Converting Nanosuspension into Inhalable and Redispersible Nanoparticles by Combined *In-situ* Thermal Gelation and Spray Drying

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ABSTRACT

While nanoparticulate drugs for deep lung delivery hold promise for particular disease treatments, their size-related physical instability and tendency of being exhaled during breathing remain major challenges to their inhaled formulation development. Here we report a viable method for converting drug nanosuspensions into inhalable, stable and redispersible nano-agglomerates through combined *in-situ* thermal gelation and spray drying. Itraconazole (ITZ) nanosuspensions were prepared by flash nanoprecipitation, and co-spray dried with two different grades of the gel-forming polymer, methylcellulose (MC M20 and MC M450) as protectants. MC M20 was found superior in protecting ITZ nanoparticles against thermal stress (through nanoparticle entrapment within its gel network structure) during spray drying. In terms of redispersibility, an S_f/S_i ratio (i.e., ratio of nanoparticle sizes after and before spray drying) of unity (1.02±0.03), reflecting full particle size preservation, was achieved by optimizing the suspending medium content and spray drying parameters. Formulation components, nanosuspension concentration and spray drying parameters all showed a significant impact on the aerosol performance of the resulting agglomerates, but an absence of defined trends or correlations. Overall, the MC-protected nano-agglomerates displayed excellent *in-vitro* aerosol performance with fine particle fractions higher than 50% and mass median aerodynamic diameters within the 2-3μm range, which are ideal for deep lung delivery.

Keywords: Itraconazole, nanoparticles, gelation, spray drying, inhalable and redispersible nano-agglomerates, aerosol performance, deep lung delivery

1. INTRODUCTION

Recent advances in nanotechnology have led to a resurgence of research interest in the development of novel pulmonary drug delivery systems for treatment of various diseases.[1-2] In addition to solubility and bioavailability enhancement, nanoparticles hold promise for specialized drug delivery via the lungs, including deep lung delivery, sustained drug release, and active drug targeting. [3] Upon arrival at the alveoli, inhaled particles in the micron-size range tend to retain on the epithelial surface, whereas their nanosized counterparts are capable of penetrating through alveolar epithelium into the interstitium [4]. Interestingly, a fraction of these nanoparticles can return to the epithelial surface, and such continuous penetration and re-entrainment cycles serve to maintain a steady drug concentration throughout the alveoli.[5] Moreover, translocation of the deposited particles from the alveolar space to the systemic circulation is only possible for particles in the nanosize range. [6] All these unique properties render nanotherapeutics particularly useful for treating certain respiratory diseases, e.g., invasive aspergillosis, tuberculosis and lung cancers; all of which can readily spread to other parts of the body if left untreated.

Despite the aforementioned merits, the utilization of solid nanoparticles in inhalation therapy is not without issues compared to their liquid injectable counterparts. Currently, there are three main types of aerosol-generating devices, namely, nebulizer, pressurized metered-dose inhaler (pMDI) and dry powder inhaler (DPI). [7] With regards to the first two types of devices, although drugs formulated as nanosuspensions can be atomized into respirable mists, the metastable nature of nanoparticles as well as the shear stress induced during atomization could destabilize the emitted aerosols. In comparison to their liquid counterparts, dry powders formulated for use with DPI offers superior stability both physically and chemically. In addition, unlike pMDI that requires propellants for aerosolization, DPI is a portable self-actuated inhalation device which is free of any ozone-depleting propellants, e.g., chlorofluorocarbons and hydrofluoroalkanes. Nevertheless, regardless of the method of aerosol administration, strict control of particle size to within the aerodynamic diameter (d_A) range of 1-5 μm is necessary for optimal pulmonary delivery. Particles with $d_A > 5$ μm are mostly deposited on the walls of the upper respiratory tract by inertial impaction while particles with $d_A < 1$ μm tend to remain air-borne in the airways and are exhaled during the normal breathing cycle. Consequently, to ensure

delivery into the lungs, nanoparticles need to be agglomerated into this respirable particle size range by an appropriate technology, for which spray drying would appear to be a pragmatic and efficient choice.

Over the past decade, spray drying has become increasingly popular for manufacturing DPI formulations owing to its ease of operation and capability of offering tight particle size control. However, the application of this technology to drying of nanosuspensions (or agglomeration of nanoparticles) is deemed challenging as the physical stability and primary particle size of nanosuspensions can significantly influence the redispersibility of the final products. In addition, the selection of protectants and their quantities used in nanosuspensions are often required to be determined in a trial-and-error manner. This could be attributed to the relatively passive protection mechanism of the protectants whose sole functions are to shield the nanoparticles from heating stress and minimize their physical contact with one another. Here we present a novel drying method for converting a drug nanosuspension into a redispersible nanoagglomerate powder through a one-step process termed "combined *in-situ* thermal gelation and spray drying". In this process, a gel-forming polymer is employed to actively agglomerate nanoparticles via thermal gelation to the optimal aerodynamic particle size during spray drying. Itraconazole (ITZ), a poorly water-soluble BCS II drug, was chosen as the model compound in the present study because of its well-established therapeutic effectiveness against fungal infections and documented potentials for combating influenza and lung cancers [8-10]. In addition, we have previously demonstrated that stable ITZ nanosuspensions can be successfully prepared by flash nanoprecipitation (FNP) with the aid of d-α-tocopheryl polyethylene glycol 1000 succinate as primary stabilizer and cholesterol as co-stabilizer.[11]

