JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1625–1630

Received June 25, 2009
Accepted September 16, 2009
DOI 10.1211/jpp/61.12.0006
ISSN 0022-3573

Research Paper

Evaluation of supercritical fluid engineered budesonide powder for respiratory delivery using nebulisers

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Abstract

Objectives Currently, suspensions prepared from micronised drug substances are the only delivery system marketed for nebulisation of steroids, and reported inconsistent or low bioavailability arising from their use provides a rationale for researching alternative formulations. Supercritical fluid processing of drug substances to obtain respirable-sized particles has been used over the last decade to formulate dry powder inhalers. We aimed thus to process budesonide powder to improve its deposition characteristics.

Methods In an attempt to overcome the limitations of nebuliser suspensions when prepared from micronised drug particles, budesonide powder was processed using a supercritical fluid based process and suspended using Tween 80 as a surfactant to provide an aqueous nebuliser formulation. The in-vitro characteristics of the emitted dose on nebulisation for the prepared suspension were then compared to a commercially available suspension formulation of budesonide using a jet and a vibrating mesh nebuliser.

Key findings The results showed a significant improvement of the in-vitro deposition properties of the suspension containing supercritical fluid engineered budesonide particles.

Conclusions The results indicated the benefit of such materials compared with traditionally micronised drug powders.

Keywords budesonide; in-vitro aerosolisation characteristics; nebuliser; SEDS; supercritical fluid

Introduction

Currently, the use of budesonide in nebulisers is limited to a single drug delivery formulation. The suspension for nebulisation (Pulmicort Respules 500 and 1000 μg/2 ml single dose units)[1] is marketed for use with jet nebulisers. However, several drawbacks have been identified when using such formulations with nebulisers. When nebulising suspensions, relatively large droplets are required to carry the suspension particles and it is expected that some aerosol droplets will contain no, or low concentrations of, drug particles.[2] Furthermore, such formulations are microsuspensions of steroids and because of the microparticulate nature use of them is associated with limited bioavailability of the drug due to low levels of drug solubility, short residence time for the drug in the lungs because of ciliary movements and unwanted deposition patterns.[3]

Since micronised drug powders are routinely used for inhalation drug delivery formulations, including metered dose inhalers (MDIs) and dry powder inhalers (DPIs), the widely reported damaging effects of this high-energy milling process on resulting particles may contribute to the relatively poor aerosolisation behaviour of nebulised suspension formulations using such materials.[4–6]

In addition, inconsistency of in-vitro deposition data when the commercial suspension product is used in different nebulisers[7] indicates a need to research alternative preparations to nebulise budesonide.

Supercritical fluid (SCF) processing to prepare micron-sized drug particles was first introduced in the 1970s. Since then, different SCF techniques such as rapid expansion of supercritical solutions (RESS), supercritical anti-solvent (SAS) and, to a lesser extent, particles from gas saturated solutions (PGSS) have been developed. These processes enable

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improved control over the properties of produced particles, such as particle size, particle size distribution and surface characteristics.[8] The procedure of solution enhanced dispersion by supercritical fluids (SEDS) is a technique that offers increased mass-transfer during the anti-solvent-based SCF process with improved control over the particles size by changing the process conditions[8] compared with high-energy milling operations.

The successful processing of a range of drug substances, including paracetamol, carbamazepine and salbutamol sulfate, and biomaterials, such as insulin and plasmid DNA, has been reported.[8] Budesonide was first processed using SEDS in 1998.[9] Since then a number of reports have compared the in-vitro performance of SEDS-engineered budesonide with micronised powder and a commercial product of budesonide. Improved deposition of budesonide powder particles in the lower stages of a Multistage Liquid Impinger,[10] improved content uniformity and emitted dose, reduced variability in the emitted dose[11] and enhanced batch-to-batch reproducibility[12] of SEDS-engineered budesonide have been reported in in-vitro studies using DPIs containing SEDS-engineered budesonide compared with micronised powder or commercial product.

