Engineered spray-dried nanosuspension of budesonide with enhanced fine particle fraction and dissolution rate

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Summary

Background. The interest in nanoparticles for pharmaceutical purposes as well as their importance in pulmonary drug delivery is rising. By producing drug nanoparticles, for instance by wet media milling, the dissolution rate can be enhanced, which is especially valuable for poorly water-soluble drugs. Spray drying enables embedding of nanoparticles into microparticles, which improves the nanoparticles' stability and application. A particle engineering approach to the spray drying process allows the production of particles with superior properties by better understanding of the particle formation mechanism.
Methods. A stabilised nanosuspension of budesonide was produced by wet media milling. Subsequently, the nanosuspension was spray-dried using the particle engineering approach to tune the particles' aerodynamic properties and to increase the nanosuspension's stability. The particles were co-sprayed using additives such as mannitol, leucine, and glycine. The resulting powders were characterised in terms of geometric and aerodynamic particle size, morphology, and shape. Furthermore, the dissolution rate of aerodynamically classified particles was measured.
Results. The samples containing leucine showed the most favourable aerodynamic and dissolution behaviour. With leucine we achieved the highest fine particle fraction (70.2%), which was ~ 40 % more compared to raw budesonide. Such formulation dissolved approximately two times faster than pure budesonide.
Conclusions. In this work, we present powders with enhanced aerodynamic properties and dissolution rate, and high fine particle fraction compared to raw budesonide. The particle engineering proved as a valuable tool for production of nanoparticle-containing microparticles with improved properties relevant for pulmonary drug delivery.

Introduction

The number of approved nanotherapeutics available on the market increased significantly in the last twenty years[1]. Most of the formulations encapsulate the drug into liposomes or polymers, and are administered intravenously, subcutaneously, or orally. Only few formulations are made of the drug itself in form of a nanosuspension. Formulating a nanosuspension is advantageous especially for drugs insoluble in water as well as in oils[2] and presents a universal and robust technique. However, all nine nanosuspension formulations on the market are for oral application[3]. Pulmonary administration of nanoparticles offers a convenient, non-invasive alternative for systemic drug delivery owing to the very thin air-blood barrier in the alveolar region of the lungs. It also avoids the first-pass effect, which is a common hurdle of orally delivered drugs. Pulmonary application of poorly water-soluble drugs is suitable since these drugs often have poor oral bioavailability[4].

Moreover, the number of poorly water-soluble drugs emerging from combinatorial screenings is more than 50% and can be expected to rise[5]. Therefore, the techniques for formulations of these "brick-like" drugs are in the spotlight of pharmaceutical industry. Many approaches exist for their processing, and they are commonly classified as bottom-up, where the nanoparticles are built from molecular structures, and top down, where the nanoparticles are formed by particle size reduction.

Nanosuspension can be applied to lungs either in form of the suspension itself, using a nebuliser or metered dose inhaler, or as a dry powder[6]. Conversion of nanosuspension into dry powder, e.g. by spray drying, is desirable for long-term stability of the nanosystem and for improved handling.

The aim of this study was to spray dry the nanosuspension using the particle engineering approach. Our goal was to prepare powders with embedded nanoparticles with improved aerosolisation properties and dissolution rate compared to unprocessed drug of approximately same geometric diameter.

Materials and Methods

Spray drying of wet milled nanosuspension
The spray-dried nanosuspension comprised of budesonide, which was chosen as a model drug due to its insolubility in water[7]. Based on previous studies, the suspension was stabilised using D-α-tocopherol polyethylene glycol 1000 succinate (TPGS). The nanosuspension was prepared by wet media milling in a DYNO®-MILL Multi Lab (Willy A. Bachofen Maschinentechnik, Switzerland) and the nanoparticles had median particle size of 258 ± 17 nm. The fine particle fraction (FPF) of the raw unprocessed material was 34.2%.

