EVALUATION OF THE FLOW PROPERTIES OF POLLEN SHAPE HYDROXYAPATITE PARTICLES FOR DRY POWDER INHALATION

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Abstract

The use of pollen shape (spiked sphere) particles for dry powder inhalation is studied. Pollen shape hydroxyapatite (HA) particles are synthesized to cover both size ranges of typical drug and carrier particles for pulmonary drug delivery. The HA particles produced have geometric diameter range from 5-50 μm and effective density range from 0.2-0.72 g/cm^3. The flow properties of the powders are characterized by the Carr's compressibility index (CI). The aerodynamic properties of the particles are studied in vitro by using an eight-staged Anderson Cascade Impactor with a Rotahaler®. HA samples of different sizes are used as single formulation in the experiment. The HA particles are found to have lower CI values than commonly used lactose carrier particles with similar size range. The pollen shape particles also show high emitted dose and fine particle fraction and correlates well with the corresponding mean aerodynamic diameter.

Introduction

Pulmonary drug delivery is attracting interests as an alternative route of drug administration. Aerodynamic diameter is commonly used to characterize aerosol drug particles for inhalation delivery. Researchers showed that particles with an aerodynamic diameter (d_a) lower than 5 μm have the highest possibility to get deep into the lung[1]. However, shape irregularity affects drag force and terminal settling velocity of the particles. Therefore, a shape correction factor is needed for the estimation of their aerodynamic diameter, which eventually demonstrates their distinct aerodynamic behavior[2,3]. Researchers found an increase in respirable fraction by lowering the aerodynamic diameter of the particles through surface treatment and turn the particles into elongated shape[2,4]. Large geometric diameter and low density particles have also been used as a means of improving the delivery of inhaled therapeutics. Particles having large geometric diameter and low-density can have the same aerodynamic diameter as small and high density particles but the particle dispersion is better due to the lower van der Waals forces. Studies also showed that large geometric diameter and low density particles have the advantages of reduced effect of diffusion and reduced macrophage clearance for better bioavailability [5,6].

Another solution to improve pulmonary drug delivery is to mix drug particles with larger sized carrier particles. In that case, the morphology of these carrier particles has important effects on deposition. Zeng et al. [7] showed that increasing the elongation ratio of lactose carrier particles can increase the respirable fraction of drug from dry powder formulation for inhalation. Most researchers [8-10] found that increasing the surface roughness of the carrier particles can enhance the particle respirable fraction while Zeng et al. [7] found otherwise.

The literature results show that shape and surface features of drug/carrier particles can affect the efficiency of dry powder inhalation. However, systematic studies of these effects are limited. Theoretically, a pollen shape structure can attribute both the characteristics of large geometric diameter and low-density. The spiky surface structure will limit the approach of two particles, increase the effective separation and reduce their contact area and van der Waals force[11]. Therefore, pollen shape particles are expected to have good dispersion properties. In this study, pollen shape hydroxyapatite (HA) particles are synthesized with different size range. The particle properties are measured and the dispersion and deposition properties of these particles are studied in vitro using a cascade impactor. .

Experimental

Preparation for HA

The HA particles are prepared according to Wang et al.[12] by hydrothermal reaction at a temperature range of 100-200°C. Pollen shape HA particles are synthesized using KH_2PO_4, (Panreac), $Ca(NO_3)_2 \cdot 4H_2O$ (Sigma-Aldrich), PSS (poly(sodium-4-styrenesulfonate), 30 wt% solution in water, Sigma-Aldrich, MW = 70,000) and urea (Sigma-Aldrich). 30 ml of KH_2PO_4 (0.02 M) solution is mixed with 50 ml of Ca $(NO_3)_2.4H_2O$ (0.02 M) solution. Then PSS is added to the mixture to get a PSS concentration of 30-40 g/liter. Sufficient amount of urea is also added to get different urea concentration. This mixture is then kept for half an hour under gentle stirring. After the urea is dissolved in the liquid, the solution is poured into an autoclave and then kept in an oven for 6-12 hours for hydrothermal reaction. After the reaction, the precipitate is collected and washed few times with de-ionized water to remove unreacted reactants and then dried at 70°C for 24 hours.

Preparation of Lactose

α-Lactose monohydrate (Sigma) is used as the control carrier particles to compare against the performance of pollen shape HA particles. Lactose is separated into three different size ranges by sieving. The ranges are 38-75 μm, 20-38 μm and <20 μm. Sieves (Retsch, Haan, Germany) with a mesh size of 20 μm, 38 μm and 75 μm are used for the separation.

