Optimizing Excipient Formulation and Spray Freeze Drying Conditions to produce Nanoparticulate Aerosols with Good Morphology and Aqueous Re-dispersibility

Lim Zongsheng Samuel

Hwa Chong Institution (College Section)

Optimizing Excipient Formulation and Spray Freeze Drying Conditions to produce Nanoparticulate Aerosols with Good Morphology and Aqueous Re-dispersibility

Lim Zongsheng Samuel[1], Katherine Kho[2] and Kunn Hadinoto Ong[2]

[1]NRP student, Hwa Chong Institution (College Section)
[2]School of Chemical and Biomedical Engineering, Nanyang Technological University

ABSTRACT

Poly-caprolactone (PCL) nanoparticles loaded with antibiotics can be inhaled to treat infections of the respiratory tract, like tuberculosis. These nanoparticles must first aggregate to form nano-aggregates before delivery into the respiratory tract. The aggregates must have an aerodynamic diameter of approximately 2-4μm and must have high re-dispersibility in aqueous media. These characteristics depend largely on the formulation of excipients used to consolidate the nanoparticles to form the nano-aggregates, as well as the spray freeze drying conditions. Excipients used in this study are mannitol, leucine, PVA, PEG, lactose, trehalose and pluronic. The excipients are mixed with PCL nanoparticles in various ratios to form a feed solution, which is then spray-freeze dried to obtain dry-powder nano-aggregates. Lactose and trehalose aggregates are unstable as they absorb moisture and collapse easily. PEG has poor redispersibility while pluronic has an aerodynamic size of greater than 4μm. PVA, leucine and mannitol are then used in subsequent runs in various ratios to determine the effect of these 3 excipients on the effective density, dg, da and aqueous re-dispersibility of the nano-aggregates produced. The most favourable ratio is the PCL:mannitol:leucine ratio of 1:6:1 at total solid concentration of 5.5% w/v and atomization flow rate of 443L/h.

INTRODUCTION

Nanoparticles have many applications. In particular, biocompatible nanoparticles like silica and poly(lactic-co-glycolic acid) (PLGA) have received much attention as viable vectors in pulmonary drug delivery[1], from anti-cancer drugs to antibacterial drugs to treat lung infections such as cystic fibrosis[2]. Drug delivery via the inhalation of nanoparticles has various advantages as compared to traditional oral medication as it allows tissue targeting, decreases side-effects and improves therapeutic efficacy[3].

Polycaprolactone (PCL) is a biodegradable polyester which is suitable for pulmonary drug delivery. As its degradation in physiological conditions is slower than other biodegradable polyesters [4], this interesting property can be exploited in the controlled release of drugs in target tissues over a prolonged period of time. This is useful in the treatment of tuberculosis, which is a lengthy process that involves oral combination antibiotic therapy administered daily for at least 6 months. An alternative to this is the steady delivery of antibiotics directly to infected cellular tissue[5]. When nanoparticles with slow degradation like PCL is used, antibiotic encapsulated within the PCL can be released slowly over an extended period of time, during which levels of drug concentration in the infected tissues will remain fairly constant. This may reduce dosing frequency and shorten treatment time.

In inhaled drug delivery, the aerodynamic diameter (d_a) of a particle determines where the particle will end up. PCL nanoparticles have extremely small d_a and will suspend in the air for long periods of time, and are consequently exhaled from the lungs. Particles with large d_a, however, will fail to enter the lungs and deposit in the oropharyngeal regions instead, as seen in Figure 1[6].
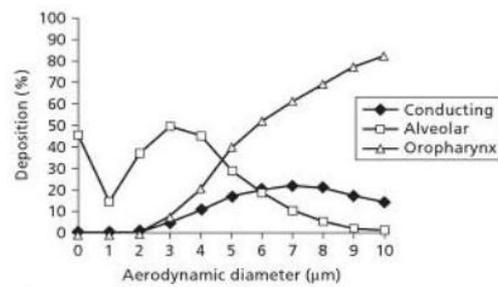
Fig 1. Percentage deposition of nanoparticles in the conducting airways of the lungs, the alveoli and the oropharyngeal regions[6].

