Characterisation of jet-milled and spray dried isoniazid for pulmonary administration

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Summary

The aim of this study was to develop a dry powder isoniazid formulation with no or a limited amount of excipients for pulmonary administration. Milled isoniazid showed an excellent particle size distribution for inhalation, however dispersion was poor. In 78% of the dispersion measurements the inhaler blocked, retaining most of its dose. Pure spray dried isoniazid yielded particles too large for pulmonary delivery, but the addition of 5% of L-leucine resulted in spray dried particles of inhalable size. DSC data showed complete crystallinity for all samples, while TGA analysis showed that isoniazid sublimates around 100°C. SEM imaging showed that pure jet milled and spray dried isoniazid particles fused together. Isoniazid spray dried with L-leucine resulted in spherical particles with no fusion visible. The most likely explanation for particle fusion is that isoniazid crystalizes, resulting in solid bridge formation. L-leucine however, forms a coating around isoniazid particles, thereby preventing this phenomenon. Further experiments are needed to show why isoniazid fuses together in the jet mill. A possible explanation is that some isoniazid sublimates due to heat generation during particle collisions, and causes solid bridge formation between particles when it ripens. Further experiments have to show whether isoniazid co-spray dried with L-leucine disperses efficiently and is stable over time.

Introduction

The World Health Organization (WHO) estimates that one third of the world population is (latently) infected with tuberculosis (TB).[1] Isoniazid is an antibiotic used in the first-line treatment of TB. However, in 15% of all TB cases worldwide resistance to isoniazid is encountered.[2] Thus far only oral and injectable forms are available. Pulmonary administration of isoniazid can provide significant advantages over oral and parenteral administration. Inhaled drug is administered directly to the target area (port of entry), which means that with the same dose administered a higher local concentration can be achieved, eradicating bacteria considered resistant.[3] Since most TB cases are in third world countries, a stable and cheap formulation is needed.[1] A dry powder combined with a cheap inhaler would fit these requirements.[3] In the scientific literature, experimental isoniazid formulations consist either of drug loaded liposomes which only contain 10% drug, or of a combination formulation in which isoniazid only makes up 12.5% of the powder.[4]–[6] These formulations are of limited use since anti-TB drugs have to be given in high doses, as recommended by the WHO.[7] For these reasons we aimed to develop an isoniazid powder formulation suitable for pulmonary delivery with no or only a limited amount of excipients.

Materials and Methods

Isoniazid and L-leucine used during spray drying were purchased from Sigma Aldrich (St. Louis, United States). Isoniazid used during jet milling was provided by Fluka (St. Louis, United States).

Spray drying was performed using a B-290 mini spray dryer supplied by Büchi (Flawil, Switzerland). A range of parameters were investigated, as shown in Table 1. All samples were produced in duplicate. The first sample (50 mg/mL concentration and 60°C inlet) was also spray dried with 5% leucine w/w. Samples were always prepared in 20 mL demineralised water before spray drying. Before each run the outlet temperature was stabilised by spraying demineralised water in the system.

Concentration isoniazid (mg/ml)	Inlet temperature (°C)	Feed rate (ml/min)	Atomizing air flow (mm)	Atomizing air flow (%)
50	60	2.5	50	100
50	40	1	50	100
50	160	2.5	50	100
50	160	12.5	50	100
50	20-30	0.1	50	100
25	60	2.5	50	100
75	60	2.5	50	100

Table 1 - spray drying conditions with the B-290 mini spray dryer. All samples were produced twice.

Milling was performed using a 50 AS spiral jet mill supplied by Alpine (Augsburg, Germany). The mill was equipped with a 0.8 mm nozzle. Table 2 shows the three different pressure settings tested, the milling gas used was nitrogen.

	Nozzle pressure (bar)	Mill pressure (bar)
Low pressure	4	0.5
Medium pressure	5	2
High pressure	7	2

Table 2 - jet milling conditions with the 50 AS spiral jet mill. All samples were produced twice.

