International Journal of Pharmaceutics 430 (2012) 98–103

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Fabrication of inhalable spore like pharmaceutical particles for deep lung deposition

Zhi-Gang Shen[a,*], Wen-Hao Chen[b], Nital Jugade[b], Ling-Yan Gao[b], William Glover[b], Jin-Ye Shen[b], Jimmy Yun[b,**], Jian-Feng Chen[a]

[a] Sin-China Nano Technology Center, State Key Laboratory of Organic–Inorganic Composites, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] NanoMaterials Technology Pte Ltd, 28 Ayer Rajah Crescent #03-03, Singapore 139959, Singapore

ARTICLE INFO

Article history:
Received 27 December 2011
Received in revised form 15 February 2012
Accepted 25 March 2012
Available online 1 April 2012

Keywords:
Spore like particle
Dry powder inhalation
High gravity controlled precipitation

ABSTRACT

An innovative strategy of fabricating uniform spore like drug particles to improve pulmonary drug delivery efficiency was disclosed in the present study. Spore like particles were prepared through combination of high gravity controlled precipitation and spray drying process with insulin as model drug first, showing rough surface and hollow core. The shell of such spore-like particle was composed of nanoparticles in loose agglomerate and could form nanosuspension upon contacting antisolvent. Further characterization confirmed secondary structure and bio-activity was well preserved in spore like particles of insulin. Stable aerosol performance at different dosages with fine powder fraction (FPF) of 80% and comparable FPF (69–76%) for formulated powder were achieved, significantly higher than marketed product Exubera. On the other hand spore like particles of bovine serum albumin, lysozyme and salbutamol sulfate showed similar high FPF of 80%, regardless of different shape of primary nanoparticles, indicating various application of this new process in significant improvement of pulmonary drug delivery.

1. Introduction

Micro and nanometer scale structures present on biological surface of plant and animal show specific properties which inspire the researchers to craft inventions and open up new horizons for scientific research and applications (Sanchez et al., 2005). For instance, based on the study on the wettability of lotus leaves (Jiang et al., 2004) or insect organs (Gao and Jiang, 2004; Gao et al., 2007; Zheng et al., 2010), superhydrophobic or superhydrophilic material was developed for oil/water separation (Feng et al., 2004), filtration or textile applications (Sun et al., 2005). In current pulmonary drug delivery systems, a lung deposition ratio of only 5.5–40.5% is achieved (Newman and Busse, 2002). Therefore, investigation of novel strategies to improve lung drug deposition is pertinent. Our research to enhance lung deposition of drugs has taken clue from bioaerosols, such as spores and pollens, observed in nature (Supplementary Fig. S1). For example, puffball (*Calvatia excipuliformis*) spores have a deposition of 67.2% within the alveoli (Geiser et al., 2000). The high lung deposition could be attributed to small aerodynamic size of 2–3 μm and rough spherical surface of spores. Based on these findings we hypothesized that the spore like particles may achieve deep lung depositions. Therefore, it is worthwhile to fabricate drug particles similar to spores to improve pulmonary drug delivery efficiency particularly in dry powder inhalations (DPI).

To fabricate spore like drug particles for deep lung deposition, particle size and particle morphology should be fabricated precisely to achieve optimal aerodynamic performance. Compared to conventional particle size reduction methods such as milling and homogenization, spray drying of drug solution or emulsion to obtain uniform microparticles is simpler and more efficient (Okuyama et al., 2006; Vehring, 2008). Particle size, particle morphology and density have been reported to influence aerosol performance significantly (Adi et al., 2008; Crowder et al., 2002; Hassan and Lau, 2009). In this way Edwards et al. developed large porous particles (LPP) which have low mass density with geometric size greater than 5 μm and enter the lower respiratory tract due to small aerodynamic size (Edwards et al., 1997; Tsapis et al., 2002; Vanbever et al., 1999). However, the excipient-included LPP resulted in slow drug release in some cases (Dailey et al., 2003). Therefore, preparation of LPP formulations may not be suitable for drugs which require rapid onset of action. In this article, we demonstrate a "bottom-up" approach, spray drying of nanosuspension from high gravity controlled precipitation (HGCP), to prepare pure drug particles similar to spores with high fine powder fraction

* Corresponding author. Tel.: +86 10 64447274/+65 68734893; fax: +86 10 64423474/+65 68723372.
** Corresponding author. Tel.: +65 68730411; fax: +65 68723372.
E-mail addresses: shenzg@mail.buct.edu.cn (Z.-G. Shen), jimmy.yun@nanomt.com (J. Yun).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.03.044

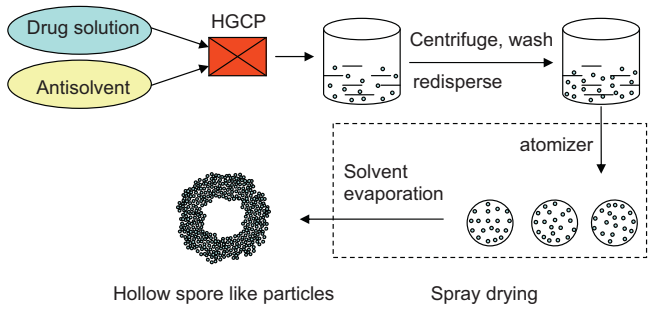

Fig. 1. Schematic of process for the preparation of spore like particles.