Accordingly, the purpose of this study was three-fold: (a) to evaluate the protective effects of two different grades of the gel-forming polymer, methylcellulose (MC M20 and MC M450) on the integrity of ITZ nanoparticles spray-dried from an optimized FNP-produced nanosuspension; (b) to examine the impact of critical formulation and processing parameters on the redispersibility and aerosol performance of the spray-dried ITZ

nanoagglomerates; and (c) to establish an optimal gelation and spray drying protocol for generating redispersible nanoparticle agglomerates in the respirable particle size range.

2. MATERIALS AND METHODS

2.1. Materials

Itraconazole (ITZ) with purity >99% was purchased from Yick-Vic Chemicals and Pharmaceuticals Limited (Hong Kong SAR, China). Cholesterol (CLT), d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), D-mannitol (mannitol), fructose, trehalose dihydrate and α-lactose monohydrate (lactose) were supplied by Sigma Aldrich (USA). Hydroxypropyl β-cyclodextrin (HPBCD) was supplied by Roquette (France), and methylcellulose (MC) of two different grades (MC M20 and MC M450) and sucrose were obtained from Wing Hing (Hong Kong SAR, China). Sodium chloride (NaCl) was purchased from BDH Laboratory Supplies (UK). Dimethylformamide (DMF) and tetrahydrofuran (THF) of analytical grade were received from RCI Labscan Limited (Thailand). Acetonitrile (ACN) of HPLC grade and acetic acid of analytical grade were purchased from Duksan Pure Chemicals (Korea) and BDH Laboratory Supplies (UK), respectively. All chemicals and solvents were used as received. Water used was collected from a Millipore water purification system (Direct-QTM, USA).

2.2. Preparation of ITZ Nanosuspension

ITZ nanosuspension with TPGS as a primary stabilizer and CLT as a hydrophobic co-stabilizer (ITZ: TPGS: CLT = 1:1:0.2 w/w/w; ITZ = 0.25 mg/ml) was prepared by flash nanoprecipitation using a four-stream multi-inlet vortex mixer, as reported previously. [11]

2.3. Centrifugal Ultrafiltration

Fifteen mL of ITZ nanosuspension was centrifuged in an Amicon® ultra-15 30K centrifugal filter device (Millipore Corp., Billerica, MA, USA) under 4000 × g. For the removal of DMF, the centrifugal ultrafiltration was conducted twice, each with replacement with pure cold water, whereas for the adjustment of the nanoparticle concentration with maintenance of the same DMF concentration, the centrifugal ultrafiltration was performed only once, followed by replacement with cold water

containing 5% v/v DMF (Fig. A1). The concentration of nanoparticles was adjusted by varying the volume of the replacement fluid as required. The effectiveness of the above ultrafiltration protocol was verified by monitoring the change in particle size with a nanoparticle size analyzer (see Section 2.7) and the change in drug concentration by HPLC (see Section 2.13).

2.4.Spray Drying

Selected protectants including mannitol, sucrose, lactose, and HPBCD were each dissolved in separate ITZ nanosuspensions (ITZ: protectant = 1: 80 w/w) kept in an ice bath. Each sample was then fed into a spray-dryer (B-191, Büchi, Switzerland). The aspirator of spray dryer was fixed at 100% for all formulations, and the air flow was set at 600L/h. Unless otherwise specified, the inlet temperature of spray dryer and suspension feed rate were maintained at 110°C and 2.5 ml/min respectively. The dried powder was collected in a product vessel.

2.5. Gelation

MC M20 solution (containing 5 mg/ml MC M20 and 5% v/v DMF) was mixed with ITZ nanosuspension in 1:1 volumetric ratio at room temperature. Agglomeration was achieved by mixing the diluted nanosuspension (ITZ: MC = 1:20 w/w; 5% v/v DMF) with a concentrated sodium chloride solution (250 mg/ml) in a 1 to 3 volume ratio. The suspension was allowed to stand for 30 min before vacuum-filtration through a filter paper of pore size of 0.8µm. The resulting powder was collected and dried in a desiccator.

2.6. Combined *In-Situ* Thermal Gelation and Spray Drying

MC solution with known concentrations of MC and DMF was mixed with the ITZ nanosuspension in 1:1 volumetric ratio under ice-cold condition. The resulting nanosuspension was spray-dried using the same parameter settings as described in Section 2.4.

2.7. Determination of Particle Size Change of ITZ Nanosuspension

The change in intensity-weighted particle size of ITZ nanosuspension was monitored by a Delsa™ Nano C particle size analyzer (Beckman Coulter Inc., USA) upon completion of each step in the drying process and in the subsequent testing of the dried

products, e.g., after addition of protectant and redispersion of the product. Nanoparticle redispersibility was tested by reconstitution of the dried products with water at room temperature.

