Optimization and Characterization of Dry Powder of Fanhuncaoin for Inhalation Based on Selection of Excipients

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In this study, dry powder formulations for inhalation of fanhuncaoin, a newly discovered antiinflammatorily active compound isolated from Chinese herb, were designed to optimize the composition and further explore the relationship between the composition, the physical properties and the aerosolization performance. Dry powders were prepared by spray-drying using leucine, chitosan, chitosan oligosaccharide and dipalmityl phosphatidylcholine (DPPC) as excipients. Following spray-drying, resultant powders were characterized using scanning electron microscopy, tapped density analysis, laser diffractometry, thermogravimetric analysis and differential scanning calorimetry. The aerosol behaviour of the powders was studied in a Twin Stage Impinger at an airflow rate of 60 l/min using a HandiHaler® inhaler device. Results revealed that the nature and the relative proportion of the excipients greatly influenced the physical characteristics of the powders and their aerodynamic behavior. Among the combinations tested, the composition ratio of fanhuncaoin/leucine/chitosan/chitosan oligosaccharide/DPPC of 10/45/33.75/11.25/0.4 (w/w/w/w/w) prepared in a total solid mass of 1% (w/v) formulation was found to be particularly optimal and exhibited a tapped density of 0.44 g/cm³, an aerodynamic diameter of 2.24 μm and an respirable fraction of 51.29%. In conclusion, optimization of the aerosolization properties of inhalation dry powders could be achieved by appropriately selecting the composition of the particles.

Key words inhalation; optimization; spray-drying; excipient; chitosan; chitosan oligosaccharide

Drug delivery to the lungs is highly desirable, especially in patients with specific pulmonary diseases such as chronic pulmonary infections. It can reduce systemic side effects and increase doses of the applicable medication at the site of drug action. It’s also a good idea to choose inhalation for systemic therapies because the respiratory region of the lung has an enormous surface area (80—100 m²/adult), a highly permeable membrane (0.1—0.2 μm) for the absorption of medication into the blood and a blood flow about 5 l/min, which rapidly transports molecules throughout the body.1) Large protein molecules, which degrade in the terrible gastrointestinal conditions, can be delivered via the pulmonary route. In addition, it is a needle-free delivery system capable of increasing the compliance of patients.

The 1—5 μm aerodynamic diameter range is needed for particles to be inhaled in order to avoid deposition in the oropharynx and maximize deposition in the lower respiratory tract.2) Generally, drugs for inhalation in dry powder inhaler (DPI) systems are micronized to achieve the requisite size; however, particles in this size range show strong interparticulate cohesion, leading to poor powder flow properties.3,4) In addition, factors that are known to influence the aerosolization properties of dry powders, such as particle morphology, density and surface composition, can not be well controlled by the micronization process.5)

Spray drying, an alternative approach to the generation of potentially respirable powders with respect to local pulmonary drug delivery, offers advantages to manipulate and control a variety of parameters such as temperature and relative humidity, solute concentration, solvent composition, solution and gas feed rate, droplet size, etc. This allows optimization of particle characteristics such as particle size, size distribution, density and morphology, and then macroscopic powder properties such as bulk density, flowability and dispersibility.6)

Senecio cannabifolius Less. (Kuan Ye Fan Hun Cao in Chinese), as one species of Senecio genus of Compositae family, shows the effects on dispelling ecchymoma, anastalisis and algesic. As one kind of folk medicine, it’s usually used to treat the diseases, such as virus influenza and many kinds of inflammatories. Unfortunately, In 2004, the importation of many kinds of preparations made from the species of Senecio genus from China had been prohibited by American and Europe drug administrative departments, because of the existence of one of its chemical constituents named pyrrolizidine alkaloids, which exhibited the chronic hepatotoxicity (liver toxicity) and carcinogenic possibility to the human beings. In order to deal with the contradiction between the toxicity and efficiency on the treatment, 52 compounds were obtained from the herb by many kinds of isolation method, such as silica gel chromatography, Lobar column, semi-preparative HPLC (PHPLC) and so on. Among the 52 compounds, 14 of them were isolated from the Senecio genus for the first time. The general pharmacological studies and the tests on acute toxicity and chronic toxicity indicated that the high, medium and low dose of several compounds had no influence on the central nervous system, cardiovascular, respiratory system and showed no toxicity.7—9) Fanhuncaoin, a discovered antiinflammatorily active pheno-lic acid isolated from the water extract of the aerial parts of Kuan Ye Fan Hun Cao, which exhibited the best result in studies was chosen to act as a model drug in our dry powder inhalation for the first time.