Particle characterization

The size distribution and surface morphology are examined by scanning electron microscopy (SEM) (JEOL JSM-5600) images. The SEM images are taken randomly from different area of the sample. The largest dimension of the particle is considered as the geometric diameter. At least two hundred particles are measured to get the mean diameter of the particles. The surface morphologies of the particles are assessed qualitatively based on the SEM images. The bulk (ρ_{bulk}) and tap (ρ_{tap}) densities of all the samples are measured according to Shi et al.[13]. The flow behavior of the samples are characterized by the Carr's compressibility index (CI) [9,14,15]. CI can be determined by:

$$CI = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \times 100\%$$

In Vitro dispersion and deposition study

The in vitro aerosol dispersion is determined using an eight staged Anderson cascade impactor (ACI) with a preseparator (Copley, UK) operating at an airflow rate of 30 liter/min.

The preseparator contained 8 ml of solvent (2% HNO_3) to prevent particle bounce and re-entrainment. The powder mixtures are aerosolized using a dry powder inhalation device (Rotahaler®, GSK, U.K.). 20mg of each sample is loaded into a hard gelatin capsule (Gelatin Embedding Capsules, size 4, 0.25 cc, Polysciences, Inc., Warrington, PA) manually. An actuation time of 20 seconds is allowed for each capsule for complete dispersion of all the particles. Experimental runs are conducted in triplicate. The emitted dose (ED) and fine particle fraction (FPF) are determined.

Results and Discussions

Physical properties of the HA particles

Pollen shape HA particles are prepared by a hydrothermal reaction. The size and morphology can be varied by controlling the PSS concentration, the urea concentration and the reaction temperature. The physical properties of different HA particles are shown in Table 1. The aerodynamic diameter is defined as the diameter of a sphere of unit density that has the same terminal settling velocity as the particle under consideration, $d_a = d_g\sqrt{\frac{\rho_e}{\lambda\rho_S}}$ where ρ_s= 1 g/cm^3, d_g is the particle geometric diameter, ρ_e is the effective particle density and λ is the particle shape factor [1,3,16]. The maximum dimension of a particle is considered as its geometric diameter. The effective density is approximated by the tap density, ρ_{tap}. The shape factor is defined as the ratio of the particle surface area to the surface area of a sphere with same volume as the particle. Since the pollen shape closely resembles a spherical particle, a shape factor of 1 is assumed.

Table 1. Physical properties of HA particles

Sample	d_{mean} (µm)	σ (µm)	ρ_{bulk} (g/cm^3) (n=0)	ρ_{tap} (g/cm^3) (n=2500)	$d_{a,theoretical}$ (µm)	CI (%)
HA-1	5.70	0.53	0.346	0.719	4.83	51.88
HA-2	13.1	1.7	0.217	0.357	8.56	39.16
HA-3	21.1	3.3	0.233	0.411	13.51	43.17
HA-4	48.55	10.21	0.215	0.289	26.64	18.72
HA-5	28.2	4.02	0.105	0.218	13.18	51.95

Table 2. Physical properties lactose samples

Sample	Size range (µm)	ρ_{bulk}(g/cm^3) (n=0)	ρ_{tap} (g/ cm^3) (n=2500)	CI (%)
Lactose1	< 20	0.217	0.645	57.98
Lactose2	20-38	0.316	0.760	58.42
Lactose3	38-75	0.586	0.874	32.95

