**Validation of a diffusion-based single droplet drying model for encapsulation of a viral-vectored vaccine using an acoustic levitator**

Blair A. Morgana, Elina Niinivaarab,c,Zhou Xingd, Michael R. Thompsona,Emily D. Cranstona,b,e,\*

a Department of Chemical Engineering, McMaster University, Hamilton, Ontario, L8S 4L7, Canada

b Department of Wood Science, University of British Columbia, 2424 Main Mall, Vancouver, BC, V6T 1Z4, Canada

c Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, P.O. Box 16300, FI-0076 Aalto, Espoo, Finland

d McMaster Immunology Research Centre and Department of Medicine, McMaster University, Hamilton, Ontario, L8S 4L7, Canada

e Department of Chemical and Biological Engineering, University of British Columbia, 2360 East Mall, Vancouver, BC, V6T 1Z3, Canada

\*Corresponding author: Emily Cranston, [emily.cranston@ubc.ca](mailto:emily.cranston@ubc.ca)

**Abstract**

Development of thermally stable spray dried viral-vectored vaccine powders is dependent on the selection of a proper excipient or excipient blend for encapsulation, which can be a time and resource intensive process. In this work, a diffusion-based droplet drying model was developed to compute droplet drying time, size, and component distribution. The model predictions were validated using an acoustic levitator to dry droplets containing protein-coated or fluorescently labelled silica nanoparticles (as adenoviral vector analogues) and a range of excipient blends. Surface morphology of the dried particles was characterized by atomic force microscopy and the distribution of silica nanoparticles was quantified by confocal microscopy. The modelled distributions of adenovirus agreed with the microscopy results for three mannitol/dextran excipient blends with varying molecular weight dextrans, verifying the equations and assumptions of the model. Viral vector activity data for adenovirus in a range of (poly)saccharide/sugar alcohol formulations were also compared to the model outputs, suggesting that viral activity decreases when the model predicts increasing adenovirus concentrations near the air-solid interface. Using a validated model with excipient property inputs that are readily available in the literature can facilitate the development of viral-vectored vaccines by identifying promising excipients without the need for experimentation.

**Keywords**

Acoustic levitation, modelling, spray drying, droplet drying, excipient, viral-vectored vaccine development

**1. INTRODUCTION**

Spray drying has the potential to play a major role in the production of thermally stable vaccine powders and next generation pharmaceutics with stability at high temperatures and particle characteristics suitable for pulmonary delivery. Compared to other popular drying technologies such as freeze drying or vacuum drying, spray drying allows for more control over the final product, is more cost effective, and can more easily process large amounts of product (Amorij et al., 2008; Ré, 1998). By controlling the input parameters during spray drying, dry powder characteristics such as particle size, morphology, and moisture content can be controlled (Mondragon et al., 2011). For these reasons, there are many examples in the literature of droplet and spray drying models that can be used to assist in particle engineering by predicting powder properties without experiments (Langrish, 2009; Mezhericher et al., 2010; Schutyser et al., 2012). However, when formulations containing biologic materials, such as proteins or viral vectors, are spray dried, the ability to retain the effectiveness of the biologic is the most vital characteristic. Component distribution (the weight fraction variance of each chemical species with respect to the radius of a droplet or solid particle) is known to have a significant impact on the performance of a final dry powder, particularly in pharmaceutical products where exposure of biologic materials to the air-solid interface can lead to a decrease in activity (Devineni et al., 2014; LeClair et al., 2016a; Xu et al., 2014). Despite this, there is a lack of predictive models in the literature that are intended to guide the encapsulation strategies for stabilizing biologics.

In addition to the lack of models describing the effectiveness of biologics after spray drying, experimental methods to link biologic material distribution/location within a powder to activity loss are limited. Standard characterization techniques such as microscopy and surface elemental analysis are inadequate for powders containing viral vectors because of the relatively small volume of virus particles compared to the total volume of the powder, and the low probability of finding a viral particle at the surface. The sensitivity and spatial coverage and resolution of these techniques is often insufficient and, in some cases, working with biologically active/infectious agents is prohibited in instruments and facilities due to biosafety concerns. Employing an inactive “analogue” in the formulation to represent the biologic material can facilitate powder characterization and, as shown here, model validation. In the present study, we aim to develop an appropriate theoretical model to give formulators added insight into the location and activity of a viral vector in the encapsulating matrix of a drying droplet – this is beyond the apparent chemical interactions and makes for a cost-saving resource in an otherwise expensive development process.

The spray drying process in its entirety is complex, involving mass and heat transport within a droplet along with progressive phase transitions, all of which occur on a time scale of milliseconds. This makes it extremely difficult to collect drying data during the spray drying of samples. To best approximate the process, it is desirable to simplify the system to an individual droplet containing dissolved or suspended solids that is drying in a controlled environment (Sadek et al., 2015). This simplified system can be broken down into two drying stages: the first stage of drying occurs as solvent begins to evaporate from the droplet surface at a constant rate, dependent on the surrounding temperature and humidity, known as the *constant rate period* (Mezhericher et al., 2011). The radius of the droplet begins to shrink, and as more solvent evaporates, the concentration of the dissolved solutes will eventually reach saturation at the surface and precipitation will occur. This causes the formation of a solid shell around a liquid core. During the second stage, the evaporation rate slows significantly as water must now diffuse through the solid shell to reach the air-solid interface and evaporate, known as the *falling-rate* *period* (Sloth et al., 2006). Over time, the shell thickness will increase as water is removed from the droplet until a dry particle is formed. Depending on the rate of diffusion, the evaporation rate, and the mechanical strength of the shell, the particle may either remain the same size, or buckle and fold into a smaller particle during the falling rate period (Vehring, 2008). Two-stage models are the most common in the literature, as they make simulating the system less complex.

There are several models available in the literature that are specific to droplet drying, including semi-empirical models, deterministic models, and reaction engineering approach models (Mezhericher et al., 2010). Many of these models have been developed with spray drying in mind, and all can predict droplet or particle bulk characteristics such as size, drying time, and temperature, while several models can also predict the component distribution (Farid, 2003; Huang, 2011; Porowska et al., 2016; Sazhin et al., 2018). However, most of these models present computed results without experimental validation, or provide validation for basic predictions such as droplet size and drying time but do not verify their more complex predictions such as component distribution (Grasmeijer et al., 2016). This detailed level of particle engineering is of critical importance to new dry powder vaccines (our focus), making it necessary to create a comprehensive model of droplet drying where the component distribution is reasonably predicted and its results are experimentally verified. As such, the goal of this work was to explain the diminished activity of a human serotype-5 adenoviral vector (AdHu5) seen in our previous spray drying studies where the loss of effectiveness in this dry powder vaccine was hypothesized to be related to the virus proximity to the particle surface (LeClair et al., 2016a).