The primary function of the lungs is respiration. Many compounds that could enhance drug delivery outcomes may also potentially injure the lungs.10) Polymers such as methyl-
cellulose or anionic surfactants which are usually employed in oral formulations cannot be delivered to the lungs because of their irritating nature or non-degradability. It should be noted that the current excipients approved by the Food and Drug Administration for respiratory drug delivery are very limited in number and not accepted worldwide.

Recently, amino acids have been shown to charge density, decrease hygroscopicity and improve the dispersibility of particles. The addition of various amino acids to formulations for inhalation generated by spray drying has been demonstrated to significantly improve the aerosolization performance of a dry powder. And different studies have showed that the addition of leucine yields the best results in term of aerosolization. It looks like that addition of leucine results in less cohesive particles due to the surfactant behavior of leucine.

Chitosan and chitosan oligosaccharide, which are cationic polysaccharides, are the deacetylated form of chitin. They have been extensively explored as pharmaceutical excipients in the last few years. A study indicated that chitosan did not adversely affect the viability of A549 and Calu-3 cells in an in vitro cell culture model of toxicity. Chitosan has the ability to modify the particle morphology and surface of drugs, subsequently, increase the deposition of drug in the lungs due to the improved aerosolization properties. What is more, the bioadhesive effect of chitosan particles may be useful in enhancing drug absorption subsequent to inhalation.

Dipalmitoyl phosphatidylcholine (DPPC), which are endogenous, represents 40% by weight of lung surfactant and contributes substantially to the unique properties of pulmonary surfactant. Therefore, it can be considered as a generally recognized as safe (GRAS) excipient in the inhalation field.

In this study, a group of dry powders were prepared by co-spray drying fanhuncaoin with leucine, chitosan, chitosan oligosaccharide and DPPC in different ratios. Based on the characterization of these powders, the dependence of the in vitro aerosolization performance on the particle component and their physical characteristics were evaluated with a view to produce an optimum formulation suitable for inhalation.

Experimental

Materials Fanhuncaoin (melting point: 147—152°C, solubility in water: 5 g/100 ml, purity: >90%) was supplied by Laboratory of Natural Medicine Chemistry (Shenyang Pharmaceutical University, China); tetra-butyl ammonium bromide and leucine were obtained from Bodi Chemicals (Tianjin, China); Chitosan (deacetylation degree: >95%, molecular weight (MW): ca. 400 kDa) and Chitosan Oligosaccharide (MW: 5000 Da) were purchased from Ruibio (Anhui, China). Dipalmitoyl phosphatidylcholine (purity: ca. 99%) was purchased from Sigma-Aldrich Chemicals (Shanghai, China). Other reagents were of analytical or chromatographic grade. Ultra-pure water was used throughout the study.

Preparation of Spray-Dried Powders Chitosan solution was prepared by mixing 2 g chitosan with 100 ml of 1% (v/v) glacial acetic acid aqueous solution for 4 h and allowed to stand overnight before use. To prepare DPPC suspension (1 mg/ml), DPPC (200 mg) was weighed into a 500 ml round bottom flask and dissolved in a 40 ml of chloroform–water: 5 g/100 ml, purity: 98%) was supplied by Laboratory of Natural Medecine Chemistry (Shenyang Pharmaceutical University, China) and ultrapure water at 55 °C and rotated for 2 h. The warm suspension was sonicated at 55 °C for 1 h in a sonic bath (SCQ-50, Shenbo ultrasonics, Shanghai, China). The resultant DPPC suspension was stored at 4 °C prior to use.