In recent years, a new generation of nebulisers has been developed to overcome many of the problems associated with conventional jet/ultrasonic nebulisers.[13] The vibrating mesh nebulisers with multiple apertures in general work on the basis of vibration of a mesh or plate in contact with the liquid. The liquid passes through the mesh generating a fine-particle and low-velocity aerosol with a high efficiency for delivering aerosol to the lungs, such that the dose of drug to be put in the device can be substantially reduced. The vibrating mesh nebulisers also are usually pocket-sized, extremely quiet in operation, are able to nebulise solutions and suspensions efficiently and have also been used for successfully aerosolising peptides, such as insulin and fragments of DNA.[13,14] In addition, unlike jet and ultrasonic nebulisers, they do not cause changes in the temperature of the medication during nebulisation.[15]

Although there are several reports on the use and aerosol performance of SCF-prepared particles in DPIs,[10–12] such materials have not been examined for nebulisation suspension formulations. The aim of this study is to prepare a suspension of SCF-engineered budesonide particles suitable for nebulisers with improved in-vitro aerosolisation characteristics when compared with the commercially available product. The in-vitro performance is assessed after nebulisation using two types of nebulisers – a jet and a vibrating mesh nebuliser.

Materials and Methods

Materials

Budesonide (Pharm. Eur.) was purchased from Industriale Chimica s.r.l. (Saronno, Italy), Pulmicort Respules suspensions for nebulisation were purchased from AstraZeneca (Loughborough, UK). Pharmaceutical grade polysorbate 80 was from Fluka (Buchs, Switzerland). All other chemicals were HPLC grade and purchased from Sigma-Aldrich (St Louis, MO, USA).

Solution enhanced dispersion by supercritical fluids

Micron-sized particles of budesonide were prepared using the SCF-SEDS method with a 50-ml vessel, by pumping a solution of 0.5% (w/w) of budesonide in acetone using a reciprocating pump (Jasco, Great Dunmow, UK). The SCF anti-solvent was CO_2 and was pumped at a rate of 20 ml/min into the vessel via a coaxial nozzle. The pressure in the vessel was controlled using a Jasco 880-81 back pressure regulator (Jasco, Great Dunmow, UK). After complete delivery of the drug solution and particle formation, residual solvent was removed from the drug particles by flushing excess amount of SCF CO_2 through the vessel. The dried budesonide, collected at the base of the precipitation vessel, was then collected after releasing the pressure. The processing conditions used to obtain a range of products are summarised in Table 1. After processing, all products were stored at 25°C in sealed containers. All other details of the equipment and process have been reported previously.[16]

Particle size analysis

Individual samples of the powders, prepared as described above, were suspended in 0.02% w/v solution of polysorbate 80 in normal saline, to give a final concentration of 1 mg budesonide/2 ml in the suspending liquid. The size of the drug particles was then measured at 25°C, 30 min after preparation of the samples, by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The cumulative mean for zeta-average values was considered as the particle size. The calibration of the instrument had been confirmed using polystyrene latex beads, 800 nm (Sigma-Aldrich). No dilution was performed on the samples before measurement. Dispersant viscosity was set to 0.887 cP at 25°C.

Scanning electron microscopy

The morphology and appearance of particles from the SCF process were examined using an environmental Quanta 400 Scanning Electron Microscope (FEI, Eindhoven,

Table 1 Processing conditions for SEDS preparation of budesonide powders and the measured mean particle size

Batch No.	Temperature (°C)	Pressure (bar)	Solution flow rate (ml/min)	Particle size (nm)
1	90	140	0.2	>4000
2	75	125	0.2	>4000
3	75	125	0.6	3370
4	75	133	0.2	3860
5	75	133	2.0	>4000
6	75	145	0.2	>4000
7	65	105	0.2	3880
8[a]	60	105	0.2	1670
9	60	117	0.2	2320
10[b]	60	125	0.2	>4000
11	55	105	0.2	2710
12	45	96	0.2	2905
13	45	105	0.2	2620

[a](i.e. S1); [b](i.e. S2).

Netherlands). A small proportion of powder was deposited directly on the carbon adhesive sheet and observed after removing any excess amounts of powder.

