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Characterization and aerodynamic evaluation of spray dried recombinant human growth hormone using protein stabilizing agents

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Abstract

The effect of the protein stabilizers on the stability and aerosol performance of spray dried recombinant human growth hormone (SD rhGH) was investigated. rhGH solution was spray dried alone, with polysorbate 20 (at three concentrations of 0.05%, 0.01%, and 0.005%), Zn^{2+} (by Zn^{2+}:rhGH molar ratio of 2:1 and 4:1), and/or lactose (by lactose:rhGH weight ratio of 2:1). Size exclusion chromatography (SEC) analysis of spray dried powders demonstrated that of all the potential protein stabilizers, the combination of polysorbate 20 (0.05%), Zn^{2+} (Zn^{2+}:rhGH molar ratio of 2:1) and lactose (lactose:rhGH weight ratio of 2:1) was the most effective at protecting rhGH against aggregation during spray drying. The results of circular dichroism (CD) analysis revealed that using of polysorbate 20 (in all concentrations) and Zn^{2+} (by Zn^{2+}:rhGH molar ratio of 2:1) together in the formulations would preserve rhGH conformational stability during the process. The particle size distribution data obtained by laser diffraction method showed all SD rhGH formulations had volume median diameter and mean diameter below 5 μm. The characterization of the aerosol performance of the spray dried powders by Andersen cascade impactor (ACI) showed that by increasing the concentration of polysorbate 20 in the formulations the aerodynamic efficiency of the resultant particles was reduced. In conclusion, the optimum amounts of polysorbate 20, Zn^{2+} and lactose satisfied both physical stability during spray drying process (2.37% aggregation) and good aerosol performance (fine particle fraction; FPF = 38.52%).

Keywords: Spray drying; Dry powder inhaler; Fine particle fraction; Recombinant human growth hormone; Protein stabilizers

1. Introduction

Human growth hormone (hGH) is an endocrine hormone produced and stored in the anterior pituitary gland containing 191 amino acids (molecular weight, 22 125 Da) (Katakam et al., 1995; Hahn et al., 2004). It is currently delivered by subcutaneous injection to children with short stature due to growth hormone deficiency (GHD), Turner's syndrome or chronic renal failure, and has also been recently approved for treatment of adults with GHD (Vance and Mauras, 1999).

A non-invasive route of administration for hGH would increase good compliance, eliminate injection-related pain and be more convenience for these patients. Systemic delivery of macromolecules by inhalation is attracting considerable attention since a decade because a number of peptides or proteins are more efficiently absorbed from the lung than from the oral, nasal, or transdermal routes (Wearley, 1991; Adjei and Gupta, 1997). This efficient systemic absorption results from unique physiological features of the lung: the large absorptive surface area, the very thin diffusion path to the blood stream, the elevated blood flow, the relatively low metabolic activity locally as well as the avoidance of first-pass hepatic metabolism.

Dry powder inhalers (DPIs) present advantages over nebulizers and metered-dose inhalers for the administration of peptide and protein therapeutics to the lung. DPIs are portable, easy to operate (breath-actuated), inexpensive, propellant-free, and

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show improved stability of the formulation as a result of the dry state (Timsina et al., 1994; Niven, 1997).

In the recent years, the spray drying of these labile pharmaceutical proteins has been successfully developed to production of inhalable dry powders. Judicious selection of machine size and process conditions leads to preparation of protein-containing powders having suitable size and aerodynamic properties (Maury et al., 2005).

Unfortunately in most cases, spray drying of an aqueous solution of a pure protein results in production of aggregates (Tzannis and Prestrelski, 1999) and/or loss of activity (Alder and Lee, 1999). Aggregation may involve covalent bonds, such as disulfide bridges, or non-covalent forces, such as hydrophobic interactions or charge–charge complexation (Pearlman and Beweley, 1993). In addition, aggregates may be either soluble (such as dimers and trimers) or insoluble. Protein aggregation and deactivation may be induced by shearing stresses in the nozzle (Maa et al., 1998), thermal stress during droplet drying (Broadhead et al., 1993; Alder and Lee, 1999), and adsorption at the air–liquid interface of the spray solution during atomization (Niven et al., 1994).

Becker et al. (1987) showed that aggregation phenomena reduced the bioactivity of rhGH. The dimer form revealed about one-third the potency of monomeric rhGH in the rat weight gain bioassay.