Formulations for spray-drying were prepared by the addition of aqueous suspension containing fanhuncaoin, leucine, chitosan oligosaccharide and DPPC to chitosan solution with different total solid mass of 0.5%, 0.67%, 0.85%, 1% and 2% (w/w). fanhuncaoin (model drug) was employed in all formulations with a constant dry weight loading (10%, w/w). The formulations consisted of leucine/chitosan/oligosaccharide/DPPC were in different ratios. The prepared formulations were subsequently spray-dried using an Eyela® SD-1000 spray-dryer (Tokyo Rikakikai Co., Ltd., Japan), under the following standard operating conditions: inlet temperature, 120°C; atomization pressure, 190 kPa; feed rate, 7.3 ml/min; aspirator setting, 0.6 m3/min. These conditions resulted in an outlet temperature of 74—82°C. The resultant powders were stored in a desiccator at ambient temperature until analysis.

Powder Characterization. Spray-Drying Yield and Drug Content The yields of spray-dried powders were quantified as the percentage of anticipated total powder yields. The fanhuncaoin content of the powders was measured in triplicate by high-performance liquid chromatography (HPLC) and expressed as the percentage of nominal load.

Scanning Electron Microscopy Spray-dried powders were mounted on double-faced adhesive tape and sputter-coated with a thin (approximately 10 nm) layer of gold in a Balzers SCD 050 (Balzers Union, Liechtenstein) coating unit at 20 mA for 2 min using an argon gas purge. The specimens were examined using a Quanta 200FEG scanning electron microscope (SEM) operated at high vacuum with an accelerating voltage of 20 kV and a specimen working distance of 10 mm.

Amorphous Nature and Water Content The degree of amorphous material was determined using differential scanning calorimetry (DSC) and the water content in the spray-dried powders was detected using thermogravimetric analysis (TGA). DSC (DSC60, Shimadzu Co., Ltd., Tokyo, Japan) was performed on 1—5 mg samples in aluminum pans using a nitrogen purge at 20 ml/min (temperature range: ambient-300 °C, heating rate 10 °C/min). TGA (TGA50, Shimadzu Co., Ltd., Tokyo, Japan) was performed on 1—5 mg samples in platinum pans using a nitrogen purge at 10 ml/min (range: ambient-150 °C, heating rate 10 °C/min).

Particle Size and Powder Tapped Density The particle size was measured with a laser diffractometer (LS230, Beckman Coulter, U.S.A.) in dry powder form after dispersing with compressed air. To achieve the required obscuration of 5%, about 100 mg of powder was needed, and each sample was measured in triplicate. The data obtained were expressed as the volume weighted mean particle size.

A self-made tapped density analyzer was used to determine the powder density (ρ). Measurements, which involved 1000 strokes to allow the density to reach a plateau, were performed in triplicate. Assuming a perfect packing, the tapped density of monodisperse spheres is approximately 21% under-estimate of the particle density due to the void spaces between particles. In the case of polydispersed particles, the void spaces are reduced but this is probably counterbalanced by incomplete packing. Carr’s Index (CI) values for each spray-dried powder were derived from poured density and tapped density data, according to Eq. 1.

\[
CI(\%) = 100 \times \frac{\text{tapped density} - \text{poured density}}{\text{tapped density}} \times 100
\]

The Carr’s Index value gives an indication of powder flow; a value less than 25% indicates a fluid powder, whereas a value greater than 25% indicates a cohesive powder. Theoretical estimates of the particle primary aerodynamic diameter (\(d_{\text{ae}}\)) can be derived from the particle sizing and tapped density data, according to Eq. 2.

\[
d_{\text{ae}} = d_0 \times \left( \frac{\rho_{\text{sample}}}{\rho_{\text{air}}} \right)^{1/3} \text{ where } \rho_{\text{sample}} = 1 \text{ g/cm}^3
\]
to the recovered dose, was expressed as a percentage and corrected for the actual fanhuncaoin content of each powder.\textsuperscript{14}

Formulation Optimization Overall desirability (OD), a key index in response optimization, was used to evaluate dry powder inhalations instead of respirable fraction (RF) evaluation. The overall desirability is a measure of how well combined goals satisfied for all the responses can be obtained. The optimum formulation was selected based on the criteria for attaining the minimum Carr’s Index and aerodynamic diameter response variables, while maximizing yield and RF response variables. Maximum and minimum acceptable values of the response variable are shown in Table 1. An overall desirability was derived from the formulation response variables to predict the ranges of variables where the optimum formulation may occur\textsuperscript{24}, according to Eq. 3:\textsuperscript{25}

$$d_i = \frac{(Y_i - Y_{min})}{(Y_{max} - Y_{min})}$$

or

$$d_i = \frac{(Y_{max} - Y_i)}{(Y_{max} - Y_{min})}$$

or

$$OD = \left( d_1 d_2 d_3 \cdots d_n \right)^1/n$$

Where $Y_i$ is the observed value of the response variable; $Y_{max}$, $Y_{min}$ is the acceptable maximum and minimum values of the response variable, respectively; and $n$ is the number of the response variables.