(FPF) (Fig. 1). HGCP in the form of a rotating packed bed (RPB) is a more efficient, cost-effective and easy to scale up method over milling, homogenization, spray freezing into liquid and supercritical antisolvent precipitation to prepare nanosuspension and has been employed to produce nanoparticles for various applications in recent years (Chen et al., 2004; Date and Patravale, 2004; Zhang et al., 2009). In the HGCP reactor drug solution is mixed with antisolvent in a high gravity environment to achieve micromixing and induce nanoprecipitation, thus nanosuspension with narrow size distribution could be obtained for subsequent spray drying so that uniform particles size and controlled morphology can be achieved.

2. Materials and methods

2.1. Materials and reagents

Human insulin (rDNA origin) was purchased from Biocon Ltd. Lactohale LH100 was purchased from Frisland food DOMO, the sieved fraction (63–90 μm) used to prepare the blended formulation powders. Salbutamol base was obtained from Vamsi labs. Bovine serum albumin (BSA) and lysozyme were obtained from Sigma Aldrich. Isopropyl alcohol (IPA, Tedia), concentrated hydrochloride (37%, HCl, Merck), sulfuric acid (2 M, Honeywell), deionized water (Fisher scientific) and ammonium acetate (99.8%, NH_4OAc, Riedel-de-hoen) were all of AR grade.

2.2. Preparation of spore like particles

1 g insulin raw was dissolved in 95 ml 0.01 M HCl (0.5 ml 37%HCl to 500 ml H_2O). 5 ml 20 mg/ml NH_4OAc was added to the above solution to obtain nearly saturated insulin solution. Lysozyme solution at 20 mg/ml was prepared in DI water including 50 mM NH_4OAc. BSA solution was prepared in the same conditions to lysozyme. The precipitation of above drug was conducted in the same way by mixing with IPA through HGCP. The drug solution and IPA was pumped into continuous HGCP at 30 ml/min and 270 ml/min, respectively, with RPB running at 30 Hz, milky slurry formed immediately and flowed out from the exit. The slurry was centrifuged at 5000 rpm for 5 min and the wet cake was washed by IPA twice to remove the impurity.

Preparation of salbutamol sulfate was different from the above method and aging step was introduced. 5 g salbutamol base was dissolved in 500 ml IPA at 60 °C with the aid of ultrasonication, then the solution was cooled to room temperature. The salbutamol base solution (10 mg/ml) was loaded into batch HGCP reactor, and 5 ml of 2 M sulfuric acid was introduced quickly into the HGCP reactor through inlet while the reactor was running at 50 Hz. After 20 min, the slurry was released and centrifuged at 5000 rpm for 10 min. The cake was washed with IPA twice to remove the impurities.

The above wet cake was redispersed in IPA at 1% which was kept under stirring and in ice bath during spray drying. The spray drying conditions were as follows: aspiration rate 100%, feed rate 4 ml/min, inlet and outlet temperature of 70 °C and 40 °C, respectively, atomization nitrogen flow rate 8 l/min. The collected powder was vacuum dried at 25 °C for 2 days.

2.3. Characterization of spore like particles

The particle size distribution (PSD) of the precipitated insulin slurry (without treatment after precipitation) and redispersion of insulin spore like particles in IPA (10 mg drug in 10 ml IPA by ultrasonic 1 min) was determined using dynamic light scattering particle size analyzer (DLS, LB-550, Horiba). The detailed parameters of DLS are: refractive index 1.6; refractive index of IPA: 1.378; result was expressed in volume.

For morphology observation, the samples were mounted to double-sided carbon tapes on aluminum sample stubs and Au sputtered at 35 mA for 1 min. The particle morphology was then viewed using scanning electron microscopy (S4200, Hitachi) at 10 kV. For the cross-sectional observation, the particle was cut using an ion beam.

Circular dichroism was performed to verify the structure stability of insulin particles. Raw insulin and spore like insulin powders were dissolved in 0.01 M HCl (5 mg/ml). 60 μl sample solution was loaded to the cuvette (0.1 mm path length) and was scanned using circular dichroism spectrometer (Jasco, J-710) in the wavelength range of 180–260 nm. The scans were obtained at bandwidth of 1 nm, step size of 0.5 and time per step of 3 s. Deconvolution of the spectrum was performed using CDNN software and the percentage of alpha-helix and random coil was given.