2.8. Determination of Geometric Particle Size of the Nanoparticle Agglomerates

A laser diffraction particle size analyzer equipped with a tornado dry powder dispersion system and a vacuum generator (LS13 320, Beckman Coulter, USA) was used to determine the particle size distribution of the dried nanoparticle agglomerates. The vacuum process generates an air flow to disperse the sample powder and direct the powder to a suction channel and then a measuring cell. In the measuring cell, the dispersed sample interacts with an illuminating light beam mainly generated by a 5mW laser diode with a wavelength of 750 nm and a fiber optic spatial filter. The resulting scattered light intensity patterns are then collected and analyzed using Fraunhofer theory to obtain volume particle size distribution. Median geometric diameter is the particle size at which 50% volume fraction of particles are undersize.

2.9. Differential Scanning Calorimetry (DSC)

The thermal properties of the dried powder product were characterized by DSC (DSC 250, TA Systems, USA). The equipment was calibrated with pure indium before use. Accurately weighed sample (1-5 mg) was placed in a hermetically sealed aluminum pan and scanned at 10°C/min under nitrogen purge from 25 to 250°C.

2.10. Powder X-ray Diffraction (PXRD)

The dried powder product was packed in an aluminum holder and analyzed using a powder X-ray diffractometer (PW1830, Philips, Netherlands) operating with a 3kW Cu anode over a 2θ interval of 3° to 40°. The step size was 0.02° and the dwell time was 2 seconds per step.

2.11. Scanning Electron Microscopy (SEM)

The morphology and surface features of the dried powder were studied by scanning electron microscopy (SEM). Samples were placed on double-sided adhesive tapes stuck on aluminum stubs. Samples were gold-coated in an ion sputter coater (SC 502, Polaron) at an electrical potential of 2.0 mV, 20 mA. The coated samples were examined under a

scanning electron microscope (JSM 6300F, JEOL, Japan) operating under vacuum at an accelerating voltage of 10-20 kV.

2.12. *In Vitro* Evaluation of Aerosol Performance

A Next Generation Impactor (NGI, Copley Scientific, UK), consisting of an induction port, 7 stages and a micro-orifice contactor (MOC), was employed to assess the aerosol performance of the dried nanoagglomerates. Silicone grease (LPS Laboratories, USA) was deposited on all cups of the NGI to minimize bouncing of particles. Accurately weighed sample (~8 mg) was separately loaded into three size-3 hydroxypropyl methylcellulose capsules (Capsugel, Australia). The first capsule was mounted and pierced in an inhaler (Osmohaler, Plastiape, Italy), which was connected to the NGI through a mouthpiece adapter. The sample was dispersed from the pierced capsule and aerosolized at a flowrate of 90 L/min for 2.7 s so that 4 L of air passed through the inhaler with a pressure drop of 4 kPa. The process was repeated for the other two capsules. Each part of NGI and capsules were then individually washed with acetonitrile/water (60:40 v/v) solution. This slightly different procedure was performed to avoid the acetonitrile from corroding the plastic inhaler. Water was used to rinse the inhaler and adapter separately, followed by dilution with acetonitrile to a final acetonitrile/water volume ratio of 60:40. The amount of drug collected in each rinse or wash was determined by HPLC.

For the calculations, recovered dose (RD) is defined as the total amount of drug recovered from capsules, inhaler, adapter, throat and all cups of NGI while emitted dose (ED) is the total amount of drug recovered excluding those from the capsules and inhaler. Powder emission efficiency is defined as ED with respect to RD. Stage cut-off diameters and cumulative mass fraction of drug less than the stated aerodynamic diameters were calculated according to the method in the latest edition of the British Pharmacopoeia. Cumulative drug mass fractions under size were plotted against aerodynamic diameters on a log probability scale, and the data were analyzed by the best-fit linear line. The fine particle fraction (FPF) is the mass of dose for the particles whose aerodynamic size is less than 5 μm with respect to the RD. Mass median aerodynamic diameter (MMAD) is the aerodynamic diameter for which 50 wt% of particles are below or above the MMAD.

2.13. High-Performance Liquid Chromatography (HPLC)

ITZ was assayed by HPLC using a previously reported method [12]. The calibration curve of ITZ constructed with different drug concentrations exhibited excellent linearity with $R^2 > 0.999$. The retention time of ITZ was ~ 10 min. Quality control samples with known ITZ concentrations were injected in between and after analyses to ensure data accuracy.

2.14. Statistical Analysis

All material testing and measurements were conducted in triplicate with separate batches of samples, and the collected data were analyzed statistically by unpaired Student's t-test at a significant level of 0.05.