HPLC Analysis of Fanhuncaoin Fanhuncaoin was determined using an Agilent Technologies 1200 series HPLC system. The chromatographic separation was achieved on a 4.6$\times$150 mm column (Eclipse XDB-C18, U.S.A.) maintained at 30°C with a mobile phase composed of methanol–5 mM NaH2PO4 solution containing 6 mM tetrabutyl ammonium bromide (30:70, v/v) delivered at a flow rate of 1 ml/min. The detection wavelength was set at 230 nm; injection volume was 20 μl. The fanhuncaoin was eluted with a retention time of 5 min.

Statistical Analysis Significance between groups was evaluated by one-way analysis of variance (ANOVA) followed by a Dunnett post hoc test and the tests were carried out using SPSS 11.0 software. Differences were considered significant at a level of $p<0.05$.

Results and Discussion Preparation of the Dry Powders Spray-dried yields of the 20 formulations investigated varied considerably (range: 31.4—56.88%), with no evidence of a connection between formulation composition and spray-dried yield. However, it looks like that the existence of leucine, chitosan, chitosan oligosaccharide and DPPC in one formulation is necessary to achieve higher yields. HPLC analysis of drug contents revealed that all formulations, with the exception of the leucine/chitosan/chitosan oligosaccharide (90/0/0, w/w/w) formulation (71.2% of total dose), showed similar drug contents with a range of 91.97—98.81% (partial data shown in Table 2), indicating that chitosan and chitosan oligosaccharide may both play an important part in providing the protection during the spray drying process.

Scanning electron microscopy was used to visualize the particle diameter, structural and surface morphology of the spray-dried powders (Fig. 2). Results showed that the powder...
components significantly influenced the particle shape and morphology. The relative proportion of leucine in the dry powders changed particle morphology as visualized from smooth and spherical (Fig. 2A) to Wrinkled and orange-like shape (Figs. 2B, C) and then wizened and raisin-like shape (Fig. 2D). The morphology of spray-dried particles is mostly affected by the rate of solvent evaporation during the spray-drying process, and the wrinkled particles may result from the build-up of vapor pressure within the particle leading to its collapse. And this phenomenon may be caused by the formation of an impervious crust through which the solvent cannot easily pass. Seville et al.12 noted that the surfactant-like properties of leucine may lead to its accumulation at the air–liquid interface and therefore at the surface of the droplet. And it is therefore conceivable that a layer consists of leucine molecules could be formed on the surface of droplets that inhibits the passage of water vapor and, as a consequence, the surface layer expands like a soap bubble. When the water fully evaporates, the leucine surface layer collapses, resulting in the observed wrinkled structure.26 Most striking about the micrographs of the leucine/chitosan/chitosan oligosaccharide (900/1350/1350, w/w/w) powders is the appearance of whisker-like material clinging to the surface of the particles (Figs. 2B, C). It is unclear whether the fanhuncanoin undergoes capillary condensation in our spray-dried powders, or whether the surface-deposited material is excess leucine or chitosan or chitosan oligosaccharide which precipitated from the spray-drying formulation during spray-drying.