The d_a of a particle is given by Eq. (1)[7] where d_g is the geometric particle diameter, ρ_{unit} is the unit density of the particle which is fixed at 1g/cm^3 while ρ_{eff} is the effective density of the particle, defined as the particle mass divided by its total volume including the open and closed pores.

$$d_a = d_g \sqrt{\frac{\rho_{eff}}{\rho_{unit}}} \qquad (1)$$

As individual PCL nanoparticles have very low d_a, it is necessary to formulate micron-size aggregates of drug-bearing nanoparticles with a d_a of approximately 2-4µm for increased efficiency in delivering the drugs into the lungs. Once delivered to the lungs, though, these aggregates must re-disperse into its nanoparticular constituents in the interstitial fluid of the lungs. This is done because drug dissolution from the micron-size aggregates is much slower than that from individual nanoparticles, as the latter has a larger surface area to volume ratio for the drugs to be delivered more efficiently (Fig. 2).
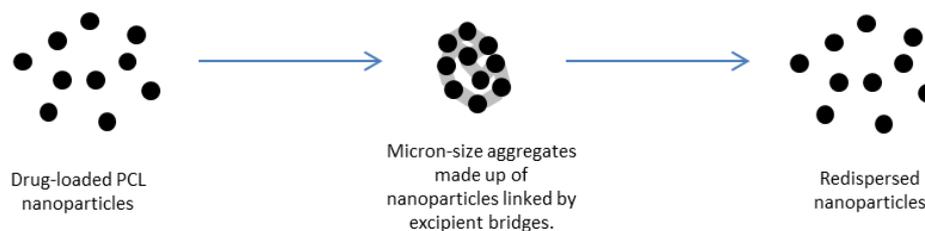

Fig 2. Diagram showing PCL nanoparticles consolidated to form nano-aggregates, which is then redispersed back into its primary particles

The degree of redispersibility in an aqueous medium is dependent on the ease of wetting of the aggregates, as well as on the strength of the excipient bridges holding the nanoparticles together. In order to produce these micron-size aggregates, spray freeze drying is employed. Spray freeze drying also results in porous aggregates with a low density which is advantageous in the thorough wetting of the particles due to an increased surface area to volume ratio. Meanwhile, water-soluble excipient bridges can ideally dissolve in aqueous media, allowing the aggregates to re-disperse into the primary nanoparticles. The degree of redispersibility can be quantified by taking the ratio of the size of the redispersed nanoparticles (S_f) divided by the size of the initial nanoparticles before spray freeze drying (S_i). A S_f/S_i value of 1-2 indicates good aqueous redispersibility.

The solubility of the excipient bridges depends on the formulation of these bridges. There are many inert excipients which are widely used in the pharmaceutical industry today due to their biocompatibility[8]. In this study, 7 of these excipients (lactose, trehalose, leucine, polyethylene glycol (PEG), polyvinyl alcohol (PVA), mannitol and pluronic) are selected and used to form the excipient bridges in order to investigate the effect of the excipients on the morphology and the aqueous redispersibility of each resulting aggregate. The desired aggregates should have a d_a of 2-4µm with a S_f/S_i value of 1-2.

MATERIALS AND METHODOLOGY

MATERIALS

Polycaprolactone (Sigma Aldrich 440744, M_n 70,000-90,000 by GPC), levofloxacin (Sigma 28266), poly(vinyl alcohol) (Aldrich 363170, M_n 13,000-23,000, 87-89% hydrolysed), and dichlorometane (Fisher Scientific D/1856/17, HPLC grade) are used in the preparation of antibiotic – loaded nanoparticles. The various excipients used are D-mannitol (Sigma Aldrich M9647, ACS reagent), L-leucine (Sigma Aldrich L8000), poly(vinyl alcohol), polyethylene glycol (Sigma P2139), pluronic F-68 (Sigma P1300), α-Lactose (Supelco 47287-U) and α,β-Trehalose (Sigma T0299).

PREPARATION OF NANOPARTICLES

An emulsification-solvent-evaporation method of Sung et al. (2009) is employed to prepare PCL nanoparticles loaded with levofloxacin (LEV). All mass measurements are used using a AS220/C/2 weigh balance (Radwag, Poland). 80mg of PCL and 8mg of LEV are dissolved in 2mL of dichloromethane. This is then poured into 6mL of aqueous 1% w/v PVA and the mixture is sonicated for 60s using a Vibra-Cell probe sonicator (VC 5040, Sonics and Materials, USA). An ice bath is used during the course of sonication to keep the solution cool. A resulting oil-in-water nano-emulsion is formed. This nano-emulsion is added to 10mL solution of aqueous 0.1% w/v PVA and stirred for 24 hours at room temperature to allow the DCM to evaporate. This transforms the nano-emulsion into a nanoparticulate suspension in which LEV is encapsulated inside the PCL matrix. After evaporation, the solution is centrifuged at 13,000rpm for 12 minutes to remove the non-encapsulated LEV and excess PVA. The resulting solution will contain approximately 4.8% w/v of PCL nanoparticles loaded with LEV. The PCL nanoparticles are mixed with excipients measured to the desired ratios by mass. The remaining volume is topped up to 7mL with deionized water.