Application of disaccharides (Broadhead et al., 1993) as stabilizers that form amorphous glasses on spray drying is widely currently used (Alder and Lee, 1999). The mechanism for their stabilizing action during the spray drying process is most likely water replacement (Tzannis and Prestrelski, 1999). Some pharmaceutical proteins such as hemoglobin (Labrude et al., 1989), β-galactosidase (Broadhead et al., 1993), lactate dehydrogenase (LDH) (Alder and Lee, 1999) and trypsinogen (Tzannis and Prestrelski, 1999) could be successfully spray dried in this way.

Previous works investigating the influence of surface active agents on the stability of rhGH demonstrated that surfactants such as pluronic, brij and polysorbate derivatives could preserve rhGH physical stability upon exposure to air–water interface created by vortex mixing or shearing (Katakam et al., 1995; Maa and Hsu, 1997; Bam et al., 1998).

Maa et al. showed that the presence of surfactant can reduce the aggregation of rhGH during spray drying process. They concluded that adsorption of protein at the air–liquid interface is the most important causative factor in protein aggregation and that two other factors including shear and thermal stresses have less importance in this field. Surfactant molecules cover the air–liquid interface rather than proteins, thus reducing the amount of protein adsorbed and hence unfolded and aggregated (Maa et al., 1998). Also zinc, like other divalent metal ions such as cobalt and copper, stabilizes rhGH by associating with protein molecules to form a dimer (Cunningham et al., 1991).

Alder and Lee presented quantitative evidence to support this model of protein/surfactant adsorption at the liquid–air interface using electron spectroscopy for chemical analysis (ESCA). They demonstrated that by addition of 0.1% (w/w) polysorbate 80 to the spray solution of LDH, no more protein molecules were detected in the surface of the spray dried particles, indicating exclusion of LDH by the surfactant (Alder and Lee, 1999).

In the present study, the effect of various concentrations of polysorbate 20 and/or zinc chloride on rhGH stability during spray drying was determined. The aerodynamic behavior of the spray dried powders was also evaluated to obtain a DPI formulation of rhGH with an appropriate aerosol performance and stability.

2. Materials and methods

2.1. Materials

Recombinant human growth hormone (rhGH) was purchased from Brasegen (Thebarton, Australia) by molecular mass of 22.13 kDa and was produced from the bacterial fermentation products of a strain of *Escherichia coli*. It was supplied as a 5 mg/ml solution of protein in the ammonium bicarbonate buffer and frozen at −80 °C temperature.

Polysorbate 20, phosphate buffer, Tris–HCl buffer, 2-propanol and propanol were purchased from Merck (Darmstadt, Germany). Zinc chloride was obtained from Fluka (Buchs, Germany). Lactose monohydrate (pharmatose® 325 M) was supplied by DMV International (Veghel, The Netherlands) and hard gelatin capsule shells (size 2) were provided by Cipla (Mumbai, India).

2.2. Spray drying

Spray drying was performed using a lab scale spray dryer (Büchi 191, Büchi, Switzerland) with an inlet temperature of 90° C, outlet temperature of 53–56 °C, air flow rate at 600 NL/h, liquid feed rate of 1.7 ml/min, and aspiration setting at 60. The initial solutions for spray drying were prepared by dissolving various amounts of polysorbate surfactant, zinc chloride and lactose with ammonium bicarbonate (pH 7.4) and adding the resulting solution to the protein solution to reach into a concentration about 2 mg/ml of rhGH (Table 1). All formulated protein solutions were prefiltered with a 0.22 μm filter (Chromafil CA-20/25S, Macherey-Nagel). The spray dried samples were transferred into tightly closed glass bottles and placed in a desiccator at 25% relative humidity and 4 °C until required for further studies.

2.3. Size exclusion chromatography (SEC) analysis

The amount of soluble aggregates of the spray dried rhGH (SD rhGH) powders was quantified by size exclusion chromatography. SEC–HPLC was performed on a pump (Waters, MA, USA; Model 501) equipped with a UV detector (Waters, MA, USA; Model 481), at 214 nm. rhGH was quantified using a Waters Bio Suite (7.8 mm × 300 mm, 5 μm) column, eluted with a mixture consisting of 3 volume of isopropanol and 97 volume of 0.063 M phosphate buffer (pH 7.0), at a flow rate of 0.6 ml/min. The concentration of rhGH sample was approximately 1 mg/ml and the injection volume was 20 μl.

