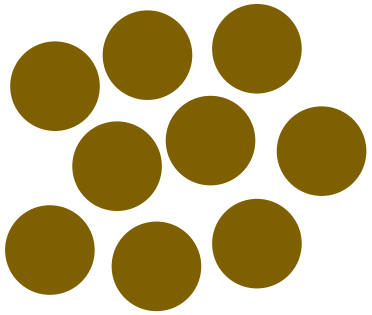
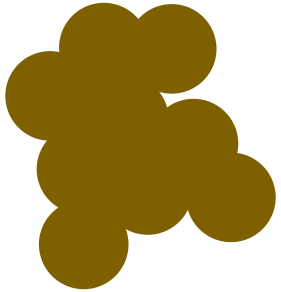
Graphical Abstract (for review)

Trehalose spray-dried particles

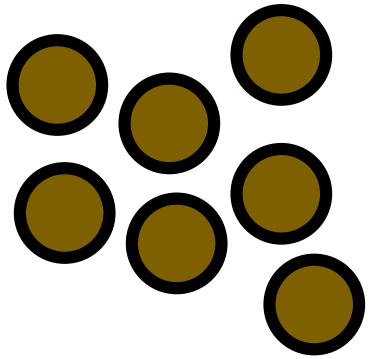


Exposed to 90% relative humidity and 25 °C

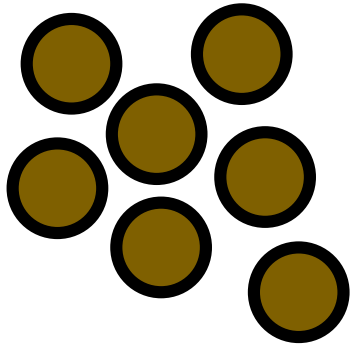
Trehalose spray-dried particles



Trehalose combined with shell-forming excipients (leucine, pullulan, and trileucine)



Trehalose combined with shell-forming excipients (leucine, pullulan, and trileucine)



Original Article

Leucine Enhances the Dispersibility of Trehalose-Containing Spray-Dried Powders on Exposure to a High-Humidity Environment

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Abstract

This study investigates the ability of various shell-forming excipients to preserve the dispersibility of dry powder dosage forms, e.g., nasally administered vaccines, upon exposure to a high-humidity environment. Trehalose combinations using leucine, pullulan, or trileucine were selected as the candidate excipient systems, and the powder dispersibility of these systems was compared with that of pure trehalose particles. Scaled-up monodisperse spray drying was used to produce sufficient quantities of uniform-sized particles for powder dispersibility analysis. Particle size, crystallinity, and morphology of the powders before and after exposure to moisture were characterized by an aerodynamic particle sizer, Raman spectroscopy, and scanning electron microscopy, respectively. Three two-component particle systems composed of trehalose/trileucine (97/3 w/w), trehalose/pullulan (70/30 w/w), and trehalose/leucine (70/30 w/w) were first formulated and their dispersibility, characterized as the emitted dose from dry powder inhalers, was then compared with that of trehalose particles. The formulation containing 30% leucine maintained the highest emitted dose (90.3 ± 10%) at a 60 L/min flow rate after 60 min exposure to 90% RH and 25 °C, showing its superior protection against exposure to humidity compared with the other systems. Further investigations under more challenging conditions at 15 L/min flow rate on the trehalose/leucine system with various compositions (70/30, 80/20, 90/10 w/w) showed that a higher leucine concentration generally provided better protection against moisture and maintained higher powder dispersibility, probably due to higher surface coverage of crystalline leucine and a thicker leucine shell around the particles.

The study concludes that leucine may be considered an appropriate shell-forming excipient in the development of dry powder formulations in order to protect the dosage forms against humidity during administration.

1. Introduction

The nasal route of drug delivery has been used not only for local disease treatment, but also for systemic drug delivery (Illum, 2003). Recently, nasal delivery of vaccines, especially those protecting against respiratory infectious disease such as influenza and COVID-19, has attracted increasing research interest. One reason for this interest lies in the potential of nasal vaccines to obtain a local immune response in addition to a systemic immune response, with the combination of the two responses theoretically providing superior protection against respiratory infections (Illum, 2002; Trows and Scherließ, 2016). A further reason lies in the fact that, compared with parenterally administered vaccines, nasally delivered vaccines are easier and safer to administer (Trows and Scherließ, 2016). Dry powder formulations of vaccines for nasal delivery have attracted especial interest recently because unlike the liquid vaccines used for nasal delivery, which typically require low-temperature storage and transport, i.e. a functional cold chain (Wang et al., 2012), dry powder formulations demonstrate increased storage stability without the need for preservatives or extensive cold chain infrastructure (Bartos et al., 2018; Garmise et al., 2007; Ishikawa et al., 2002; Pozzoli et al., 2016; Wang et al., 2012). Hence, dry powder formulations are particularly attractive for global distribution of vaccines (Garmise et al., 2006).

Along with the advantages they carry over their liquid counterparts, dry powder formulations of vaccines for nasal delivery also bring specific requirements to powder development and production. For example, dry powder formulations for nasal delivery must contain an appropriately large particle size (diameter > 10 μm) to allow sufficient drug deposition in the nasal cavity while minimizing lung deposition (Schroeter et

al., 2015, 2011). Moreover, the particle size distribution should be reproducible for consistent product performance (Garmise et al., 2006). The powders should also demonstrate high stability at room temperature for long-term storage and shipping (Wang et al., 2012). Particularly important for nasal delivery is powder dispersibility, which is defined as the ability of powders to disperse into individual particles or flowable agglomerates with external dispersion forces (Vehring, 2008), since this property can influence the drug delivery efficiency, i.e. the delivered dose. Factors affecting powder dispersibility have been studied extensively, with particles featuring corrugated surfaces having been found the most dispersible because of their lower density and smaller radius of curvature at the contact zones (Weiler et al., 2010). However, powder dispersibility can decrease sharply due to capillary forces or material bridging when powders are exposed, even briefly, to a high-humidity environment. If unprotected dry powder dosage forms are removed from their packaging in a humid environment but not administered to patients immediately, powder dispersibility may decrease quickly and an incorrect or no dose may be delivered if some or all of the undispersed powder remains in the delivery device. It is therefore desirable to design dry powders to maintain a relatively high dispersibility at least on brief exposure to a high-humidity environment. There are several studies investigating this powder property; however, the powders in these studies are designed for drug delivery to the lung, with a particle size in the range of 1-5 μm (Cui et al., 2018; Li et al., 2017, 2016; Sibum et al., 2020; Zhou et al., 2014). For the large particle size required for nasal delivery, the shell formation kinetics are different (Ordoubadi et al., 2020), so that the results from these studies are not directly applicable here. Strategies to achieve out-of-package robustness for dry powder dosage forms with large particle size for nasal delivery require further investigation.

Trehalose is a disaccharide often used as an excipient to stabilize biological actives for pharmaceutical applications (Feng et al., 2011). However, sugar excipients like trehalose become very cohesive in atmospheric conditions (Feng et al., 2011), likely requiring additional protection against humidity. Previous studies have shown that powders containing leucine or trileucine demonstrate enhanced dispersibility

(Boraey et al., 2013; Lechuga-Ballesteros et al., 2008), and continue to do so when the powders are exposed to moisture (Li et al., 2017, 2016; Shetty et al., 2018; Sibum et al., 2020). Formulations containing pullulan, a linear exopolysaccharide, have been shown to feature corrugated particle surfaces (Carrigy et al., 2019), which are known to improve powder dispersibility (Baldelli and Vehring, 2016). Furthermore, because of the high glass transition temperature of pullulan (Carrigy et al., 2019), formulations containing pullulan are expected to undergo less plasticization and material bridging than unprotected trehalose when exposed to moisture, thus potentially maintaining its dispersibility.

In this study, the dispersibility on exposure to high humidity of three two-component particle systems, trehalose/leucine, trehalose/trileucine, and trehalose/pullulan, was compared to the dispersibility of unprotected trehalose particles. For formulation and process design, particle engineering via spray drying was utilized because it provides the theoretical tools for accelerated design of complex structured microparticles (Vehring, 2008). Conventional spray drying produces polydisperse droplets and particles, which complicate calculation of the shell formation process and can directly influence the powder dispersibility. Polydisperse droplet sizes may lead to variations in particle morphology, including shell thickness, which can also impact powder dispersibility and robustness. To eliminate these complexities, the effect of moisture on powder dispersibility is better studied in a monodisperse model. Monodisperse spray drying uses a vibrating orifice generator instead of a twin-fluid atomizer (Carrigy and Vehring, 2019) and has recently been scaled up (Wang et al., 2021) to produce sufficient powder quantities for the dispersibility tests planned in this study.