In the efficacy study, six female Wistar rats (Vital River Laboratory Animal Technology Co. Ltd, approximately 7–8 weeks of age and 150–250 g of body weight) were randomized and assigned to negative group (blank vehicle), raw insulin and spore like insulin group. Insulin injection solution (either control or HGCP insulin) was prepared to a 1 mg/ml solution using diluent. The diluent consists of 0.2 g phenol dissolved in 100 ml physiological saline water (<1.3% according to FDA) with pH adjusted to 2.5 using HCl. This stock solution can be diluted with the diluent to the desired concentration. The stock solution should be prepared fresh and used soonest possible.

Blood samples were collected from study rats at 4 time points (pre-dose and 30, 60, and 120 min post dose). The dosing solutions of insulin were administered to study rats via subcutaneous injection at 100 μg/kg. Clinical observations, mortality/moribundity and body weights of the animals were recorded according to the schedules. Each rat was food-fasted overnight prior to dosing and sample collection. Specifically, blood samples were collected from the jugular vein. Glucose (GLU) was analyzed using Hitachi 7080 chemistry analyzer. Comparisons of all data collected at each interval on body weights, and clinical pathology data (Glucose concentration) were performed using one-way analysis of variance (ANOVA) method, followed by Dunnett's method (Provantis™ 7.0.3, which is integrated with the industry standard SAS statistical analysis tool). The efficacy study was conducted in the immunology department, approved by the IAUCU of Vital Bridge which is licensed with accreditation number 001209 by AAALAC.

Other characterization methods could be referred in Supplementary data.

2.4. Aerosol performance of spore like particles and formulation

The aerosol performance of spore like particles of various compounds was investigated by loading approximately 10 mg pure drug powders into a HPMC size 3 capsule (Capsugel). The dispersion was performed with the Aeroliser dry powder inhaler (Plastiape) at 60 l/min for 4 s into a multistage liquid impinger (MSLI, Copley, UK) pre-loaded with 20 ml of solvent. For insulin