The batch of SCF-processed budesonide composed of particles that had the smallest average size (i.e. batch 8, SN1) as well as the batch that showed a different morphology (i.e. batch 10, SN2) were chosen for further study of in-vitro characteristics of nebulised suspensions.

In-vitro characteristics of nebulised suspensions

Suspensions of the two selected SCF-engineered budesonide batches were prepared in 0.02% w/v solution of polysorbate 80 in normal saline to obtain a final concentration of 500 μg/ml. The in-vitro characteristics of the emitted dose from these suspensions were compared with that of Pulmicort Respules 500 μg/ml (P) using two different nebulisers. Four millilitres of each preparation was nebulised using the Sidestream jet nebuliser driven by a Porta-neb compressor (Profile Therapeutics plc, Bognor Regis, UK) and 1 ml was used in the Microair U-22 vibrating mesh nebuliser (Omron, Tokyo, Japan). The total output of each system and aerodynamic characteristics of the respirable fraction were determined according to European Committee for Standardization (CEN) methodology,[17] except that the amount of budesonide was determined instead of a fluoride tracer. A Marple Series 298 low-flow cascade impactor (Graseby, Watford, UK) was used for the aerodynamic particle size studies. The details of equipment and process have been reported previously.[18]

The geometric standard deviation (GSD) and mass median aerodynamic diameter (MMAD) were calculated and the respirable fraction taken as cumulative amount of particles < 5 μm.

The fine particle fraction (FPF) was calculated using the following equation:

$$\text{FPF} = \text{percentage of emitted dose} \times \text{respirable fraction} \quad (1)$$

Statistical methods

Statistical analysis was performed to compare the results obtained from in-vitro studies of nebulised suspensions (i.e. total output and aerodynamic characteristics) using the Kruskal–Wallis test. Individual differences between the values were then examined using Dunn's test. $P < 0.05$ was considered as a significant difference in all cases.

Results

Analysis of size and morphology of particles

Table 1 shows the particle size of samples obtained by PCS. Particle sizes over 4000 nm were outside the size analysis range of the particle size instrument and therefore the dispersed samples with particle sizes exceeding this limit were reported as > 4000 nm. Figure 1 compares the SEM image of powder number 1, 8 and 10.