2. Materials and Methods

2.1. Materials

Trehalose dihydrate (Cat. No. BP2687-1, Fisher Scientific, NJ, USA), trileucine (Product No. L0879, Sigma-Aldrich Corp., Missouri, USA), pullulan (Product No. J66961, Alfa Aesar, MA, USA), and L-leucine (Cat. No. BP385-100, Acros Organics BVBA, Geel, Belgium) were dissolved in demineralized water to prepare the feed solutions for monodisperse spray drying.

2.2. Monodisperse Spray Drying

A monodisperse spray drying setup utilizing a dual-orifice plate (Wang et al., 2021) was used to manufacture model particles for this study. Briefly, monodisperse droplets were generated using a custom vibrating orifice atomizer (Azhdarzadeh et al., 2016), with the liquid feed being pressurized to form jets through the micro-orifice plate. The dual-orifice plate, manufactured by gallium focused ion beam milling, produced two separate liquid jets simultaneously, thus doubling the liquid throughput and, consequently, the powder production rate of a conventional single-orifice plate (Wang et al., 2021). A piezoelectric ceramic ring attached to the head of the atomizer vibrated at a chosen frequency controlled by a function generator. Controlling the driving frequency and the jet exit velocity via the pressure difference across the orifice achieved disintegration of the liquid jets into monodisperse droplets in the Rayleigh breakup regime (Azhdarzadeh et al., 2016). To minimize droplet collisions, pressurized air was used to disperse the droplets in a turbulent flow from a disperser cap. The monodisperse droplets were then dried in a custom spray dryer (Ivey et al., 2018) into solid particles. Compressed air was selected as the drying gas since no flammable chemicals were used. The spray-dried particles were separated in a stainless-steel cyclone and collected in a glass collection bottle. The inlet and outlet drying gas temperatures were 80 ± 1 °C and 56 ± 1 °C, respectively. The drying gas flow rate was set to 500 standard liters per minute (SLPM) so that particles > 1 µm could be efficiently separated and collected. The outlet relative humidity (RH) was predicted to be

~2%. Based on the predicted outlet RH, the dry basis moisture content of trehalose was predicted to be less than 1% from the moisture sorption isotherms for amorphous trehalose (Hancock and Dalton, 1999). Since all the formulations in this study contain at least 70% trehalose, the moisture contents of all the spray-dried particles were expected to be comparable with that of trehalose, which were considered to be minimal. The details of the chosen spray drying parameters are shown in Table 1.

Table 1. Spray drying parameters

Parameter	Value
Pressure difference across orifice	100-200 kPa
Orifice diameter	30 μm
Inlet temperature	80 ± 1 °C
Outlet temperature	56 ± 1 °C
Predicted outlet RH	~2%
Disperser cap orifice diameter	3.0 mm
Dispersing gas pressure	275 kPa
Drying gas flow rate	500 SLPM
Piezoelectric driving frequency	85 ± 5 kHz

2.3. Two-Component Particle System Design

For nasal drug delivery, the particle size distribution should be large enough so that only a small fraction of particles have aerodynamic diameters of less than 10 μm, to avoid delivery to the lung (Schroeter et al., 2015, 2011). The particle size resulting from spray drying can be easily designed according to a mass

balance consideration. For a two-component particle system, the particle morphology and internal distribution can also be predicted and explained by particle formation theory.

The aerodynamic diameter of spray-dried particles can be expressed as

$$d_{\mathrm{a}} = \left(\frac{\rho_{\mathrm{P}}}{\rho_0}\right)^{\frac{1}{6}} \left(\frac{c_{\mathrm{F}}}{\rho_0}\right)^{\frac{1}{3}} d_{\mathrm{D}} \tag{1}$$

where ρ_{P} is the particle density, ρ_0 is the unit density, c_{F} is the feed solution concentration, and d_{D} is the droplet diameter.

The time necessary to dry the droplets, i.e. the droplet drying time, τ_{D}, can be approximated by

$$\tau_{\mathrm{D}} = \frac{d_0^2}{\kappa} \tag{2}$$

where d_0 is the initial droplet diameter, and κ is the evaporation rate, which depends on the chosen process parameters.

A dimensionless Peclet number, Pe, is very useful in predicting the internal distribution of the formulation components and particle morphology and is defined as

$$Pe_i = \frac{\kappa}{8D_i} \tag{3}$$

where D is the diffusion coefficient, and i represents component i. The Peclet number describes how quickly the droplet evaporates compared with the diffusional motion of the solutes.

For the design of core-shell type particles the surface enrichment of component i, E_i, is a useful parameter. It is defined as the ratio of the surface concentration, $c_{\mathrm{s},i}$, to the average concentration, $c_{\mathrm{m},i}$, and can be approximated by

$$E_i = \frac{c_{\mathrm{s},i}}{c_{\mathrm{m},i}} = 1 + \frac{Pe_i}{5} + \frac{Pe_i^2}{100} - \frac{Pe_i^3}{4000} \tag{4}$$

Equation (4) is accurate within 1% for $Pe < 20$, assuming an asymptotic state (Boraey and Vehring, 2014).

For crystallizing components or excipients with low solubility that precipitate via spinodal decomposition (Ordoubadi et al., 2021), the time for a component to reach a critical surface concentration, $\tau_{c,i}$, is another important parameter, expressed as

$$\tau_{c,i} = \tau_D\left(1 - \left(\frac{c_{0,i}}{c_{c,i}} E_i\right)^{\frac{2}{3}}\right) \quad (5)$$

where $c_{0,i}$ and $c_{c,i}$ are the initial concentration and critical concentration of component i. Upon reaching the critical surface concentration, the component starts to crystallize or precipitate via spinodal decomposition. The precipitation window, or the time available for crystallization, is defined as

$$\tau_{p,i} = \tau_D - \tau_{c,i} = \tau_D\left(\frac{c_{0,i}}{c_{c,i}} E_i\right)^{\frac{2}{3}} \quad (6)$$

Using equations (1)-(6), the spray-dried particle size, the internal component distribution during evaporation, and the kinetics of phase separation and shell formation for all excipients in this study can be predicted.

In monodisperse droplet generation, the droplet diameter is approximately twice the orifice diameter (Wu et al., 2007). From a 30 µm-orifice and a 10 mg/mL initial feed solution concentration for all the candidate formulations, the aerodynamic diameter of the spray-dried particle in this study is estimated to be in the range of 10-20 µm, which is at the lower end of particle sizes encountered in nasal delivery. These particles were chosen for the study because they are likely more difficult to protect from the effect of moisture uptake than the bigger particles due to the higher specific surface area. For the spray drying conditions in this study, trehalose has a relatively low Peclet number around 1, leading to an even distribution throughout the droplet, while pullulan has a relatively high Peclet number on the order of 10. Therefore, pullulan has a large surface enrichment and can form a shell covering the trehalose core. Since pullulan has a relatively high glass transition temperature of ~261°C (Carrigy et al., 2019), the pullulan shell is hypothesized to protect the trehalose core on exposure to high relative humidity, thus enhancing the powder dispersibility.

In the trehalose/trileucine system, trehalose and trileucine have similar and relatively low Peclet numbers around 1. However, trileucine has a much lower solubility (7 mg/mL) than trehalose (690 mg/mL) (Wang et al., 2019), which causes the trileucine to reach the critical concentration for phase separation early, enabling the formation of a shell. The high surface activity of trileucine may also aid the process (Vehring, 2008). It has been shown that particle systems containing trileucine have low density and corrugated particle surfaces, both of which traits reduce cohesiveness and enhance aerosol performance (Lechuga- Ballesteros et al., 2008). The trehalose/trileucine system has also shown improved dispersibility on exposure to moisture (Sibum et al., 2020). In the trehalose/leucine system, leucine also has a relatively low Peclet number of around 0.8, as well as a relatively low solubility of 22 mg/mL. If the initial saturation is sufficient, leucine can reach supersaturation early and may crystallize at the droplet surface, leading to the formation of a crystalline shell of leucine (Ordoubadi et al., 2020). It has been shown that leucine not only enhances powder dispersibility, but also protects powders against moisture (Li et al., 2017, 2016).

In this study, the total feed solution concentration for all formulations was 10 mg/mL. Three two-component particle systems at different compositions including trehalose/trileucine (97/3 w/w), trehalose/pullulan (70/30 w/w), and trehalose/leucine (90/10, 80/20, 70/30 w/w) were formulated and their dispersibility characterized as the emitted dose from dry powder inhalers compared with that of trehalose particles. These compositions were chosen because, according to previous studies (Carrigy et al., 2019; Li et al., 2017; Sibum et al., 2020) and the results of particle formation calculations, the protective excipients of these particle systems were likely to provide enough surface coverage around the trehalose core to enhance powder dispersibility. It is worth noting that only 3% trileucine formulated in the trehalose/trileucine system instead of 30% as in other systems was due to the lower solubility of trileucine in an aqueous solution.