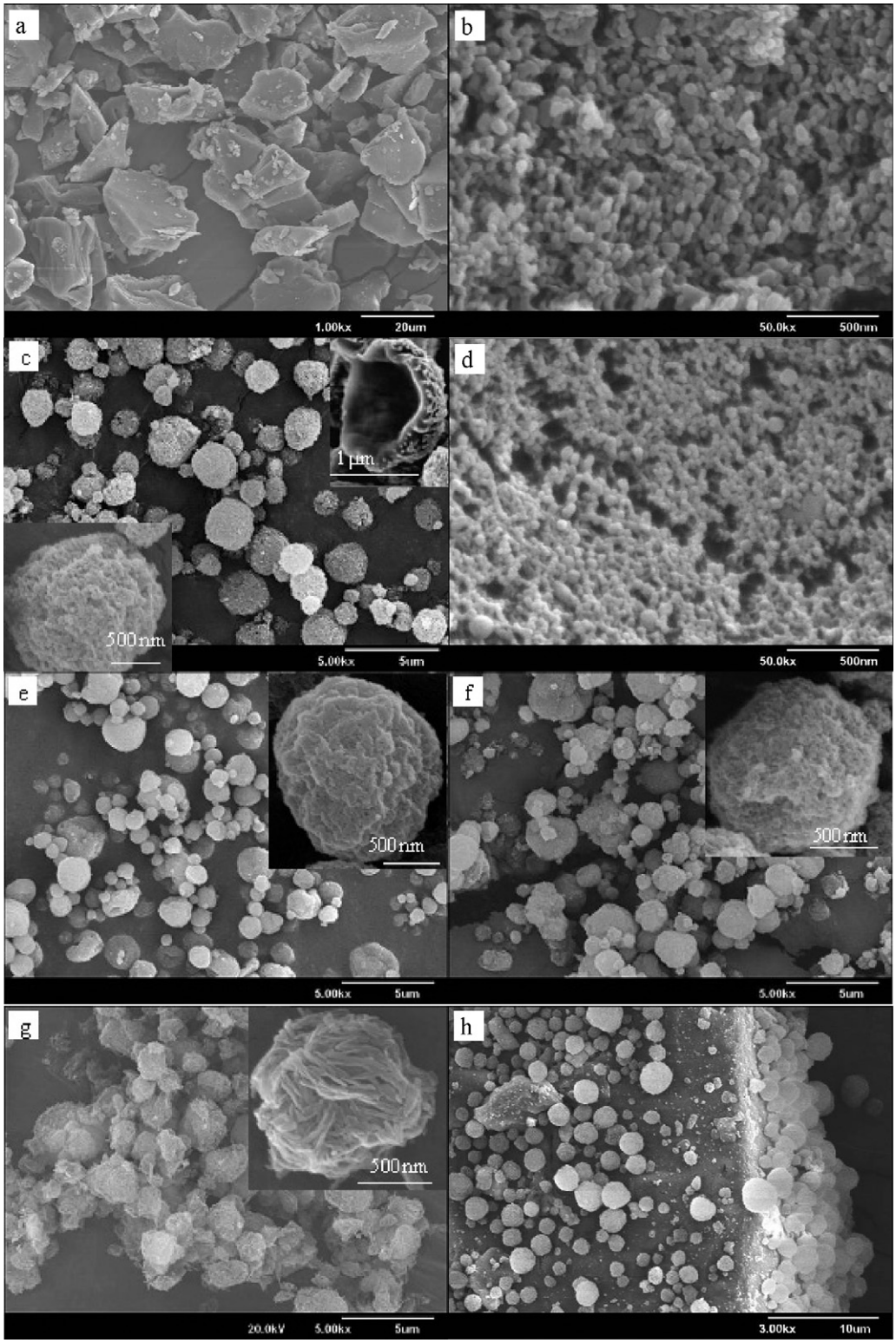

Fig. 2. SEM images of raw insulin (a), HGCP precipitated insulin slurry (b), spray dried spore like insulin particles (c), redispersion of spore like insulin particles (d), spray dried spore like lysozyme (e), spore like BSA (f), spore like salbutamol sulfate (g) and formulated spore like insulin with lactose (h).

or lysozyme, the solvent is 0.01 M HCl. For BSA or salbutamol sulfate, water is used. Drug deposition in the capsule, inhaler, throat, four stages and the filter was extracted with certain solvent, for insulin or lysozyme using 0.01 M HCl and for BSA or salbutamol sulfate using water, respectively, and analyzed using UV absorption at 276 nm (UV2450, Shimadzu). Standard curve of insulin $A_{276} = 0.9512^{*}C\ (mg/ml) - 0.003$, $R = 0.9999$; Lysozyme $A_{276} = 2.5721^{*}C\ (mg/ml) - 0.0081$, $R = 0.9995$; BSA $A_{276} = 0.6174^{*}C\ (mg/ml) + 0.0068$, $R = 0.9999$; Salbutamol sulfate $A_{276} = 5.5288^{*}C\ (mg/ml) + 0.0207$, $R = 0.9991$. FPF is defined as the fraction of drug particles in stage 3, stage 4 and filter relative to the total recovered drug mass.

The effects of capsule size (size 3, 4) on aerosol performance of insulin particles and formulated powder at different dosages (1 mg, 3 mg) were investigated further. The formulated powder was prepared by blending 1 g spore like insulin particles with 9 g lactose (63–90 μm) sieved from lactohale LH100 in a glass bottle by an Inversina Powder Mixer for 1 h at the speed of 40 rpm. 10 mg of the formulated powder was weighed out to check its blend uniformity (relative to labeled content). Insulin deposition at each stage was analyzed by HPLC purity method. Each test was performed in triplicate.

2.5. Stability investigation

Pure spore like insulin particles and raw insulin sealed in glass bottle were stored at 25 °C/60% RH (RT) and fridge (4–8 °C), respectively. The chemical stability including purity, degradation and aggregation, as well as the dispersion performance were investigated up to 3 months with raw insulin as reference. Each test was performed in triplicate.

3. Results and discussion

3.1. Preparation and characterization of spore like particles

We began our work with insulin as model drug. Uniform insulin nanosuspension with mean particle size of 200 nm was obtained subsequent to precipitation through HGCP (Figs. 2b and 3a). However, post spray drying, it was evident that insulin nanoparticles contributed to form the rough surface of spherical micron size spore like particles with hollow core (Fig. 2c, inset). Rough surface is advantageous for the particles to release and form fine aerosol as it is thought to lower adhesive forces and decrease inter particular agglomeration (Tang et al., 2004). In addition, the hollow core of spore like insulin particles resulted in lower particle density and improved aerosol performance (Edwards et al., 1997). The effective particle density was 0.36 g/cm^3, calculated from tap density measurement (Tsapis et al., 2002), which was lower than particle density 0.78 g/cm^3 of the spores (*C. excipuliformis*) (Geiser et al., 2000). Therefore higher lung deposition compared to natural spores (*C. excipuliformis*) can be anticipated. The rough shell was composed of layers of nanoparticles, thicker than LPP. Thus, spore like insulin particle had greater structural integrity and uniform redispersion with size and morphology similar to that of precipitated nanoparticles was observed when the particles were dispersed in IPA (Figs. 2d and 3a). It indicated that insulin spore like particles were in the form of loose agglomerates rather than hard aggregation after spray drying, which were locked by van der Waals forces (Velev et al., 1996). Specific surface area (SSA) of 116 m^2/g suggests that nanoparticles were in loose agglomerates. This could be advantageous since fast dissolution and rapid action could be achieved in pulmonary drug delivery.