DSC showed all formulations to be amorphous with no crystalline endothermic fusion peaks (Figs. 1A—D). Thermogravimetric analysis of the spray-dried powders indicated that the water content of the powders ranged from 0.17 to 7.72% (Figs. 1E, F). As excipient crystallization, flowability and dispersion performance can be significantly affected by the water content of powders, so relatively low water content is of great importance. Water content should be influenced by many factors including the relative proportion of excipients, inlet temperature during the spray-drying process and surface area of spray-dried powders. In addition, there may be no way to discriminate the residual water from the water adsorbed during the storage. Even so, these values are in line with other studies that show water content of spray-dried powders to be up to 7.5% (w/w).13,27

All powders showed high emission, with at least 90% of the capsule contents being emitted during aerosolization testing. Statistical analysis indicated that there was no difference in the ED values of the different powders (p>0.05, data not shown), with the exception of the leucine/chitosan/chitosan oligosaccharide (0/45/45, w/w/w) formulation (ED: 85.39%, p<0.05, data not shown), and it was found that the residual particles adhered to the wall of inhaler and/or capsule shells due to electrostatic attractions. This high emission is clearly a reflection of the aerodynamic properties of our spray-dried powders, owing to the inclusion of chitosan, chitosan oligosaccharide, DPPC and leucine.14

Optimal Total Solid Mass In the first step, we would like to optimize the total solid mass of formulations to be spray-dried. The fanhuncanoin/leucine/chitosan/chitosan oligosaccharide/DPPC (10/22.5/33.75/33.75/0.2, w/w/w/w/w) were maintained in equivalent amounts. An aqueous suspension containing a total solid mass of 0.5%, 0.67%, 0.8%, 1% and 2% (w/v) were prepared, respectively, before spray-drying.

The presence of chitosan in the formulation increased the viscosity of the liquid being spray-dried which could result in a larger droplet at the spray-drying nozzle. As a result, a larger particle would be given.28,29 Leucine, a particularly hydrophobic amino acid with surfactant-like properties,30,31 usually used as a dispersibility enhancer in preparation of spray-dried respirable powders, has the ability to migrate to
the droplet surface during the rapid drying phase in spray-drying, and hence affect the surface characteristics of the resultant particles, resulting in highly dispersible particles with optimal aerosolisation properties. Thus, it is feasible that inclusion of chitosan with long chains would influence the ability of leucine to migrate to the surface of the droplet during the spray-drying process. This could therefore have an adverse effect on the surface properties of the resultant spray-dried particles, resulting in increased interparticle cohesion, decreased deaggregation of the particles during aerosolisation and a following lower RF. Interestingly, in our study, scanning electron microscopy showed that the total solid mass involved did not play an important part in the geometric size and overall morphology of the powders (Figs. 2B, C).

Each of the all five formulations had a good spray-dried yield as high as 50% of the expected powder mass. It looks like that the formulation with a total solid mass of 1% (w/v) had the lowest geometric size as well as aerodynamic size, and the highest RF ($p<0.05$, Fig. 3). Varying total solid mass from 0.5 to 2% (w/v) led to a poorer flowability and denser particles ($p<0.05$, Fig. 3). We were unable to find the reason why the formulation with a total solid mass of 0.67% (w/v) had the lowest RF (29.46%, Fig. 3) and the lowest OD value.

The OD values finally revealed that the optimal total solid mass in our study is 1% (w/v).

**Optimal DPPC Content**

Having selected a total solid mass of 1% (w/v) formulation, we then proceeded with the investigation of the optimal DPPC content. DPPC, as a surfactant, is present at the air-liquid interface of the droplet during atomization. Decreased inter-particle cohesion would be generated because the presence of DPPC at the particle surface might reduce surface energy. In our study, DPPC was incorporated in variable content (0%, 0.2%, 0.4%, 0.6%, w/w, respectively), and 10% of fanhuncaoin, 45% of leucine, 22.5% of chitosan and 22.5% of chitosan oligosaccharide in equivalent amounts (w/w). Spray-drying was carried out with an aqueous suspension of 0.5% total solid mass (w/v).
Drug was maintained at a proportion of 10% and DPPC 0.2% (w/w). The relative proportion of chitosan and chitosan oligosaccharide was always 1 : 1 (w/w). Spray-drying was carried out with an aqueous suspension of 0.5% total solid mass (w/v). The formulation led to powders with lowest spray-dried yield and highest water content. As the percentage of DPPC in the spray-dried powders was increased from 0.4 to 0.6%, an increase in tapped density from 0.44 to 0.48 g/cm³, mean geometric diameter from 4.89 to 5.55 μm, primary aerodynamic diameter from 3.5 to 4.25 μm, Carr’s Index from 22.31 to 27.95% and a decrease in RF from 45.19 to 38.04% were observed (p<0.05, Fig. 4). The increased DPPC and the relatively decreased leucine at the surface of the spray-dried powders might be responsible for this phenomenon.

