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Note

Drug/lactose co-micronization by jet milling to improve aerosolization properties of a powder for inhalation

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Abstract

The aim of this work was to formulate a powder for inhalation with fusafungine, a drug substance initially highly cohesive. The classical approach based on micronization by jet milling to prepare respirable drug particles and then blending with a carrier was first applied. A fractional factorial experimental design was implemented to screen six formulation parameters. The effect of drug/lactose co-micronization on aerosolization was then evaluated. *In vitro* deposition studies were performed with the twin stage glass impinger and the inhaler Spinhaler®. Micronization did not induce DSC-detectable amorphization and gave a highly cohesive, poor flowable powder with a theoretical aerodynamic diameter of 5 μm. The powder was then blended with coarse lactose and optionally fine lactose. Unfortunately, the respirable fraction could not be optimized and remained below 10%. On the other hand, a co-micronized powder drug/fine lactose 50:50 gave a respirable fraction of 16%. Following blending with a carrier, the respirable fraction and the emitted dose fraction reached 23% and 69%, respectively. The use of a fine lactose grade for co-micronization was essential. In conclusion, this study demonstrated that co-micronization with a fine lactose is an efficient and simple strategy to formulate a powder for inhalation with enhanced aerosolization properties, especially for highly cohesive drug substance.

Keywords: Dry powder inhaler; Co-micronization; Jet milling; Lactose; Respirable fraction; Agglomerate

Dry powder inhaler (DPI) development requires the preparation of the drug substance with a particle size distribution suitable for inhalation, the subsequent formulation of a free flowing non-cohesive powder mixture and the design of a convenient inhaler with adequate dispersing properties to aerosolize the powder formulation. Many efforts have focused on improving the inhaler, especially to design a system independent from inspiratory flow rate and to enhance the aerosolization process during inhalation. Nevertheless, the use of an inexpensive unit-dose device remains well adapted in the case of drug substances intended for short period of treatment (Newman and Busse, 2002). For such device, the powder formulation has to be filled in a gelatin or hypromellose hard capsule which is then loaded inside the device before puncture of the shell. Whatever the inhaler type, aerosolization performance remains firstly dictated by the physical properties of drug particles. Recent research on particle engineering focused on large porous particles (Lotan et al., 2003), spheronization (Borgström et al., 2002), supercritical fluid-based techniques (Reverchon and Della Porta, 2003; Rehman et al., 2004) and controlled crystallization techniques (Steckel et al., 2003). A more classical and simple approach is based on micronization. Drug micronization is known to give relevant theoretical aerodynamic diameter but the resulting powders are often highly cohesive and display poor aerosolization and flowability properties. Thus, further blending of the micronized drug with a carrier, typically a coarse lactose, is classically proposed in the literature. Adhesion forces between the carrier and the micronized drug particles must be able to create a stable ordered mixture but should remain sufficiently low to ensure the release of drug particles in a turbulent air flow. In addition, numerous studies evidenced higher powder dispersion and deagglomeration when a ternary mixture was prepared, i.e. coarse carrier/micronized drug/fine lactose (Zeng et al., 1999, 2000; Tee et al., 2000; Louey and Stewart, 2002). The effect of fine lactose remains unclear, but it is generally thought that its presence saturates high energy sites on coarse lactose, decreases drug/carrier adhesion forces and subsequently facilitates the separation of the drug particles in a turbulent air flow.

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Table 1
Particle size distribution for mannitol (Pearlitol®SD200) and for the different lactose grades

Application in the present study	Trade name and supplier	Particle size distribution
Carrier for micronized drug substance or co-micronized product	Pearlitol®SD200 (Roquette frères) Pharmatose®325M (DMV) Inhalac®120 (Meggle) Inhalac®70 (Meggle)	1% > 315 μm 94% > 75 μm Average particle size 50 μm $d_{0.5,4\ bar}$ = 121 μm $d_{0.5,4\ bar}$ = 144 μm
Fine lactose used to prepare ternary mixture	Lactochem®Microfine (Borculo Domo)	90% < 10 μm
Lactose used for co-micronization with the drug substance	Lactochem®Microfine (Borculo Domo) Lactose Fast-Flo® (Foremost Farms) Tablettose®80 (Meggle)	62% < 150 μm40–75% < 180 μm

Median diameters for Inhalac® were determined in-house by laser diffraction. Other values are specifications from the suppliers.

The aim of this study was to investigate micronization in order to prepare aerosolizable drug particles of fusafungine, a highly cohesive drug substance intended for short and local treatment of bronchial infection. Micronized fusafungine was formulated with a carrier or as a ternary mixture. Moreover, an original approach was investigated: the co-micronization of fusafungine and lactose followed by blending with a coarse carrier. In the field of DPI, co-milling using a jet mill micronizer was reported to coat active particles with various additives called force control agents, preferably leucine (Staniforth et al., 2004). However, to our knowledge, the interest of drug:lactose binary mixture co-milling has never been reported. Our goal was to reach a particle mean aerodynamic diameter between 3 and 5 μm, a respirable fraction above 20% and an emitted dose fraction above 70%.