Powder characterization was performed using X-ray powder diffraction (XRD, peak pattern), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and Fourier transform Infrared spectroscopy (FTIR) (Supplementary Fig. S2a–d). No significant differences were observed between raw insulin and spore like insulin particles. Hence, high resolution XRD analysis was used. The raw insulin showed ring patterns (Supplementary Fig. S2e), which rely on the crystal size and crystallinity of particles (Lim et al., 2009). In contrast, ring like patterns were not observed in spore like insulin particles (Supplementary Fig. S2f). This confirms the amorphous nature of the spore like insulin. Subsequent to HGCP and spray drying process, spore like insulin had purity of 99%, with A21 deamidation (0.45%) and aggregation

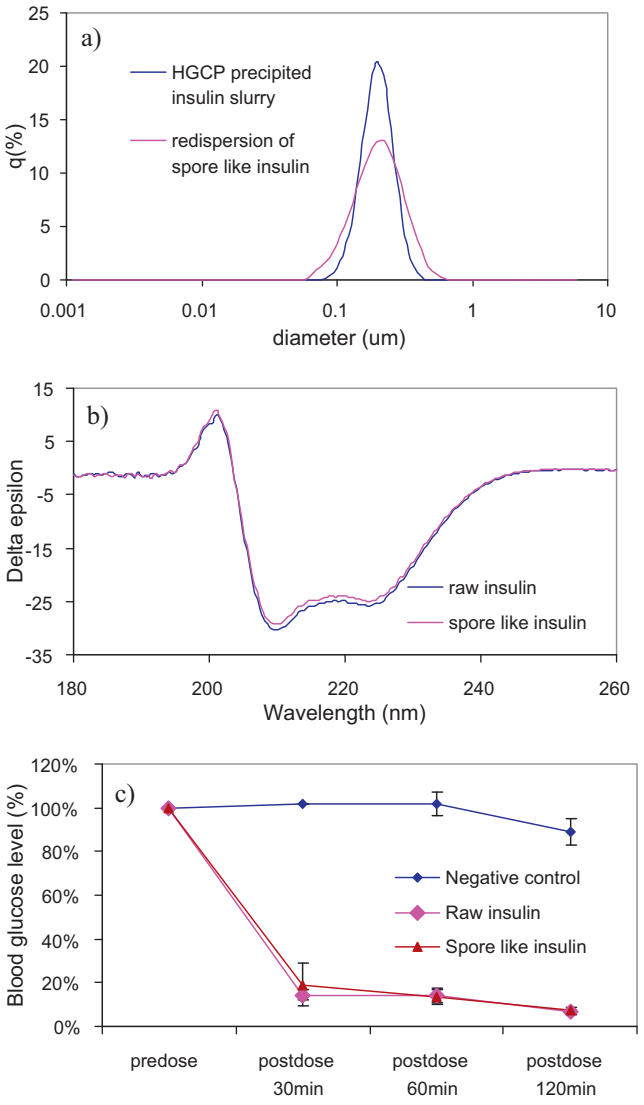

Fig. 3. (a) Particle size distribution of HGCP precipitated insulin slurry and redispersion of spore like insulin. (b) Circular dichroism spectrums of raw insulin and spore like insulin. (c) Serum glucose level at different post-dose time point after administration of insulin raw and spore like insulin in rats ($n = 2$).

(0.59%) was achieved. The spore like insulin particles were within the USP limits (Supplementary Table S1). Furthermore, circular dichroism analysis showed similar α-helix and random coil (97.2% and 0.6%, 96.8% and 0.8% for raw insulin and spray dried powders, respectively) indicating the secondary structure of insulin was retained (Fig. 3b). IPA residue in spore like insulin particles was 0.06%, which was within the USP limit of 0.5%. A simple efficacy test in Wistar rats showed that spore like insulin decreased serum glucose level to the same extent as raw insulin, which indicates that efficacy was preserved well in the spore like insulin particles (Fig. 3c). Based on the above characterization, it can be concluded that spore like insulin particles were prepared using our methodology without loss of chemical purity, stereoscopic structure and efficacy.

3.2. Aerosol performance and stability

The aerosol dispersion test of spore like insulin was performed via monodose DPI inhaler (Plastiape) through piercing the drug loaded capsule. It was found that dosage (1–10 mg) and capsule size (size 3 or 4) did not influence the FPF and deposition at