2.4. Aerodynamic Particle Sizer (APS)

For in-process particle monodispersity monitoring and measurement of the particle size distribution, an aerodynamic particle sizer (APS) (Model: 332100, TSI, Shoreview, USA) was used to isokinetically sample particles and repeatedly measure size distribution for 20 s every 15 minutes during the entire spray drying process, which typically lasts 3-4 hours. The driving frequency to the microjet atomizer needs to be adjusted to restore monodispersity in case the in-line measured particle size distribution shifts towards polydispersity and the geometric standard deviation exceeds 1.2.

2.5. Preconditioning of Powder Samples

The sample powders were filled into size 3 hydroxypropyl methylcellulose capsules (Quali-V®-I, Qualicaps, Inc., Madrid, Spain) in a dry environment (<2% RH) that was verified using a hygrometer (MI70 Measurement Indicator with HMP77B humidity and temperature probe, Vaisala, Vantaa, Finland). The powder mass filled into each capsule ranged from 20 to 40 mg. The filled capsules were then closed and kept in a dry environment or in a humid environment for 10, 20, 30, and 60 min before being pierced and actuated from a dry powder inhaler. For the humid environment group, the filled capsules were placed in open petri dishes inside an environmental chamber (Photostability Test Chamber Model No. CEO910W-4, Thermal Product Solutions, PA, USA) set to 90% RH and 25°C.

2.6. Emitted Dose Measurements

Dispersibility of the preconditioned powder samples was characterized using the emitted dose from a dry powder inhaler (Seebri® Breezhaler®, Novartis International AG, Basel, Switzerland). The dry powder inhaler was connected to an Alberta Idealized Throat with a custom 3D-printed mouthpiece adapter for actuation and flow rate control. The actuation flow rate was controlled by a critical flow controller (Critical

Flow Controller Model TPK 2000, Copley Scientific Limited, Nottingham, UK) for a constant flow rate of 60 or 15 L/min for 2.4 s. The capsules were pierced manually in the dry powder inhaler to prepare for actuation. Emitted dose from the device, defined as the percentage of powder mass emitted from the total filled powder in the capsule, was assessed. The filled powder mass was determined by gravimetrically weighing the empty and filled capsule, and the emitted powder mass was determined by gravimetrically weighing the dry powder inhaler with the capsule installed before and after actuation. The emitted dose for each case was measured in duplicate.

2.7. Scanning Electron Microscopy (SEM)

The spray-dried particles stored in a dry box and those exposed to 90% RH and 25° C for 30 and 60 min were analyzed for particle morphology using field emission scanning electron microscopy (Zeiss Sigma FESEM, Zeiss, Jena, Germany). The particle samples were gold-coated for 120 s using a sputter coater (Desk II Gold Sputter, Denton Vacuum, NJ, USA) and then imaged using the in-lens detector set to 3-5 kV accelerating voltage at a working distance of 4-7 mm. The particles exposed to the humid environment were analyzed immediately after exposure.

2.8. Raman Spectroscopy

The sample spray-dried particles stored in a dry box and the particles exposed to 90% RH and 25 °C for 30 and 60 min were qualitatively analyzed for solid phase using a custom Raman spectroscopy instrument (Wang et al., 2017). Before the capsules were opened and the particles analyzed, they were taken out of the environmental chamber and kept at room conditions (21 ± 1°C and 30-50% RH) for less than 20 min. A 671 nm diode-pumped laser (Ventus 671, Laser Quantum, UK) was used for the Raman system, which had a maximum power of 500 mW. The sample materials were placed on the sample holder in a cavity with a volume of 0.2 µL. The sample materials were analyzed at room temperature (21 ± 1°C) and under dry

conditions (< 3% RH) in a nitrogen atmosphere. Raw materials of trehalose, leucine, and trileucine were measured directly as the crystalline references, and raw material of pullulan was measured directly as the amorphous reference. Spray-dried trehalose was measured as the amorphous reference, as described elsewhere (Wang et al., 2019). The amorphous leucine reference was achieved by subtracting the spectrum of water from the spectrum of saturated aqueous leucine solution (Feng et al., 2011).

2.9. Statistical Analysis

The emitted dose measurements were reported as mean ± standard deviation and were analyzed statistically by a two-tailed student's t-test. Statistically insignificant differences were indicated for p-values larger than 0.05.

3. Results and Discussion

3.1. Particle Size

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for all spray-dried formulations are shown in Table 2. The MMADs were in the range of 13-17 μm as designed. It is generally believed that the GSD of monodisperse spray-dried particles should be less than 1.3 (Ivey et al., 2018). The GSDs for all formulations prepared in this study were in the range of 1.09-1.14, demonstrating high uniformity in the size of the spray-dried particles produced by the dual-orifice atomizer.

Table 2. MMAD and GSD for all spray-dried formulations

Formulation	MMAD (μm)	GSD

Tre	16.1	1.09
Tre97Tri3	13.3	1.12
Tre70Pu30	14.2	1.14
Tre70Leu30	15.1	1.11
Tre80Leu20	16.3	1.09
Tre90Leu10	15.3	1.11

MMAD: mass median aerodynamic diameter

GSD: geometric standard deviation

3.2. Crystallinity

The Raman spectroscopy analysis results for the spray-dried trehalose/pullulan 70/30 and trehalose/trileucine 97/3 formulations are shown in Figure 1. As reported elsewhere (Wang et al., 2019), trehalose does not crystallize in typical spray drying conditions. To verify the solid phases of pullulan and trileucine, the amorphous trehalose reference was subtracted from the spectra of spray-dried formulations. After the subtraction process, the residual spectrum of trehalose/pullulan 70/30 was found to be very similar to the amorphous pullulan reference, and the residual spectrum of trehalose/trileucine 97/3 showed no evidence of crystalline trileucine since no characteristic fingerprint peaks could be detected. Therefore, as expected, pullulan and trileucine were confirmed to be amorphous, forming amorphous shells covering the trehalose core.

The Raman spectroscopy analysis results for spray-dried trehalose/leucine 90/10, 80/20 and 70/30 are shown in Figure 2. After the amorphous trehalose reference has been subtracted, the three residual spectra are all similar to the crystalline leucine reference with a lattice mode at ~180 cm^{-1} and without any evidence of amorphous leucine, confirming that leucine was crystalline in all three spray-dried trehalose/leucine formulations. It is worth noting that as little as 10% leucine in the formulation leads to fully crystalline leucine in these spray-dried particles. A previous study (Feng et al., 2011) showed that a minimum of 25%

leucine was necessary to achieve fully crystalline leucine in the spray-dried trehalose/leucine particles under the conditions of this study. This deviation may stem from the different particle sizes and, consequently, the different times available for crystallization. Instead of using equation (6), the time available for crystallization of leucine in the trehalose/leucine system was determined more precisely as the window from the time for leucine to reach the critical concentration to the time for trehalose to reach the critical concentration, i.e. $t_{\mathrm{c,tre}} - t_{\mathrm{c,leu}}$ (Ordoubadi et al., 2020). The critical concentration of leucine, which is reached at a supersaturation of 3.5 (Ordoubadi et al., 2020), is 77 mg/mL, and the critical concentration of trehalose is 830 mg/mL (Ordoubadi et al., 2020). Under the chosen conditions in this study, the Peclet number of leucine and trehalose was around 0.8 and 1 respectively, and the droplet evaporation rate was approximately 4.9 μm^2/ms. With these values inserted into equation (1)-(5), the time available for crystallization can then be determined. In Feng et al.'s study the spray-dried particles containing 10% leucine, of which the crystallinity was 53%, had a MMAD of 2.0 µm and time available for crystallization of 0.1 ms, while in this study the spray-dried particles with the same leucine fraction, yet completely crystalline, had a MMAD of 15.3 µm and a time available for crystallization of 4.1 ms. Longer time available for crystallization gives more time for the solute to crystallize, thus leading to a higher degree of crystallinity.

