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Use of recrystallized lactose as carrier for inhalation powder of interferon *α2b*

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The aim of the present investigation was to evaluate the effects of physical properties of the carrier on the *in vitro* deposition performance of dry powder inhalations (DPIs) of recombination human interferon *α2b* (IFN *α2b*). Recrystallized lactose was used as the carrier. Inverse gas chromatography (IGC) was used to assess the surface energy, and atomic force microscopy (AFM) was used to assess the roughness and topography of the carrier. *In vitro* performance of the powder blends was strongly correlated to the physical properties of the carrier. Plotting emitted dose (%) vs. flow rate and fine particle fraction vs. surface energy, yielded an R^2 value of 0.9621 and 0.9146, respectively.

The physical properties of carrier particles play an important role in determining the deposition of dry powder inhalations (DPIs) (Young and Cocconi 2002; Zeng et al. 2001). But how to obtain an appropriate carrier and how physical properties of a carrier, especially flowability, roughness and surface energy, affect the *in vitro* deposition of protein-containing dry powder aerosols needs further investigation. To obtain lactose carrier particles with different physical properties, four batches of lactose particles were prepared by recrystallization since the properties of lactose may change with the crystallization conditions (Zeng et al. 2000).

IFN *α2b*-containing spray dried powder was chosen as a model protein and was prepared using a proprietary process (Jiang et al. 2004). The spray dried interferon was blended with lactose carrier at a ratio of 1 : 12.5 (w/w) and then filled in a capsule (size 3) containing 30 ± 1 mg of powder mixture.

The aerosolization of blends was investigated using a twin stage impinger (TSI, Chinese Pharmacopoeia), containing 7 ml of dilution solvent in stage one and 30 ml of dilution solvent in stage two. Powderhaler (Shanghai Tianping Pharmaceutical Factory, China) was used as the delivery device at a rate of 60 ± 2 L/min. IFN *α2b* was analyzed by ELISA. Fine particle dose (FPD) was defined as the amount of drug deposited in stage two, and emitted dose (ED) was the quantity of drug emitted from the inhaler device. Fine particle fraction (FPF) was the ratio of FPD to ED. For each of the five formulations, the deposition tests were performed 6 times and every time 30 capsules of powder were tested.

The majority of the crystals obtained were either tomahawk shaped or prismatic and appeared more regular, with an apparent decrease in the ratio of length to width when comparing the recrystallized and untreated lactose particles (data not shown here). The Fig. shows the representative AFM topography images of the five batches of lactose. The root mean square roughness (RMS) values were 75.1 nm, 65.8 nm, 30.2 nm, 26.4 nm and 71.3 nm for the control lactose and recrystallized lactose induced by ethyl acetate, ethanol, water, and glycerin, respectively, suggesting that a smoother surface can be obtained by recrystallization.

Surface asperities may increase the contact area and subsequently increase van der Waals forces, and sometimes act as a shelter for the entrapped drug, rendering it non-available to the drag forces generated by the inhaled air stream and thus further reducing drug detachment. A smooth surface would be expected to have smaller surface crevices than a rough surface, and consequently, entrapment of a drug particle within the crevices of a surface may be less likely to occur. This would reduce the adhesion forces between the drug and the carrier particles and hence increase the portion of drug particles that are dissociated from the carrier particle surface during inhalation. The re-

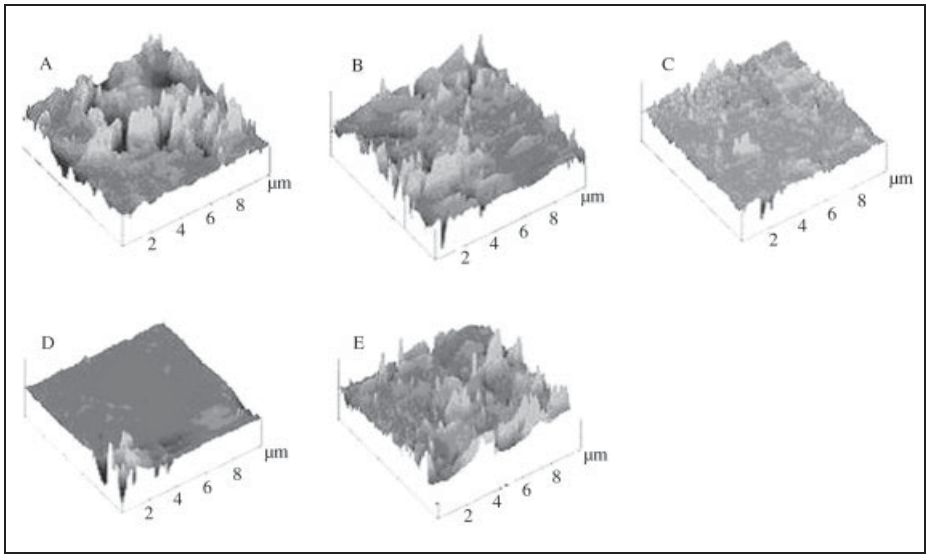

Fig.:
Representative AFM images of commercially available lactose (A), lactose recrystallized in the presence of ethyl acetate (B), ethanol (C) and glycerin (E), and lactose recrystallized in the absence of organic solvents (D). x, y and z axes are 10 μm, 10 μm and 0.3 μm, respectively