Therefore, considering the OD values, we selected the powder containing 0.4% of DPPC (w/w) as the optimal DPPC proportion.

Optimal Leucine Relative Proportion We then investigated the influence of the relative proportion of leucine on dry powders physical and aerodynamic characteristics. We formulated six different powders in which fanhuncaoin were maintained at a proportion of 10% and DPPC 0.2% (w/w). The relative proportion of chitosan and chitosan oligosaccharide was always 1 : 1 (w/w). Spray-drying was carried out with an aqueous suspension of 0.5% total solid mass (w/v).

As the percentage of leucine in the spray-dried solution was decreased from 90 to 0%, an increase in water content from 0.17 to 7.72% was observed (Fig. 5). This could be due to water being retained in the increasing chitosan matrix during spray-drying. Additionally, when leucine proportion was decreased from 90 to 0%, denser particle formation was observed (0.06 g/cm³ for 90% leucine particles compared with 0.56 g/cm³ for 0% leucine particles, p<0.05, Fig. 5). That chitosan is heavier than leucine may be the predominant reason for this phenomenon.

The leucine/chitosan/chitosan oligosaccharide formulation (0/45/45, w/w/w) formulation presented the poorest flowability (Carr’s Index: 51.91%) and worst aerosolization properties (RF: 15.31%, p<0.01, Fig. 5). The incorporation of leucine into a spray-dried formulation as an aerosolization enhancer has been demonstrated to obviously increase the RF of particles in a dry powder formulation.14,34)

Scanning electron microscopy indicated that decreasing the leucine content of the spray-dried powders led to increasingly smooth and spherical particles (Figs. 2A—D). The increasing surface smoothness usually increases adhesion forces between particles arising from an increased contact area between the interacting species. Thus, a rough surface of powders may be a good choice for inhalation. The advantage of wrinkled particles is the less contact between particles, thereby resulting in a better in vitro deposition. What is more notable for the leucine/chitosan/chitosan oligosaccharide (0/45/45, w/w/w) powder is that the SEM showed that it consisted of particles of diameter below 5 μm, but the measured mean particle size of the powder was 12.37 μm. Similar results have also been reported by other researchers, who suggested that the larger size obtained during particle sizing reflects the cohesion of individual particles to form larger aggregates that fail to disperse during the sizing procedure.

The leucine/chitosan/chitosan oligosaccharide (45/22.5/22.5, w/w/w) powder possessed the highest OD value, so we selected the powder containing 45% of leucine as the optimal leucine proportion.

Optimal Chitosan/Chitosan Oligosaccharide Relative Proportion In the last step, we studied in which ratio the remaining 45% of the powder composition should be divided over chitosan and chitosan oligosaccharide. In our study, fanhuncaoin was still maintained at a proportion of 10%. The optimal leucine proportion 45% (w/w) and DPPC 0.4% (w/w) were employed in 5 different formulations. Chitosan and chitosan oligosaccharide were incorporated in variable amounts. Spray-drying was carried out with an aqueous suspension of 1% total solid mass (w/v).

A trend suggesting that increasing the relative proportion of chitosan from 0 to 45% increased the tapped density from 0.27 to 0.53 g/cm³ (p<0.01, Fig. 6) was observed. The tapped density is influenced by many factors such as particle shape and morphology, interparticulate forces and so on. In our study, the fact that chitosan is heavier than chitosan oligosaccharide may be responsible for this phenomenon to a great extent. The addition of chitosan or chitosan oligosaccharide to the dry powders did affect mean geometric diameter and
primary aerodynamic diameter \((p<0.05, \text{Fig. 6})\). Removing chitosan from the formulation led to the poorest flowability (Carr’s Index: 43.66, \(p<0.05, \text{Fig. 6}\)). SEM showed that powders without chitosan consisted of semispherical particles (Fig. 2E), and the particles appear to have undergone fusion. This may have occurred possibly at some stage during SEM processing. Researchers have shown that chitosan can enhance the dispersibility of spray-dried powders.\(^{35}\) and it is feasible the chitosan is modifying the surface of the powder particles, decreasing interparticulate cohesion and thereby improving powder dispersibility. When chitosan oligosaccharide concentration was decreased from 11.25 to 0%, poorer flowability \((p<0.05, \text{Fig. 6})\) and lower RF \((p<0.01, \text{Fig. 6})\) were observed, which could indicate that chitosan oligosaccharide concentration is crucial for optimal powder flowability and dispersibility.
charide can act as a dispersibility enhancer, too.

