High concentrated protein particles for needle-free ballistic powder delivery prepared via spray-freeze-drying

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Introduction

The technique of direct particle injection via high velocity gas jets was originated and developed at Oxford University and its efficacy and safety in the area of vaccination and drug delivery is well established [1]. There is no bleeding or cross contamination and the injection is pain free. The elimination of needle and syringe re-use, needle-stick injuries to the administration personnel or the cold chain of refrigeration are further advantages of this technique. A wide range of therapeutic compounds, especially biopharmaceuticals and vaccines, can be delivered via this method. The powder characteristics preferred for use with current device designs are robust, monodisperse particles with a large diameter (≥40µm) and high density (≥0.7g/ml) [1]. Spray-freeze-drying has been shown to be a suitable method to produce particles for needle-free ballistic delivery of a reformulated flu vaccine [2]. However, current devices in this area are limited to the injection of 0.5 to 3mg powder which can be critical for reformulation of poorly water soluble drug substance. The aim of this research was to demonstrate the feasibility of spray-freeze-drying to produce robust and stable insulin loaded powders for particle injection from high concentrated, aqueous protein suspensions as model for the formulation of glucagon particles usable in the emergency administrations in diabetic patients with severe hypoglycemia.

Materials and Methods

Materials: Trehalose-dihydrate, mannitol and dextran (10kDa and 100kDa) (Sigma, UK) were dissolved in concentrations from 250mg/g to 350mg/g in double distilled water at a pH value of 2.0 and 7.0. Insulin from bovine pancreas (Sigma, UK) was dissolved and suspended at different concentrations in the sugar matrix solution using a vortexing system or an ultra-turrax homogenizer at 20 000rpm for one minute in order to achieve insulin concentrations of up to 50% in the final spray-freeze-dried particles.

Spray-freeze-drying: Spray-freezing was performed by atomization of the liquid solutions or suspensions at a liquid flow rate of 0.5ml/min into a separation funnel filled with liquid nitrogen using a 25kHz or 48kHz ultrasound nozzle (SonoTek, USA). The spray-frozen droplets were transferred into precooled 20ml standard freeze-drying vials and the vials positioned onto the precooled shelves (-45°C) of the freeze-drying system (FTS Lyostar 1, USA). Primary drying was performed at -10°C and 100mtorr for 2160min and secondary drying at 25°C and 100mtorr for 1440min.

Particle analyses: Particle size distributions were determined from a liquid suspension of the final powder in cyclohexane using a Malvern Mastersizer S. Analysis of true powder density was performed using a Pycnomatic ATM He-Pycnometer (Porotec, UK). Tap density testing was carried out according to PhEur. The residual water content of the powders were determined using Karl-Fischer titration (Mettler-Toledo DL39 with Stromboli). SEM pictures of the particles were taken with an Amray 1810T scanning electron microscope (Amray Bedford, USA).

Protein stability: Determination of protein aggregation was performed via size-exclusion HPLC using a ProteinPak 125Å column (Waters, UK) and UV-detection at 276nm (Varian, UK) according to PhEur. The amount of protein degradation was measured via reverse phase HPLC using a Jupiter 5µm C-18 300Å column (Phenomenex, UK) and UV-detection at 214nm according to PhEur.

FTIR: Changes in protein secondary structure were determined using a Magna IR 550 spectrophotometer (Thermo Fisher). Spectra, taken from aqueous solutions, were focused on the area-normalized, non-deconvolved Amid I region and the corresponding 2nd derivative spectrum.

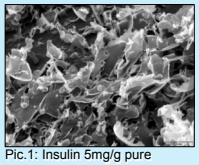
Results and Discussion

Spray-Freezing: Ultrasound atomization led to narrow droplet size distributions with mean volume diameters between 45 and 60µm (span 0.6 to 1.1) than two-fluid nozzle atomization. Quantitative insulin incorporation from the liquid formulation into the spray-freeze-dried particles was possible up to 150mg insulin in 1.0g TMD (3:3:3:1) 35% (w/w). Higher amounts of insulin led to difficulties during atomization, e.g. non-atomizing interval, resulting in larger differences between theoretical and actual insulin content in the particles. However, it was possible to produce particles from a suspension containing a maximum of 250mg insulin.

