunity. There are several benefits to nasal targeting over lung targeting beyond reduced losses to oropharyngeal deposition. As discussed in the previous section, particles designed for nasal delivery must be larger than those intended for pulmonary delivery. Larger particles have a lower surface-area-to-volume ratio, which may promote improved stability through reduced opportunity for surface-based degradation mechanisms. Furthermore, powders with larger particle size are much more flowable and easier to disperse than powders for inhalation into the lung. Nasal powder delivery devices considered for vaccination purposes are active devices that do not require patient coordination or a synchronized breathing maneuver, which could make them suitable for vaccination in infants.

Several nasal dry powder devices have been developed, with some already in use commercially, for instance Teijin's Puvlizer Rhinocort, a nasal dry powder delivery device to treat rhinitis. This device employs mechanically generated airflow activated by the user through a bellows-like system to aerosolize the reserved powder.[82] Another nasal dry powder device is the Unidose DP, developed by Bespak. This hand-held device also uses a bellows-like system that is compressed and released to disperse the powder.

A study of this device using an experimental spray-dried immunoglobulin G formulation combined with an inhalation rate of 25 L/min for 12 seconds showed a high emitted dose, with 95% of the powder delivered into a nasal cavity model.[83] This device was also used in a clinical trial to deliver a powder-based norovirus vaccine intranasally.[84] Aptar's Unit Dose Spray is another inexpensive, single-use intranasal powder delivery device that uses a spring-loaded mechanism to eject the powder. This device has been used in a preclinical trial for delivery of a dry powder norovirus vaccine to guinea pigs.[85] It has also been commercialized to deliver glucagon as rescue medication for low blood sugar emergencies (Baqsimi®; Eli Lilly & Co, Indianapolis, IN).

Concluding Remarks

Extensive experimental work and the remarkable progress in ongoing vaccine development projects have now demonstrated that the development of a thermostable dry form of a vaccine for global distribution is feasible. The use of spray drying as a drying method in combination with advanced particle engineering methods results in the production of a flexible dry powder dosage form that can be used to improve thermostability for a variety of vaccine types, including lipid-based adjuvant systems, subunit vaccines, live-attenuated vaccines, etc. These studies have also introduced several promising stabilizing formulation systems with excipients that pose a low toxicity risk. The dry powder format can be reconstituted and then injected like a liquid vaccine but also presents an excellent opportunity for other methods of administration such as inhalation.

The potential for inhalable routes of administration for vaccines has been clearly demonstrated, and the tools are now available to target dry powder intranasal or pulmonary delivery through a number of commercially available devices. The development of dry powder vaccines for nasal or pulmonary administration will benefit from decades of applicable experience in the development, manufacture, and scale-up of respiratory drug products. The implementation of thermostable, inhalable vaccines with improved out-of-package robustness is hoped to greatly improve distribution in resource-poor settings, where the greatest burden of infectious diseases is carried.

Authors' Contributions

M.G. wrote the initial article draft. R.V. provided critical feedback to the article. Both authors approved the final article.

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Author Disclosure Statement

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