Table 1
Percent of the aggregates and chemical impurities (desamido and oxidized) of the spray dried rhGH formulations at 2 mg/ml protein concentration

Materials	Lactose (weight ratio to rhGH)	Polysorbate (%, w/w)	Zn^{2+} (molar ratio to rhGH)	Aggregation (%)	Chemical impurities (%)
F_1	No	No	No	9.67	2.8
F_2	No	0.05	2	2.49	3.48
F_3	2:1	0.05	2	1.96	0.67
F_4	2:1	No	2	6.79	1.57
F_5	2:1	0.005	2	5.25	1.93
F_6	2:1	0.01	2	2.37	1.5
F_7	2:1	0.01	No	2.97	1.8
F_8	2:1	0.01	4	8.87	0.9

2.4. Reversed-phase HPLC (RP-HPLC) analysis

Contents of impurities, such as desamido and oxidized forms of rhGH, could be determined by RP-HPLC. The spray dried rhGH powders were analyzed using a pump (Waters, MA, USA; Model 600) equipped with a UV detector (Waters, MA, USA; Model 486), at 220 nm. rhGH was quantified using a Grace, Vydac (4.6 mm × 250 mm, C_4, 5 μm) column that maintained at 45 °C by column oven (Younglin; Model CT530). As a mobile phase, 0.05 M Tris (pH 7.5)/*n*-propanol (71/29) was used at a flow rate of 0.5 ml/min. The concentration of rhGH samples was approximately 1 mg/ml and injection volume was 20 μl.

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

To determine protein fragmentation and whether the protein aggregates were covalently bond or non-covalently bond, blue silver stained SDS-polyacrylamide gel electrophoresis (SDS-PAGE, Mini-Protean, Bio-Rad) was performed on a Bio-Rad gel (were prepared discontinuously with stacking and running gel of 5%, 0.5 M Tris–HCl, pH 8.8 and 12%, 1.5 M Tris–HCl, pH 8.8, respectively; 10-well comb) at 200 mV for 40 min at the ambient temperature under both 2-mercaptoethanol (2-ME)-reduced and non-reduced conditions. Each protein sample was loaded at 5 μg per lane.

2.6. Circular dichroism (CD) spectroscopy

In order to determine the conformational change of SD rhGH, far-UV spectra were taken using a spectropolarimeter (Jasco J-810, Tokyo, Japan). Quartz cuvettes of 0.1 mm in path length at 22 °C were used. All SD rhGH powders were dissolved in distilled water to make approximately 0.3 mg/ml rhGH solutions. The absorbance of each sample was then measured every 3 min over the course of 30 min. Data were collected at 1-nm intervals over the range of 240–190 nm with a collection time of 5 s/data point. To eliminate contributions from excipients, a mixture of all excipients used in preparation of the SD rhGH powders was spray dried and was prepared in the same manner described above and its spectra substracted from the protein spectra to obtain the spectra which are corrected for excipients contributions.

2.7. Particle size measurement

The particle size distribution of the samples was determined by laser diffraction (Malvern Mastersizer X, Malvern, UK). Five milligrams of each formulation was dispersed in 5 ml of appropriate medium (2-propanol containing one drop of 1% (v/v) polysorbate 20 solution) and sonicated (Starsonic 60, Liarre, Italy) for 2 min. A few droplets of each sample were poured into the small volume cell of the instrument to obtain an obscuration of between 18% and 20%. The analysis was carried out in triplicate for each sample.

2.8. Scanning electron microscopy (SEM)

The particle shape of the samples was evaluated by a scanning electron microscope (SEM) (CamScan MV 2300, Cambridge, UK). Particles were coated with gold under vacuum (E5200 Auto sputter coater, Bio-Rad, England) and examined at an acceleration voltage of 25 kV.

2.9. In vitro deposition test

One capsule filled by 8 mg of each SD rhGH powders was introduced to Andersen cascade impactor (ACI, Copley, Nottingham, UK) via a Spinhaler®. After aerosolization of the powders for 4 s at a flow rate of 60 l/min, the inhaler, capsule shell, throat, preseparator, the seven stages and plates and filter were washed with purified water. The concentration of rhGH in each solution was determined using a spectrofluorophotometer (Shimadzu RF-5000, λ_{ex} = 275 nm, λ_{em} = 335 nm) (Pearlman and Beweley, 1993). For all assays, rhGH from the same batch was used as protein standard and samples were analyzed in duplicate. Calibration plots for protein were linear over the range of 1–10 μg/ml.