For the spray drying process, we used Büchi Mini Spray Dryer B-290 coupled with Dehumidifier B-296 and molecular sieve (all Büchi Labortechnik, Switzerland). The inlet temperature, the feed flow rate, and the aspirator flow rate were set to 170°C, 9 mL/min, and 35 m^3/h, respectively. The atomising gas flow setting varied between 30 and 55 mm (corresponding to 7.3 and 22.9 L/min). The total concentration of the feed was 1% w/v. Budesonide concentration was 50% (i.e. the first 0.5% w/v) of the solid. The second 0.5% w/v of the total concentration was made by one of the matrix formers: mannitol, glycine, leucine, or a 1:1 mixture of mannitol and leucine.

Laser diffraction (LD)
LD was used to determine the particle size of the spray-dried powders. The instrument comprised of the HELOS sensor and the dispersing system RODOS equipped with the micro dosing unit ASPIROS (all Sympatec Corp, Germany). Approximately 150 mg of the powder was filled in glass vials; each sample was measured in triplicate. The measurement range was 0.1/ 0.18 to 35 µm. All measurements were performed with an injector primary pressure of 1 bar.

Scanning electron microscopy (SEM)
SEM was used to observe the particle size and morphology, and to evaluate the aggregation of the particles. Images of the spray-dried powders were taken with Supra 40VP microscope (Carl Zeiss, Germany) and InLens detector at an accelerating voltage of 5 kV. The working distance was 5 mm. Before the measurements, the samples were coated for 45 s with gold, using ThermoVG Scientific Polaron Sputter Coater.

Next Generation Impactor (NGI)
The Next Generation Impactor (Copley, United Kingdom) was used to determine the aerodynamic diameter of the spray-dried powders. The powders were weighed into two-piece hard gelatine capsules of size 3 (gifted from CAPSUGEL®, France). For each sample measurement, five capsules containing approximately 1 mg of the powder were prepared. An inhaler of Aerolizer® type was used and the powder was lead through the impactor at airflow of 60 L/min for 4 s, according to manufacturer's recommendation. We washed both the induction port (IP) and the pre-separator (PS) with 25 mL of ethanol, and each NGI stage with 10 mL of ethanol. Each of these ethanolic solutions was collected and quantified using an HPLC. The FPF was calculated as the fraction of particles with an aerodynamic diameter < 4.46 µm.

High performance liquid chromatography (HPLC)
An HPLC system 1100 Series from Agilent Technologies, USA was used for budesonide quantification. The HPLC analysis was carried out using a reversed phase column HyperClone ODS (C18) with the dimensions 150 x 4.6 mm, 5 µm (Phenomenex®, USA). The HPLC method elaborated by Hou et al.[8] was employed to determine the amount of budesonide in each stage of the NGI and in the powders.

Dissolution testing
For the dissolution tests, the modified USP method developed and improved by Son et al.[9] was applied Three spray-dried samples and the raw material were tested to assess and compare the dissolution rates. All samples for dissolution were taken from stage 3 (cut-off diameter 4.46 - 2.82 µm). In total, 300 mL of phosphate buffered saline with pH 7.4 was used as the dissolution medium. After the deposition in the impactor, we placed a pre-soaked polycarbonate membrane (pore size 0.05 µm; Whatman Nuclepore™, USA) on the membrane holder. The dissolution was tested under sink conditions at 37.0 ± 0.5°C. Samples were taken after 20, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 minutes, and additionally after 24 hours. The samples were filtered using nylon filters with pore size 0.45 µm and analysed with the abovementioned HPLC method.

Results and Discussion

First, samples with budesonide and mannitol in ratio 1:1 were spray dried. The median particle size measured by LD was 8.2 µm, which corresponds well with the SEM images (Figure 1**Error! Reference source not found.**-M). The particles were crumpled and had seamless surface. Despite the crumpled morphology, which is favourable for increased FPF[10], the FPF of the sample was 13.1% (Figure 2**Error! Reference source not found.**). Most powder deposited in the induction port (IP) and the deposited amount decreased exponentially with increasing stage number.