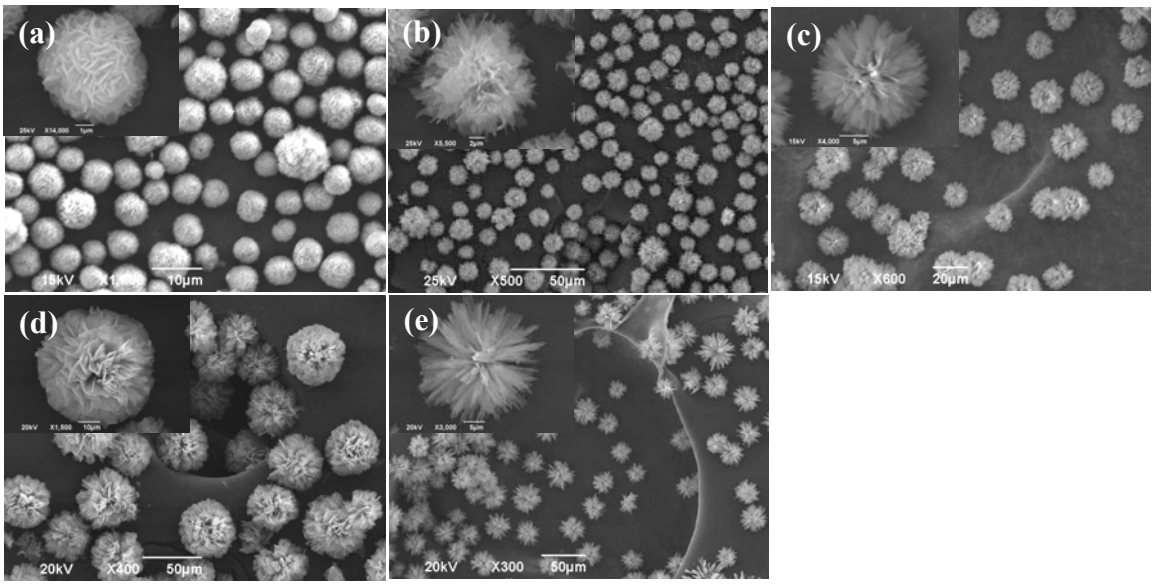


Figure 1. The SEM image of HA particles produced by using (a) PSS-40 g/liter & urea- 7.5M, at 200^0C (HA1); (b) PSS-40 g/liter & urea- 3M, at 200^0C (HA2); (c) PSS-40 g/liter & urea- 0.5M, at 150^0C (HA3); (d) PSS-40 g/liter& urea- 0.5M, at 100^0C (HA4); (e) PSS-30 g/liter & urea- 0.5M, at 200^0C (HA5).

Figure 1 shows the SEM images of the five HA samples synthesized in this study. It can be seen from the SEM images that with PSS concentration of 40 g/liter, particles with denser surface structure are produced. They comprise petal like surface structure whereas the particles produced with PSS 30 g/liter have needle like surface structure (HA5). At a temperature of 100 ^{o}C larger HA particles (HA4) are found. With higher urea concentration of 7.5 M and 3 M at 200 ^{o}C temperature, HA particles with smaller size ranges are generated (HA1, HA2). It can also be seen that the HA particles are well segregated which is very effective in dispersing them into air.

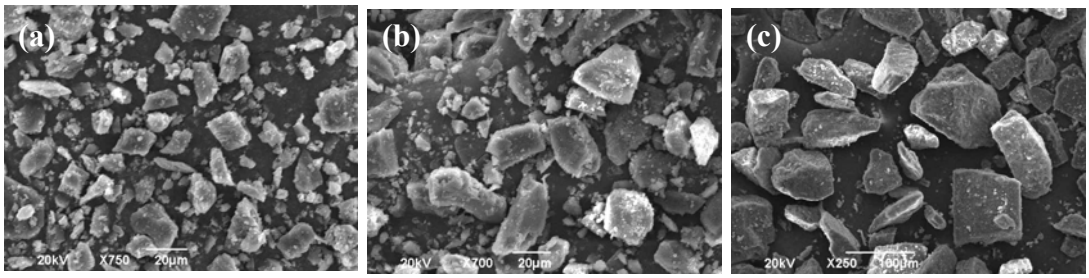


Figure 2. SEM images of lactose monohydrate with a size range of (a) less than 20 μm (lactose1); (b) 20-38 μm (lactose2); and (c) 38-75 μm (lactose3).

The flow behavior of the HA particles are compared with commonly used lactose particles of similar size range. The lactose particles are sieved into three different size ranges and their SEM images are shown in Figure 2. It is to note that sieving alone is not possible to completely separate all the smaller particles from the larger ones. A fraction of fine particles is present in the

samples. The fine particles may affect the flow behavior of the samples by forming large aggregates. Their physical properties are stated in Table 2.

Powder Flow

Carr's compressibility index (CI) is commonly used to characterize powder flow. CI value quantifies the powder flow based on the concept that compressible powders are more cohesive and would have poor flow property. Cohesive particles can form aggregates and through tapping, these aggregates may collapse. The overall volume of the particles will be reduced and the powder will appear to be compressible. Non-cohesive particles, on the other hand, will not collapse and the powder would appear to be incompressible. From Figure 3, it can be seen that, particles with lower size range (< 38 μm) have higher CI values. Interparticle forces are dominating in small particles and therefore these particles normally exhibit poor flow property. Nonetheless, HA particles still have lower CI than lactose particles in a similar size range. This can be attributed to the surface structure of the HA particles that the interparticle forces are weakened and less aggregates are formed. It can be seen from the SEM images that the HA samples (HA1, HA2 and HA3) are well segregated despite the small particle size.

A comparison of the different HA particles in a similar size range indicate that a petal like surface structure would give a lower CI than a needle like surface structure. The needle like surface may induce particle interlocking and cause particle aggregation. Therefore, the flow property of needle surface can be expected to be poorer than petal like surface.