SPRAY FREEZE DRYING

In this set-up, a 1L polypropylene beaker is used as a spraying chamber. The beaker contains 400mL of liquid nitrogen kept agitated at 500rpm. The nozzle of the two-fluid atomizer from the B-290 mini spray-dryer (BÜCHI Laboratory-Techniques, Switzerland) is positioned 10 cm above the initial level of the liquid nitrogen. During the process of the spray freeze drying, each feed solution is mixed with a magnetic stirrer to ensure a homogenous feed entering the atomizer. A peristaltic pump is used to drive the feed suspension into the atomizer at 4.5 mL/min. The atomization flow rate is set according to a desired value. After spraying about 7 ml of the feed suspension, the pump is stopped. The slurry of excess liquid nitrogen and frozen droplets is poured into a freeze-drying container, pre-chilled at 4 ^{o}C. A layer of HDPE film is used to cover the opening of the container after most of the liquid nitrogen has evaporated. A small opening in the film allows remaining liquid nitrogen to escape the container during freezing. The slurry is lyophilized for 24 h in a freeze dryer Model Alpha 1-2 LD plus (Christ, Germany) at -50 ^{o}C and 0.05 mbar. This process produces porous spherical dry-powder PCL nanoparticle aggregates.
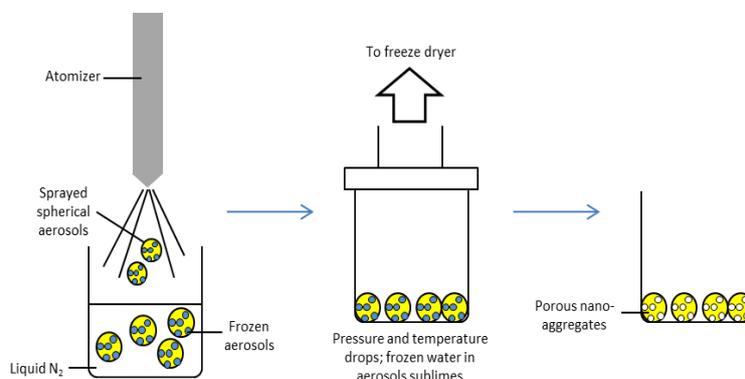
Fig. 3 Diagram showing how spray freeze drying works

ANALYSIS OF POWDER

The morphology of the SFD dry-powder nano-aggregates is characterized in terms of ρ_{eff}, d_g, and d_a. The bulk density (ρ_{bulk}) of the powder is calculated by obtaining the freely-settled volume of a weighted sample using a 5 ml graduated measuring cylinder. The powder is then tapped using a Quantachrome Autotap density meter. The tapped volume of the spray-freeze-dried particles is measured after 2000 taps to determine the tapped density (ρ_{tap}). ρ_{eff} is then taken to be a 21% underestimate of the tapped density[9], as shown in Eq. (2). A powder sample with lower effective particle density is likely to exhibit greater porosity. The shape and surface morphology of the powder was qualitatively observed using a Scanning Electron Microscope (SEM) model JSM-6700F (JEOL, USA). Prior to SEM, the powder sample is sputter coated with platinum for 60 s, 20 mA. The d_g is determined from the SEM images using the image processing software, Image J. A sample size of at least 1000 particles is used to obtain the average volume diameter of the SFD powder. The d_a of the particles can then be calculated according to Eq. (1).

$$\rho_{eff} = \frac{\rho_{tap}}{0.79} \qquad (2)$$

RESULTS AND DISCUSSION

In the initial run, each of the seven excipients was mixed individually with the PCL in a ratio of 1:1 up to a total solid concentration of 2.5% w/v. 7mL of this feed solution was fed into the atomizer at approximately 4.5mL/min and an atomization flow rate of 357L/h.