3. Results and Discussion

3.1. Spray Drying

Saccharides are commonly used as protectants against shear force and elevated temperature in spray drying. As TPGS (melting point ~38°C) was employed as the primary stabilizer for the ITZ nanoparticles, it would be expected that it could not withstand the high-temperature environment during spray drying. Selection of protectants with high melting points might mitigate the issue as they could protect the primary nanoparticles from the heating stress and minimize the physical contact between primary nanoparticles. For this purpose, lactose, mannitol, sucrose and HPBCD were selected as they have relatively high melting points compared with the studied inlet temperature of spray dryer.

It was found that without removal of the DMF from the nanosuspension, the spray-dried products containing ITZ-TPGS-CLT nanoparticles with various selected protectants could not be redispersed back into individual nanoparticles upon reconstitution with water. To eliminate the effect of organic solvent, DMF was removed by centrifugal ultrafiltration. As shown in Fig. A2 and Table A1, the ultrafiltration process had minimal impact on the integrity and stability of the nanoparticles, as the particle size, encapsulation efficiency, and drug loading remained essentially unchanged after the process.

For the spray drying of the nanosuspension without DMF but with a protectant added, only HPBCD was effective for producing redispersible powder (Table A2). The particle size of spray-dried powder with HPBCD was confirmed by SEM to fall within the micron-size respirable range (Fig. A3). In addition, DSC and PXRD results confirmed its

amorphous nature (Fig. A4). However, the ratio of the size of primary nanoparticles following spray drying and reconstitution (S_f) to the initial particle size of the nanosuspension before spray drying (S_i) was significantly larger than the generally accepted range for nanoparticle stability (i.e., 1.47 ± 0.09 vs 0.7-1.3).[13-14] Hence, it was necessary to seek a better formulation strategy to minimize the particle size increase of primary nanoparticles during spray drying.

3.2.Gelation

It has been shown that conventional protectants are not sufficiently effective for protecting drug nanoparticles during spray drying, indicative of their relatively passive protection mechanism at an early stage of the spray drying process. To resolve the issue, alternative protectants capable of offering the nanoparticles active protection need to be sought. The desired protectant should actively and efficiently encase the nanoparticles at the initial stage of the spray drying process instead of remaining uniformly dispersed in the nanosuspension droplets. The protectant should also exhibit stronger and more specific interactions with the surface of the nanoparticles so as to lower the amount of the protectant required for protecting the nanoparticles. Ideally, such a protectant should actively bind the nanoparticles together to form stable agglomerates so that the total exposed surface area of nanoparticles is minimized to a level sufficient for long-term stability. The agglomerates are also required to dissociate back into individual nanoparticles after arrival at the alveoli.

Gel-forming polymers with sol-gel transition temperature (T_{gel}) above body temperature (37°C) are potential protectants that fulfill the aforementioned requirements. A typical example of such polymers is methylcellulose (MC) with T_{gel} falling within the range of 40°C to 50°C. MC is generally considered safe for human consumption, and has been approved by regulatory authorities (e.g., US-FDA) for use as an excipient in various pharmaceutical formulations. With a suitable T_{gel}, gelation of MC can start upon heating inside the heating chamber of a spray dryer. The T_{gel} of MC depends on the degree of substitution (DS) of the hydroxyl groups. The higher the DS, the lower the T_{gel}. At the molecular level, MC joins via their hydrophobic moieties to form a gel. Since the surface of the ITZ nanoparticles has been shown to be not fully covered by hydrophiles right after production,[12] the nanoparticles might also attach to the hydrophobic segments of MC,

283 resulting in nanoparticles being entrapped inside a gel network. As an agglomerating agent
284 for drug nanoparticles designed for delivery to the alveoli, MC possesses the advantage of
285 being readily degraded in the alveolar fluid once dissolved. [15] The desired MC should be
286 one which is not only soluble at body temperature to release the agglomerated nanoparticles
287 in the alveoli, but is also able to undergo gelation upon heating in a spray dryer. MC M20
288 (DS = 1.6) with a T_{gel} of 48°C [16] was selected for further evaluation in this part of the study.

289 In order to verify if the gel-forming MC is capable of entrapping the nanoparticles,
290 it is important to determine the nanoparticle content in the gel immediately after its
291 formation from the nanosuspension prior to drying. However, for MC to form a gel, such
292 a study has to be conducted at an elevated temperature, i.e., T_{gel} (40-50°C) of MC, which
293 can potentially destabilize the nanoparticles, depending on the duration of the study. To
294 circumvent this temperature-dependent stability problem, an electrolyte can be added to
295 the MC solution to effect gelation at room temperature (i.e., salting-out effect). It has been
296 reported that the T_{gel} of MC can be lowered by addition of sodium chloride. [17] Gelation of
297 MC can then occur at ambient conditions when T_{gel} is adjusted to room temperature or
298 below. The aqueous solubility of MC is generally moderate due to the substitution of some
299 polar hydroxyl groups with methoxide groups. To determine the entrapment of
300 nanoparticles by the gel-forming MC M20, the present study has employed a drug-to-MC
301 M20 ratio of 1:20 w/w, which denotes a much lower proportion of MC compared with the
302 conventional protectants used in spray drying. After addition of the MC M20 solution to
303 the nanosuspension, the size of the nanoparticles remained unchanged, suggesting that MC
304 M20 exerts no significant adverse impact on the nanoparticles. When the nanosuspension
305 with dissolved MC was added to sodium chloride solution at room temperature, sizable
306 gels were formed and then collected by conventional filtration after 30 minutes. The
307 nanoparticles which were not entrapped in gels should pass through the filter membrane in
308 conventional filtration. No particle was detected in the collected filtrate by DLS particle
309 sizing, suggesting all the nanoparticles were entrapped inside the gels. Upon contact with
310 water (without salt), the gels were de-agglomerated. The size of primary nanoparticles after
311 de-agglomeration of gels was found to increase by 1.46 ± 0.02 times, indicative of
312 redispersibility of primary nanoparticles. The size increase of primary nanoparticles may
313 be attributed to partial destabilization of the nanoparticles by the high electrolyte