Herein, predictions were made using a continuous, diffusion-based droplet drying model based on the deterministic model of Grasmeijer et al. (Grasmeijer et al., 2016) and compared to observations of experimentally produced particles using an acoustic levitator, which slows down the drying process and allows for real-time data to be collected. We have previously demonstrated that acoustic levitation of drying droplets mimics crucial aspects of the spray drying process (Morgan et al., 2019). The distribution of components and their relationship to viral activity loss were studied using an adenoviral analogue, specifically, protein coated silica nanoparticles (pSiNPs) to mimic AdHu5 (Pang et al., 2014). To the best of our knowledge, this is the first work that spatially locates adenovirus or adenoviral analogues within a dried particle, which is an important step towards understanding viral deactivation routes and excipient performance in thermally stable vaccine powders, leading to products with improved viral stability and effectiveness.

**2. MATERIALS AND METHODS**

**2.1 Preparation of protein coated silica nanoparticles (pSiNPs)**

The particle size and coating were chosen to mimic AdHu5 without the associate biohazard. Carboxylated silica nanoparticles (SiNPs; 100 nm diameter) were purchased from Creative Diagnostics (New York, NY), at concentrations of 25 mg/mL. The SiNPs were covalently coupled to α1-acid-glycoprotein (Millipore-Sigma; Oakville, Canada) using a two-step 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) reaction based on Pang et al. and a protocol from Bangs Laboratories (Bangs Laboratories, 2010; Pang et al., 2014). Two rounds of centrifugation were used to wash 1 mL of SiNPs. In each round, 10 mL of 2-ethanesulfonic acid (MES) buffer (Millipore-Sigma; Oakville, Canada; pH 6.0) was added to the SiNPs, followed by centrifugation at 10,000 G for 15 minutes and removal of the supernatant. After the second round of centrifugation, the SiNP pellet was re-suspended in 2.5 mL MES buffer (pH 6.0) using 15 minutes of bath sonication. After re-suspension, 100 mg of EDC was added while stirring the SiNP suspension continuously for 15 minutes at room temperature (between 23°C and 25°C) to allow the reaction to occur. The SiNPs were then washed again with two rounds of centrifugation in MES buffer (pH 7.2) and the pellet was re-suspended in 5 mL of MES buffer (pH 7.2) using bath sonication for 15 minutes. The α1-acid-glycoprotein (1 mg, Millipore-Sigma; Oakville, Canada) was dissolved in 5 mL of MES buffer (pH 7.2) was added to the SiNP suspension, and this mixture was agitated for 4 hours at room temperature to allow the coupling reaction to occur. In the final steps, the pSiNP suspension was washed in 10 mL of purified water while being centrifuged at 10,000 G for 15 minutes, and the pellet re-suspended in 10 mL of purified water using bath sonication for 15 minutes. The final pSiNP suspension was stored at 4°C until use, and the same batch of pSiNPs was used throughout all experiments. All purified water used had a resistivity of 18.2 MΩ cm. Dynamic light scattering, zeta potential measurements, atomic force microscopy (AFM), and Fourier-transform infrared (FTIR) spectroscopy were all used to confirm that the coupling reaction was successful (Supplementary Information, Table S1 and Figures S1 and S2).

**2.2 Droplet drying by acoustic levitation**

Excipients for the experiments included D-mannitol and dextran of three different molecular weights (Mr 10 kDa, 40 kDa, and 500 kDa), all purchased as USP grades from Millipore-Sigma (Oakville, Canada) and used as received. Mannitol was dissolved into purified water with a dextran of chosen molecular weight at a ratio of 75:25 by mass, respectively, at a total solids concentration of 4 wt%. Two different concentrations of pSiNPs were examined with this excipient solution, either at the so-called ‘normal’ loading, and a ‘high’ loading (50 times normal, which was more easily detected in dry particles). For the normal loading concentration, 16 µL of pSiNP suspension at a concentration of pSiNPs/mL was added to 10 mL of the excipient solution to give an equivalent loading of particles to the viral vector in dry powder vaccines (Morgan et al., 2020, 2019). For the ‘high’ loading concentration, 800 µL of pSiNP suspension at a concentration of pSiNPs/mL was added to 10 mL of excipient solution.

In some cases, fluorescent carboxylated SiNPs (fSiNPs; 100 nm diameter) purchased from Creative Diagnostics (New York, NY) were used instead of pSiNPs, for visualization in confocal microscopy. The received fSiNPs (10 mL at 25 mg/mL concentration) were diluted to a concentration of fSiNPs/mL in purified water and combined with mannitol/dextran equivalent to the ‘high’ loading (500 µL of fSiNP suspension for 10 mL of excipient solution) for pSiNPs, i.e., 50-fold the normal AdHu5 loading.

A model 13D10 ultrasonic levitator with single-walled process chamber (tec5USA; Plainview, NY) was used to prepare particles; levitated particles are about 50 times larger than spray dried particles but have been shown to exhibit similar viral activity trends when drying conditions are replicated and are much easier to characterize for their surface morphology (Morgan et al., 2019). A 10 µL glass syringe (Hamilton; Reno, NV) with a 26G needle (Becton Dickinson; Mississauga, Canada) was used to place liquid droplets of approximately 2.5 μL in the standing acoustic wave of the levitator as well as to collect dried particles. The levitator was operated in a custom-made glove box with a programmable heat gun (Steinel; Bloomington, MN) to control the surrounding temperature of the droplet. Temperature and relative humidity (RH) measurements were measured continuously throughout the drying process using a USB sensor (Dracal; Brossard, Canada). The RH in the glove box was not controlled, varying with temperature, from approximately 30% RH at room temperature, to approximately 4% RH at the set temperature between 55°C and 60°C. A Celestron MicroDirect USB microscope and accompanying software (Celestron; Torrance, CA) was used to take a series of time-lapsed images during the drying process. In order to increase the sharpness of the droplet edge during imaging, droplets were backlit using an LED ring light passed through a light diffusing sheet. Custom-made Python software was used to measure both major and minor droplet axis lengths in pixels from the time-lapse images, as pressure from the acoustic wave flattens the droplet in the vertical direction so that the cross-sectional area is elliptical in shape. These values were converted into micrometers using a calibration.