The different excipients used, their role and their specifications are presented in Table 1. Micronization was performed with a jet mill micronizer (Ceca) at a feeding rate of 20 g h^{-1}, a feed pressure of 3 bar and a pressure within the micronizer chamber of 6 bar. Powders were manually sieved before and after micronization (0.4 and 0.25 mm, successively). In the case of co-micronization, the drug and the excipient(s) were mixed for 10 min at 46 rpm with a Turbula® mixer prior to micronization. The formulation of micronized fusafungine with carriers or as ternary mixture was studied through an asymmetrical fractional factorial experimental design $2^5/3^1//12$ (Lewis et al., 1999). Calculations were performed using the computer software NEMROD® (LPRAI, Marseille, France). During the mixing step with the carrier, an intermediate sieving (0.25 mm) was optionally performed at half time (Table 2). When fine lactose was used to prepare ternary mixtures, a carrier/fine lactose (10:1) powder mix was first prepared before addition of the drug substance and further mixing. Mixing equipment was a Turbula® mixer (46 rpm). The mixing of the co-micronized product with a coarse lactose (Inhalac® 70) as carrier was performed with a drug to carrier ratio of 1:60, a total mixing time of 20 min and an intermediate sieving (0.25 mm) after the first 10 min of mixing. All formulated powders were stored in glass bottles at room temperature.

Powder densities were determined according to European Pharmacopoeia and Carr index was defined as: Carr index = (tapped density − bulk density)/tapped density.

Pycnometric density of powders (d) was determined according to European Pharmacopoeia using the helium pycnometer Micrometer ACCUPYC 1330 (Micromeritics).

Particle size distributions were determined by laser diffraction (Mastersizer 2000, Malvern Instruments) using the dry powder dispersing system Scirocco 2000 at a pressure of 0 or 4 bar. Particle size distributions were characterized by the vol-

Table 2
Formulation of the micronized drug substance (mDS) with a carrier and optionally fine lactose: studied parameters, description of the experiments and results in term of respirable fraction

	Use of fine lactose	mDS/fine lactose mixing time[a] (min)	Carrier	mDS/carrier ratio	Mixing with the carrier (min)	Intermediate sieving during mixing with the carrier	Respirable fraction (%)
1	No	5	Inhalac® 120	1:15	5	No	7.5
2	No	5	Pharmatose®325M	1:15	20	Yes	5.9
3	No	5	Pearlitol®SD200	1:15	5	No	2.9
4	No	20	Pearlitol®SD200	1:60	5	Yes	4.3
5	No	20	Inhalac® 120	1:60	20	No	4.0
6	No	20	Pharmatose®325M	1:60	5	No	5.1
7	Yes	20	Pharmatose®325M	1:15	5	No	6.6
8	Yes	20	Pearlitol®SD200	1:15	20	No	4.2
9	Yes	20	Inhalac® 120	1:15	5	Yes	4.5
10	Yes	5	Pharmatose®325M	1:60	5	No	10.5
11	Yes	5	Pearlitol®SD200	1:60	20	No	5.9
12a	Yes	5	Inhalac® 120	1:60	5	Yes	5.8
12b	Yes	5	Inhalac® 120	1:60	5	Yes	4.3
12c	Yes	5	Inhalac® 120	1:60	5	Yes	8.0

[a] For experiments without fine lactose, the drug substance alone was subjected to agitation with Turbula® mixer.

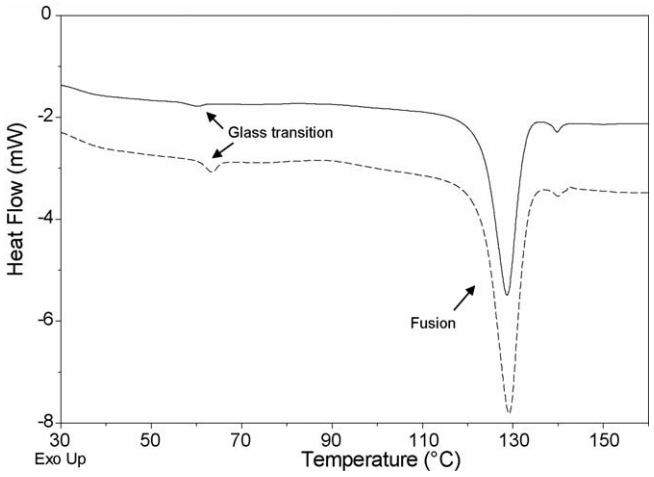

Fig. 1. Comparative DSC profiles for non-micronized (dotted line) and micronized drug substance (solid line).