concentration in the aqueous suspending medium which can alter the aqueous solubilities of the nanoparticle components. [18] Since gelation of MC appeared effective for entrapment of nanoparticles, subsequent studies had focused on the utilization of spray drying in conjunction with gelation to produce nanoparticle agglomerates with improved redispersibility, stability and aerosol performance.

3.3.Production of nanoparticle agglomerates by combined gelation and spray drying

It has been demonstrated that gelation with MC is capable of actively entrapping nanoparticles to form redispersible nanoparticle agglomerates. Hence combined spray drying and gelation would appear to hold promise for the production of redispersible nanoparticle agglomerates within the proper particle size range for deep lung delivery. This section focuses on the development of a suitable combined spray drying and gelation protocol for the production of nanoparticle agglomerates. A systematic approach was adopted to investigate the impact of the properties of MC, concentration of the primary nanoparticles and MC, concentration of organic solvent, as well as spray drying conditions on the properties and aerosol performance of resulting nanoparticle agglomerates. In these spray drying studies, an equivalent volume of MC solution was added to each ITZ-TPGS-CLT nanosuspension so that the original concentration of the latter was reduced by half (ITZ concentration = 0.125 mg/ml after dilution) prior to spray drying. The ratio of drug to MC in the nanosuspensions was fixed at 1:20 w/w initially. A summary of different tested agglomerate formulations with their median geometric diameters, redispersibility, MMAD and FPF is provided in Table 1.

3.3.1. Effect of viscosity of MC

Apart from MC M20 [viscosity ~20 mPaS for 2% aqueous solution at 20°C; DS~1.6][16], MC M450 [viscosity ~ 450 mPaS for 2% aqueous solution at 20°C, DS~1.5][19] of similar DS but higher viscosity was also assessed for its ability to protect primary nanoparticles in spray drying. It could be seen from Fig. 1 that the particle size of primary nanoparticles after redispersion of the agglomerates with MC M20 was significantly smaller than that using MC M450 regardless of the DMF content ($p<0.05$), implying that a lower viscosity of MC offers better protection to primary nanoparticles. This can be linked to the higher molecular mobility or diffusivity of the less viscous MC M20. Having

a higher diffusivity, MC M20 can move faster to encapsulate the nanoparticles during spray drying, thus reducing the exposure time of unprotected nanoparticles to the hot environment, and therefore, it was selected for further assessment in subsequent studies.

3.3.2. Effect of concentration of organic solvent

In the presence of DMF, co-spray drying of the nanosuspensions with conventional protectants failed to yield redispersible agglomerates, whereas similar spray drying treatment with the gel-forming agent, MC, instead yielded stable and redispersible dried nano-agglomerates. This suggests that MC offered the nanoparticles better protection or stronger resistance against the destabilization by DMF during spray drying. Increasing the DMF content in the range of 0-5% v/v in the nanosuspension was seen to reduce the growth of primary nanoparticles during spray drying (Fig. 2). With the nanoparticles being protected within the MC gel and the presence of DMF which possesses relatively low surface tension and viscosity in the nanosuspension, the exposure of nanoparticles towards shear stresses during atomization would be alleviated. A previous study demonstrated DMF could form intermolecular hydrogen bonds with MC and interact with the methoxy groups of MC via a dipole-dipole interaction,[20] suggesting the presence of DMF could reduce the overall system enthalpy. Another work also revealed that the incorporation of a small amount of DMF (even at a 0.05-0.1 mole fraction) in water could significantly lower the molal heat capacity of a DMF-water mixture.[21] Thus, the drying time of sprayed droplets as well as the exposure time of nanoparticles towards various drying stresses would be reduced. However, the protective effect of MC on the nanoparticles (produced at ITZ:MC M20 = 1:10 w/w) began to subside when the DMF content in the nanosuspension was raised beyond 5% v/v, as shown by an increase in S_f/S_i thereafter ($p < 0.05$; Fig. 6.2).