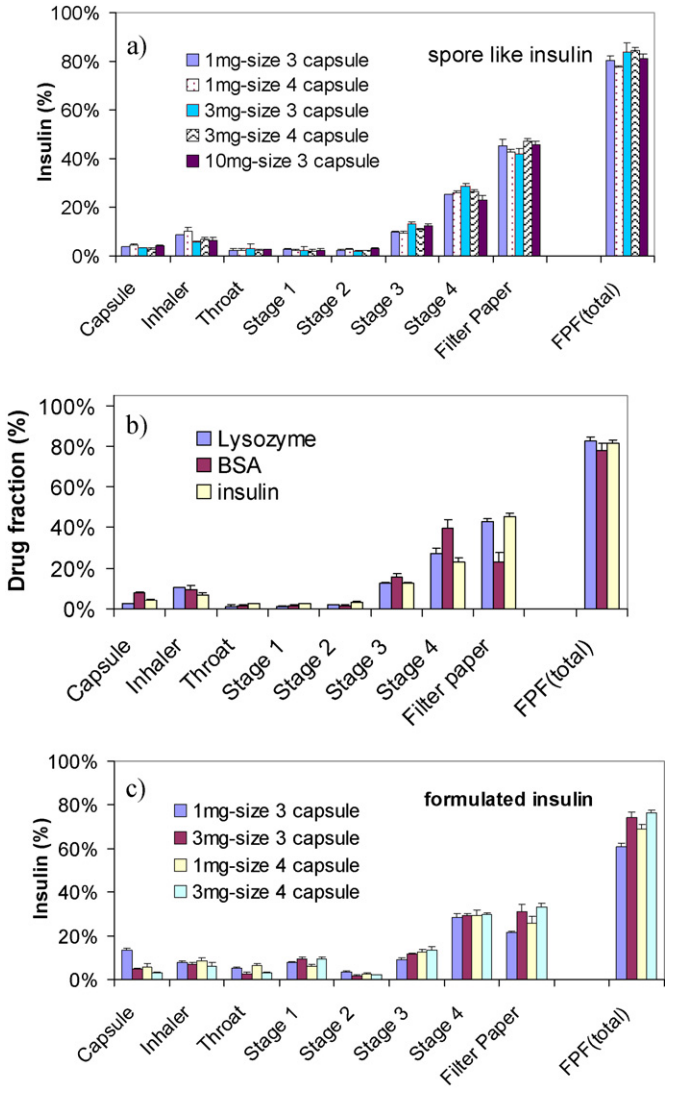

Fig. 4. (a) Effects of dosage and capsule size on the aerosol dispersion of spore like insulin ($n = 3$). (b) Aerosol performance of spore like lysozyme, BSA and salbutamol sulfate particles ($n = 3$). (c) Effects of dosage and capsule size on the aerosol performance of insulin DPI formulation ($n = 3$).

each stage significantly when tested at 60 l/min (Fig. 4a). Spore like insulin particles could achieve FPF above 80%, with mass mean aerodynamic diameter (MMAD) about 1.6 μm. During stability investigations, spore like insulin particles sealed in glass bottle were found to be stable for 3 months at room temperature (Supplementary Table S1). It was evident that amorphous spore like insulin was more stable than the crystalline raw insulin, as indicated by lower amount of A21 deamidation and aggregation. This was in agreement with Pikal et al.'s observation for insulin particles (Pikal and Rigsbee, 1997). Spore like insulin particle did not show any significant change in aerosol performance when stored at room temperature or in refrigerator for 3 months (Supplementary Fig. S3).

The fabrication strategy of spore like particles could be applied to other pharmaceutically active molecules, such as lysozyme, bovine serum albumin and salbutamol sulfate (Fig. 2e–g). As shown in Fig. 4b, high FPF of above 80% was achieved in different spore like particles produced by our methodology, regardless of morphology of primary nanoparticles. Previously salbutamol sulfate spherical agglomerates composed of mixture of nano and microparticles with FPF between 43 and 54% (Chiou et al., 2007) were reported. It is clear that uniform nanoparticles are the key to achieve high FPF.

3.3. Formulation development and evaluation

Although, it is possible to regulate and load as low as 1 mg of pure drug into capsules or blisters during industrial production, carrier is often necessary at such lower dosage. In the following trials, insulin powder was selected as model drug to be blended with lactose carrier to check dosage flexibility. Blending with sieved lactose (63–90 μm, from lactohale LH-100) in the ratio of 1:9 in the mixer for 1 h, uniform formulation could be obtained with content uniformity of 93.87% ± 3.53% ($n = 10$). From SEM observations, it was found that spore like insulin particles adhered on to surface of lactose. No obvious powder fragments indicated that the shell of insulin powders was strong enough to withstand attrition in mixing process (Fig. 2h). Formulated powder equivalent to 1 mg or 3 mg of insulin was loaded into capsule (HPMC, size 3 or 4) to compare the aerosol performance with pure spore like insulin. As shown in Fig. 4c, no significant difference in FPF was observed in size 3 and 4 capsule for 3 mg dosage. FPF of 74% and 76% was observed for formulated powders, compared to 84% noted for spore like insulin (Fig. 4a). Although, lower FPF was observed at 1 mg dosage, FPF might be improved in smaller capsule. Formulation in size 4 capsules reached FPF of 69%. The FPF performance of formulated powder of spore like insulin was much better as compared to the marketed insulin formulation Exubera®, FPF was only 33% and 45% for 1 mg and 3 mg, respectively (White et al., 2005).