Table 3
Physical properties of the drug substance

	Non-micronized drug substance	Micronized drug substance
Volume diameters for dispersion at 0 bar (μm)		
$d_{0.1}$	14	55
$d_{0.5}$	30	272
$d_{0.9}$	76	866
Volume diameters for dispersion at 4 bar (μm)		
$d_{0.1}$	4	2
$d_{0.5}$	12	5
$d_{0.9}$	28	10
Agglomeration ratio	2.5	59.9
Carr index (%)	27	33
Theoretical aerodynamic diameter (μm)	nd	5.2
Respirable fraction (%)	nd	4

nd: not determined.

ume median diameter $d_{0.5}$ and by $d_{0.1}$ and $d_{0.9}$ values ($n = 3$). The agglomeration ratio $d_{0.5,\,0\,\mathrm{bar}}/d_{0.5,\,4\,\mathrm{bar}}$ was considered as representative of the degree of powder cohesiveness. The theoretical aerodynamic diameter of the individual particles ($\mathrm{dae_{th}}$) was calculated as follows: $\mathrm{dae_{th}} = \sqrt{d} \times d_{0.5,\,4\,\mathrm{bar}}$.

A JEOL JSM 6100 scanning electron microscope was used to examine the powder blend.

Thermograms were acquired by differential scanning calorimetry (DSC) with a heating rate of 5 °C/min (Thermal Analysis Instruments, MDCS 2920).

In vitro deposition test was performed with the twin stage glass impinger (Apparatus A, European Pharmacopoeia) connected to a vacuum pump (60 l $\mathrm{min^{-1}}$), the Spinhaler® inhalator device and size 2-hard gelatin capsule. Where a carrier was present in the formulation, capsules were filled with 20 mg of powder. In absence of a carrier, capsules were filled with 2 mg of powder. For each powder to be analysed, three capsules are successively placed inside the inhalator and the pump was switched on for 10 s twice to aerosolize the powder from each punctured capsule. The total collected dose included the amount of fusafungine found in both stages of the impinger, the amount of fusafungine found in the inhalator and the amount of fusafungine remaining in the three capsules. The respirable fraction (RF) was calculated by dividing the amount of fusafungine found in the lower stage of the impinger (cut-off aerodynamic diameter: 6.4 μm) by the total collected dose. The emitted dose fraction was calculated by dividing the amount of fusafungine found in both stages of the impinger by the total collected dose. Fusafungine was assayed by HPLC with UV detection.

DSC profiles for both non-micronized and micronized fusafungine were similar showing that micronization did not induce any detectable amorphization (Fig. 1). Drug micronization decreased the median particle size from 12 to 5 μm, as determined with a pressure of 4 bar (Table 3). The theoretical aerodynamic diameter of the micronized drug substance was 5.2 μm. However, the agglomeration tendency was markedly increased. SEM analysis of the micronized drug substance confirmed the presence of large agglomerates and the absence of well-individualized small particles (Fig. 2). In addition, the micronized powder was characterized by very low respirable fraction (4%) and a Carr index of 33%, showing poor flowability properties.

To improve the flowability and the dispersion of drug particles during aerosolization, a blending step of the micronized drug with a coarse carrier was evaluated. It is well recognized that the physical properties of the carrier have a significant influence on formulation dispersibility and aerosol performance (Larhib et al., 2003). Thus, three different marketed carriers were tested in the present work, namely two different lactose grades (Inhalac® 120 and Pharmatose® 325 M) and one mannitol grade (Pearlitol® SD200). The particle size distribution for these excipients is presented in Table 3. In addition, Lactochem® Microfine was optionally incorporated in the formulation to prepare a ternary mixture. Basically, five formulation parameters were screened to evidence active formulation parameters (Table 2). Unfortunately, the respirable fraction was between 2.9% and 10.5%, which did not comply with our requirement (>20%). The statistical analysis of the data revealed that the difference between the observed results for the respirable fraction

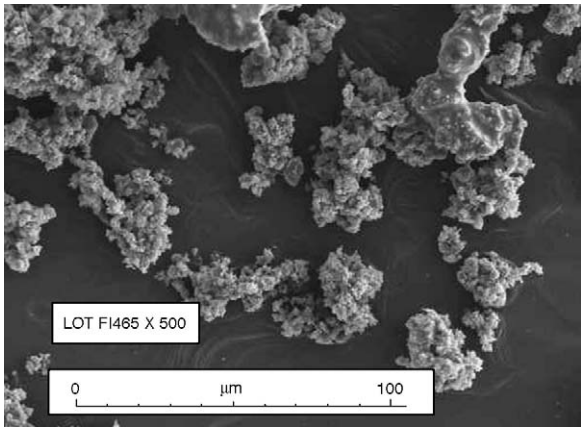

Fig. 2. Microscopic appearance of micronized drug substance as revealed by SEM analysis.