3.3.3. Effect of drug to MC ratio

For the spray drying studies employing DMF-free nanosuspensions, an increase in the ITZ:MC M20 ratio from 1:20 w/w to 1:10 w/w (i.e., decreasing MC M20 concentration) increased the S_f/S_i ratio from 1.53 ± 0.02 to 2.43 ± 0.10 (Fig. 2). In contrast, repetition of the above study for DMF-containing nanosuspensions revealed a decrease in the S_f/S_i value from 1.51 ± 0.06 to 1.29 ± 0.02 (at 2.5% v/v DMF) and from 1.33 ± 0.08 to 1.13 ± 0.05 (at 5% v/v DMF) upon raising the ITZ:MC M20 ratio from 1:20 w/w to 1:10 w/w. It is

important to note that the concentration of MC M20 exerts a significant impact on the drying time of sprayed droplets and hence the exposure time of nanoparticles to various drying stresses. This effect poses stability threat to the nanoparticles regardless of the presence of DMF. Higher concentration of MC affords higher viscosity of the medium during gel formation, thereby prolonging the drying time of the droplets and exposure time of nanoparticles to drying stresses. In the case of DMF-free nanosuspensions, as the initial evaporation rate of sprayed droplets was indeed low, the viscosity effect induced by different ratios of ITZ to MC M20 did not significantly retard the initial evaporation rate. However, less amount of MC M20 relative to nanoparticles (i.e., ITZ:MC M20 = 1:10 w/w) would imply less protection being provided for the primary nanoparticles, resulting in a larger final size of the nanoparticles. In the case of nanosuspensions containing 2.5% v/v and 5% v/v DMF, the initial evaporation rate was accelerated by the presence of DMF. While the nanosuspension formulated with ITZ to MC M20 ratio of 1:10 w/w did not afford unacceptably high viscosity, the extension on the solvent evaporation time by MC M20 at a higher concentration in the nanosuspension with ITZ to MC M20 ratio of 1:20 w/w could be significant. Thus in the presence of DMF, the nanosuspensions containing a higher proportion of MC M20 (i.e., with ITZ to MC M20 ratio of 1:20 w/w) consistently displayed poorer stability of the nanoparticles redispersed from the spray-dried agglomerates (Fig. 2). However, in the extreme case when the ITZ to MC M20 ratio in the nanosuspensions was further raised to 1:5 w/w in the presence or absence of DMF (5% v/v), non-redispersible products were obtained, indicative of an insufficiency in the amount of protectant required for nanoparticle stabilization.

3.3.4. Effect of ITZ nanosuspension concentration

The ITZ to MC M20 ratio was fixed at 1:10 w/w for all studies reported in this section. The concentrations of both nanoparticles and MC M20 in the nanosuspensions with 5% v/v DMF were adjusted to double to significantly amplify the effect of water content, if any, on the final size of primary nanoparticles. As shown in Fig. 3, the final size of primary nanoparticles remained unchanged ($p > 0.05$) with increases in both ITZ and MC M20 concentrations. Since the evaporation rate of droplets was indeed high due to the presence of DMF, any further reduction in the drying time by increasing the nanosuspension concentration or reducing the water content might not have any additional

benefit. In addition, the positive effect of the latter could be offset by an increased tendency to particle aggregation in the more concentrated nanosuspension. To further substantiate the significance of drying time reduction, similar studies were conducted on DMF-free nanosuspensions, which would display a slower solvent evaporation rate compared with the DMF-containing counterparts. Moreover, S_f/S_i was lower for the more concentrated DMF-free nanosuspension ($p < 0.05$), reflective of the importance of drying time reduction in maintaining the stability of nanoparticles.

3.3.5. Effect of inlet temperature of spray drying

Nanosuspensions containing ITZ and MC M20 in the ratio of 1:10 w/w and DMF content at 5% v/v were employed to determine the optimal spray drying conditions for producing redispersible nanoparticle agglomerates based on the calculated S_f/S_i ratios. Higher inlet temperature of spray dryer can increase droplet evaporation rate and thereby reduce the drying time, but it can also augment the heating stress on nanoparticles. Hence there exists an optimal drying temperature at which a desirably high drying efficiency can be achieved for the agglomerates with no or minimal damage to the nanoparticles. The final size of primary nanoparticles was improved when the inlet temperature increased from 100°C to 110°C ($p < 0.05$), but further increase in inlet temperature to 120°C (Fig. 4) offered no further improvement in reducing the final size of primary nanoparticles ($p > 0.05$). Hence, 110°C was taken as the optimal inlet temperature.

3.3.6. Effect of feed rate of the ITZ nanosuspension into spray dryer

A descending trend for S_f/S_i was observed with a decrease in feed rate of the nanosuspension into the spray dryer (Fig. 5). This can be explained by generation of smaller droplets with a slower feed rate. The drying time was shorter with a smaller droplet size, resulting in smaller final particle size. With an S_f/S_i ratio close to unity obtained at the feed rate of ~1 and ~1.5ml/min (the S_f/S_i values at these two feed rates were statistically indistinguishable; $p > 0.05$), the feed rate of ~1.5ml/min was deemed optimal since it could significantly reduce the processing time. In summary, the optimal protocol for constraining the size change of primary nanoparticles ($S_f/S_i = 1.02 \pm 0.03$) was spray drying of the nanosuspension at an inlet temperature of 110°C and a feed rate of ~1.5ml/min.