**2.3 Particle microscopy**

Samples mounted on a magnetic metal specimen support disc with double sided tape were imaged by AFM using tapping mode on a Bruker Multimode 8 AFM (Bruker; Santa Barbara, CA) at a scan rate of 0.25 Hz. Al coated silicon probes were used (NCHR probes from Asylum Research, Oxford Instruments, Santa Barbara, CA) with a nominal 42 N/m spring constant and 30 kHz resonance frequency. All images were processed with a standard third-order polynomial flattening technique in the NanoScope analysis software v.8.10 (Bruker; Santa Barbara, CA).

Confocal laser scanning microscopy was done using an Olympus FV1000 laser scanning/two-photon confocal microscope (Waltham, MA) with an excitation wavelength of 559 nm. 75 scans spaced 2 µm apart were taken for a total depth of 150 µm for each levitated particle (dried in the acoustic levitator at 60°C and 4% RH; one particle for each mannitol/dextran blend was imaged) using the Olympus 20x NA 0.75 air objective. Particles were held in place using double sided tape. The images were captured using Fluoview FV100 software (Olympus; Waltham, MA) and image analysis was done using the Fiji package for ImageJ software (National Institutes of Health; Bethesda, MD) according to a previously published method (Schindelin et al., 2012).

**3. NUMERICAL MODELLING – DROPLET DRYING**

The droplet drying model used in this work was developed in Matlab (Mathworks Inc.; Natick, MA), adapted from a published model (Grasmeijer et al., 2016). In this model, the droplet was comprised of 40 concentric subshells of progressively decreasing thickness and increasing distance from the droplet center but equal volume, each composed of a uniform composition that changed as a function of time. For every iteration in the modelled droplet drying process, as the solvent evaporated from the outer surface of the droplet, the diameter of each shell was recalculated to keep the volume of each of the 40 subshells equal.

The original model was modified to improve its accuracy for the current set of results, and to improve the ease of use when modelling new materials. Due to ‘acoustic streaming’ caused by the ultrasonic wave used for droplet levitation, the mass transfer from droplet to surroundings is higher than drying in stagnant air, as assumed in the original model (Kawahara et al., 2000). To account for this, a temperature-dependant dimensionless Sherwood number was added to the term calculating evaporative mass transfer of water from the droplet to its surroundings. At each temperature, a constant Sherwood number was assumed for the duration of drying, using reported Sherwood numbers at 25°C and 60°C in the literature (Schiffter and Lee, 2006). Diffusion coefficients for the original model’s various dissolved components came from fitting data (Ekdawi-Sever et al., 2003; Grasmeijer et al., 2016). To reduce reliance of the model on experimental diffusion data, which may not be available for all excipients, subsequent coefficients of all solutes were calculated using the Stokes-Einstein equation:

where is the Boltzmann constant (J/K), *T* is the droplet temperature (K), is the viscosity of the solvent (Pa s), and is the hydrodynamic radius of solute *i* (m). Although experimental diffusion coefficients may be more accurate, the use of Equation (1) to determine diffusion coefficients allows users to get preliminary predictions from the model without any experimental data.The diffusion coefficient of water was also adjusted using a correlation from Mendoza and Schmalko, who measured the diffusion coefficient of water for the osmotic dehydration of papaya (Mendoza and Schmalko, 2002):

where is the mass fraction of water. This correlation was selected to increase the robustness of the model at predicting the drying behaviour of carbohydrate-based formulations, as this equation was used to model the diffusion of water in a variety of carbohydrates of similar molecular weight (Mendoza and Schmalko, 2002) to those used in this study.

To calculate the viscosity of systems containing dextran, an equation from Carrasco et al. was used to correlate the viscosity to temperature, concentration of dextran, and the molecular weight of dextran (Carrasco et al., 1989):

where is a pre-exponential factor at infinite dilution (Pa s), and are constants for a given molecular weight, *c* is the concentration of dextran (%), is the activation energy of viscous flow at infinite dilution (J/mol), and *R* is the universal gas constant (. The predicted viscosities for a 4% mannitol/dextran solution (1.6 m Pa s) and an 8% mannitol/dextran solution (1.9 mPa s) were comparatively similar to measured viscosities at 25°C for the same solutions (1.1 mPa s for a 4% solution and 1.3 mPa s for an 8% solution) (Morgan et al., 2020). This correlation was chosen as dextran was the largest excipient molecule modelled in this study and was considered to be the dominant cause for changes in viscosity, whereas the mannitol was assumed to have a relatively negligible influence on the modelled system viscosity. This assumption can be verified by the data of Moulik and Khan, who reported a change in viscosity of approximately 0.4 mPa s over a range of mannitol concentrations at 27°C, whereas Equation (3) predicts a change in viscosity of nearly 100 mPa s over the same range of concentrations at 25°C for dextran (Moulik and Khan, 1977).

Additionally, the model was modified to significantly decrease the diffusion of solutes once the radius of the droplet stopped shrinking (i.e., once a solid shell had formed). This was to prevent the unrealistically rapid self diffusion of excipients in the highly concentrated media. The diffusion of water was allowed to continue as predicted by equation (2), since it reportedly accounts for the mobility of water through carbohydrates.

In order to run the model, measured values for surrounding air temperature, RH, and initial cross-sectional area of the droplet were required as inputs. Several other material properties were required as inputs for each excipient or solute being modelled, namely dry density, molecular weight, hydrodynamic radius, and solubility in water. These properties can be readily found in the literature or in material data sheets provided by suppliers for most common excipients, including those used for these experiments. The model inputs for hydrodynamic radius, density, and molecular weight used in this work for the three dextrans, mannitol, and AdHu5 are provided in Table 1. We note that the density and radii of pSiNPs and fSiNPs are similar to AdHu5 (Pang et al., 2014). Code for the model is available upon request by emailing the authors.