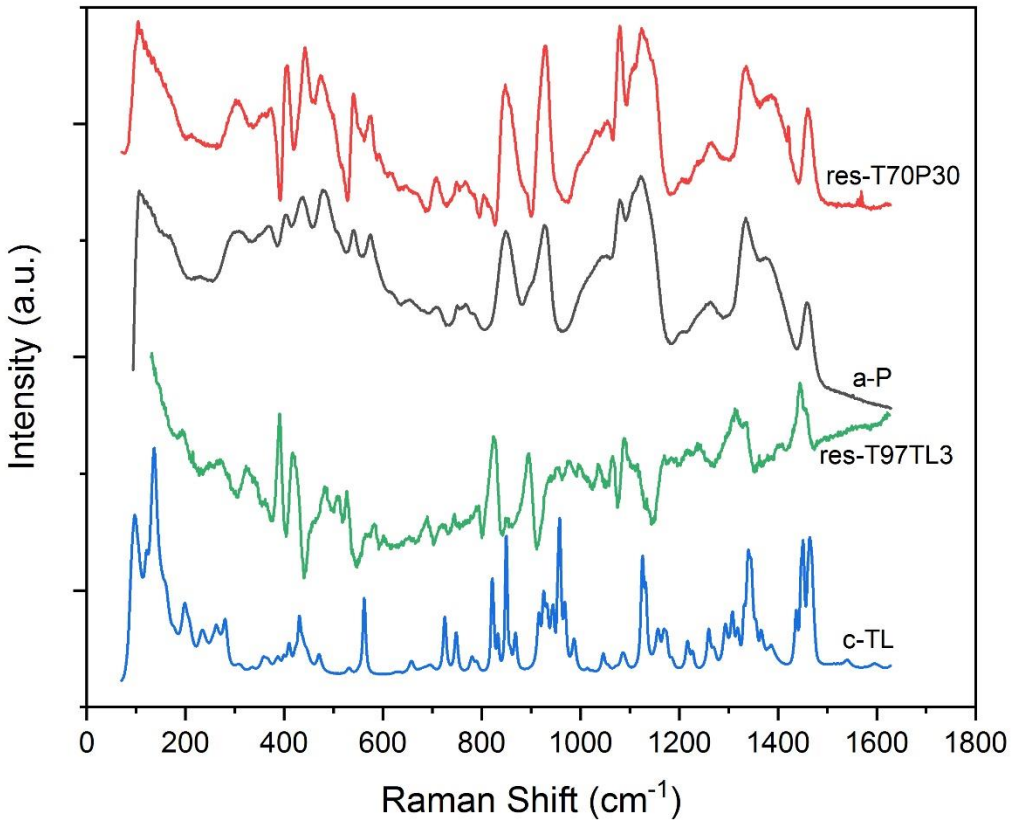


Figure 1. Raman spectroscopy analysis of spray-dried trehalose/pullulan and trehalose/trileucine particles. Legend: "res-T70P30" is the residual spectrum after the amorphous trehalose reference has been subtracted from the spray-dried trehalose/pullulan 70/30, "a-P" is the raw amorphous pullulan reference, "res-T97TL3" is the residual spectrum after the amorphous trehalose reference has been subtracted from the spray-dried trehalose/trileucine 97/3, and "c-TL" is the raw crystalline trileucine reference.

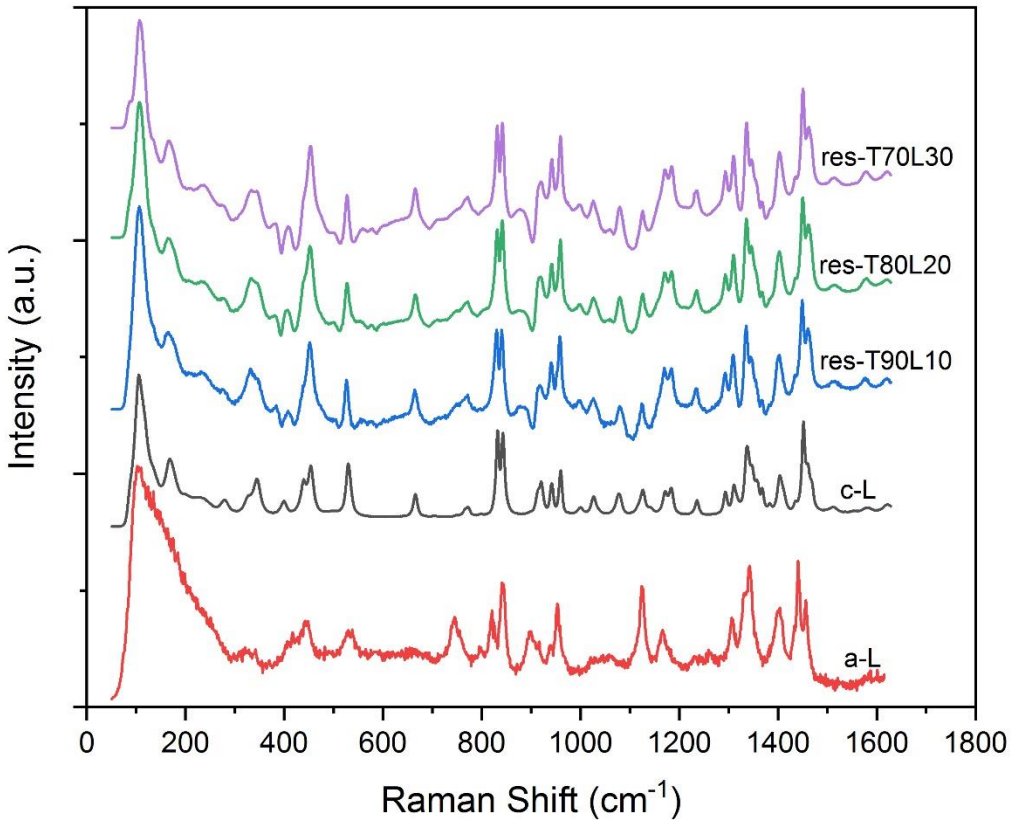
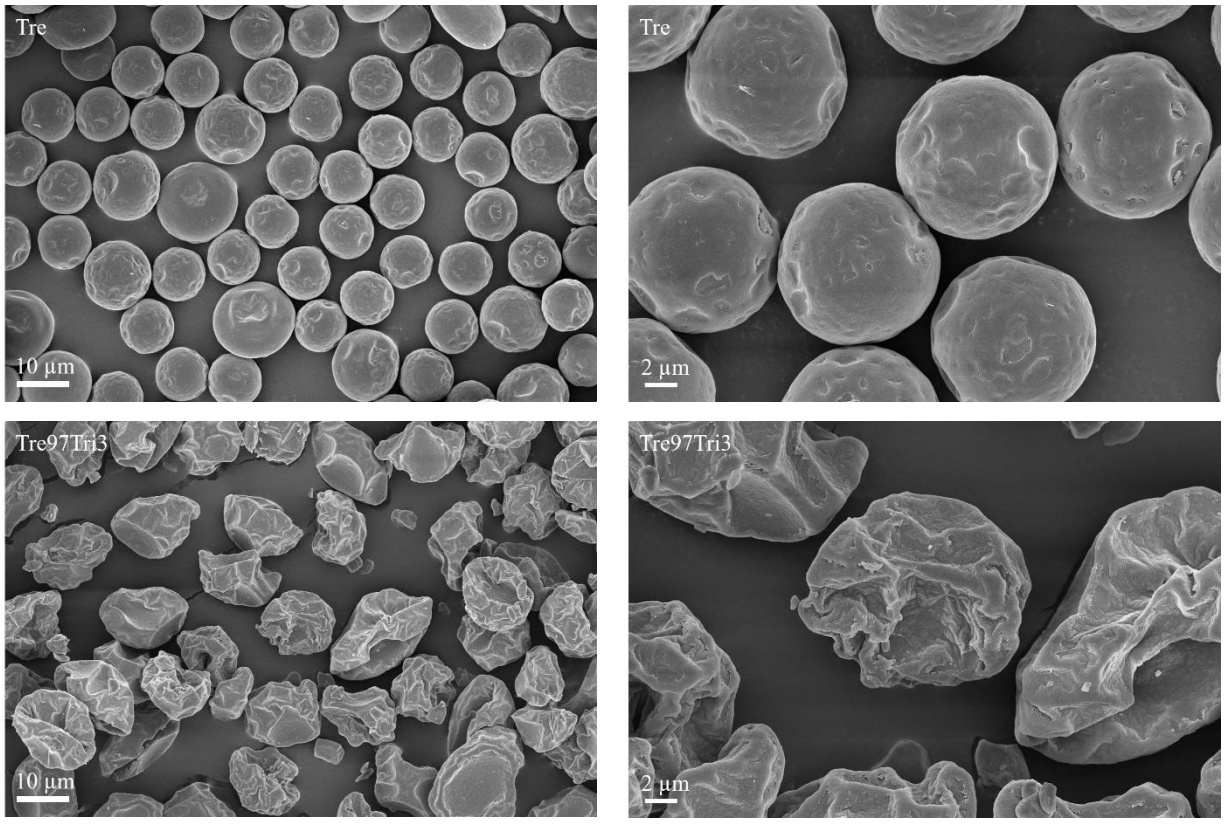


Figure 2. Raman spectroscopy analysis of spray-dried trehalose/leucine particles. Legend: The spectra labeled "res-" are the residual spectra after the amorphous trehalose reference has been subtracted from the three different spray-dried trehalose/leucine formulations, "c-L" is the raw crystalline leucine reference, and "a-L" is the amorphous leucine reference.