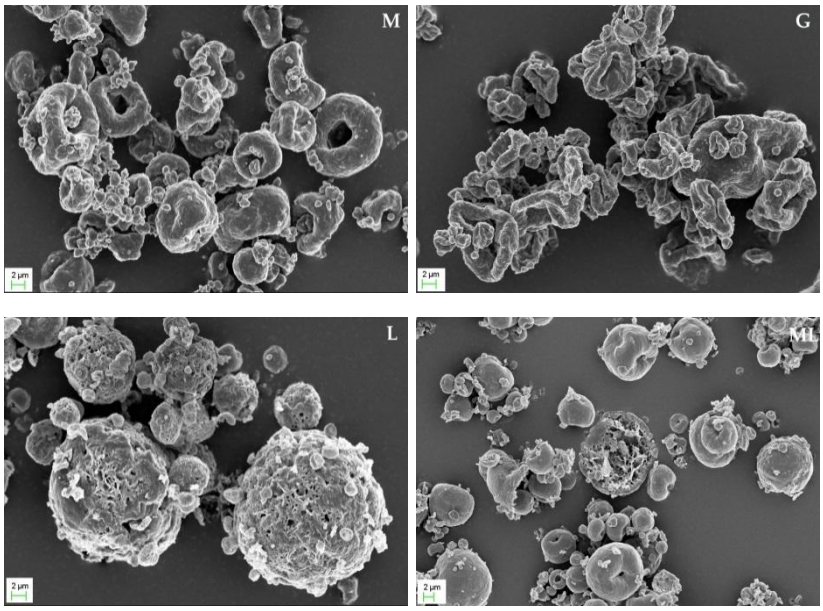

Figure 1 SEM images of spray-dried samples with different matrix formers: M - mannitol, G - glycine, L - leucine, and ML –mannitol: leucine (1:1). Scale bar 2 µm.

In further experiments, we substituted mannitol with shell-forming agents namely leucine[11] and glycine[12]. Under the same spray drying conditions as in previous experiment, particles with median particle sizes of 7.35 µm (leucine) and 7.61 µm (glycine) were formed. The morphology of the particles with glycine appeared to be even more folded than the particles with mannitol (Figure 1**Error! Reference source not found.**-G). However, the FPF of the sample with glycine increased only by 5%, and reached 18.7% (Figure 2). Instead, the addition of leucine resulted in spherical particles with porous shell (Figure 1-L). Although the particle size was comparable with other samples, the FPF increased to 26.7%. The deposition was highest in the induction port but did not decrease exponentially; the deposition in stage 1 (S1) was higher than in the pre-separator. Finally, the nanosuspension was combined with mannitol and leucine mixture. After spray drying, the particles had smaller both the median particle size (5.33 µm) and FPF (22.6%). The particles had smooth surface with only little indents. However, they showed sponge-like internal structure (Figure 1-ML).

Since leucine proved to perform best in terms of FPF among the tested matrix formers, further experiments were carried out with this substance. High deposition in the "upper" part of the NGI (induction port, pre-separator, stage 1) indicated that the particle size is too large at the given density. Since the increase of the atomising pressure generally leads to the formation of smaller droplets[13], the particle size was lowered by increasing the atomising pressure in the spray dryer. The atomising gas flow setting was increased with 5 mm steps from 30 mm (the starting setting) to 55 mm. The median particle size was 5.59 µm at 35 mm setting, 3.80 µm at 40 mm setting, 2.90 µm at 45 mm setting, 2.42 µm at 50 mm setting, and 2.39 µm at 55 mm. Further increase was not possible due to technical limitation of the spray dryer. Each 5 mm increment increased the FPF by approximately 10% (Figure 3Figure). Eventually, FPF of 70.2% was reached with the powder prepared at 50 mm setting. Further increase to 55 mm setting led to lower FPF.

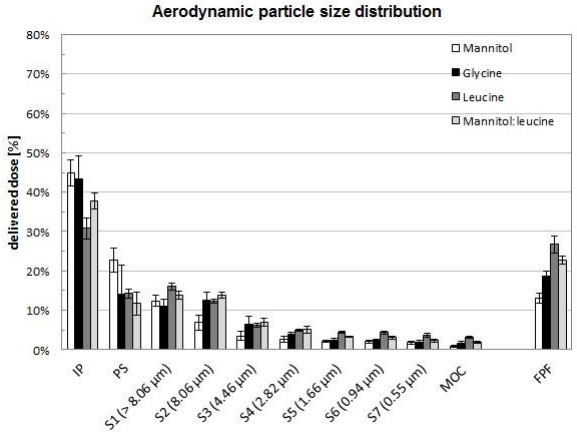

Figure 2 Aerodynamic particle size distribution of spray-dried samples with different matrix formers. IP-MOC denotes impactor stages with corresponding upper aerodynamic cut-off diameter in parentheses. Data presented as mean ± standard deviation (n=2).