**Table 1:** Model input values for hydrodynamic radius, density, and molecular weight for dextrans, mannitol, and AdHu5.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Hydrodynamic Radius (m) | Density (kg/m3) | Molecular Weight (kg/mol) | References |
| 10 kDa Dextran |  | 1050 | 10 | (Armstrong et al., 2004; Yamaguchi et al., 2018) |
| 40 kDa Dextran |  | 1050 | 40 |
| 500 kDa Dextran |  | 1050 | 500 |
| Mannitol |  | 1490 | 0.182 | (Better et al., 1997; Jenkins and Bell, 1987; Zhu and Yu, 2017) |
| AdHu5 |  | 1335 |  | (Kennedy and Parks, 2009; Ruigrok et al., 1984; Wilcox and Ginsberg, 1963) |

**4. RESULTS AND DISCUSSION**

**4.1 Droplet drying time, droplet size, and evaporation rate**

To ensure that the model could accurately predict drying time, final particle size, and solvent evaporation rate, experimental drying results for pure mannitol, pure dextran, and a 3:1 blend by mass of mannitol/dextran (40 kDa) were compared to drying model predictions at both ~23°C (room temperature, measured on each day experiments were conducted) and 60°C. For comparisons to spray drying, 60°C was the more important temperature to consider, as it matches the outlet temperature of the spray dryer when drying powders containing AdHu5 (LeClair et al., 2016b). The 2D projected droplet areas seen by the USB microscope for these formulations were compared to their predicted areas by the model with respect to drying time, shown in Figure 1. Cross-sectional areas of the droplets rather than droplet diameters were examined to account for the slightly oblong shape of the droplets caused by the acoustic field in the experiments. Discontinuous gaps in the experimental data were caused by fluctuations of the droplet position within the acoustic field, leading to blurry images that could not be measured by the Python software and were removed from the drying curves.



**Figure 1:** Comparisons of experimental data collected from an acoustic levitator to predictions made by the model for (a) 4% mannitol at 23°C and 26% RH; (b) 4% 40 kDa dextran at 23°C and 32% RH; (c) 4% mannitol and 40 kDa dextran at a 3:1 ratio at 23°C and 28% RH; (d) 4% mannitol at 60°C and 3% RH; (e) 4% 40 kDa dextran at 60°C and 3% RH; and (f) 4% mannitol and 40 kDa dextran at a 3:1 ratio at 60°C and 3% RH. Levitator images were collected every 10 seconds during drying. Irregularities in the experimental data plots are caused by fluctuations of the droplet position in the acoustic levitator while drying.

In all 12 drying curves (Figure 1), the constant and falling rate stages can be identified by the two sloped lines in each plot. These comparisons show that the model accurately predicted the time to dry, indicated by the matching inflection point on each curve, for all excipients at both temperatures studied. The model predictions are most accurate for single excipient formulations and lower temperature systems. The model also accurately matched the evaporation rate of water from the droplets, as the slope of the constant rate stage (region with the highest negative slope) is the same for both experimental and modelled results in all 6 samples. The model under-predicted the final particle size when experiments were performed at 60°C, possibly due to early precipitation of excipients experimentally at the increased evaporation rate. Since the droplet is shrinking faster than the diffusion of excipients away from the surface, there is an increase in excipient concentration at the surface, leading to faster shell formation (Vehring et al., 2007). This would cause the formation of particles with interior voids which are not accounted for in the model, leading to a bigger experimental particle than predicted by the model. Although drying time, particle size, and evaporation rate are not novel predictions for modelling a droplet drying process, it was important to validate the accuracy of the model before moving forward with more complex predictions. We also note that this is one of only a few examples in the literature where such a model has been matched to drying curves of acoustically levitated droplets containing dissolved solids.

**4.2 Model validation of the component distribution**

One of the most important and novel aspects of this modelling effort was the ability to predict the distribution of up to four different components, plus water, throughout a final dry particle. Although there are models in the literature that can predict component distribution, they are limited to single component systems or surface composition only, and none of these models have been validated with experimental results (Grasmeijer et al., 2016; Grosshans et al., 2016; Meerdink and van’t Riet, 1995; Porowska et al., 2016). There are also no examples of models in the literature that predict the distribution of particulate suspended in solution, like a viral vector, nor any that link model predictions to viral activity. The ability of an excipient or blend of excipients to encapsulate a viral vector, avoiding its exposure to a solid-air interface, is a critical factor to consider in formulation and process development. Biologics that are at or near the solid-air interface are more exposed to degradation stresses, such as heat, and more likely to undergo damaging chemical reactions or aggregation (Crowe et al., 1998). There are several examples in the literature of the link between increased biologics at the air-solid interface and their decreased activity and stability, and the amount of protein at the air-solid interface has been reported to be the dominant factor that determined protein degradation in freeze-dried samples (Devineni et al., 2014; Xu et al., 2014). LeClair et al. compared three different excipients or excipient blends for their ability to stabilize AdHu5, and found that the worst-performing excipient was the only formulation to have visible surface nodules, which were hypothesized based on their size to be viral particles (LeClair et al., 2016a).

To explore the capability of the model to accurately reflect component distribution, three excipient formulations based on the 3:1 mannitol/dextran formulation containing AdHu5 were studied with the different molecular weight dextrans (10 kDa, 40 kDa, and 500 kDa). The dextrans were chosen to significantly vary the AdHu5 distribution in a particle, making for easier experimental validation. Figure 2 shows the component distributions in final dry particles of the three formulations, modelled at 60°C and 4% RH, for the same initial droplet diameter. These results are presented as mass fractions normalized for each component *i* (i.e., mass of component *i* in shell *x/*total mass of component *i*) so that all excipient distributions can be viewed on the same scale, as the AdHu5 makes up a very small portion of the overall mass in a particle and would be indistinguishable otherwise.



**Figure 2:** Predicted component distributions for a 3:1 mannitol/dextran excipient blend containing AdHu5 dried at 60°C and 4% RH in the acoustic levitator for three different molecular weight dextrans: (a) 10 kDa, (b) 40 kDa, and (c) 500 kDa.

Among these three formulations, the greatest component change was the distribution of AdHu5 in each particle. Going from 10 kDa to 40 kDa to 500 kDa dextran caused a decline in AdHu5 particle concentration at the surface of a final dry particle, changing from 18% (10 kDa dextran) to 10% (40 kDa dextran) to 5% (500 kDa dextran). This is caused by a nearly ten-fold increase in hydrodynamic radius of dextran as its molecular weight increased from 10 to 500 kDa. As reported by Armstrong et al., the corresponding hydrodynamic radii for 10 kDa, 40 kDa, and 500 kDa dextrans are 1.9 nm, 4.8 nm, and 15.9 nm, respectively (Armstrong et al., 2004). This increase in hydrodynamic radius caused slower diffusion, and thus the larger dextran became concentrated at the air-liquid interface as the particle dried. The increase in dextran at the interface excluded the more freely mobile mannitol molecules and AdHu5 particles from concentrating at the same location. A similar segregation of the two excipients, mannitol and dextran, was also expected based on the differences in diffusion coefficients for mannitol and dextran in water reported in the literature (Meerdink and van’t Riet, 1995). This segregation can be seen in the component distributions shown in Figure 2, caused by the differences in diffusion rate discussed previously.