3.3. Particle Morphology

The SEM images of spray-dried particles for all the formulations are shown in Figure 3. In all cases, the particles showed uniform particle size, confirming the results from the APS measurements. Some particles of a slightly larger size can be observed in the images. These particles were dried from doublets or triplets due to droplet coalescence, which is a known phenomenon reported previously in monodisperse spray drying using a microjet atomizer (Ivey et al., 2018; Wang et al., 2019). The trehalose particles generally had spherical shapes with small dimples on the particle surface, a trait that has also been observed in previous studies (Carrigy et al., 2019; Ordoubadi et al., 2019; Wang et al., 2019). Spray-dried particles of

trehalose/trileucine 97/3 and trehalose/pullulan 70/30 had highly corrugated particle surfaces, which are caused by the early formation and subsequent collapse of amorphous trileucine and pullulan shells when they still lack mechanical strength. Spray-dried particles of trehalose/leucine 70/30, 80/20, and 90/10 were generally spherical without much corrugation. Some well-defined domains, which are likely individual leucine crystals, can be observed on the particle surfaces. An interesting particle morphology can be found on some particles of trehalose/leucine 80/20, which had multiple protrusions on the particle surface. These are possibly larger individual crystals, and only some particles in this batch exhibited this morphology, which points to the randomness of the onset of nucleation in the particle formation process.



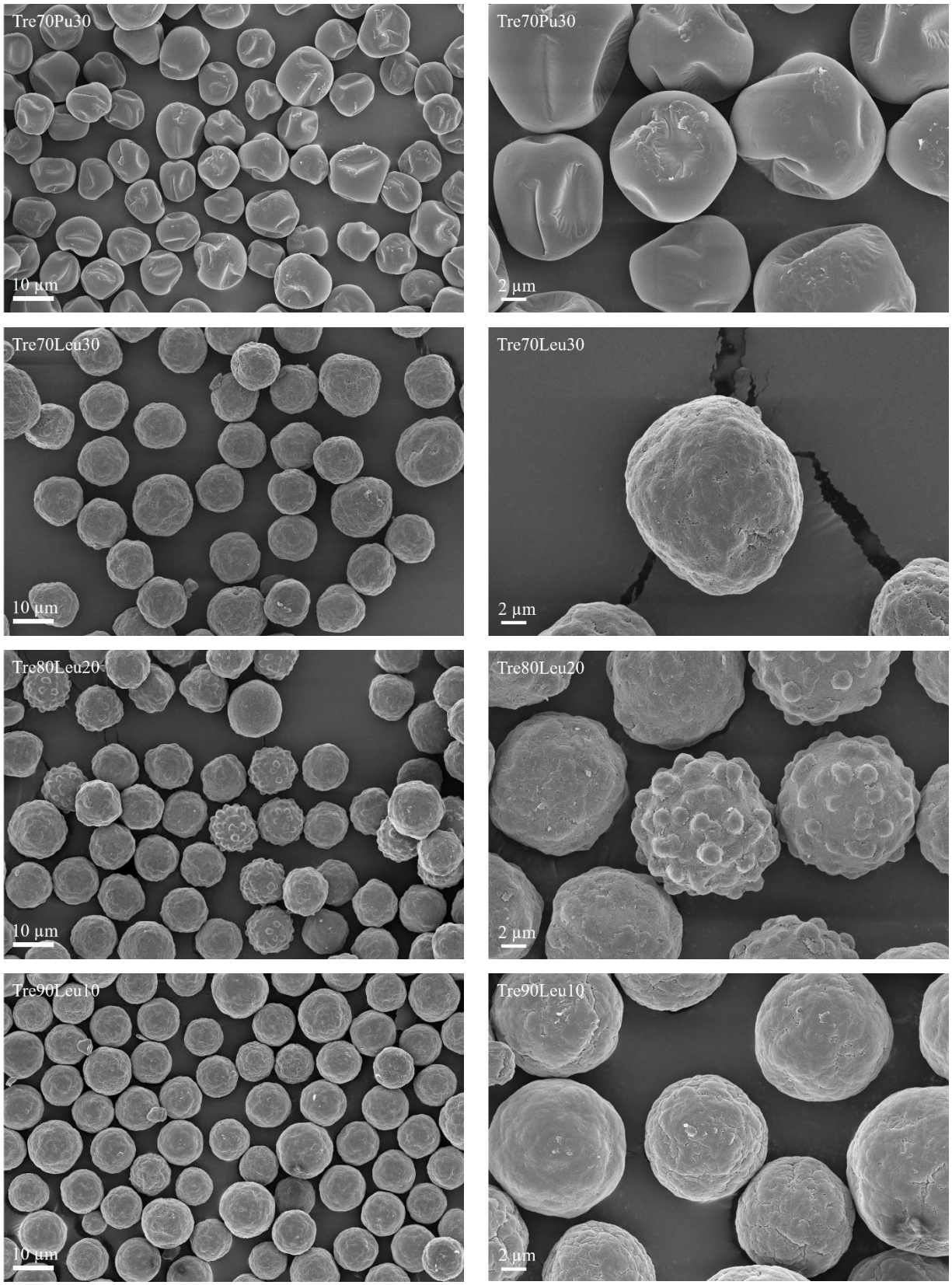


Figure 3. SEM images of all spray-dried formulations: trehalose, trehalose/trileucine 97/3, trehalose/pullulan 70/30, and trehalose/leucine 70/30, 80/20, and 90/10.

3.4. Powder Dispersibility

The emitted dose measurements for trehalose, trehalose/pullulan 70/30, trehalose/leucine 70/30, and trehalose/trileucine 97/3 are shown in Figure 4. The DPI actuation flow rate was set to 60 L/min, and the capsules filled with the spray-dried powders were exposed to 90% RH and 25 °C for 0 min, 30 min, and 60 min before being measured. From Figure 4, we can see that at a 60 L/min flow rate all the formulations had relatively high emitted doses (>90%) prior to humidity exposure with statistically insignificant differences, which is not surprising due to the large particle size of the powders. After 30 min exposure to 90% RH and 25 °C, the emitted dose of trehalose decreased to 68.9 ± 6.9%, while all three two-component systems maintained high emitted doses (>90%) with statistically insignificant differences. This shows that after 30 min exposure to a relatively high-humidity environment the three two-component systems were all able to maintain powder dispersibility, as measured by emitted dose, compared with pure trehalose particles. After 60 min exposure to 90% RH and 25 °C, hardly any trehalose particles were emitted from the device. The emitted dose for the Trehalose/pullulan 70/30 was also significantly reduced, to 7.5 ± 0.2%, a result likely due to the plasticization of the amorphous pullulan shell. Since pullulan has a higher glass transition temperature than trehalose (Carrigy et al., 2019), the pullulan shell may maintain dispersibility for the shorter period of time, but in the extreme case of an hour's exposure to high humidity the pullulan shell may still undergo sufficient plasticization to increase cohesion in the powder. Trehalose/trileucine 97/3 showed a greater decrease in the emitted dose, to 42.8 ± 23%. The addition of trileucine to the dry powder formulation led to corrugated particle surfaces, which may provide more protection than the fairly smooth pullulan surfaces. However, because of the relatively low concentration of trileucine in the formulation, the trileucine shell might be relatively thin and likely to plasticize due to its amorphous nature, thus explaining the limited protection it offers against humidity.

The emitted dose of trehalose/leucine 70/30 after 60 min exposure to humidity showed only a slight decrease to 90.3 ± 10%. The hydrophobic, crystalline leucine shell is able to reduce cohesive forces, thus enhancing the powder's dispersibility (Boraey et al., 2013). In addition, the crystalline leucine shell has very low water uptake and no plasticization, which reduces the interactions between particle surface and moisture, thus providing protection against moisture (Li et al., 2016). This improvement in powder dispersibility was shown to remain quite effective for a very long exposure time to humidity, demonstrating that the trehalose/leucine 70/30 particle system had the best powder dispersion performance against moisture of the three two-component particle systems. Therefore, the trehalose/leucine system was selected as the optimal formulation system to maintain powder dispersibility on exposure to high humidity for further investigations into the effectiveness of lower mass fractions of leucine, which will be discussed later.