Table: Flow rate, surface energy of carriers and in vitro deposition of the DPIs

Lactose carrier	Physical properties of carrier[a]		*In vitro* performance of IFN[b]	
	Flow rate (mg/s)	E_{SP} (mJ/g)	ED (IU)	FPF (%)
Commercially available	32.3 ± 1.2	35.97 ± 1.91	82883 ± 7982	46.1 ± 5.4
Ethyl acetate-induced	84.3 ± 1.4	22.02 ± 0.89	111683 ± 7982	58.7 ± 3.2
Ethanol-induced	54.6 ± 1.6	19.82 ± 0.80	91578 ± 5414	66.7 ± 1.7
Water-induced	74.2 ± 1.8	18.32 ± 0.76	105233 ± 7982	68.9 ± 5.2
Glycerin-induced	50.4 ± 1.3	23.94 ± 1.4	92536 ± 7982	57.1 ± 2.4

[a] n = 5, [b] n = 6

crystallized lactose, with a smoother surface than the control, is hence desirable to improve the aerosolization behavior of DPIs.

Before aerosolization each of the capsules was pricked an orifice of 1.3 mm in diameter, which was similar to the size of the orifice when the flowability of the carrier particles was tested (d = 1.6 mm). With this approach we are able to examine the impact of the carrier's flowability on the emitted dose. As shown in the Table, four batches of recrystallized lactose were shown to have a significantly better flowability than the control ($P < 0.01$), which is possibly attributable to the more regular shape and smoother surface of recrystallized lactose. A trend exists between the flowability of the carrier and the emitted dose of the drug, with a linear coefficient, R^2 of 0.9621, suggesting that a better flowability of the carrier can lead to a higher emitted dose.

There is a substantial difference between the surface energy obtained for the original lactose and recrystallized lactose ($P < 0.01$). The different surface energy reflects different orientations and spacing between the surface molecules, giving rise to different energy states. We could see a decrease in the fine particle fraction with an increased surface energy, with a linear coefficient, R^2 of 0.9146. Carrier particles with a high surface energy have a high tendency to adsorb respirable fine particles onto their surfaces and form strong attractive forces with adhered particles, which makes it difficult for the drug particles to become dislodged from the carrier particles under normal inhalation conditions.

Experimental

Lactose (200 g) was dissolved in 200 ml of distilled water at 95 °C with constant stirring. The solution was filtered through a filter paper (pore size 0.45 μm) while hot and kept at 40 °C for 1.5 h with constant stirring and then filtered again followed by cooling to room temperature without any disturbance. 25 ml of ethyl acetate, ethanol and glycerin was then added respectively, and the mixture was immediately homogenized at approximate 8000 rpm until the majority of the crystals had grown to a size range of 63 to 90 μm. The crystals were immediately filtered and washed twice with 70% (v/v) ethanol and three times with absolute ethanol. All five batches of products were fractioned so that the particle size was between 63 and 90 μm.

Detailed topographical information of the lactose samples was investigated using AFM (Nanoscope IIIa, Digital Instruments, USA) using a tapping mode with a silicon probe over 10 μm 10 μm area at a scan rate of 1.0 Hz.

The flow rate is expressed in terms of the quantity of powder flowing through an orifice in a unit period of time (mg/s). For each sample 10 g powder was allowed to flow through an orifice of 1.6 mm in diameter.

IGC was used to study the surface energies and the basic relationship employed is: $RT \ln V_n = 2N(\gamma_S^D)^{1/2} \alpha(\gamma_L^D)^{1/2} + C$ (Newell et al. 2001). A series of n-alkanes, C_6, C_7, C_8, C_9, C_{10}, were injected as nonpolar probes, and methane was injected as reference. Plotting a graph of $RT \ln V_n$ against $\alpha(\gamma_L^D)^{1/2}$ allows the dispersive component of the surface energy of the test powder (γ_S^D) to be calculated from the slope. IGC was carried out using Shimadzu (GC-9A) gas chromatograph with flame ionization detection (FID). The temperature of column was maintained at 35 °C, and the injection port temperature was held at 100 °C and FID temperature at 250 °C. The lactose (about 6 g) was packed into the glass columns (5 mm in diameter, 100 cm in length) by vertical tapping.

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