Computed drying histories showing the distribution of components in the droplet as a function of time for the three cases depicted in Figure 2 can be found as time-lapse videos in the Supplementary Information, Videos S1, S2, and S3. In all three videos, the composition changes rapidly until a shell forms at approximately 400 seconds, at which point water is the only component that diffuses at a noticeable rate. The videos also demonstrate that AdHu5 is initially driven to the interface, then begins to diffuse throughout the droplet and becomes more evenly distributed as the droplet nears shell formation. The change in the composition profiles over time for the three molecular weight dextrans are fairly similar making the component distributions of the final dried particle the most relevant for the discussion here.

To experimentally validate these modelling results, the three different formulations with increasing dextran molecular weights and containing the ‘high’ loading of pSiNPs were tested in the levitator at 60°C and 4% RH, and the corresponding particle surfaces were imaged using AFM. Particles with ‘normal’ pSiNP loading proved ineffective in highlighting the component distribution differences, as no particles were detected at the surface likely due to the low probability of finding pSiNPs in the scanned region by AFM (data not shown). A control sample of 3:1 mannitol/dextran (10 kDa) containing no pSiNPs was imaged for comparison to establish a baseline for surface morphology. Figure 3 shows a side-by-side comparison of the surface of four samples: the control and three different formulations containing pSiNPs. Insets in Figure 3 highlight visible pSiNPs or clusters of pSiNPs as inferred from their spherical shape and dimensions.



**Figure 3:** Surface images captured using AFM (amplitude images in tapping mode) of levitated particles at 60°C and 4% RH. All particles were composed of 3:1 mannitol/dextran, with various molecular weight dextrans: (a) 10 kDa dextran with no pSiNPs; (b) 10 kDa dextran with ‘high’ pSiNP loading; (c) 40 kDa dextran with ‘high’ pSiNP loading; and (d) 500 kDa dextran with ‘high’ pSiNP loading. Insets indicate examples of individual or clusters of pSiNPs. All scale bars are 1 µm. AFM height images with their color scales indicated are provided in the Supplementary Information, Figure S3, and all features measured are from height images, not the amplitude images shown here.

ThepSiNPs were seen on the levitated particle surfaces for all formulations except for the control, although the limited area probed by AFM (7 µm 7 µm) made it impossible to quantify differences in their surface content as a function of dextran molecular weight. Image analysis confirmed the diameter of these spherical surface features was between 115 – 125 nm, matching the size of pSiNPs dried from suspensions without excipients (also determined by AFM, Supplementary Information, Figure S1). Long shard-like crystalline regions of mannitol with well-defined edges could be seen as well as smoother regions corresponding to the amorphous dextran, indicating the heterogeneity of the particle surface; x-ray diffraction (XRD) analysis of levitated mannitol/dextran particles in a previous study showed that mannitol is the only crystalline component in this formulation (Morgan et al., 2019). We note that AFM does not allow for quantification of the mannitol and dextran surface composition and differences in the visible amount of crystalline mannitol in Figure 3 are an artefact of the specific regions selected and should not be overinterpreted. XRD patterns of the three formulations were indistinguishable supporting that changing the molecular weight of dextran does not influence mannitol crystallinity or the overall particle crystallinity (Supplementary Information, Figure S4).

Importantly, the detected pSiNPs were only present in the dextran regions and were excluded from the mannitol crystals (Figure 3); we believe the pSiNPs mimic the surface chemistry and mobility of adenoviral particles in an evaporating droplet sufficiently well to compare to experiments with AdHu5 and the model developed here. Although this experimental result appears to support the hypotheses of others that crystalline materials are less likely to encapsulate and stabilize biologic materials, the importance of amorphous phases for stabilization of AdHu5 requires further research quantifying the long term (thermal) stabilization capabilities of crystalline and semicrystalline excipients (LeClair et al., 2016a; Weers et al., 2007).

In attempts to experimentally quantify the distribution of components at the air-solid interface for the three formulations, we focused on the AdHu5 component, whose location is likely the most crucial for viral stability and vaccine effectiveness. Fluorescently tagged fSiNPs were substituted into the samples at the same ‘high’ loading and imaged by confocal laser scanning microscopy, shown in Figure 4. The samples were imaged in air to limit the amount of fluorescent signal from inside the particle, although some signal penetration into the particle interior was still evident. The large images in Figure 4 were generated by creating a z-stack of average pixel intensity of 75 slices for each particle, representing the 2D flattened surface density of fSiNPs for an entire sphere. The small images at the bottom of Figure 4 are slices taken 0 µm, 50 µm, 100 µm and 150 µm from the outer top surface of the particle (left to right). Control samples of each formulation without fSiNPs confirmed that the levitated particles did not display any intrinsic fluorescence (data not shown).



**Figure 4:** Confocal laser scanning microscopy images of mannitol/dextran particles levitated at 60°C and 4% RH with various molecular weight dextrans, all containing a ‘high’ loading of fSiNPs: (a) 10 kDa dextran; (b) 40 kDa dextran; (c) 500 kDa dextran. Large images are z-stacks created from average pixel intensities of 75 images taken 2 µm apart; smaller bottom images are (left to right) 0 µm, 50 µm, 100 µm, and 150 µm from the top of the particle. All scale bars are 100 µm.

This distribution of fSiNPs in Figure 4 is in agreement with the predicted AdHu5 distributions in Figure 2. The fSiNPs were highly concentrated at the surface of the sample with the 10 kDa dextran particles showing much more fluorescence (Figure 4a) than the other two formulations (Figure 4b and 4c). Despite the weak fluorescent signal from the interiors, there appeared to be many more fSiNPs distributed deeper in the dried particle as the molecular weight of dextran increased. To quantify this representation of the AdHu5 distribution, the overall emitted fluorescent signal was calculated from the average pixel intensity in each large image in Figure 4. The 10 kDa sample had the highest average pixel intensity of 20441, the 40 kDa sample had an average intensity of 9812, and the 500 kDa sample had the lowest average intensity of 6214. The ratios of fluorescent intensity are very similar to the model: for the 40 kDa sample the fluorescent intensity was 48% of the 10 kDa sample and the modelled surface mass fraction was 56% of the 10 kDa model; similarly, the 500 kDa sample had ratios of 30% and 28% for fluorescent intensity and modelled surface mass fraction, respectively. We note that fSiNPs and pSiNPs were equally representative of AdHu5 in this model since the assumed motion is calculated based solely on diffusion and does not account for chemical interactions between excipients and AdHu5/SiNPs, which could be added to future iterations of the model to increase the accuracy at predicting AdHu5/SiNP distribution with excipients of similar hydrodynamic radii.