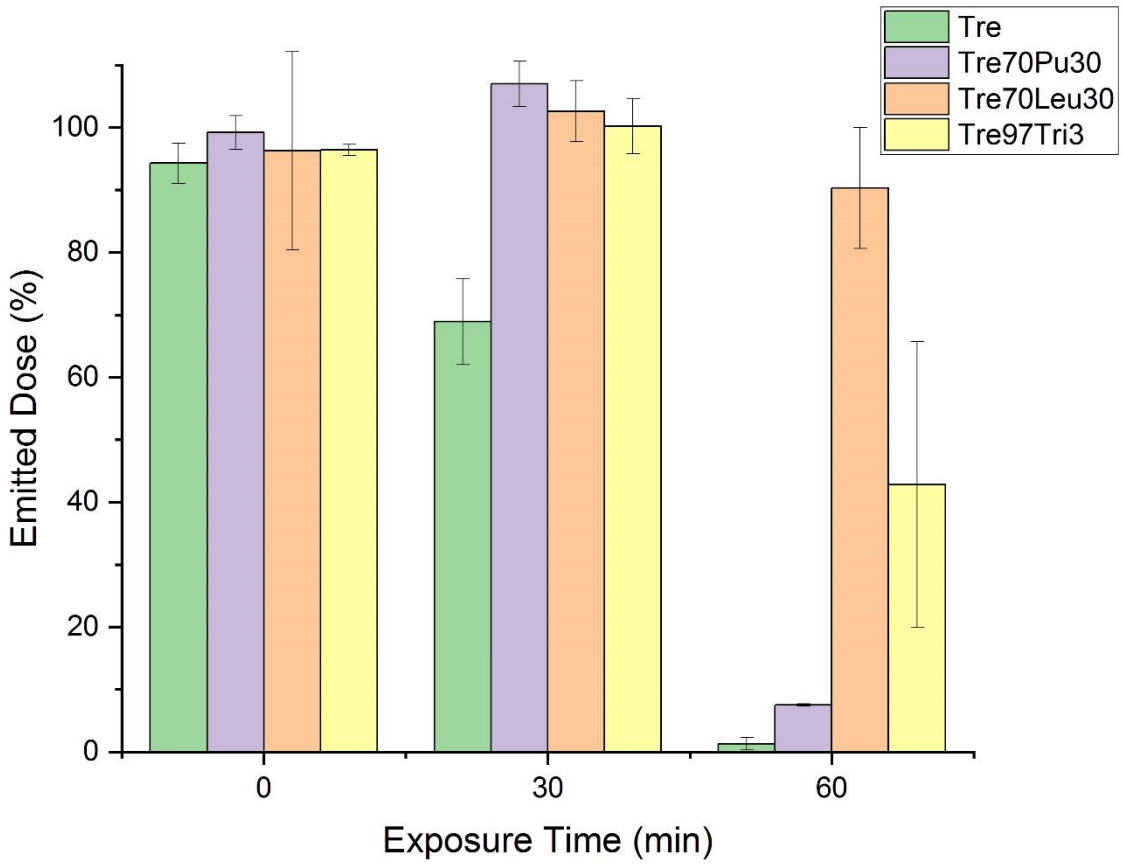


Figure 4. Emitted dose measurements at 60 L/min actuation flow rate for trehalose, trehalose/pullulan 70/30, trehalose/leucine 70/30, and trehalose/trileucine 97/3. These dry powder formulations were exposed to 90% RH and 25 °C for 0 min, 30 min, and 60 min. The error bars represent the percentage difference between the two measurements.

The emitted doses of trehalose/leucine 90/10, 80/20, 70/30, as well as those of the pure trehalose particles, are shown in Figure 5. The actuation flow rate was reduced to 15 L/min to provide a more difficult scenario, and the humidity exposure time was set to 0, 10, 20, 30, and 60 min. From Figure 5, we can see that at the 15 L/min flow rate neat trehalose powder indeed shows a reduced emitted dose of 65.6 ± 8.4% as compared to the > 90% at 60 L/min. Yet, all trehalose/leucine formulations had higher emitted doses than neat trehalose even without exposure to humidity, showing that the addition of as little as 10% leucine in the dry powder formulation enhanced the powder dispersibility. Among the three trehalose/leucine formulations, surprisingly, trehalose/leucine 80/20 had the highest emitted dose (102.5 ± 5.5%). This may be due to the observed surface morphology of some trehalose/leucine 80/20 particles, whose many protrusions on the particle surface may greatly increase the corrugation of the particle surface and enhance the powder dispersibility.

After 10 min exposure to 90% RH and 25 °C the emitted dose of trehalose decreased from 65.6 ± 8.4% to 42.8 ± 23%, while the emitted doses of the three trehalose/leucine formulations were preserved. These results show that the powder dispersibility of trehalose particles is affected rather quickly, after only 10 min exposure to humidity under these conditions, while the coverage of a crystalline leucine shell provides very effective protection. After 20 min exposure to humidity, the emitted dose of trehalose decreased further to 15.4 ± 6.4%, while the emitted doses of trehalose 70/30 and 80/20 remained unchanged. Trehalose/leucine 90/10 showed a slight decrease in the emitted dose, to 71.0 ± 0.7%, which may be due to the thinner leucine shell. After 30 min exposure to humidity, all the trehalose/leucine formulations showed moderate decreases in the emitted doses. However, even trehalose/leucine 90/10, with the lowest emitted dose among the three trehalose/leucine formulations, still had a much higher emitted dose (42.7 ± 5.7%) than trehalose (10.0 ± 3.3%), showing the improvement relative to pure trehalose particles. After 60 min exposure to humidity,

trehalose/leucine 90/10 was no longer able to provide protection and maintain the powder dispersibility. Trehalose/leucine 80/20 and 70/30 showed decreases in the emitted doses to 36.5 ± 16.2% and 23.5 ± 3.3% respectively, indicating that these formulations were still able to provide some protection after 60 min exposure. It is worth noting that in all cases trehalose/leucine 80/20 had a comparable emitted dose to that of trehalose/leucine 70/30 with statistically insignificant differences, despite its lower leucine mass fraction. Although trehalose/leucine 70/30 is supposed to provide higher surface coverage of leucine and a thicker leucine shell for better protection against moisture, the observed morphology of trehalose/leucine 80/20, with its higher surface corrugation, enhances powder dispersibility yet further.

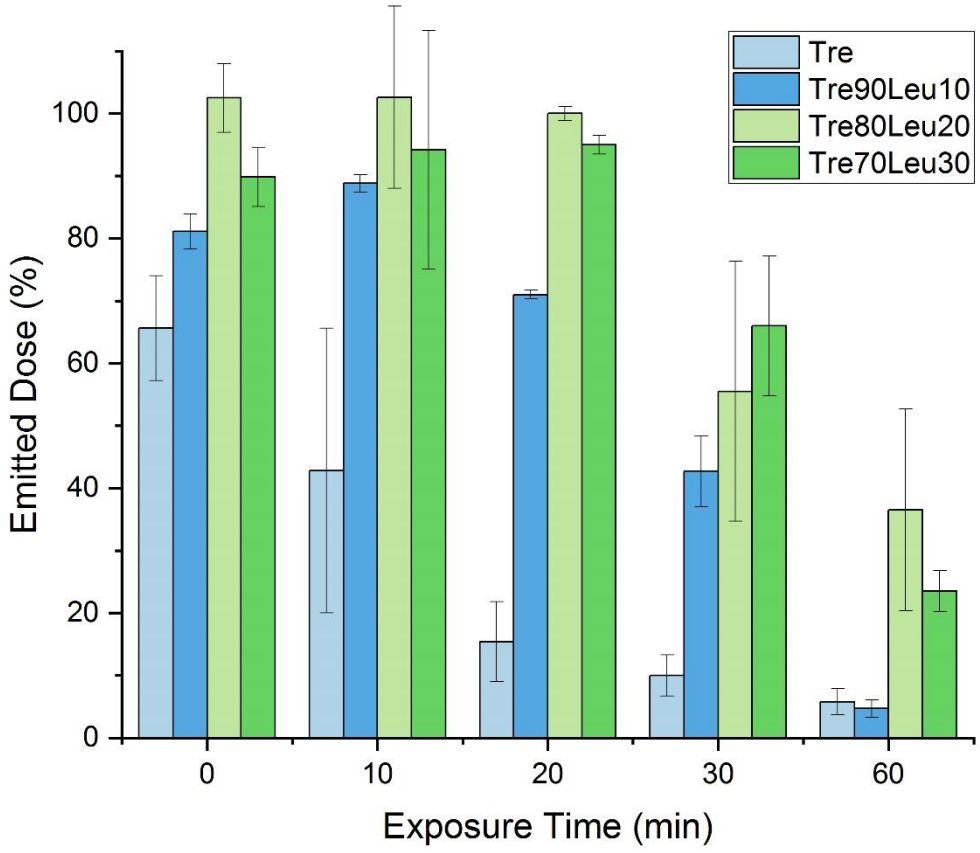
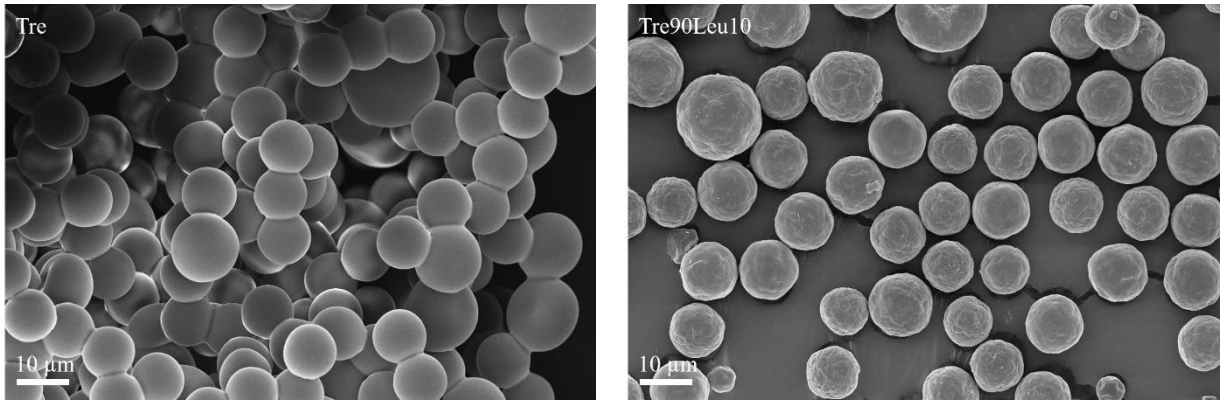