Particle sizes were measured using a HELOS BF diffraction unit with a RODOS dry disperser, both provided by Sympatec (Clausthal-Zellerfeld, Germany). The HELOS diffraction unit was equipped with an R3 lens, resulting in a measurement range of 0.1 µm-175 µm. Powders were dispersed with the RODOS at 0.5, 3 and 5 bars. All measurements were performed two times for the milled samples and three times for the spray dried samples. The average with standard deviation was then calculated.

Dispersion measurements were performed using the HELOS diffraction unit equipped with the INHALER 2000 attachment, also provided by Sympatec (Clausthal-Zellerfeld, Germany). The Twincer® dry powder inhaler (DPI), provided by PureIMS (Roden, the Netherlands), was used as test inhaler. Measurements were performed with a pressure drop of 2, 4 and 6 kPa and with a dose of 25 mg. All samples were measured twice.

Scanning electron microscopy (SEM) images were obtained using a JSM 6301-F microscope provided by JEOL (Tokyo, Japan). Samples were placed on carbon tape and coated with 10 nm of gold using the JFC-1300 Auto Fine Coater, also provided by JEOL (Tokyo, Japan), while purged with argon gas. Imaging itself took place under high vacuum, with an acceleration voltage of 10kV, a working distance of 10 mm and a spot size of 25. The secondary electron detector was used.

Differential scanning calorimetry (DSC) was performed with the Q2000 supplied by TA instruments (New Castle, United States). A sample of 1-4 mg was placed in a Tzero pan and hermetically sealed. Pans were loaded into the DSC at 20°C and a ramp from 20°C until 210°C was made at a rate of 20°C /min. Samples were always measured the same day as they were spray dried or milled, each sample was measured twice.

Thermogravimetric analysis (TGA) was performed on the isoniazid starting material for spray drying. A sample of 6-8 mg was placed on a platinum pan. The sample was heated at 80°C for 4 hours after which the temperature was increased with 20°C and again the temperature was kept stable for 4 hours. This process was repeated until 140°C was reached. Non-cumulative weight loss percentage over these 4 hour periods was then calculated.

Results

In Table 3 the laser diffraction data of the jet milled isoniazid samples is given. The particle sizes are consistently smaller compared to the spray dried samples. Furthermore, the higher the pressure the smaller the particles, as is to be expected.

	X10 (µm)	X50 (µm)	X90 (µm)
Low pressure	1.59 ± 0.01	2.91 ± 0.06	5.34 ± 0.63
Medium pressure	1.26 ± 0.42	2.50 ± 0.58	4.57 ± 1.19
High pressure	1.11 ± 0.26	2.22 ± 0.26	3.82 ± 0.28

Table 3 - the jet milled isoniazid laser diffraction data. Depicted the X10, X50 and X90 at 3 bars (average ± SD, n = 2).

Table 4 shows the amount of 'blockages' (i.e. where agglomerates in the formulation cause almost complete inhaler retention) during the dispersion measurements. At a pressure drop of 2 kPa all measurements showed blocked Twincers®, at 4 kPa the situation improves for the low and high pressure milled samples with 1 blockage for every 2 measurements. However, at 6 kPa these samples show again blockages in all measurements except for the medium pressure sample, there no blocked Twincers® were seen.

	2 kPa	4 kPa	6 kPa
Low pressure	2/2	1/2	2/2
Medium pressure	2/2	2/2	0/2
High pressure	2/2	1/2	2/2

Table 4 - amount of measurements that showed blockages in the Twincer® dpi during dispersion measurements (n=2).

Concentration isoniazid (mg/ml)	Inlet temperature (°C)	Feed rate (ml/min)	Outlet temperature (°C)	X10 (µm)	X50 (µm)	X90 (µm)
50	60	2.5	37	3.09 ± 0.29	10.95 ± 0.16	30.90 ± 1.62
50	40	1	31	2.93 ± 0.15	9.68 ± 0.93	23.27 ± 3.20
50	160	2.5	94	3.39 ± 0.56	20.32 ± 4.18	127 ± 11.53
50	160	12.5	57	3.42 ± 0.17	16.06 ± 3.85	86.96 ± 31.91
50	20-30	0.1	20-27	2.52 ± 0.37	9.61 ± 1.46	30.98 ± 8.51
25	60	2.5	37	2.72 ± 0.16	9.22 ± 0.20	24.54 ± 0.83
75	60	2.5	37	3.66 ± 0.27	14.08 ± 0.92	44.61 ± 6.19
50 + Leucine	60	2.5	37	0.99 ± 0.06	2.57 ± 0.13	5.08 ± 0.22