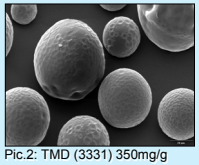
Freeze-Drying: Primary drying at -30°C and 100mtorr resulted in spherical particles with almost smooth surface (Pic.2). Increase of shelf temperature to -10°C during primary drying led to particle collapse with highly wrinkled particle surface morphology (Pic.3-4). The product temperature exceeded the T_g' of -29.1°C determined for the TMDD (3:3:3:1) 35% (w/w) formulation for most of the duration of primary drying. The tap density of the powders increased from 0.704±0.023g/ml (at -30°C) to 0.791±0.013 (at -10°C) (48kHz). The true powder density decreased from 1.49±0.03g/ml for the pure matrix particles to 1.40±0.04g/ml for the particles with 36.7% insulin. The powder tap density remained almost constant between 0.79 and 0.81g/ml for all formulations atomized at 48kHz.

Particle Characteristics: Spray-freeze-drying of a 0.5% (w/w) insulin solution at pH 2.0 led to a powder morphology similar to regular freeze-dried products (Pic.1). Resuspending of the product resulted in break-up of the structure into insulin microparticles with a mean volume diameter of 7.13µm (Fig.1). Spray-freeze-drying of any of the formulations 1 to 8 (Table 1) resulted in free flowing distinct particles stable upon resuspension. The mean volume diameter of the final particle size distribution (48kHz) increased from 46.81µm for the pure TMD (3:3:3:1) matrix formulation to 50.21µm for the matrix with 36.7% insulin with slightly increasing span from 0.921 to 1.023. A further increase in insulin concentration of the final particles could only be achieved by decreasing the concentration of the matrix formulation but resulted in a powder with wider size distribution (76.21µm / span 1.379) and a lower tap density of 0.636g/ml.

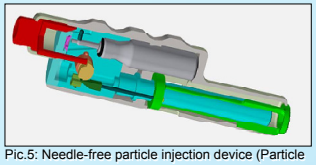
Insulin stability: Size exclusion chromatography showed a decrease of insulin monomer of -0.193±0.102% after spray-freeze-drying of a 5mg/g pure insulin solution at pH 2.0. The amount of A-21 desamido insulin increased during freeze drying from 1.680% to 2.931±0.235%. Formulation with trehalose, mannitol and dextran at pH 7.0 decreased the amount of insulin monomer loss compared to the pure spray-freeze-dried insulin microparticles to -0.104±0.013% as well as the formation of A-21 desamido insulin to 1.887±0.089%. No changes in insulin stability could be observed in samples stored at 25°C for three months. FTIR spectroscopy of the spray-freeze-dried pure 0.5% insulin show small changes in the spectrum of the Amid I region in comparison to untreated insulin. No changed could be seen for any of the formulated and stored spray-freeze-dried products.



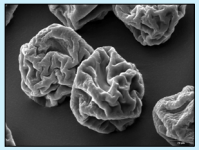
Pic.1: Insulin 5mg/g pure after SFD at -30°C



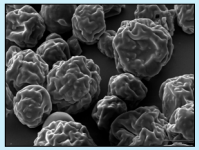
Pic.2: TMD (3331) 350mg/g after SFD at -30°C



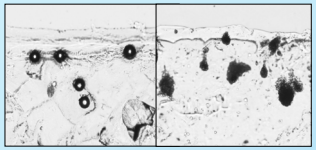
Pic.5: Needle-free particle injection device (Particle Therapeutics Ltd)



Pic.3: TMD (3331) 350mg/g after SFD at -10°C



Pic.4: TMD (3331) 350mg/g plus 25% insulin after SFD at -10°C



Pic.6: Comparison of particle penetration: a) 48µm polystyrene; b) SFD – Formulation 4

Conclusions

Spray-freeze-drying of suspensions has shown to be a feasible method to produce high concentrated insulin particles for needle-free ballistic delivery. The use of trehalose, mannitol and dextran provided a suitable matrix for stabilizing the suspension and the protein during drying and for forming dense and robust particles that penetrated well into agar test beds.