In our process, uniform nanosuspension was obtained through HGCP which later formed spore like particles on spray drying. The process is simple, efficient and easy to scale up, which offers uniform particle size and morphology. Spray drying step in the process contributes to rough surface, hollow core and low density of powder. Compared to LPP (Edwards et al., 1997) or powders obtained from direct spray drying of drug solutions (Adi et al., 2008), rough shell of spore like particles comprises of multiple layers of nanoparticles. Multilayer structures of spore like particles offer several advantages particularly they are stronger compared to LPP hence withstand blending process. Rough surface was reported to decrease adhesion forces thereby decreasing inter particular agglomeration, allow particles to be easily released from the inhaler or the carrier and form aerosol with high FPF (Adi et al., 2008; Tang et al., 2004). Low particle density was found to decrease the aerodynamic diameter of particles and improve aerosol performance (Edwards et al., 1997). The above mentioned characteristics of spore like particle might have resulted in high FPF and comparable aerosol performance in formulated powder after blended with lactose carrier. In our process, the precipitated slurry was centrifuged; particles were then washed and redispersed in IPA prior to spray drying rather than directly spray dried, to minimize the effect of dissolved or un-precipitated drug. It is reported that the dissolved drug fraction may cause stability concerns, such as recrystallization or protein degradation and aggregation (Maltesen et al., 2008) in powder produced from direct spray drying drug solution. No significant bridging-effect existed during spray drying due to the absence of dissolved drug. Further the nanoparticles constitute the shell of spore like particles in loose agglomerate and formed nanosuspension when contacted anti-solvents (Fig. 2d). It is assumed that such distinct advantage of spore like particles may lead to less macrophage clearance (Todoroff and Vanvever, 2011), fast dissolution and rapid action if applied in vivo. With respect to high FPF of 80% in spore like particles of various drugs (Fig. 4b) and well-preserved bio-activity in spore like insulin, our process provides a simple and efficient way for pulmonary drug delivery especially for therapeutic proteins.

4. Conclusion

In conclusion, we have presented a strategy of fabricating inhalable spore like particles through combined HGCP and spray drying process. This method supports the large scale production of spore like particles. The shell of hollow spore like particles composed of nanoparticles which are in loose agglomerates thus possibly have better therapeutic effects. The major advantages of such spore like particles include high FPF of 80%, small MMAD of 1.6 μm and stable aerosol performance at different dosages. In addition, flexible dosage can be achieved by blending with lactose carrier, with desirable FPF comparable to pure spore like drug particles. In summary, spore like particles achieved through HGCP and spray drying offers significant improvement in pulmonary drug delivery.

Acknowledgments

This work was partially supported by National Natural Science Foundation of China (No. 20976008) and the Talent Training Program of Beijing City (2007B022).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2012.03.044.

References

Adi, H., Traini, D., Chan, H.K., Young, P.M., 2008. The influence of drug morphology on aerosolisation efficiency of dry powder inhaler formulations. J. Pharm. Sci. 97, 2780–2788.

Chen, J.F., Zhou, M.Y., Shao, L., Wang, Y.Y., Yun, J., Chew, N.Y., Chan, H.K., 2004. Feasibility of preparing nanodrugs by high-gravity reactive precipitation. Int. J. Pharm. 269, 267–274.

Chiou, H., Li, L., Hu, T., Chan, H.K., Chen, J.F., Yun, J., 2007. Production of salbutamol sulfate for inhalation by high-gravity controlled antisolvent precipitation. Int. J. Pharm. 331, 93–98.

Crowder, T.M., Rosati, J.A., Schroeter, J.D., Hickey, A.J., Martonen, T.B., 2002. Fundamental effects of particle morphology on lung delivery: predictions of Stokes' law and the particular relevance to dry powder inhaler formulation and development. Pharm. Res. 19, 239–245.

Dailey, L.A., Kleemann, E., Wittmar, M., Gessler, T., Schmehl, T., Roberts, C., Seeger, W., Kissel, T., 2003. Surfactant free, biodegradable nanoparticles for aerosol therapy based on the branched polyesters DEAPA-PVAL-g-PLGA. Pharm. Res. 20, 2011–2020.

Date, A.A., Patravale, V.B., 2004. Current strategies for engineering drug nanoparticles. Curr. Opin. Colloid Interface Sci. 9, 222–235.

Edwards, D.A., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M.L., Mintzes, J., Deaver, D., Lotan, N., Langer, R., 1997. Large porous particles for pulmonary drug delivery. Science 276, 1868–1871.