Table 5 - the spray drying settings used in the B-290 spray drier and the resulting X10, X50 and X90 as measured with laser diffraction at 3 bars (average ± SD, n = 2). Atomizing air flow was 50 mm while the aspirator flow was 100% for all samples.

Table 5 shows the different spray drying settings tested. The settings that have not been changed are the aspirator flow and the atomizing air flow, these were 100% and 50 mm respectively. Particle size seems to be mostly affected by the isoniazid concentration in the feed solution and the inlet temperature, while the feed rate has a negligible effect. Only with L-leucine particles small enough for pulmonary administration were obtained.

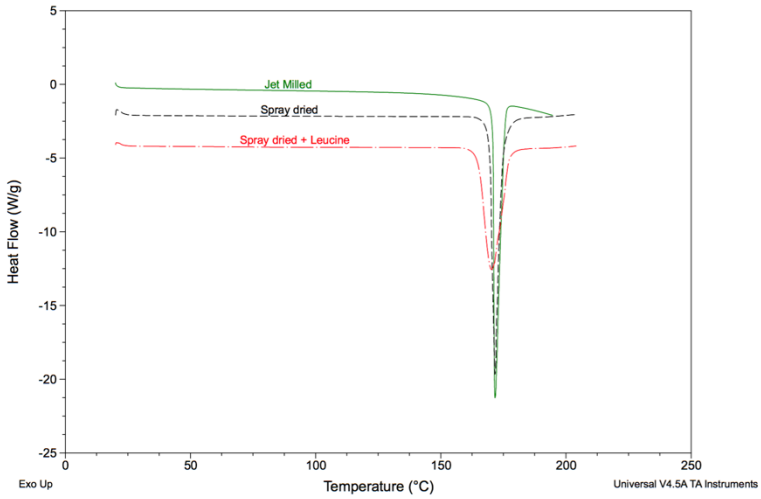

Figure 1 shows representative DSC data from the jet milled, spray dried and spray dried with 5% L-leucine samples. All samples show a sharp melting peak at 171°C except for the samples containing L-leucine, which have a broader melting peak at 170°C. All samples are completely crystalline.

Figure 1 - representative DSC data from the jet milled and spray dried samples. All samples were measured twice.

Temperature (°C)	Mass loss (°C)
80	0.13
100	1.13
120	8.18
140	45.10

In Table 6 the TGA data is shown. At 80°C no mass loss is seen with a decrease over a 4-hour period of only 0.13%. However, at 100°C isoniazid starts to show signs of sublimation, with a decrease of 1.13% in mass. As expected, the rate of sublimation increases with increasing temperature. At the highest temperature tested, 140°C, 45.10% of the sample has sublimated in a 4-hour period.

Table 6 - the TGA data showing the temperature and the mass loss at that temperature. Mass loss was measured over a 4 hour period.

Scanning electron microscopy images of the low and high pressure mill samples are depicted in Figure 2, together with the 50 mg/mL 60°C inlet spray dry samples with and without 5% L-leucine. The two jet milled samples unexpectedly showed highly smoothed particles. Furthermore, the high pressure sample shows this to a greater extent than the low pressure one, the particles look almost completely spherical and some particles seem molten together. This 'fused' appearance is even more pronounced for the pure isoniazid spray dried sample in Figure 2C. One large particle is visible seemingly consisting of multiple particles fused together. The spray dried sample that contains 5% L-leucine consists of smaller particles compared to the formulation of pure isoniazid. These particles are spherical, which is expected from spray dried powders. The SEM images qualitatively corroborate the laser diffraction data shown in Table 1.