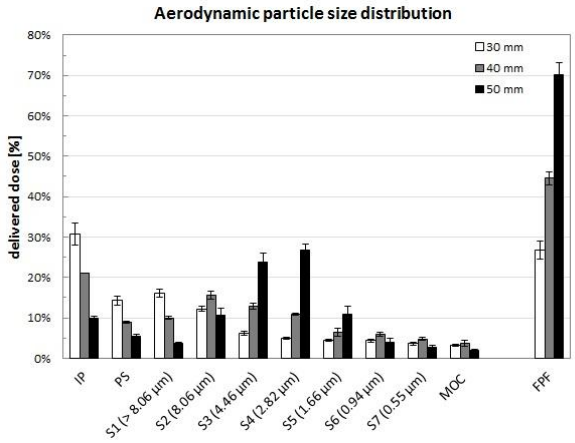

Figure 3 Aerodynamic particle size distribution of spray-dried samples with leucine at different atomising pressure settings (30, 40, and 50 mm). IP-MOC denotes impactor stages with corresponding upper aerodynamic cut-off diameter in parentheses. Data presented as mean ± standard deviation (n=2).

The dissolution test of raw budesonide (median particle size of 3.12 ± 0.03 µm) as well as spray-dried samples with leucine, with mannitol, and with the mannitol and leucine mixture was performed. Figure 4Figure shows the dissolution profiles of all tested samples from stage 3. Although mannitol's solubility is ten times higher than leucine's, the sample with leucine dissolved faster. This sample was also the only one that dissolved completely within eight hours. A fast dissolution can be due to particles' porosity. Therefore, larger area compared to spherical particles is available for the action of the dissolution medium which increases the dissolution rate[14]. The sample containing budesonide and mannitol dissolved from 50% in one hour and reached 80% of dissolution after 7 hours. Interestingly, the sample containing the mannitol and leucine mixture was the slowest to dissolve among the tested spray-dried samples. This was probably caused by the particle shape and morphology. Unlike powders with mannitol, these particles were spherical with a smooth surface, which did not allow the penetration of the dissolution medium into the particles. As expected, raw budesonide exhibited the slowest dissolution.

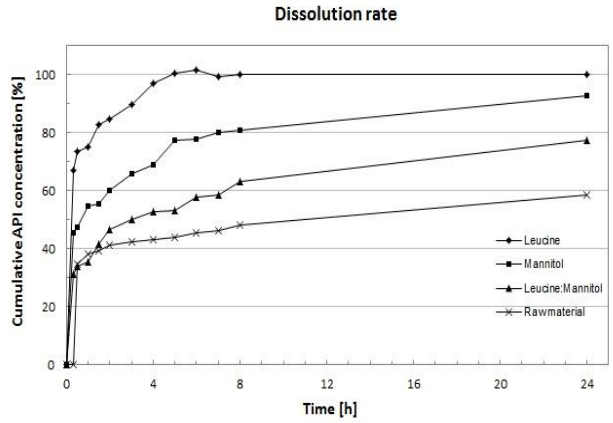

Figure 4 Dissolution profiles of raw budesonide and of spray-dried samples, all aerodynamically classified from stage 3

Conclusion

In our study, we prepared nanobudesonide-containing powders with enhanced aerodynamic properties and dissolution rate, compared to budesonide microparticles. The particle engineering approach was useful and valuable to produce powders with the above named properties. From the investigated additives, especially leucine improved the performance of the samples in the NGI and enhanced the dissolution rate, presumably due to the formation of more porous particles. The particles with leucine showed better aerosol behaviour than particles with glycine, mannitol, or a mannitol:leucine mixture.

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