Feng, L., Zhang, Z., Mai, Z., Ma, Y., Liu, B., Jiang, L., Zhu, D., 2004. A super-hydrophobic and super-oleophilic coating mesh film for the separation of oil and water. Angew. Chem. Int. Ed. 43, 2012–2014.

Gao, X.F., Yan, X., Yao, X., Xu, L., Zhang, K., Zhang, J.H., 2007. The dry-style antifogging properties of mosquito compound eyes and artificial analogues prepared by soft lithography. Adv. Mater. 19, 2213–2217.

Gao, X.F., Jiang, L., 2004. Water-repellent legs of water striders. Nature 432, 36.

Geiser, M., Leupin, N., Maye, I., Im Hof, V., Gehr, P., 2000. Interaction of fungal spores with the lungs: distribution and retention of inhaled puffball (*Calvatia excipuliformis*) spores. J. Allergy Clin. Immunol. 106, 92–100.

Hassan, M.S., Lau, R.W., 2009. Effect of particle shape on dry particle inhalation: study of flowability, aerosolization, and deposition properties. AAPS Pharm. Sci. Tech. 10, 1252–1262.

Jiang, L., Zhao, Y., Zhai, J., 2004. A lotus-leaf-like superhydrophobic surface: a porous microsphere/nanofiber composite film prepared by electrohydrodynamics. Angew. Chem. Int. Ed. 43, 4338–4341.

Lim, S.H., Park, H.W., Shin, C.H., Kwon, J.H., Kim, C.W., 2009. Human insulin microcrystals with lactose carriers for pulmonary delivery. Biosci. Biotechnol. Biochem. 73, 2576–2582.

Maltesen, M.J., Bjerregaard, S., Hovgaard, L., Havelund, S., van de Weert, M., 2008. Quality by design-spray drying of insulin intended for inhalation. Eur. J. Pharm. Biopharm. 70, 828–838.

Newman, S.P., Busse, W., 2002. Evolution of dry powder inhaler design, formulation and performance. Respir. Med. 96, 293–304.

Okuyama, K., Abdullah, M., Wuled Lenggoro, I., Iskandar, F., 2006. Preparation of functional nanostructured particles by spray drying. Adv. Powder Technol. 17, 587–611.

Pikal, M.J., Rigsbee, D.R., 1997. The stability of insulin in crystalline and amorphous solids: observation of greater stability for the amorphous form. Pharm. Res. 14, 1379–1387.

Sanchez, C., Arribart, H., Guille, M.M., 2005. Biomimetism and bioinspiration as tools for the design of innovative materials and systems. Nat. Mater. 4, 277–288.

Sun, T.L., Feng, L., Gao, X.F., Jiang, L., 2005. Bioinspired surfaces with special wettability. Acc. Chem. Res. 38, 644–652.

Tang, P., Chan, H.K., Raper, J.A., 2004. Prediction of aerodynamic diameter of particles with rough surfaces. Powder Technol. 147, 64–78.

Todoroff, J., Vanvever, R., 2011. Fate of nanomedicines in the lungs. Curr. Opin. Colloid Interface Sci. 16, 246–254.

Tsapis, N., Bennett, D., Jackson, B., Weitz, D.A., Edwards, D.A., 2002. Trojan particles. Low porous carriers of nanoparticles for drug delivery. Proc. Natl. Acad. Sci. U.S.A. 99, 12001–12005.

Vanbever, R., Mintzes, J.D., Wang, J., Nice, J., Chen, D.H., Batycky, R., Langer, R., Edwards, D.A., 1999. Formulation and physical characterization of large porous particles for inhalation. Pharm. Res. 16, 1735–1742.

Vehring, R., 2008. Pharmaceutical particle engineering via spray drying. Pharm. Res. 25, 999–1020.

Velev, O.D., Furusawa, K., Nagayama, K., 1996. Assembly of latex particles by using emulsion droplets as templates. 1. Microstructured hollow spheres. Langmuir 12, 2374–2384.

White, S., Bennett, D.B., Cheu, S., Conley, P.W., Guzek, D.B., Gray, S., Howard, J., Malcolmson, R., Parker, J.M., Roters, P., Sadrzadeh, N., Schumacher, J.D., Seshadri, S., Sluggett, G.W., Stevenson, C.L., Harper, N.J., 2005. EXUBERA: pharmaceutical development of a novel product for pulmonary delivery of insulin. Diabetes Technol. Ther. 7, 896–906.

Zhang, H.X., Zhao, H., Wang, J.X., Chen, J.F., Lu, Y.F., Yun, J., 2009. Facile preparation of monodisperse pharmaceutical colloidal spheres of atorvastatin calcium via self-assembly. Small 16, 1846–1849.

Zheng, Y., Bai, H., Huang, Z., Tian, X., Nie, F., Zhao, Y., Zhai, J., Jiang, L., 2010. Directional water collection on wetted spider silk. Nature 463, 640–643.