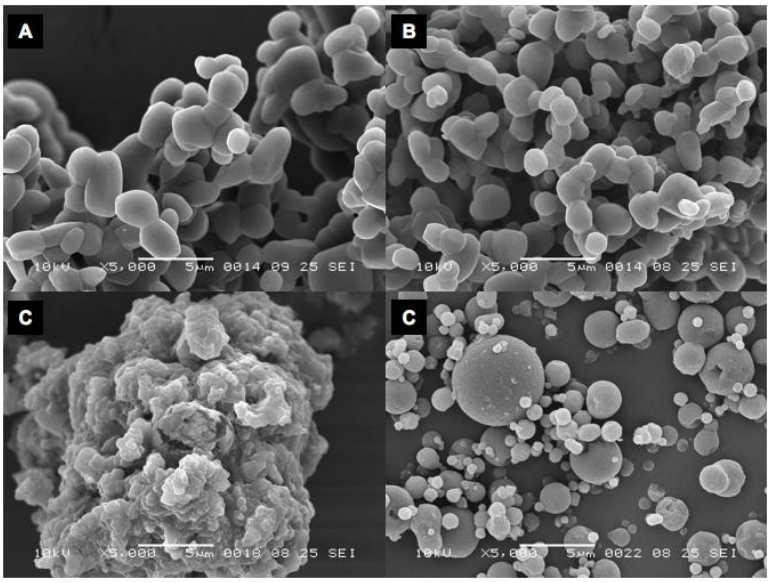

Figure 2, Scanning electron microscopy images of the spray dried and jet milled samples. A shows a jet milled sample at low pressure, while B shows a jet milled sample at high pressure. C shows the 50mg/ml 60 inlet sample, while D shows the sample containing 5% leucine. Magnification 5000 x.

Discussion

Milled isoniazid showed an appropriate particle size for pulmonary administration, with an X50 between 2.91 ± 0.06 µm and 2.22 ± 0.26 µm for all jet milled samples. However, milled isoniazid blocked the DPI during most dispersion measurements (Table 4). It is unclear how these particles gain their molten morphology. This seems to be related to milling pressure, and could result from mechanical stress or the associated increase in temperature locally on the particles surfaces. The TGA data in Table 6 showed that isoniazid sublimates at relatively low temperatures. A possible explanation is that the local increase in temperature sublimates some of the isoniazid, when it cools down the isoniazid deposits on the surface of agglomerates fusing these particles together. However, the binding force seems to be weak since the laser diffraction is able to disperse this powder into primary particles. Further experiments are needed to corroborate this theory. It would be interesting to see what the effect of excipients would be that limit the local increase in temperature, such as a lubricant like magnesium stearate.

As is visible in Table 5 the spray dried samples with pure isoniazid all have large particle sizes and are not suitable for pulmonary administration. Unexpectedly it is the inlet temperature which has the biggest influence of the parameters tested, which increases the X50 of 10.95 ± 0.16 µm at 60°C to 20.32 ± 4.18 µm at 160°C inlet temperature. These particles are larger than the droplets generated in the nozzle, which are somewhere between 8 and 9 µm. The most likely explanation for this remarkable observation is that isoniazid crystalizes in the collection vial fusing different particles together. This seems to be corroborated by DSC data (Figure 1), which are indicative of complete crystallinity, and the SEM image in Figure 2C, where different particles can be distinguished that have fused together.

Isoniazid + 5% L-leucine seems to result in a particle size distribution suitable for inhalation, with an X50 of 2.57 ± 0.13 µm and an X90 of 5.08 ± 0.22 µm. The most likely explanation is that during the spray drying process L-leucine enriched at the droplet surface and formed a coating, which L-leucine has been known to do.[8] This coating may have prevented the isoniazid cores from interacting with each other and thus prevented the fusion between multiple particles. Furthermore, no fusion between individual particles is visible.

Conclusion

Isoniazid displays some strange behaviour which may be heat related. Dispersibility of pure jet milled isoniazid is poor and pure spray dried isoniazid has an unsuitable particle size distribution. However, with the use of 5% L-leucine it is possible to improve the particle size distribution of spray dried material to an acceptable level. Further tests are needed to show if this obtained formulation is dispersible and stable over time.

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