At last, we selected the chitosan/chitosan oligosaccharide (33.75/11.25, w/w) powder, which possessed the highest OD value, as the optimal chitosan/chitosan oligosaccharide relative proportion.

**Influence of the Physical Characteristics on the Aerosolization Properties** Studies demonstrated that the aerodynamic behavior of a powder depends on multiple interrelated factors, such as particle density, primary particle size, powder crystallinity, powder composition, surface properties and powder cohesiveness.\(^3\) Hence, the influence of the physical characteristics of the powders on their RF was investigated in this study. We pooled the powders of varying proportion of excipients presented in Figs. 3–6 and plotted their RFs as a function of tapped density, geometric size, primary aerodynamic diameter and the Carr’s Index. As shown in Fig. 7, geometric size, aerodynamic diameter as well as the Carr’s Index of the dry powders could all affect the RF in the same way, *i.e.* a higher level of these factors resulting in a lower RF. Although lower R-square values were obtained from the test, the correlations between these physical properties and the RF could be fitted to linear models and the results exhibited statistically significant differences (*p* < 0.05, Fig. 7). To our surprise, the tapped density had no effect on the RF in the range of values considered (*p* > 0.05, Fig. 7), which is not in line with that presented by other researchers.\(^3\)

**Conclusion**

In this paper, dry powder formulations for inhalation of *fanhuncaoin*, a newly discovered antiinflammatory active phenolic acid isolated from Chinese herb, were appropriately designed to optimize the composition and further explore the relationship between the composition, the physical properties and the aerosolization performance. Results revealed that the composition of the particles, *i.e.*, excipient type and proportion, significantly influenced the physical characteristics of the dry powders, such as water content, tapped density, geometric and aerodynamic size as well as flowability and, thus, influenced their aerosolization performance. In addition, each excipient in the formulation helped to achieve optimal aerodynamic behavior. It was interesting to find that both chitosan and chitosan oligosaccharide can not only influence the particle shape and morphology but enhance the dispersibility of spray-dried powders. Chitosan oligosaccharide, possessing the properties of soluble, biodegradable, biocompatible, bioadhesive and nontoxic,\(^1\) has the ability to enhance mucosal absorption by opening tight junctions.\(^2\) potentially resulting in an increase of drug absorption subsequent to inhalation. In our study, the addition of chitosan oligosaccharide in formulations can achieve higher spray-dried yields (Figs. 5, 6), protect drug from destruction during the spray-drying process (*p* < 0.05, Table 2), obtain spray-dried powders with better flowability (*p* < 0.05, Fig. 6) and higher RF (*p* < 0.01, Fig. 6). In a word, chitosan oligosaccharide, which has received little attention to date, is found for the first time to be a promising useful excipient for inhalation, and it is expected that it will eventually find his way into approved products. In addition, in this study, overall desirability, a key index in response optimization, was used first time to evaluate dry powder inhalations instead of respirable fraction (RF) evaluation. OD value was determined by 4 response variables, including spray-drying yield, Carr’s Index value, aerodynamic diameter and RF. Thus, it may reflect intrinsic quality of inhalation dry powder better than RF. And there has the potentiality to obtain a more reliable result in estimating dry powder inhaler applications by using OD value evaluation. Among the combinations tested, the *fanhuncaoin*/leucine/chitosan/chitosan oligosaccharide/DPPC (10/45/33.75/11.25/0.4, w/w/w/w/w) prepared in a total solid mass of 1% (w/v) formulation was particularly optimal and exhibited RF as high as 51.29% doses emitted from the HandiHaler\(^6\) inhaler device and, So, it would be anticipated to deposit in the lower regions of the respiratory tract.

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**References**

30. WOLFENFEND R., ANDERSSON L., CULLIS P. M., SOUTHWICK C. C., *Biochem-