Sample	ρ_{eff} (g/cm3)	$d_g(\mu m)$	$d_a(\mu m)$	S_f/S_i
1.1 Lactose	NA	16.40	NA	33.42
1.2 Trehalose	NA	14.85	NA	18.50
1.3 Leucine	0.0190	19.02	2.62	8.86
1.4 PEG	0.0159	18.48	2.33	26.48
1.5 Pluronic	0.1008	14.59	4.63	2.53
1.6 Mannitol	0.0209	18.6	2.67	2.8
1.7 PVA	0.1263	13.89	4.93	2.02

On the overall, apart from pluronic, PVA, lactose and trehalose, the excipients produce particles with low densities and average d_g, which leads to excellent d_a well within the range of 2-4μm. This allows the particles produced to be efficiently delivered to the alveolar regions. In an aqueous medium, the nano-aggregates containing leucine and mannitol exhibit acceptable redispersibility characteristics, while nano-aggregates with PEG are largely not dispersible.

Lactose and trehalose are initially included into this round of testing because their high solubility (21.6g/100mL for lactose, 68.9g/100mL for trehalose) in water is thought to aid in re-dispersibility. However, they were extremely unstable, absorbing much moisture from the atmosphere and this causes the porous aggregates to collapse easily. Thus it is impossible to correctly measure the density of the powders. The collapsed aggregates exhibit poor redispersibility, as seen in the high S_f/S_i figure, probably because these collapsed structures have lower surface area to volume ratio, reducing the amount of particle wetting.

PVA and Pluronic have large d_a, making them both not suitable as excipients. However, PVA has the best aqueous redispersibility characteristics. Thus, it is still possible to include PVA into the formulation of the excipient bridges to aid in redispersibility in later tests. As such, only leucine, mannitol and PVA are used in the subsequent runs.

In the second run, mannitol and leucine are used to determine the morphology of the nanoparticle aggregates. The ratio of the PCL:excipient and mannitol:leucine for sample 2.1 are derived from past literature[11]. For the subsequent samples, the PCL:excipient ratio and the mannitol:leucine ratio are varied slightly to investigate the effect of these ratios on the morphology of the nano-aggregates formed. In this run, 7mL of 2.4% w/v solution is fed into the atomizer at an atomization flow rate of 357L/h.

Sample	PCL:excipient ratio	Mannitol:leucine ratio	ρ_{eff} (g/cm3)	d_g(µm)	d_a(µm)	S_f/S_i
2.1	1:7	6:1	0.0159	22.53	2.84	2.86
2.2	1:7	5:2	0.0100	28.43	2.85	2.81
2.3	2:6	6:1	0.0146	24.04	2.90	2.77
2.4	2:6	5:2	0.0122	27.25	3.00	3.45

All the nano-aggregate powders formed are light and not dense, and all powders are well within the desired d_a range of 2-4µm. Increased leucine concentration slightly decreases the density but increases the d_g considerably. In the end, these result in a negligible increase in d_a. At the same time, increasing the concentration of PCL nanoparticles results in slightly increased d_a of the nano-aggregates formed. Even though all the samples exhibit mediocre redispersibility properties, they are porous (Fig. 4), which increases the surface area to volume ratio. This leads to improved redispersibility results as compared to the individual excipients alone (i.e. samples 1.3 and 1.6).
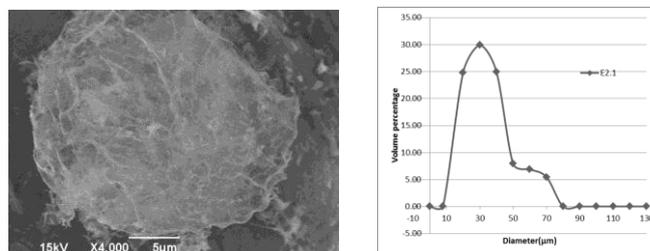

Fig. 4 A particle and size distribution curve from sample 2.1 (PCL:mannitol:leucine ratio of 1:6:1)

A noticeable characteristic with samples 2.1 to 2.4 is that they are extremely fragile and weak and easily broken. This increases the difficulty of analyzing the powder under SEM because many of the particles are already broken up. The particles produced are distributed across a large range of sizes (Fig. 4). A large particle size distribution is inefficient as the particles at the extremes of the size range do not have a suitable da and are hence not useful. A small mass per unit volume (i.e. a low density) may be an indication that the structure of these aggregates may be

weakened[12]. In order to obtain aggregates with a more robust structure, the density of the powders is increased in the next run. Thus, the total solid concentration is increased to increase the mass while the atomization flow rate is increased to decrease the volume of the aggregates.