The recovered dose (RD) was the total amount of the drug recovered from the device, capsules, and all parts of the impactor. Fine particle dose (FPD) was determined as the amount of drug deposited on stage 1 to the filter (<6.18 μm) (Zeng et al., 2001b). The effective cut-off diameter (ECD) of each stage of the ACI at 60 l/min were calculated from Stokes' law using the ECD of the stages at 28.3 l/min. Fine particle fraction (FPF) was calculated as the percentage of the ratio of FPD to the total amount of the drug recovered per capsule. The emitted dose (ED) was defined as the total drug recovered from throat, preseparator, the seven

stages and plates and filter. The percentage emitted was calculated as the ratio of ED to the total drug recovered per capsule, expressed as percentage. Dispersibility was defined as the ratio of FPD per ED percentage.

3. Results and discussion

3.1. Physical characteristics

The aggregate formation of SD rhGH powders was determined using SEC–HPLC technique. Table 1 summarizes the extent of protein aggregation for excipient-free rhGH and rhGH formulated with either or both polysorbate 20 and Zn^{2+} at protein concentration of 2 mg/ml using spray drying process. Also lactose (by lactose:rhGH weight ratio of 2:1) was co-spray dried with these protein stabilizers in the formulation of SD rhGH. The results showed that the presence of polysorbate 20 has crucial effect on preventing the formation of rhGH aggregate during spray drying process. Spray drying of rhGH alone produced a powder (F_1) containing 9.79% aggregates. The level of aggregates was reduced to 6.79% by the inclusion of lactose and zinc in the formulation (Zn^{2+}:rhGH molar ratio of 2:1, F_4). Using polysorbate 20 in the preparation of the initial feeds has resulted to the production of powders containing various amounts of rhGH aggregates (1.96–5.25%) depending on its concentration (Fig. 1). The data observed for aggregation of rhGH in F_6–F_8 formulations revealed the important role of Zn^{2+} molar ratio in the initial feed. In the presence of 0.01% (w/w) polysorbate 20, the addition of Zn^{2+} with a 2:1 molar ratio (F_6) showed a negligible reducing effect on the aggregation of the protein. However, increasing Zn^{2+}:rhGH molar ratio to 4:1 (F_8) exhibited an important negative effect on the aggregation of rhGH. The effect of Zn^{2+} binding on rhGH structure in solutions was previously reported (Yang et al., 2000). Zn^{2+} induces dimerization of rhGH molecules that are more stable than monomeric rhGH, when used with low molar ratio (up to 2:1). But presumably, at greater molar ratios of Zn^{2+} to protein, there is further crosslinking into higher order oligomeric species that are defined as aggregates.

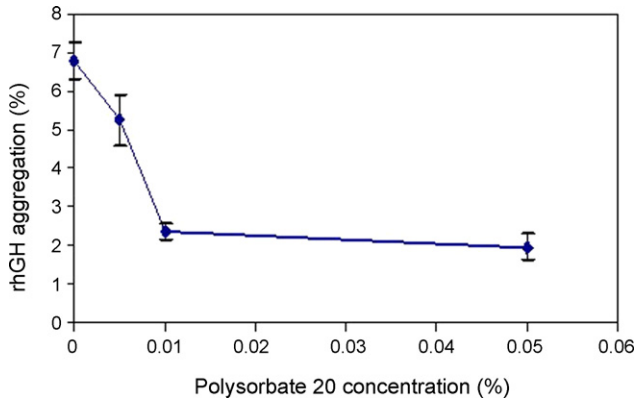

Fig. 1. Effect of polysorbate 20 concentration on aggregation of SD rhGH powders. All formulations obtained in the presence of the same amounts of lactose (by lactose:rhGH weight ratio of 2:1) and Zn^{2+} (by Zn^{2+}:rhGH molar ratio of 2:1) in the initial feeds.

Fig. 1 shows the effect of polysorbate concentration on the rhGH aggregation during spray drying. It was demonstrated that protein aggregation decreased and leveled off by increasing the surfactant concentration. The main reducing effect was observed up to 0.01% (w/w) concentration of the surfactant. The increment of polysorbate concentration from 0.01% to 0.05% exhibited only a small reduction (~0.4%) in the protein aggregation. The general conception of this phenomenon is that surfactants adsorb preferentially at air–liquid interface produced in the atomization during spray drying; therefore only a smaller interface was available for protein to unfold and aggregate (Maa et al., 1998). The F_3 which obtained in the presence of 0.05% (w/w) polysorbate 20, showed the lowest aggregation (1.96%) during spray drying of rhGH.