3.4.Structure and morphology of nanoparticle agglomerates

Nanoparticle agglomerates can exist as either solid or hollow structure. The structure depends on the movement of primary nanoparticles together with other components present in droplets during spray drying. The primary nanoparticles with dissolved saccharides or HPBCD as protectants are uniformly distributed in the atomized spherical droplets initially. Evaporation of the liquid medium starts at the surface of the droplet, leading to a high local concentration of primary nanoparticles and protectants on the surface. A concentration gradient is then developed which drives the movement of primary nanoparticles and protectants by diffusion towards the center of the droplet (inward motion). Meanwhile, when the liquid medium evaporates, the droplet surface recedes and a thermophoretic flow towards the surface is created to replenish the volume of fluid lost (outward motion).[22-23] This flow keeps the primary nanoparticles and protectants stationing on the receding surface. The net inward movement of the primary nanoparticles and protectants depends on the difference in magnitude between the inward force and the outward force. A Peclet number (Pe), a dimensionless mass transport number, can be used to describe this situation (Eq. 1).[24-25] This number shows the relative significance of the time required for the drying of the droplet (τ_d) and diffusion of the primary nanoparticles or protectants (R^2/D).

$$Pe = \frac{R^2}{\tau_d D} \quad \text{(Eq. 1)}$$

where τ_d is the drying time, R is the droplet radius, and D is the diffusion coefficient of the nanoparticle or protectant.

Fig. 6 illustrates the position of primary nanoparticles in a droplet at different Pe values. When the evaporation flux is small, the outward force is small. The net inward movement of the primary nanoparticles and protectants is faster than the radial velocity of the receding droplet surface ($Pe << 1$). The materials inside the droplet remain uniformly distributed. The end-time of the evaporation is nearly the same as when solidification starts. Solid-structured particles are formed. When the evaporation rate is high, the outward force is large. The net inward movement of the primary nanoparticles and protectants is slower than the movement of the surface ($Pe >> 1$), and thus the primary nanoparticles and protectants accumulate on the surface of the droplet. The concentrations of the protectants

on the surface quickly reach saturation, and the protectants become solidified. Simultaneously, the primary nanoparticles are driven together by capillary force.[23] This leads to the formation of a solid composite shell consisting of the primary nanoparticles and protectants and enclosing remaining liquid medium. The enclosed liquid medium is heated in the next instant and evaporates within the shell. The ability for the evaporating liquid to escape from the shell depends on the permeability of the shell. The presence of gaps on the shell increases the permeability. When the permeability is low, the rate of liquid vapour moving out from the shell is lower than the generation rate of liquid vapour inside the shell. Pressure is developed inside the droplets and pushes the remaining primary nanoparticles in the liquid medium onto the inner surface of the shell. If the pressure is increased to such a level that the shell cannot withstand, the evaporating liquid medium will puncture the shell, resulting in holes in the shell or rupturing of the shell. This process is termed thermal expansion.[25-26] When the permeability is high, the liquid vapour can escape easily from the shell through the gaps between the primary nanoparticles. As the shrinkage of the droplet continues, the liquid-vapour interfaces in the gaps reverse their curvature (from convex to concave, as illustrated in Fig. 7). This creates a tension force pulling the gaps towards the outer surface of the shell and pushing the spherical shell inward to form buckles.[27-28] Both of the above cases produce hollow nanoparticle agglomerates.

It should be noted that in using MC to entrap nanoparticles in liquid medium prior to spray drying, active aggregation of MC to form a gel during the drying process also needs to be taken into consideration. Mobility of MC will be dramatically decreased after gel formation. Since gel formation of MC on droplet surface initiates actively and rapidly upon heating in spray drying, inward movement of MC will soon slow down and solidification of MC on droplet surface will commence near the onset of heating, and will be quicker compared with conventional protectants. As it has been shown in the preceding section that primary nanoparticles are actively taken up into MC gels during gel formation, migration of primary nanoparticles towards droplet center will be seriously retarded by MC gels. Hence, hollow nanoparticle agglomerates will most likely form if MC is employed as a carrier or agglomerating agent for primary nanoparticles. This has been substantiated in the present study by the production of nanoparticle agglomerates in the form of buckled,

dimpled spheres when co-spraying the nanosuspension with MC M20 (Fig. A5). Hence, combined spray drying and gelation has proven effective for generating hollow aerosols. It could be seen that the agglomerates in Fig. A5 exhibited a smooth surface with no exposed or discernable individual nanoparticles. This is probably because all the nanoparticles were fully entrapped or covered by the MC which was present in a much larger quantity.