In the third run, the PCL:excipient and mannitol:leucine ratios are fixed as with 2.1 and 2.2. However, the total solid concentration for 3.1 and 3.2 is increased to 4.0% w/v at an atomization flow rate of 414L/h, while the total solid concentration for 3.3 and 3.4 is further increased to 5.5% w/v with an increased atomization flow rate of 443L/h. With such changes, the effects on the morphology of the particles are quite distinct.

Sample	PCL:excipient ratio	Mannitol:leucine ratio	ρ_{eff} (g/cm3)	$d_g(\mu m)$	$d_a(\mu m)$	S_f/S_i
3.1	1:7	6:1	0.0198	13.30	1.87	1.22
3.2	1:7	5:2	0.0172	14.26	1.87	1.92
3.3	1:7	6:1	0.0351	15.47	2.90	1.31
3.4	1:7	5:2	0.0361	9.11	1.73	1.35

The resulting powders from 3.1, 3.2, 3.3 and 3.4 are very much denser than those from 2.1, 2.2, 2.3 and 2.4 respectively. The particles are much sturdier and resistant to shearing forces. The higher atomization flow rate has visibly decreased the d_g of the resulting powders. This is done to compensate for the increased density so that the d_a will not increase too much. However, despite the increase in density, the d_a of the particles are still below the target range. A lower atomization flow rate could perhaps be used to obtain particles with a larger d_g, which will result in particles with larger d_a.

The decreased d_g has improved redispersibility considerably as compared to samples 2.1-2.4. The particles formed are as porous as in samples 2.1 – 2.4 (Fig. 5), but with a smaller d_g, there is a greater surface area to volume ratio which improves the degree of particle wetting, in turn increasing the degree of redispersibility in aqueous media. At the same time, with denser particles, the size distribution is more narrow (Fig. 5). This means that a larger proportion of the particles fall within the desired range, making the process more efficient.
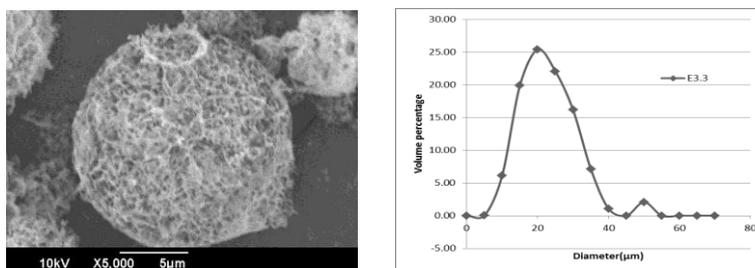

Fig. 5 A particle and size distribution curve from sample 3.1 (PCL:mannitol:leucine ratio of 1:6:1, total solid 5.5%, atomization flow rate 443L/h)

In the fourth run, the effects of PVA on the morphology of the powders are investigated. The ratios of PCL:excipients and PVA:leucine are shown below. The total solid concentration and the atomization flow rate is kept consistent with run 2 at 2.4% w/v and 357L/h respectively.

Sample	PCL:excipient ratio	PVA:leucine ratio	ρ_{eff} (g/cm3)	$d_g(\mu m)$	$d_a(\mu m)$	S_f/S_i
4.1	1:7	6:1	0.1268	14.39	5.13	1.34
4.2	1:7	5:2	0.0867	19.67	5.79	1.46

The powders generated from this run exhibit good redispersibility properties. This can be attributed to the use of PVA because PVA is a surfactant, and this may allow the nanoparticles to break apart more easily in an aqueous medium, hence aiding in redispersibility. Overall, the density of the powders has also increased from the previous runs. The use of PVA increases the density of the particles because it causes the powders to adhere more closely to each other, thus reducing effective volume. However, this increase in density increases the d_a of the powders to beyond the desired threshold. Thus, this powder is ineffective in drug delivery. To improve this, a higher atomization flow rate should be used to reduce the d_g which will lead to a lower d_a.