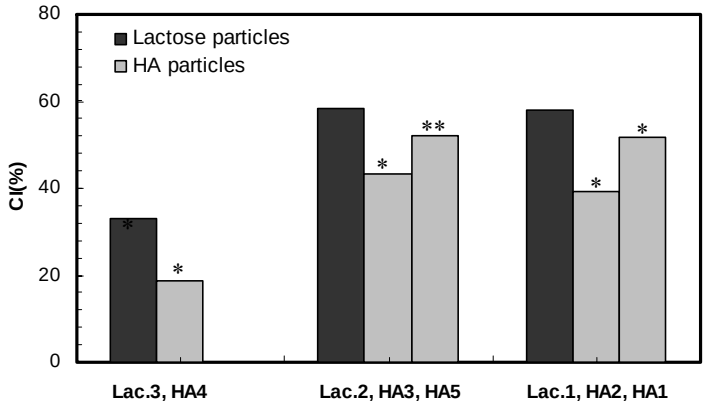
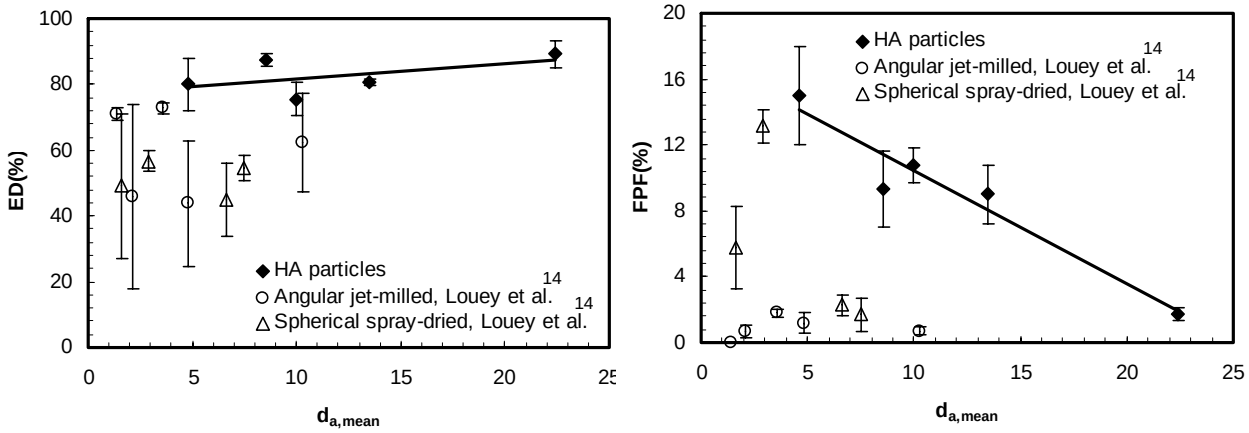


Figure 3. Comparison of the CI index of the pollen shape HA particles with lactose particles of similar size range. * = petal like surface structure, **= needle like surface structure.

In vitro deposition property of HA particles in single formulation

The deposition properties of the pollen shape HA particles are studied in terms of ED and FPF. ED is defined as the mass percentage of particles delivered from the inhaler. FPF is defined as the mass percentage of the particles deposited in stage 2 or lower in the cascade impactor. ED can be used as the index of aerosolization property. It can be seen from Figure 4(a) that the ED can correlates well with the mean aerodynamic diameter of the HA particles. There is limited dispersion and deposition study using single particle formulation in cascade impactor. The ED of the HA particles are compared with angular jet milled and spherical spray dried mannitol particles of various size and shape studied by Louey et al.[14]. The mannitol particles were aerosolized at an air flow rate of 60 liter/min whereas the flow rate used in this study is 30

liter/min. Despite the lower air flow rate, the HA particles show higher ED than the angular and spherical particles as shown in Figure 4(a). An increase in flow rate can substantially increase the total powder emission from an inhaler[17]. It is anticipated that the HA particles would exhibit even higher ED at a flow rate of 60 liter/min. Figure 4(b) shows a good correlation between the FPF and the mean HA aerodynamic diameter. The FPF of the HA particles is also compared against the FPF of the mannitol particles[14]. The improvement in FPF of HA particles over the FPF of the mannitol particles is even more obvious than the ED. It is apparent that pollen shape surface structure is highly effective in improving the ED and FPF of drug particles in inhalation delivery.



(a) (b)

Figure 4. Comparison of (a) ED and (b) FPF of the HA particles with mean aerodynamic diameter.

Concluding Remarks

Pollen shape HA particles with a geometric diameter range of 5 -50 μm are synthesized. The flow behavior of the particles is assessed with CI index. In comparison, the HA particles show better flow behavior than that of commonly used lactose carrier particles with similar size range. The cascade impactor experiment shows a good correlation of the ED and FPF with mean aerodynamic diameter of the HA particles. A comparison with literature results show that pollen shape surface structure is effective in improving the ED and FPF of drug particles in inhalation delivery.

Acknowledgement

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