SDS-PAGE analysis (under both non-reducing and reducing conditions) was performed to determine the nature of rhGH aggregates formed by spray drying (Fig. 2). The lanes related to SD rhGH powders without lactose (F_1 and F_2 samples) show darker bond patterns in the high molecular weight region (44 kDa), suggesting that lactose has reducing effect on the rhGH and has shifted the larger amounts of SD rhGH lanes to the lower molecular weight region (the place of monomer at

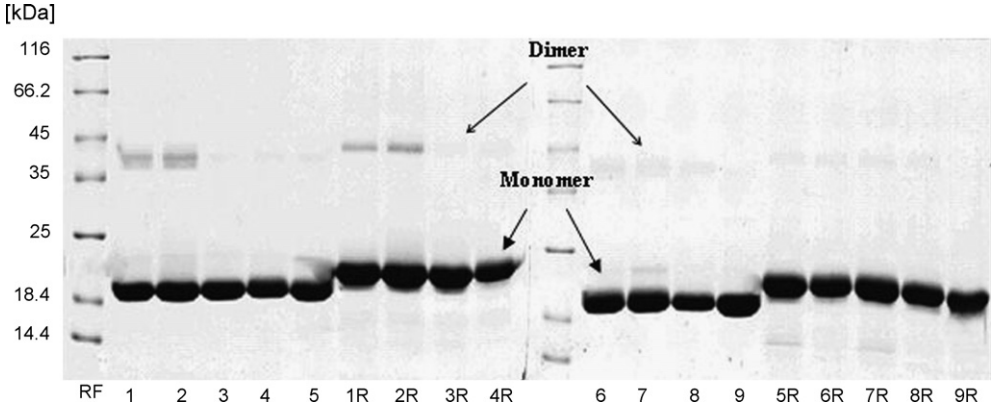

Fig. 2. Blue silver stained SDS-PAGE patterns of rhGH samples in 2-ME-reduced and non-reduced conditions: RF, molecular mass reference; 1, F_1; 2, F_2; 3, F_3; 4, F_4; 5, F_5; 6, F_6; 7, F_7; 8, F_8; 9, bulk (not spray dried), non-reduced and 1R, F_1; 2R, F_2; 3R, F_3; 4R, F_4; 5R, F_5; 6R, F_6; 7R, F_7; 8R, F_8; 9R, bulk (not spray dried), reduced.

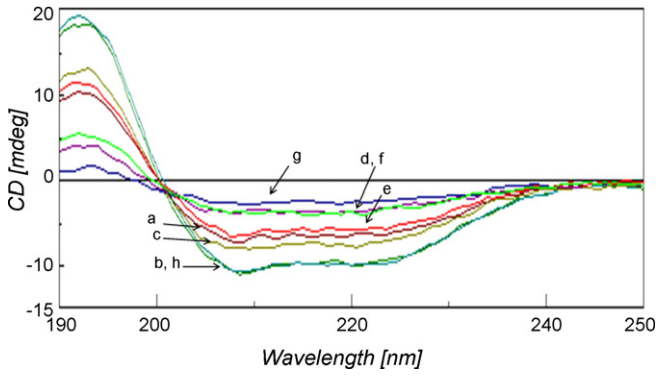

Fig. 3. The CD spectra of the various spray dried rhGH formulations and not spray dried rhGH bulk: (a) F_1, (b) F_2, (c) F_3, (d) F_4, (e) F_6, (f) F_7, (g) F_8 and (h) bulk.

22 kDa). Also, no difference was observed over the low and high molecular ranges (14 and 44 kDa) among the other spray dried samples and the original bulk. This observation indicated that some of rhGH molecules upon spray drying aggregated mainly via non-covalent bonds and were dissociable under SDS gel conditions. The native tertiary structure of hGH is composed from four α-helical columns with two large loop regions. Therefore, it is flexible in structure and non-covalent aggregation is highly probable (Katakam et al., 1995).