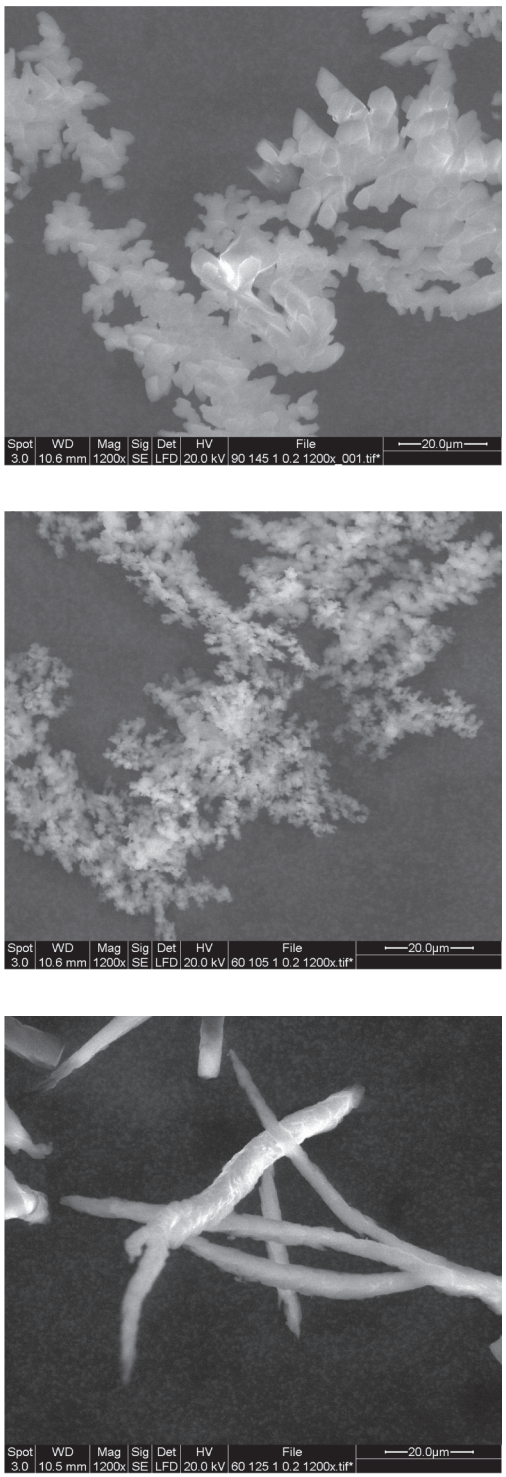

Figure 1 Scanning electron microscopy images of the samples No. 1 (a), 8 (i.e. S1) (b) and 10 (i.e. S2) (c)

In-vitro characteristics of the nebulised suspensions

Table 2 details the mean (SD) in-vitro aerosol output of the formulations of S1 and S2 and P for both jet and vibrating mesh nebulisers. Table 3 summarises the mean (SD) aerodynamic particle size data obtained from the cascade impactor for both nebulisers. Figures 2 and 3 represent the Log_{10} aerodynamic particles size of the preparations plotted against the cumulative percentage less than stated size.

Table 2 In-vitro aerosol output data

Sample	Nebuliser system	Drug left in chamber (%)	Entrained on inhalation filter (%)	Entrained on exhalation filter (%)
S1	Jet	48.61 (15.68)[1]	27.63 (8.63)	22.52 (8.48)
S2	Jet	72.50 (3.82)[1]	15.55 (2.71)[2]	11.94 (1.11)[3]
P	Jet	51.24 (7.79)	27.25 (4.77)[2]	20.10 (2.74)[3]
S1	Vibrating mesh	17.35 (7.63)[4]	40.42 (3.93)[6]	39.44 (4.27)[8]
S2	Vibrating mesh	60.85 (5.69)[4,5]	21.13 (2.06)[6,7]	18.01 (3.63)[8,9]
P	Vibrating mesh	24.88 (19.90)[5]	34.98 (9.70)[7]	33.06 (9.01)[9]

Superscript numbers in the table show a significant difference ($P < 0.05$) between corresponding values. Data are presented as means (SD). $^{1,2,3,4,5,6,7,8,9}P < 0.05$ between corresponding numbers (Dunn's test).

Table 3 in-vitro aerodynamic particle size

Sample	Nebuliser system	MMAD (μm)	GSD (μm)	Respirable fraction	FPF
S1	Jet	6.54 (2.05)	1.78 (0.27)	28.32 (6.35)	14.58 (8.20)
S2	Jet	7.15 (0.35)	1.80 (0.07)	34.55 (2.40)	9.57 (2.25)
P	Jet	7.08 (0.64)	1.83 (0.15)	30.03 (5.76)	18.73 (6.76)
S1	Vibrating mesh	3.53 (0.68)*	2.43 (0.53)	67.05 (11.29)	53.65 (14.21)
S2	Vibrating mesh	2.40 (0.20)*,[1]	1.98 (0.08)	84.38 (5.42)[2]	32.81 (1.80)
P	Vibrating mesh	6.45 (2.16)*,[1]	1.70 (0.17)	40.69 (23.76)[2]	28.02 (24.76)

GSD, geometric standard deviation; MMAD, mass median aerodynamic diameter; FPF, fine particle fraction. Data are presented as means (SD), $n = 4$. *$P < 0.05$ (Kruskal–Wallis test); $^{1,2}P < 0.05$ (Dunn's test); superscript numbers and asterisks in the table show a significant difference between corresponding values.