Table 4
Effect of drug substance (DS) co-micronization with different grades of lactose on respirable fraction and emitted dose fraction

Co-micronized lactose	DS/lactose ratio	Formulation with the carrier Inhalac®70	Respirable fraction (%)	Emitted dose fraction (%)
No	100:0	Yes	7.8	80.9
Lactochem® Microfine	50:50	Yes	23.2 ± 2.6 ($n = 3$)	69.3 ± 2.6 ($n = 3$)
	70:30	Yes	14.9	63.3
	50:50	No	15.6	36.2
Fast Flow®	50:50	Yes	6.2	68.4
	70:30	Yes	7.0	76.8
Tablettose®80	50:50	Yes	7.4 ± 1.5 ($n = 3$)	64.8 ± 6.5 ($n = 3$)
	60:40	Yes	8.0	72.4
	70:30	Yes	7.5	72.2
	80:20	Yes	3.1	75
	90:10	Yes	6.0	76

could be attributed to the experimental error (calculated from the triplicated experiment) and was not especially controlled by the variation of the formulation parameters (ANOVA $P > 0.05$). We concluded that the cohesiveness of the drug particles was too important after micronization. Thus, no ordered powder mix could be obtained from blending with a carrier, irrespective of the formulation parameters.

Co-micronization was performed using different lactose grades: Lactochem® Microfine or lactose with larger particle sizes (lactose Fast-Flo® and Tablettose®80). A deposition test was performed on the micronized product as such or after its mixing with the carrier Inhalac®70. Co-micronization of drug/fine lactose 50:50 and then mixing with the carrier gave a respirable fraction of 23.2%, an emitted dose fraction of 69.3% and good flowability properties (Carr index <20%) (Table 4). The microscopic observation by scanning electron microscopy revealed the presence of small particles of the co-micronized ingredients coating the coarse particles of carrier (Fig. 3). The respirable fraction decreased from 23.2% to 14.9% when a 70:30 drug/fine lactose ratio was used instead of 50:50. During co-micronization, it appeared that the feeding of the micronizer with the powder mix (drug substance/fine lactose) was not optimal. To improve this parameter we tried to replace fine lactose with Lactose Fast-Flo® or Tablettose®, two other grades of lactose with larger particle sizes (Table 3). Unfortunately, the respirable fraction decreased and was found below 10% (6.2% for a 50:50 drug/lactose ratio). The modification of the drug/lactose ratio for Tablettose® and Fast-Flo® did not improve the respirable fraction which remained below 10%. We believe that during co-micronization, drug particles combined with lactose particles at the same time as being fractured, thus creating an ordered powder mix and reducing drug cohesiveness. But this required the micronization of the drug with lactose particles already reduced (i.e. fine lactose). Another hypothesis would be an increase in surface energy of lactose particles in the case of Fast-Flo® and Tablettose®80, thus rendering the lactose particles too interactive. While in the case of the fine lactose, no further particle size reduction occurred that limited the apparition of surface charges.

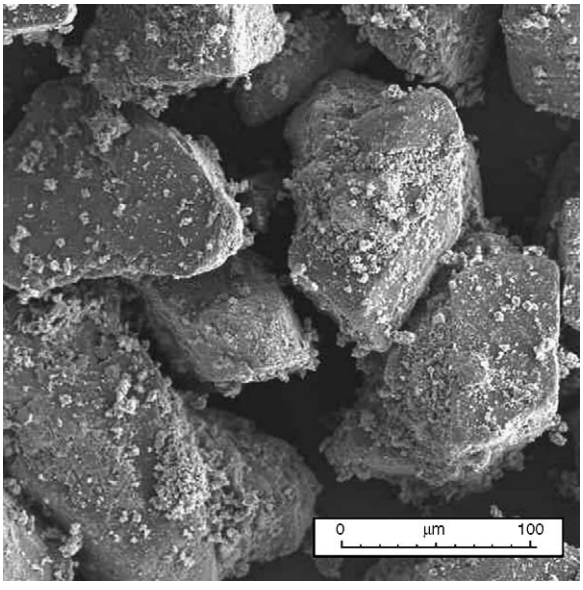

Fig. 3. SEM image of a co-micronized product (fusafungine/fine lactose 50:50) after mixing with the carrier Inhalac® 70.

In conclusion, we demonstrated that co-micronization by jet milling of a drug substance with a fine lactose grade is an efficient and simple approach to formulate a powder for inhalation, especially for an initially highly cohesive drug substance. This strategy does not require specialized equipment. Comprehensive studies by AFM and microcalorimetry should now be carried out to clarify the functional effect of the co-micronized excipient.

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