Hollow nanoparticle agglomerates have advantages over their solid counterparts. It has been shown that non-hollow polymer- and lipid-based nanoparticle agglomerates are not readily redispersible in water.[29-30] This is because the outer layer of nanoparticles needs to be wetted and redispersed first before wetting and redispersion of the inner layer can proceed. This will require a long period of time for wetting the whole solid nanoparticle agglomerates. In contrast, with a similar concentration of primary nanoparticles, hollow nanoparticle agglomerates have relatively large geometric diameters and few layers of primary nanoparticles in the agglomerate shell, resulting in a high redispersion rate of primary nanoparticles.[31-32] Since the aggregate strength decreases with an increase in geometric diameter, hollow aerosols should also show better powder dispersion. Buckled spheres of the nanoparticle agglomerates can further decrease contact area between aerosols, leading to superior powder dispersion performance.[33]

3.5. *In vitro* aerosol performance

Aerosol performance of nanoparticle agglomerates is critical for effective delivery of drug nanoparticles to the deep lungs, and can be assessed with an NGI. All formulations containing MC M20 showed extremely high powder emission efficiency (>90%; Fig. 8 and Fig.9), indicating that the aerosols were effectively discharged from the inhaler. FPFs were generally higher than 50% and reached up to 71%, which are considered high compared with other aerosol studies.[34] The superior aerosol performance can be attributed to a decrease in interparticulate contact area for buckling and hollow structure of the ITZ nanoparticle agglomerates.[35] The majority of the nanoparticle agglomerates produced using different spray drying parameters and suspension concentrations have an MMAD of 2-3μm, which is most ideal for targeting the alveoli.

As far as the formulation of nanosuspensions is concerned, no defined trend exists for both MMAD and FPF with an increase in organic solvent content or drug to protectant

ratio of the nanosuspension (Fig. 8). It has been reported that excipient (DPPC and albumin) concentration in nanoformulation of albumin-lactose-DPPC system exerts a great impact on aerosol performance although no obvious correlation between them was observed.[36] Tsapis and coworkers demonstrated that spray drying of DPPC-DMPE-lactose-nanoparticles in ethanol/water system with different nanoparticle concentrations did not show any significant influence on the aerodynamic diameter of the resulting product.[25] Similar lack of correlation between formulation component concentration and aerosol performance has been observed for nanoparticle agglomerates formulated with polyacrylate and silica.[37] Increasing nanosuspension concentration may have a positive or negative impact on aerosol performance. To investigate the effect of nanosuspension concentration on the aerosol performance of ITZ nanoparticle agglomerates with MC, the concentration of ITZ nanosuspension with MC and DMF was doubled and spray-dried. As shown in Fig. 9a, doubling the nanosuspension concentration (both ITZ nanoparticles and MC M20 concentrations) significantly worsened the aerosol performance of the agglomerates (i.e., larger MMAD and smaller FPF; $p < 0.05$).

With regard to the spray dryer parameters, feed rate also showed no correlation with aerosol performance (Fig. 9b), but an increase in inlet temperature afforded an increase in MMAD ($p < 0.05$; Fig. 9c) while having no significant impact on FPF. The ascending MMAD trend with increasing inlet temperature may only be valid for the formulations in the present study since other studies showed that the influence of inlet temperature on aerodynamic diameter of dried agglomerates was also affected by the weight ratio and size of primary nanoparticles.[38] It is worth noting that regardless of the widely documented impact of formulation and manufacturing variables on FPF and MMAD, a higher FPF should always be associated with a lower MMAD although this relationship might not be apparent in certain cases.[39] The detailed results on geometric diameters, MMAD, FPF and redispersibility of samples prepared by different tested formulation and processing conditions are provided in Table 1.

Based on the aerosol performance and redispersibility data, the optimized conditions for the production of nanoparticle agglomerates were as follows: Nanosuspensions containing ITZ and MC M20 at a mass ratio of 1:10 together with 5%

v/v DMF; spray-dried at an inlet temperature of 110°C and a feed rate of 1.5 ml/min. The optimized agglomerates powder (i.e., formulation k in Table 1) exhibited excellent *in-vitro* aerosol performance and redispersibility with FPF of 65.35 (± 1.68) %, MMAD of 2.16 (± 0.02) μm and an S_f/S_i ratio of 1.02(± 0.03). The PXRD pattern of the optimized agglomerates powder displayed no significant diffraction peak but a halo diffused pattern instead, indicative of the amorphous nature of the sample (Fig. 10a). This finding was also supported by an absence of melting events in the DSC analysis (Fig. 10b).

CONCLUSION

The problems of nanoparticle instability and poor redispersibility by direct spray drying of nanosuspension with conventional protectants could be resolved by employing *in-situ* gelation with MC in conjunction with spray drying. In the present study, we have shown that this novel drying approach could generate readily redispersible nanoparticle agglomerates in the desired aerodynamic particle size range, which is ideal for deep lung deposition. Formulation component, concentration of nanosuspension as well as spray drying parameters all showed a significant impact on the aerosol performance of resulting nanoparticle agglomerates, but an absence of defined trends or correlations. The present study offers an effective approach for simultaneously overcoming two major challenges in inhaled nanoparticle formulation development, viz. generation of inhalable and redispersible nano-agglomerates, and maintenance of the integrity and stability of individual nanoparticles.

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Declaration of interest: none

Appendix A. Supplementary material

This paper includes supplementary material which has been uploaded separately during submission.

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