**4.3. Viral activity of different formulations interpreted based on model predictions**

Previously published AdHu5 viral activity data for three types of acoustically levitated particles with different excipients were compared with the model to see if their component distributions correlated with activity (Morgan et al., 2019). Levitated particles were composed of 9:1 lactose/trehalose, 3:1 mannitol/dextran (40 kDa), or 1:3 xylitol/dextran (40 kDa), where the ratios quoted are weight fractions. Figure 5 shows the activity results for these three excipient blends and the component distributions as predicted by the model (with drying conditions of 55°C, 6% RH, and the same starting droplet size). According to our hypothesis, we expect activity losses to be proportionally higher when the virus is concentrated near the surface of the dried particles – the model was able to predict the trend in performance of different excipient formulations based on the modelled component distribution reasonably well.

The lactose/trehalose formulation with the highest reported viral activity losses (1.6 log loss) was predicted to have 17% of all AdHu5 virus at or near the surface (calculated from the outer 2 µm), while the xylitol/dextran had only 5% of the virus at or near the surface and the lowest reported loss (1.0 log loss). Mannitol/dextran, which is predicted to have moderate activity loss between the other two blends (1.4 log loss), was predicted to have 10% of AdHu5 at or near the surface. These findings appear to support our hypothesis, originating from research done linking degradation of various proteins to the amount of protein at the air-solid interface and observations of LeClair et al. that found virus-sized nodules on the surfaces of spray dried leucine and lactose/trehalose AdHu5 vaccines which demonstrated high activity losses (Devineni et al., 2014; LeClair et al., 2016a; Xu et al., 2014). While this work does not speculate on the precise mechanism of deactivation of AdHu5 at air-solid interfaces, it does indirectly support that activity is linked to viral location within a dried particle.



**Figure 5:** A comparison of experimentally determined viral activity data (top left, from reference (Morgan et al., 2019)) to component distribution predictions made by the model for lactose/trehalose (top right), mannitol/40 kDa dextran (bottom left), and xylitol/40 kDa dextran (bottom right).

**4.4 Particle engineering using modelling**

In order to make recommendations on formulation and drying conditions, the input parameters to the model were adjusted with the aim of creating a more ‘ideal’ adenovirus distribution where the AdHu5 would be less concentrated at the surface of the final dried particle. The impacts of RH, drying temperature, and the addition of a fourth component to a base formula of 3:1 mannitol/dextran (40 kDa) was examined with all other parameters remaining the same. Temperature was modelled at 10°C, 23°C, 50°C, and 80°C with a constant RH of 4%; RH was modelled at 0%, 25%, 50%, and 75% with a constant temperature of 60°C; and the addition of SiNPs with diameters of 500 nm as the fourth component was modelled at concentrations of 0, 10, 100, and 1000 times the concentration of AdHu5 at a constant temperature of 60°C and a constant RH of 4%. Figure 6 shows the modelled distributions under these various conditions where only the distribution of AdHu5 at each condition is shown for clarity. The complete component distributions for all 12 conditions explored are provided in the Supplementary Information, Figures S5–S7.



**Figure 6:** The impact of increasing (a) relative humidity, (b) temperature, and (c) addition of SiNPs on the distribution of AdHu5 within the final dried particle. All modelling was done using a 3:1 mannitol/dextran (40 kDa) formulation.

Figure 6a and Figure 6b show that a decrease in temperature or increase in RH during drying have the same effect of reducing the amount of AdHu5 at the surface, making it more evenly distributed. Both a lower temperature and a higher RH would lengthen the droplet drying time, allowing time for the components to diffuse throughout the droplet more evenly. Given slow enough drying, the distribution of components would tend toward a more uniform composition throughout the particle. However, this allows more time for AdHu5 to aggregate, which also leads to deactivation (Evans et al., 2004). Lower drying temperatures and higher RH would also lead to a final product with a higher moisture content which can lower the stability of the powder as water is a plasticizing agent that can depress the glass transition temperature (Ameri and Maa, 2006). Storage at or above the glass transition temperature can greatly increase the mobility of AdHu5 in the matrix and increase the chance of degradation; both the chemical and physical stability of the material are dependant on the glass transition temperature as well (Ameri and Maa, 2006; Hancock and Zografi, 1994). An increase in RH would also produce a larger particle as seen in Figure 6a (i.e., the modelled line ends at a larger radius for higher RH values), because the droplet does not completely dry out.

Figure 6c shows that the addition of 500 nm SiNPs can also reduce the tendency of AdHu5 to partition at the surface of the final dry particle. In this case, the larger size of the SiNPs compared to AdHu5 would cause the SiNPs to diffuse slower and concentrate near the particle surface, forcing AdHu5 towards the center (Walters et al., 2014). This is similar to the effect seen using formulation additives such as surfactants like Tween 20, which competes to partition at the air-solid interface and can limit aggregation of proteins (Kreilgaard et al., 1998). However, selection of these additive components must be done carefully to ensure that they are approved for human consumption and non-toxic. Overall, these modelling results show that there are many different ways to approach particle engineering with an end goal of creating an even distribution of AdHu5, although the route towards “ideal” particles needs to be carefully considered to maintain biologic activity, thermal/storage stability and safety of the final product.

**5. CONCLUSIONS**

These results show that the proposed diffusion-based droplet drying model can make accurate predictions for time to dry, final particle size, and component distribution within an individual drying droplet under various environmental conditions. The model also suggested trends in viral vector activity by demonstrating that activity decreases when the model predicts large amounts of AdHu5 at the particle surface, although future work should further investigate the predictive power of the model related to activity. We believe that the use of this model for particle engineering (with inputs of excipient properties easily available in the literature) is an ideal first step in formulation development and can reduce time and costs by identifying good encapsulating excipients and excipient blends without the need for experimental work. Overall, combining modelling predictions with experimental results from the acoustic levitator can substantially facilitate dry powder vaccines development without requiring large amounts of the active biologic; once a promising composition is identified, the activity of the final product can be optimized through spray drying. Future improvement to the model, including considerations for surface activity and chemical interactions, will allow predictions to be made for other active vaccine components, such as enveloped viruses and mRNA-based vaccines.