Figure 5. Emitted dose measurements at 15 L/min actuation flow rate for trehalose, trehalose/leucine 90/10, 80/20, and 70/30. These spray-dried powders were exposed to 90% RH and 25 °C for 0 min, 10 min, 20 min, 30 min, and 60 min. The error bars represent the percentage difference between the two measurements.

3.5. Effects of moisture on particle morphology and crystallinity

The SEM images of the trehalose and the trehalose/leucine 90/10, 80/20, and 70/30 spray-dried particles after 30 min and 60 min exposure to humidity are shown in Figure 6 and Figure 7, respectively. From Figure 6, we can see that after 30 min exposure to humidity many trehalose particles were fused together, while no material bridging or fusing was observed between trehalose/leucine 90/10, 80/20, and 70/30 particles. All trehalose/leucine formulations were able to prevent particle fusing after 30 min exposure to humidity, thus maintaining powder dispersibility. In Figure 7, after 60 min exposure to humidity trehalose particles were no longer discernible; the material now had the appearance of large solid blocks. A high degree of fusing and loss of particle shape was also observed for trehalose/leucine 90/10. This observation agreed with the very low emitted doses of trehalose and trehalose/leucine 90/10 particles after 60 min exposure to humidity. By contrast, a relatively low degree of fusing between particles was observed for trehalose/leucine 80/20 and 70/30, which agreed with their relatively higher emitted doses.



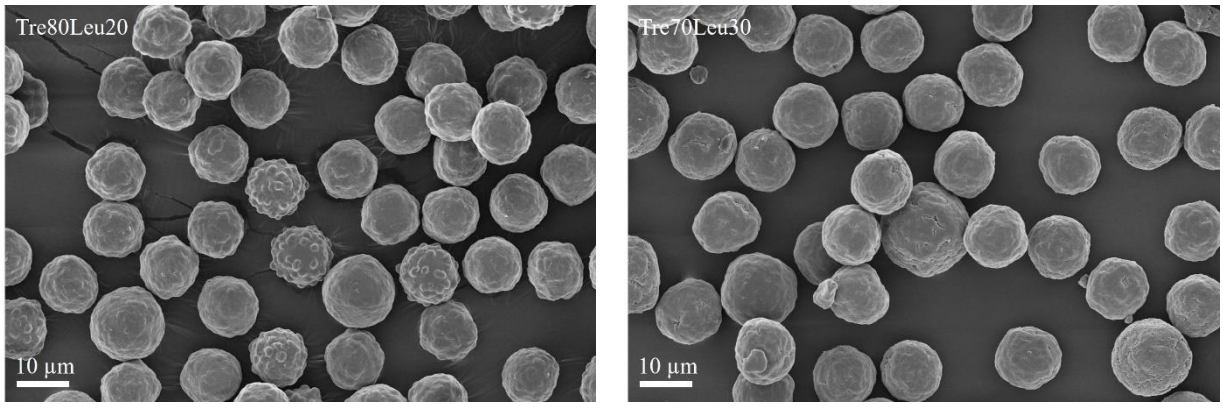


Figure 6. SEM images of spray-dried trehalose, and trehalose/leucine 90/10, 80/20, and 70/30 after 30 min exposure to 90% RH and 25 °C.

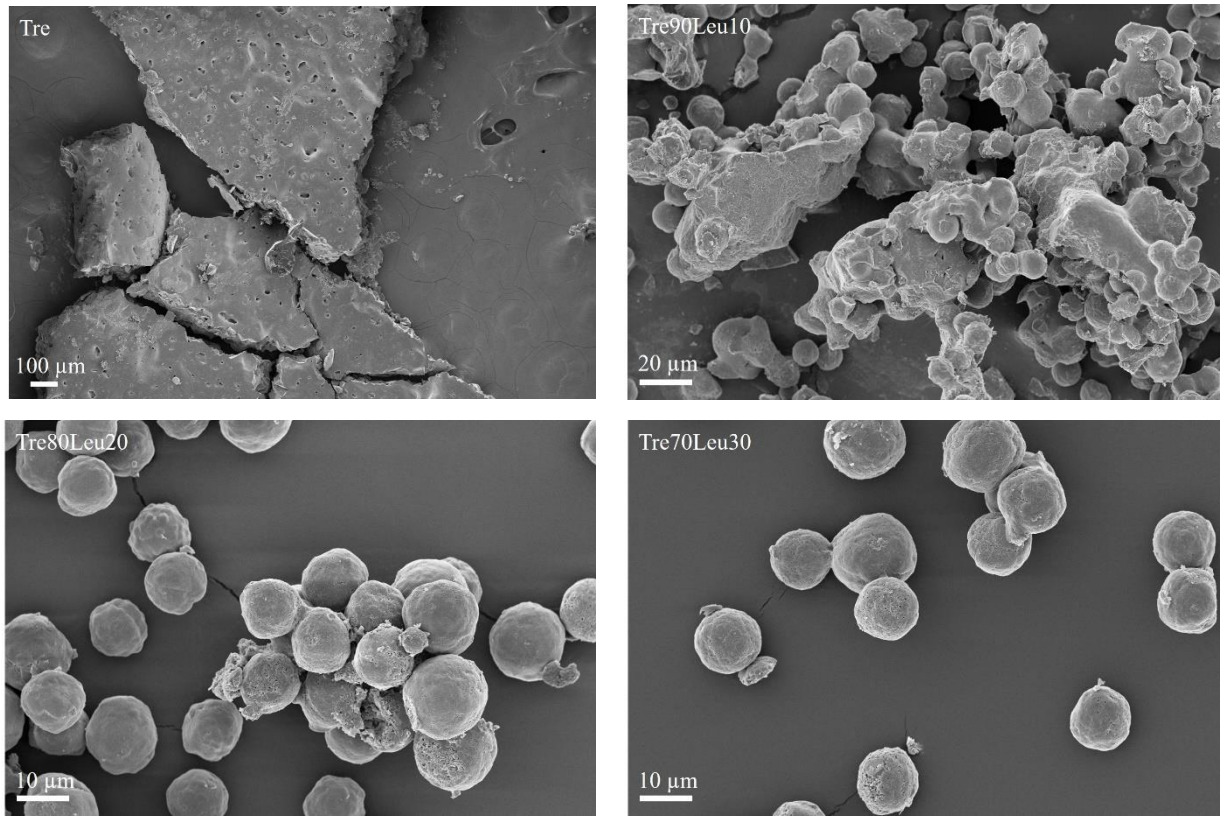


Figure 7. SEM images of spray-dried trehalose, and trehalose/leucine 90/10, 80/20, and 70/30 after 60 min exposure to 90% RH and 25 °C.