References

[1] Burkoth, T.L. et al.(1999): "Transdermal and transmucosal powdered drug delivery." Crit Rev Ther Drug Carrier Syst, 1999. 16(4): p. 331-84

[2] Maa, Y.F. et al. (2004). "Influenza vaccine powder formulation development: spray-freeze-drying and stability evaluation." J Pharm Sci 93(7): 1912-23

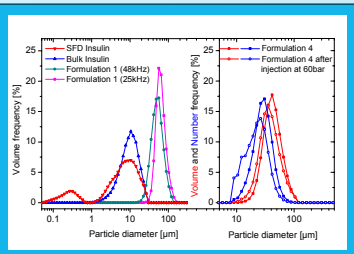


Fig.1: Particle size distribution after SFD and particle injection (60bar helium)

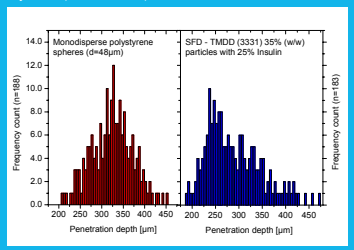


Fig.2: Distribution of particle penetration depth after injection into 3% agar test-beds (60bar helium)

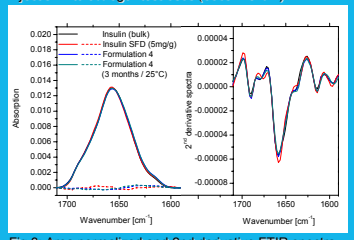


Fig.3: Area normalized and 2nd derivative FTIR spectra

Formulation		Preparation	Nozzle	UV/VIS-measured insulin content [%]	Size Distribution D(v,0.5) / Span	Physical Stability D(v,0.5) / Span	Density [g/ml]	Loss Insulin Monomer [%]	A-21 desamido insulin[%]
1	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	-	53.12 / 0.646	49.65 / 0.956	0.736	-	-
	0.000g Insulin	Solution	48kHz	-	46.81 / 0.921	43.71 / 1.215	0.791	-	-
2	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	-	-	-	-	-	-
	0.039g Insulin (bulk)	Suspension / Vortexing	48kHz	9.9 ± 0.3	47.43 / 0.987	43.32 / 1.223	0.79	-0.104	1.962
3	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	-	-	-	-	-	-
	0.088g Insulin (bulk)	Suspension / Vortexing	48kHz	19.7 ± 0.3	48.98 / 1.023	48.42 / 1.212	0.79	-0.095	1.917
4	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	25.3 ± 1.2	61.67 / 0.801	53.42 / 1.076	0.739	-0.114	1.901
	0.117g Insulin (bulk)	Suspension / Homogenizer	48kHz	25.1 ± 0.9	49.57 / 1.026	42.89 / 1.231	0.791	-0.945	1.768
5	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	29.7 ± 0.5	60.43 / 0.792	51.32 / 1.103	0.746	-0.098	1.894
	0.150g Insulin	Suspension / Homogenizer	48kHz	30.4 ± 0.9	50.01 / 1.061	44.23 / 1.273	0.789	-0.120	1.823
6	1.000g TMDD (3331) 35% (w/w)	pH 7.0	25kHz	-	-	-	-	-	-
	0.233g Insulin	Suspension / Homogenizer	48kHz	36.7 ± 1.5	50.21 / 1.031	44.01 / 1.301	0.802	-0.123	1.856
7	1.000g TMDD (3331) 25% (w/w)	pH 2.0	25kHz	-	-	-	-	-	-
	0.250g Insulin	Suspension / Homogenizer	48kHz	44.8 ± 2.2	76.28 / 1.379	41.09 / 1.643	0.636	-0.110	2.080
8	1.000g TMDD (3322) 35% (w/w)	pH 7.0	25kHz	30.3 ± 0.8	59.02 / 0.753	54.19 / 0.938	0.793	-0.094	1.871
	0.117g Insulin	Suspension / Homogenizer	48kHz	30.1 ± 0.6	51.75 / 1.005	46.98 / 1.198	0.827	-0.845	1.798

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