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**7. SUPPLEMENTARY INFORMATION**

The supplementary information contains: dynamic light scattering, zeta potential, FTIR, and AFM data for control SiNPs and pSiNPs, as well as the associated method information; XRD methods and spectra for three mannitol/dextran blends containing SiNPs; complete modelled component distributions for the 12 drying conditions in Figure 6; and videos showing the complete modelled drying histories of the mannitol/dextran blends in Figure 2. Code for the droplet drying model is available upon request by emailing the authors.

**8. REFERENCES**

Ameri, M., Maa, Y.F., 2006. Spray drying of biopharmaceuticals: Stability and process considerations. Drying Technol. 24, 763–768. https://doi.org/10.1080/03602550600685275

Amorij, J.P., Huckriede, A., Wilschut, J., Frijlink, H.W., Hinrichs, W.L.J., 2008. Development of stable influenza vaccine powder formulations: Challenges and possibilities. Pharm. Res. 25, 1256–1273. https://doi.org/10.1007/s11095-008-9559-6

Armstrong, J.K., Wenby, R.B., Meiselman, H.J., Fisher, T.C., 2004. The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation. Biophys. J. 87, 4259–4270. https://doi.org/10.1529/biophysj.104.047746

Better, O.S., Rubinstein, I., Winaver, J.M., Knochel, J.P., 1997. Mannitol therapy revisited (1940-1997). Kidney Int. 52, 886–894. https://doi.org/10.1038/ki.1997.409

Carrasco, F., Chornet, E., Overend, R.P., Costa, J., 1989. A generalized correlation for the viscosity of dextrans in aqueous solutions as a function of temperature, concentration, and molecular weight at low shear rates. J. Appl. Polym. Sci. 37, 2087–2098. https://doi.org/10.1002/app.1989.070370801

Crowe, J.H., Carpenter, J.F., Crowe, L.M., 1998. The Role of Vitrification in Anhydrobiosis. Annu. Rev. Physiol. 60, 73–103. https://doi.org/10.1146/annurev.physiol.60.1.73

Devineni, D., Gonschorek, C., Cicerone, M.T., Xu, Y., Carpenter, J.F., Randolph, T.W., 2014. Storage stability of keratinocyte growth factor-2 in lyophilized formulations: Effects of formulation physical properties and protein fraction at the solid-air interface. Eur. J. Pharm. Biopharm. 88, 332–341. https://doi.org/10.1016/j.ejpb.2014.05.012

Ekdawi-Sever, N., De Pablo, J.J., Feick, E., Von Meerwall, E., 2003. Diffusion of sucrose and α,α-trehalose in aqueous solutions. J. Phys. Chem. A 107, 936–943. https://doi.org/10.1021/jp020187b

Evans, R.K., Nawrocki, D.K., Isopi, L.A., Williams, D.M., Casimiro, D.R., Chin, S., Chen, M., Zhu, D., Shiver, J.W., Volkin, D.B., 2004. Development of Stable Liquid Formulations for Adenovirus-Based Vaccines. J. Pharm. Sci. 93, 2458–2475. https://doi.org/10.1002/jps.20157

Farid, M., 2003. A new approach to modelling of single droplet drying. Chem. Eng. Sci. 58, 2985–2993. https://doi.org/10.1016/S0009-2509(03)00161-1

Grasmeijer, N., Frijlink, H.W., Hinrichs, W.L.J., 2016. Model to predict inhomogeneous protein–sugar distribution in powders prepared by spray drying. J. Aerosol Sci. 101, 22–33. https://doi.org/10.1016/j.jaerosci.2016.07.012

Grosshans, H., Griesing, M., Mönckedieck, M., Hellwig, T., Walther, B., Gopireddy, S.R., Sedelmayer, R., Pauer, W., Moritz, H.U., Urbanetz, N.A., Gutheil, E., 2016. Numerical and experimental study of the drying of bi-component droplets under various drying conditions. Int. J. Heat Mass Transf. 96, 97–109. https://doi.org/10.1016/j.ijheatmasstransfer.2015.12.062

Hancock, B.C., Zografi, G., 1994. The Relationship Between the Glass Transition Temperature and the Water Content of Amorphous Pharmaceutical Solids. Pharm. Res. https://doi.org/10.1023/A:1018941810744

Huang, D., 2011. Modeling of Particle Formation during Spray Drying. Eur. Dry. Conf. - EuroDrying’2011 26–28.

Jenkins, R.T., Bell, R.A., 1987. Molecular radii of probes used in studies of intestinal permeability. Gut 28, 110–111.

Kawahara, N., Yarin, A.L., Brenn, G., Kastner, O., Durst, F., 2000. Effect of acoustic streaming on the mass transfer from a sublimating sphere. Phys. Fluids 12, 912–923. https://doi.org/10.1063/1.870347

Kennedy, M.A., Parks, R.J., 2009. Adenovirus virion stability and the viral genome: Size matters. Mol. Ther. 17, 1664–1666. https://doi.org/10.1038/mt.2009.202

Kreilgaard, L., Frokjaer, S., Flink, J.M., Randolph, T.W., Carpenter, J.F., 1998. Effects of additives on the stability of recombinant human factor XIII during freeze-drying and storage in the dried solid. Arch. Biochem. Biophys. 360, 121–134. https://doi.org/10.1006/abbi.1998.0948

Laboratories, B., 2010. Washing microspheres. Hum. Mol. Genet. 19, NP.

Langrish, T.A.G., 2009. Multi-scale mathematical modelling of spray dryers. J. Food Eng. 93, 218–228. https://doi.org/10.1016/j.jfoodeng.2009.01.019

LeClair, D.A., Cranston, E.D., Xing, Z., Thompson, M.R., 2016a. Evaluation of excipients for enhanced thermal stabilization of a human type 5 adenoviral vector through spray drying. Int. J. Pharm. 506, 289–301. https://doi.org/10.1016/j.ijpharm.2016.04.067

LeClair, D.A., Cranston, E.D., Xing, Z., Thompson, M.R., 2016b. Optimization of Spray Drying Conditions for Yield, Particle Size and Biological Activity of Thermally Stable Viral Vectors. Pharm. Res. 33, 2763–2776. https://doi.org/10.1007/s11095-016-2003-4

Meerdink, G., van’t Riet, K., 1995. Modeling segregation of solute material during drying of liquid foods. AIChE J. 41, 732–736. https://doi.org/10.1002/aic.690410331