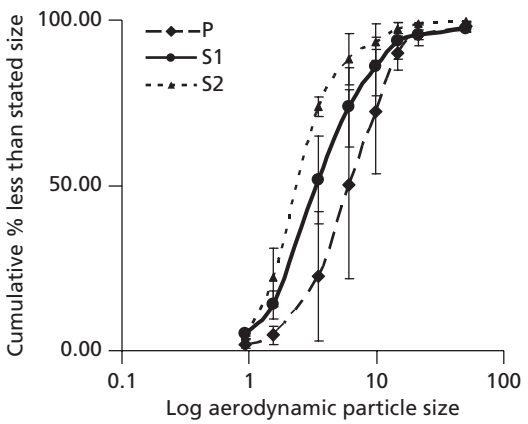

Figure 2 The aerodynamic particle size distribution of S1, S2 and P using a vibrating mesh nebuliser

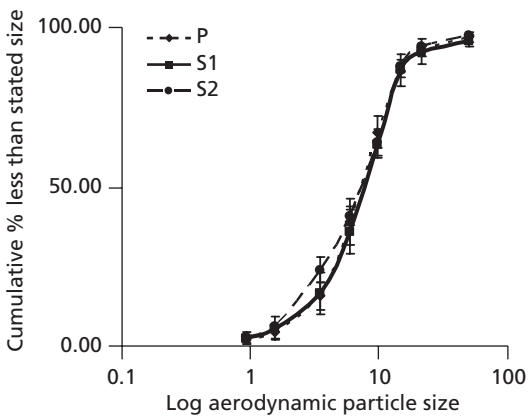

Figure 3 The aerodynamic particle size distribution of S1, S2 and P using a jet nebuliser

Discussion

Particle size analysis

The particle size results (Table 1) show that the particle size of the product changed under different processing conditions. The SCF processed batch with the smallest size, and regarded as being the most suitable for formulation for nebulisation, was observed for batch 8 (S1). This powdered material was selected and used for the remainder of the experiments.

Scanning electron microscopy

The results show alternative morphologies for powder particles obtained using different processing conditions. Images in Figure 1b confirm the small particle size for batch 8 compared with batches 1 and 10. A different morphology was observed for particles of batch 10 (Figure 1c), with ribbon-shaped particles. In an initial attempt to investigate the effect of particle morphology and shape on the aerodynamic properties of the emitted aerosol, suspensions of this batch (S2) were also examined, and compared with the performance of S1 and the commercial suspension product (P).

In-vitro characteristics of the nebulised suspensions

Data in Tables 2 and 3 (i.e. in-vitro aerosol output and aerodynamic particle size measurements) show a substantially smaller MMAD in the vibrating mesh nebuliser compared

with the jet nebuliser when using SCF-engineered products, suggesting an overall improved performance of the nebulised suspensions when delivered from a vibrating mesh rather than a jet nebuliser. These findings are consistent with previous reports of the performance of vibrating mesh nebulisers.[13,14]

From Tables 2 and 3 it was seen that no significant change ($P > 0.05$) occurred in the in-vitro characteristics of the nebulised suspensions when using the jet nebuliser for S1, S2 and P. The only exception was the significant change for the percentage of remaining budesonide suspension for S1 compared with S2 after nebulisation. This suggests an improved emitted dose when using S1 as well as amount of drug entrained on inhalation/exhalation filter when comparing S2 with P. These differences are most likely due to the larger size and more irregular shape of the particles in the S2 suspension formulation. The larger ribbon-shaped particles in S2 (i.e. > 4 μm) are unlikely to be as readily aerosolised and therefore larger amounts remain in the chamber of the nebuliser. This finding provides some initial insight into the joint roles of particle size and shape on aerosolisation performance during nebulisation. Overall, the data indicate that the performance of the S1 and P will not be significantly different but better than S2 when using this nebuliser.

When considering the vibrating mesh nebuliser, major improvements in the in-vitro properties of the nebulised suspension are observed. The MMADs for S1 and S2 are 3.53 and 2.40 μm, respectively, compared with an MMAD of 6.45 μm for P with a much reduced variation (i.e. SD 0.68 and 0.20 μm, respectively, compared with 2.16 μm). The respirable fraction (%) is 67.05 and 84.38 for S1 and S2 compared with 40.69 for P and more consistent (i.e. SD 11.29 and 5.42 compared with 23.76). The FPF values (%) for S1 and S2 are 53.65 and 32.81, respectively, against 28.02 for P, with reduced values of standard deviation. Generally, the in-vitro aerodynamic particle size of the SCF-engineered particles is preferable for nebulised formulations than the commercial product.