As compared to samples 2.1 and 2.2, which were produced under identical conditions, samples 4.1 and 4.2 showed distinctly greater density and smaller d_g. This is due to the use of PVA, which causes particles to adhere more closely to one another. Although, a surfactant like PVA improves redispersibility as compared to mannitol, it results in dense nano-aggregates, which are not as porous as the particles produced by mannitol (Fig. 6).
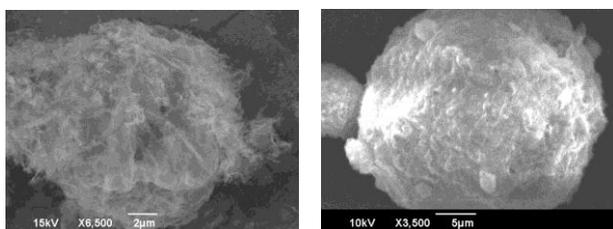

Fig 6 A particle from sample 2.2 (left) and 4.2 (right)
(PCL:mannitol:leucine ratio of 1:5:2 for sample 2.2, PCL:PVA:leucine ratio of 1:5:2 for sample 4.2, total solid 2.4%, atomization flow rate 357L/h)

In the fifth run, all three excipients, PVA, leucine and mannitol, are used. The conditions of this run is kept consistent with the rest of the other runs, at 2.4% w/v of total solid concentration and an atomization flow rate of 357L/h.

Sample	PCL:excipient ratio	PVA:leucine:mannitol ratio	ρ_{eff} (g/cm3)	$d_g(\mu m)$	$d_a(\mu m)$	S_f/S_i
5.1	1:7	1:2:4	0.0257	31.72	5.08	3.29
5.2	1:7	2.5:2:2.5	0.0749	22.01	6.02	1.58
5.3	1:7	4:2:1	0.0722	15.68	4.21	1.67

As expected, the redispersibility of the samples increases with the concentration of PVA but only to a certain point. This can be attributed to PVA's actions as a surfactant. This helps disperse the nanoparticles in an aqueous medium. When the powder is dry, however, the PVA makes the powder particles to adhere to each other more closely because of its stickiness. This clumping of particles may decrease the exposed surface of the powder (Fig 7), decreasing the redispersibility instead. On the overall, the d_g is rather large, which results in a large d_a.
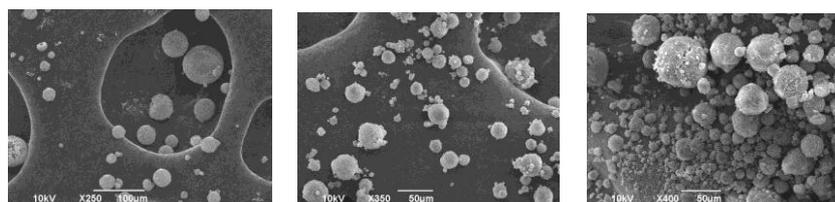

Fig 7 SEM photographs from samples 5.1, 5.2 and 5.3 (left to right). Note the increasing stickiness and clumping of the nano-aggregates owing to increased concentrations of PVA

As compared to samples 4.1 and 4.2, samples 5.1 – 5.3 are less dense and have generally larger d_g. As such, the d_a are slightly larger than in samples 4.1 and 4.2. To improve this, the atomization flow rate could be increased to

produce nano-aggregates with a smaller d_g. On the whole, particles from samples 5.1 – 5.3 are more porous than samples 4.1 and 4.2 (Fig 8). This is due to the addition of mannitol into the formulation
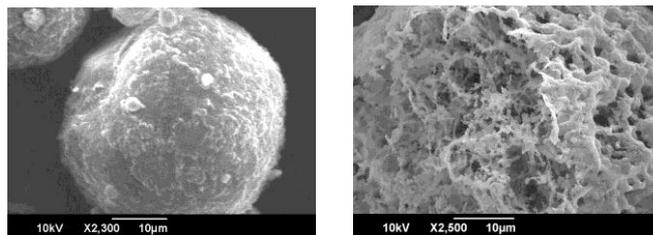

Fig 8 Particles from samples 4.1 and 5.1 (left to right)

In conclusion, from the five runs, it can be seen that lactose, trehalose, PEG and pluronic is not suitable to be used solely as excipient bridges. On the other hand, leucine, mannitol and PVA gives a few of the several desirable characteristics of the powder produced.

Leucine is mandatory in all the runs as it leads to the production of free-flowing particles instead of large chunky lumps[13]. It can be seen that an increase in mannitol concentrations increases the effective density of the powder marginally and also allow a smaller d_g of particles without affecting the density much. Consequently, a lower d_a is obtained. Mannitol also creates porous particles. On the other hand, increasing PVA concentrations improves aqueous redispersibility of the powders and produces particles with smaller d_g. However, the density of the particles increases considerably together with PVA concentrations, leading to a high d_a. PVA also tends to produce smooth-surfaced particles with low porosity.