The impurities of the rhGH spray dried samples were quantified by RP-HPLC (Table 1). F_2 showed the highest percent (3.48%) of the impurities, which was about 2.5 times higher than that was detected in the untreated bulk rhGH. However, the amounts of impurities which were determined for all SD rhGH samples were in accordance with the pharmacopoeial requirements (Ph. Eur., 2002).

In order to understand the structural changes of rhGH during spray drying the CD analysis was performed in the "far-UV" (190–240 nm) region (Fig. 3). Since rhGH is composed of α-helix and random coil structure without β-sheet structure, the α-helix content was calculated on the basis of the molar ellipticity value at 222 nm (Kim and Park, 1999). The spectra show, when rhGH was spray dried without any excipients (F_1), the α-helix content was decreased in comparison to that of the bulk rhGH. The addition of excipients showed various effects on α-helix content, depending on their composition. F_2 sample showed α-helix content close to that of the bulk rhGH. It was obtained in the presence of Zn^{2+} (2:1 molar ratio to rhGH) and polysorbate 20 (0.05%, w/w). This observation indicated the important role of Zn^{2+} and polysorbate on the conformational stability of SD rhGH. Addition of lactose (F_3) decreased the protective effect of these excipients on the structural integrity of rhGH upon the process stresses. The CD spectra of F_3, F_4 and F_6 samples revealed the detrimental effect of polysorbate on control of α-helix content. The comparison of these spectra indicated that the application of polysorbate in a specific concentration ranging from 0.01% to 0.05% (w/w) could preserve the α-helix content of rhGH close to that of F_1. Analysis of F_6, F_7 and F_8 samples also demonstrated the role of Zn^{2+} and its content on rhGH stability. As shown in Fig. 3, the presence of Zn^{2+} at 2:1 molar ratio with respect to rhGH in the initial feed exhibited higher contents of the α-helix, compared to the other ratios.

Table 2
Particle size distributions of the materials

Materials	$d_{50\%}$ (μm)	Mean (μm)	Mode (μm)
F_1	5.6	4.6	5.0
F_2	3.0	2.6	2.6
F_3	4.3	3.1	3.1
F_4	4.5	3.8	3.8
F_5	4.4	3.4	3.4
F_6	4.5	3.9	3.5
F_7	3.3	2.9	3.2
F_8	3.2	2.7	2.7

The particle size data of SD rhGH powders are reported in Table 2. All SD rhGH samples have the volume median diameter ($d_{50\%}$) of about 3.0–5.0 μm and mean diameter of about 2.6–4.6 μm. F_1 sample showed the largest particle size distribution among the other samples. The use of excipients decreased the particle size of rhGH spray dried powders.

Fig. 4 presented the SEM photographs of the SD rhGH powders. Although all samples were prepared under the same operating conditions, the polysorbate containing powders had a dimply surface, whereas surfactant-free ones showed a raisin-like morphology. A possible explanation is that during drying of the surfactant-free protein solution, the rhGH molecules located in the surface of the droplets were denaturated and formed a crust on the particle surface. The crust impeded the water diffusion from the interior, so the obtained particles collapsed to various irregular shapes (Maa et al., 1997, 1998). By incorporation of polysorbate from 0.005% to 0.05% in the spray drying solutions, protein surface coverage would be replaced by surfactant molecules, and modified the properties of the crust such that it smoothed out the surface of the particles.

3.2. In vitro deposition

The deposition data obtained from aerosolization of the SD rhGH powders are summarized in Table 3. Excipient-free SD rhGH powder (F_1) exhibited the FPF of 32.98% and dispersability of 42.42% after aerosolization through ACI at 60 l/min. Using 0.05% polysorbate 20 in the spray drying solution of rhGH (F_2

Table 3
Fine particle fraction (FPF), percentage emission (PE) and dispersibility of rhGH after aerosolization of the spray dried samples at 60 l/min

Materials	FPF (%)	PF (%)	Dispersibility (%)
F_1	32.98	77.74	42.42
F_2	7.22	18.46	39.11
F_3	7.36	21.17	34.77
F_4	57.04	64.25	88.78
F_5	50.78	61.01	83.23
F_6	38.52	45.98	83.77
F_7	40.29	52.75	76.38
F_8	43.10	56.60	76.15