Raman spectroscopy analysis results for the spray-dried trehalose/leucine particles after 30 min exposure to 90% RH and 25 °C are shown in Figure 8. To verify the solid phase of trehalose, the crystalline leucine reference was subtracted from the spectra for trehalose/leucine 90/10, 80/20 and 70/30. The residual spectra as well as the spectrum of trehalose were very similar to the amorphous trehalose reference, and the characteristic sharp peaks from the crystalline trehalose were not detected, indicating its fully amorphous nature. Therefore, after 30 min exposure to 90% RH and 25 °C, the solid phase of trehalose was confirmed to be amorphous in all cases, irrespective of the presence of leucine. Raman spectra for the same formulations after 60 min exposure to 90% RH and 25 °C are shown in Figure 9. After subtraction of the crystalline leucine reference, the residual spectra are all similar to the crystalline trehalose reference, and no evidence of amorphous trehalose can be found. Therefore, it can be concluded that after 60 min exposure to 90% RH and 25 °C, the trehalose had crystallized in all spray-dried trehalose/leucine and in the neat trehalose particles. The spectroscopy analysis shows that under these test conditions the addition of leucine does not change the crystallization behavior of trehalose. This result is not totally unexpected since the crystalline leucine shell is composed of a patchwork of individual leucine nanocrystals (Ordoubadi et al., 2020), and thus the shell does not prevent diffusion of water to the trehalose core. Although leucine may not be able to prevent the plasticization of trehalose, the crystalline leucine shell can still preserve the powder dispersibility, even at a high water content in the particles.

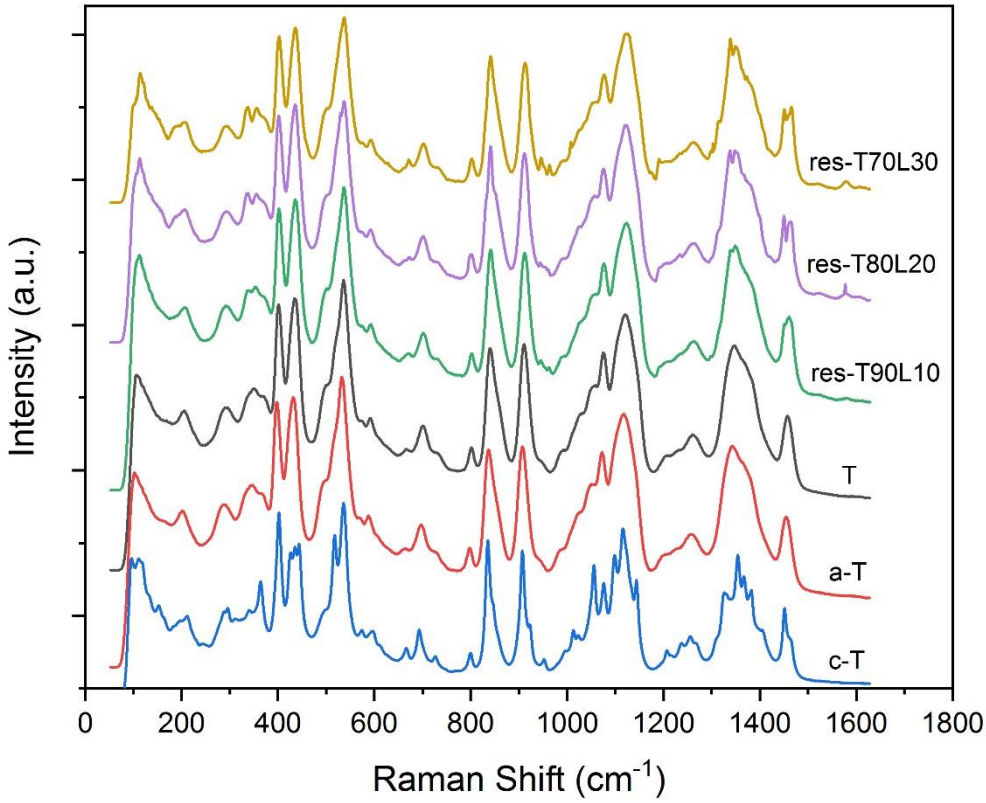


Figure 8. Raman spectroscopy analysis of spray-dried trehalose/leucine and pure trehalose particles after 30 min exposure to 90% RH and 25 °C. Legend: The spectra labeled "res-" are the residual spectra after the crystalline leucine reference has been subtracted from the three different spray-dried trehalose/leucine formulations, "T" is the spectrum of spray-dried pure trehalose particles, "a-T" is the amorphous trehalose reference, and "c-T" is the raw crystalline trehalose reference.

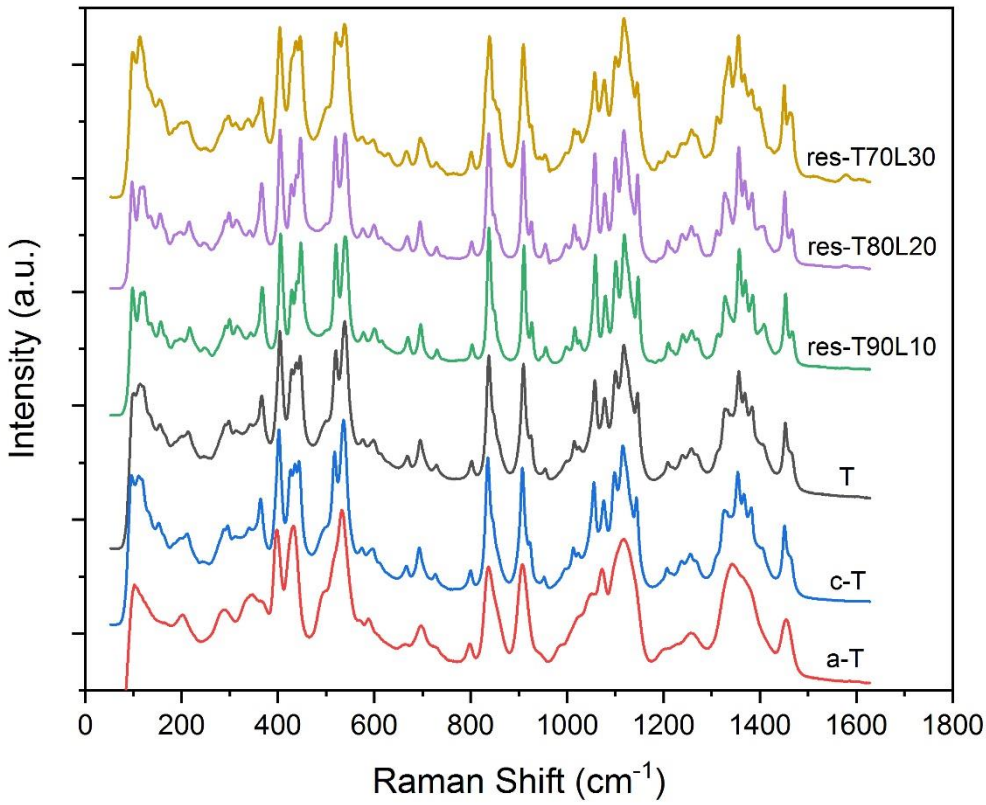


Figure 9. Raman spectroscopy analysis of spray-dried trehalose/leucine and pure trehalose particles after 60 min exposure to 90% RH and 25 °C. Legend: The spectra labeled "res-" are the residual spectra after the crystalline leucine reference has been subtracted from the three different spray-dried trehalose/leucine formulations, "T" is the spectrum of spray-dried pure trehalose particles, "c-T" is the raw crystalline trehalose reference, and "a-T" is the amorphous trehalose reference.

4. Conclusions

Unlike pure trehalose particles, leucine, trileucine, and pullulan as shell forming excipients were capable of maintaining powder dispersibility when exposed to a high-humidity environment in a simulated out-of-package scenario. The trehalose/leucine system was the most promising in protecting particles against moisture in a size range suitable for nasal delivery. The powder dispersibility of trehalose decreased quickly after 10 min exposure to 90% RH and 25 °C and decreased significantly after 30 min exposure, due to material bridging and fusing, while the trehalose/leucine system effectively reduced the effect of material

bridging and fusing and improved the powder dispersibility within 30 min exposure to a high-humidity environment. From the spectroscopy analysis, the crystalline leucine shell may not be able to prevent the water uptake and crystallization of trehalose, but it can nevertheless keep the powders dispersible even at a high moisture content. Thus, leucine may be considered an appropriate shell-forming excipient, providing robustness for nasal dry powder vaccines and other biologics for which a trehalose-based formulation platform is advantageous. Compared with particles for lung delivery, smaller amounts of leucine are needed to form a crystalline shell on large particles for nasal administration, thereby preserving a larger payload capacity in the particles. Future studies may investigate the surface composition of trehalose/leucine particles quantitatively to better understand the effect of leucine surface coverage using surface analysis techniques such as X-ray photoelectron spectroscopy.

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Zheng Wang: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – Original Draft, Writing – Review & Editing

Hui Wang: Methodology, Investigation, Writing – Review & Editing

Reinhard Vehring: Conceptualization, Methodology, Formal analysis, Project administration, Supervision, Writing – Review & Editing

Conflict of Interest

The authors of this paper declare that they have no conflict of interest to report.