CONCLUSION

This study concluded that lactose and trehalose are unsuitable for use as excipients due to their instability, while pluronic aggregates have high d_a and PEG aggregates are not redispersible and are thus unsuitable as well. Meanwhile, the effects of leucine, PVA and mannitol on the morphology of the PCL nanoparticular aggregates have been investigated in-depth. It is discovered that an increase in mannitol concentrations increases the density of the aggregates by a little, and increases the d_g slightly as well. Thus, it increases the d_a. Mannitol is not very good for redispersibility, but is crucial in producing porous particles. An increase in PVA concentrations increases the density of the aggregates significantly, but decreases the d_g a lot as well. As such, the effect on d_a is slight. PVA as a surfactant helps in aqueous redispersibility, but produces smooth-surfaced particles which are not porous. Meanwhile, leucine in required every time to ensure free-flowing particles.

This is useful for the planning of future experiments to optimize the production of such aggregates using the spray freeze drying technique. So far, the conditions producing the best results is 5.5% w/v of total solid concentration with atomization flow rate of 443L/h, and a PCL:mannitol:leucine ratio of 1:6:1. Though PVA helps in aqueous redispersibility, it is not crucial here because the high atomization flow rate leads to the production of particles with low d_g. This resulting high surface area to volume ratio promotes aqueous redispersibility. Of course, further work could be carried out on the total solid concentration of the feed solutions to vary the density, as well as on the atomization flow rate to vary the d_g of the aggregates, which will directly affect the d_a as well. The PCL:mannitol:leucine ratios can be further tweaked to improve the aqueous redispersibility as well.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Cho, K., Wang, X., Nie, S., Chen, Z., & Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research*, *14*, 1310-1316.

[2]   Gelperinak, S., Kisich, K., Iseman, M. D., & Heifets, L. (2005). The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. *American journal of respiratory and critical care medicine*, *172* (12), 1487-1490.

[3]   Gupta, R. B., & Kompella, U. B. (2006). *Nanoparticle technology for drug delivery*. United Kingdom: Taylor & Francis.

[4]   Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A (June 2004). Poly-epsilon-caprolactone microspheres and nanospheres: an overview. *Int J Pharm, 278* (1), 1–23.

[5]   Sung, J. C., Padilla, D. J., Garcia-Contreras, L., VerBerkmoes, J. L., Durbin, D., Peloquin, C. A., Elbert, K. J., Hickey, A. J., & Edwards, D. A. (2009). Formulation and pharmacokinetics of self-assembled rifampicin nanoparticle systems for pulmonary delivery. *Pharmaceutical Research*,*26*(8), 1847-1855.

[6]   Stockley, R. A. (2007). *Chronic obstructive pulmonary disease*. Oxford: Wiley-Blackwell.

[7]   Chen, W., & Fryrear, D. W. (2001). Aerodynamic and dgs of airborne particles. *Journal of Sedimentary Research*, *71*(3), 365-371.

[8]   Rowe, R. C., Sheskey, P. J., & Owen, S. C. (2006). *Handbook of pharmaceutical excipients*. APhA Publications.

[9]   Vanbever, R., Mintzes, J. D., Wang, J., Nice, J., Chen, D., Batycky, R., Langer, R., & Edwards, D. A. (1999). Formulation and physical characterisation of large porous particles for inhalation. *Pharmaceutical Research*, *16*(11), 1735-1742.

[10]   Kanig, J. L., Lachman, L., & Lieberman, H. A. (1986). *The Theory and Practice of Industrial Pharmacy* (3 ed.). Philadelphia: Lea & Febiger.

[11]   Kho, K., & Hadinoto, K. (2010). Effects of excipient formulation on the morphology and aqueous re-dispersibility of dry-powder silica nano-aggregates. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *359*(1-3), 71-81.

[12]   Ji, S., Gu Q., & Xia B. (2006). Porosity dependence of mechanical properties of solid materials. *Journal of Materials Science, 41*(6), 1757-1768.

[13] Kho, K., Cheow, W. S., Lie, R. H., & Hadinoto, K. (2010). Aqueous re-dispersibility of spray-dried antibiotic-loaded polycaprolactone nanoparticle aggregates for inhaled anti-biofilm therapy. *Powder Technology*, *203*(3), 432-439.