The differences in performance of the three suspension formulations from the vibrating mesh nebuliser are attributed to a number of factors. The drug particles in the commercial formulation are reported to have a size of 2–3 μm, from SEM analysis, with irregular morphology and shape, typical for materials subjected to high-energy milling such as micronisation.[19] The aerodynamic particle size is more relevant when considering nebulisation performance and the larger figure obtained for the commercial suspension from this study links to the observed poorer performance. However, if particle size was the only property causing this behaviour, the aerodynamic properties of the jet-nebulised aerosols should have also been similar. Another factor likely to contribute to the relative performance between different nebulisers is the effect of evaporation of aerosolised particles. When using jet nebulisers, agglomerated, dried or semi-dried particles will deposit in the cascade impactor due to evaporation.[20] However, the aerosolised suspension delivered from vibrating mesh nebulisers does not experience such extensive evaporation and the consequent reduced in-vitro performance.

In addition, the in-vitro aerosol output data for the vibrating mesh nebuliser show improved performance figures (i.e. percentage of drug left in chamber and entrained in inhalation and exhalation filters) for S1 compared with P. The amount of drug left in the chamber of the nebuliser after aerosolising S1 is reduced with a lower standard deviation than the figures for P (i.e. 17.35 vs 24.88 and SD 7.63 vs 19.90), while this value is significantly larger for the S2 formulation (60.85).

When comparing the two suspensions prepared from SCF-engineered particles, the MMAD and respirable fraction of the emitted dose from S2 are significantly improved compared with S1. However, when the FPF – which represents respirable fraction of emitted dose – is taken into account, the overall performance of S2 is less attractive, indicating the better performance of S1 than S2. The drug particles in S2 are physically larger than S1 (> 4μm vs 1.7 μm) and have a more irregular morphology. From the relatively low percentage of emitted dose from S2 samples, it is proposed that the rod-like particles with larger diameters (i.e. majority of the particles) have remained in the chamber and only the particles, or the parts of the particles, with smaller diameters have been emitted. Nevertheless, the percentage of particles in S2 that are aerosolised exhibit a ribbon-shaped morphology and thus may align themselves with the air flow and are deposited in the lower stages of the cascade impactor.

Evaporation of the nebulised solvent is a second factor that possibly affects the aerodynamic properties of the nebulised preparations.[20] To limit this effect, using a cooled next generation impactor (NGI) has been suggested[21] and thus it could be useful to use this approach in future studies.

In general, the MMAD and FPF data for the three suspension preparations examined indicate that S1 substantially outperforms S2 and P when using the vibrating mesh nebuliser, with smaller MMAD and amount of drug left in the chamber of the nebuliser, with improved respirable fraction and FPF generated in the same nebulisation. In addition large SD values observed for the in-vitro characteristics of P when using the vibrating mesh nebuliser may lead to inconsistency in the amount of drug delivered in-vivo.

Conclusions

The challenges associated with using suspensions in different types of nebulisers provide a rationale to look for alternative formulations in lung delivery of hydrophobic drugs using nebulisers. In this study, the suspension of SEDS-engineered budesonide showed a distinct improvement over the marketed suspension of budesonide in terms of its in-vitro characteristics when nebulised, using a vibrating mesh nebuliser. The smaller MMAD value and reduced in-vitro variation in measured aerosolisation parameters indicate the potential of supercritical fluid processing technologies in producing an alternative preparation of budesonide for vibrating mesh nebuliser applications.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The authors would like to express their gratitude to Ministry of Health, Treatment and Medical Education of I.R. of Iran and Iranian Nanotechnology Initiative for supporting this research.

Acknowledgements

We are grateful to Dr Lyn Daintree for her help in the supercritical fluid studies.

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