Mendoza, R., Schmalko, M.E., 2002. Diffusion coefficients of water and sucrose in osmotic dehydration of papaya. Int. J. Food Prop. 5, 537–546. https://doi.org/10.1081/JFP-120015490

Mezhericher, M., Levy, A., Borde, I., 2011. Modelling the morphological evolution of nanosuspension droplet in constant-rate drying stage. Chem. Eng. Sci. 66, 884–896. https://doi.org/10.1016/j.ces.2010.11.028

Mezhericher, M., Levy, A., Borde, I., 2010. Theoretical models of single droplet drying kinetics: A review. Drying Technol. 28, 278–293. https://doi.org/10.1080/07373930903530337

Mondragon, R., Hernandez, L., Enrique Julia, J., Carlos Jarque, J., Chiva, S., Zaitone, B., Tropea, C., 2011. Study of the drying behavior of high load multiphase droplets in an acoustic levitator at high temperature conditions. Chem. Eng. Sci. 66, 2734–2744. https://doi.org/10.1016/j.ces.2011.03.033

Morgan, B.A., Manser, M., Jeyanathan, M., Xing, Z., Cranston, E.D., Thompson, M.R., 2020. Effect of Shear Stresses on Adenovirus Activity and Aggregation during Atomization To Produce Thermally Stable Vaccines by Spray Drying. ACS Biomater. Sci. Eng. 6, 4304–4313. https://doi.org/10.1021/acsbiomaterials.0c00317

Morgan, B.A., Xing, Z., Cranston, E.D., Thompson, M.R., 2019. Acoustic levitation as a screening method for excipient selection in the development of dry powder vaccines. Int. J. Pharm. 563, 71–78. https://doi.org/10.1016/j.ijpharm.2019.03.026

Moulik, S., Khan, D., 1977. Viscosities of Concentrated Solutions of Polyhydroxy Nonelectrolytes, Glucose, Sucrose, Mannitol & Sorbitol in Relation to Solute-Solvent Interaction & a Universal Viscosity Equation. Indian J. Chem. 15, 267–272.

Pang, L., Farkas, K., Bennett, G., Varsani, A., Easingwood, R., Tilley, R., Nowostawska, U., Lin, S., 2014. Mimicking filtration and transport of rotavirus and adenovirus in sand media using DNA-labeled, protein-coated silica nanoparticles. Water Res. 62, 167–179. https://doi.org/10.1016/j.watres.2014.05.055

Porowska, A., Dosta, M., Fries, L., Gianfrancesco, A., Heinrich, S., Palzer, S., 2016. Predicting the surface composition of a spray-dried particle by modelling component reorganization in a drying droplet. Chem. Eng. Res. Des. 110, 131–140. https://doi.org/10.1016/j.cherd.2016.03.007

Ré, M.I., 1998. Microencapsulation By Spray Drying. Drying Technol. 16, 1195–1236. https://doi.org/10.1080/07373939808917460

Ruigrok, R.W.H., Nermut, M. V., Andree, P.J., 1984. The molecular mass of adenovirus type 5 as determined by means of scanning transmission electron microscopy (STEM). J. Virol. Methods 9, 69–78. https://doi.org/10.1016/0166-0934(84)90084-3

Sadek, C., Schuck, P., Fallourd, Y., Pradeau, N., Le Floch-Fouéré, C., Jeantet, R., 2015. Drying of a single droplet to investigate process–structure–function relationships: a review. Dairy Sci. Technol. 95, 771–794. https://doi.org/10.1007/s13594-014-0186-1

Sazhin, S.S., Rybdylova, O., Pannala, A.S., Somavarapu, S., Zaripov, S.K., 2018. A new model for a drying droplet. Int. J. Heat Mass Transf. 122, 451–458. https://doi.org/10.1016/j.ijheatmasstransfer.2018.01.094

Schiffter, H., Lee, G., 2006. Single-droplet evaporation kinetics and particle formation in an acoustic levitator. Part 1: Evaporation of water microdroplets assessed using boundary-layer and acoustic levitation theories. J. Pharm. Sci. 96, 2274–2283. https://doi.org/10.1002/jps

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: An open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Schutyser, M.A.I., Perdana, J., Boom, R.M., 2012. Single droplet drying for optimal spray drying of enzymes and probiotics. Trends Food Sci. Technol. 27, 73–82. https://doi.org/10.1016/j.tifs.2012.05.006

Sloth, J., Kiil, S., Jensen, A.D., Andersen, S.K., Jørgensen, K., Schiffter, H., Lee, G., 2006. Model based analysis of the drying of a single solution droplet in an ultrasonic levitator. Chem. Eng. Sci. 61, 2701–2709. https://doi.org/10.1016/j.ces.2005.11.051

Vehring, R., 2008. Pharmaceutical particle engineering via spray drying. Pharm. Res. 25, 999–1022. https://doi.org/10.1007/s11095-007-9475-1

Vehring, R., Foss, W.R., Lechuga-Ballesteros, D., 2007. Particle formation in spray drying. J. Aerosol Sci. 38, 728–746. https://doi.org/10.1016/j.jaerosci.2007.04.005

Walters, R.H., Bhatnagar, B., Tchessalov, S., Izutsu, K.I., Tsumoto, K., Ohtake, S., 2014. Next generation drying technologies for pharmaceutical applications. J. Pharm. Sci. 103, 2673–2695. https://doi.org/10.1002/jps.23998

Weers, J.G., Tarara, T.E., Clark, A.R., 2007. Design of fine particles for pulmonary drug delivery. Expert Opin. Drug Deliv. 4, 297–313. https://doi.org/10.1517/17425247.4.3.297

Wilcox, W.C., Ginsberg, H.S., 1963. Structure of type 5 adenovirus. J. Exp. Med. 118, 295–306. https://doi.org/10.1084/jem.118.2.295

Xu, Y., Grobelny, P., Von Allmen, A., Knudson, K., Pikal, M., Carpenter, J.F., Randolph, T.W., 2014. Protein quantity on the air-solid interface determines degradation rates of human growth hormone in lyophilized samples. J. Pharm. Sci. 103, 1356–1366. https://doi.org/10.1002/jps.23926

Yamaguchi, T., Ishikawa, T., Imai, Y., 2018. Microfluidic devices based on biomechanics, in: Integrated Nano-Biomechanics. Elsevier, pp. 217–263. https://doi.org/https://doi.org/10.1016/B978-0-323-38944-0.00007-3

Zhu, M., Yu, L., 2017. Polyamorphism of D-mannitol. J. Chem. Phys. 146. https://doi.org/10.1063/1.4989961