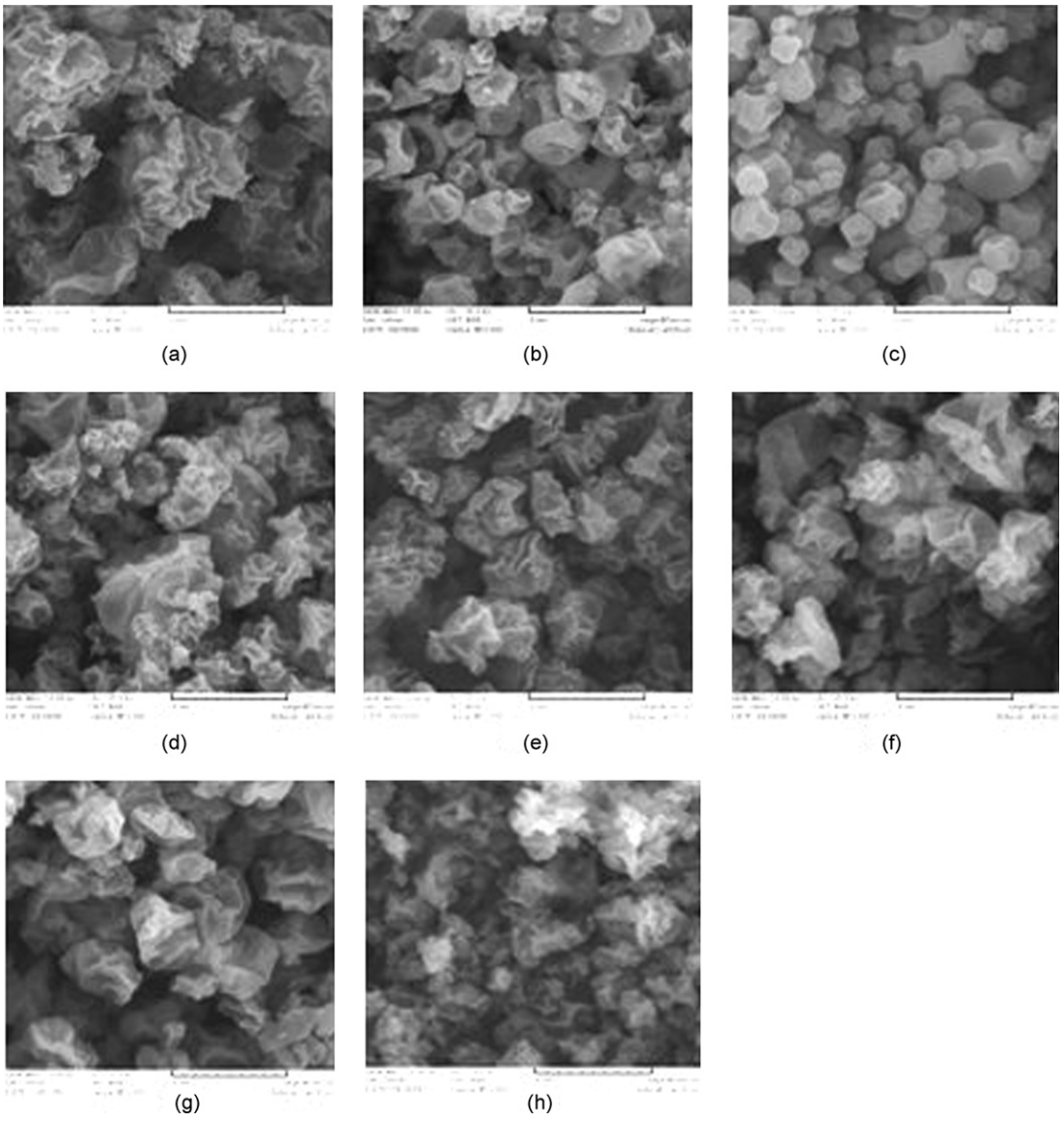

Fig. 4. Scanning electron micrographs of the SD rhGH powders: (a) F_1, (b) F_2, (c) F_3, (d) F_4, (e) F_5, (f) F_6, (g) F_7, (h) F_8. (Magnification: the scale bars represent 5 μg).

and F_3), the FPF value of the produced particles was diminished to about 7%. This poor aerosol performance presumably is explainable by the higher smooth surface of these particles, as was shown qualitatively by SEM (Fig. 4). The increasing surface smoothness usually increases adhesion forces between particles as a result of an increased contact area between the interacting species (Zeng et al., 2001a). These two formulations exhibited the lowest percentage emissions, due to the attraction between the surface of the particles and the wall of inhaler and/or capsule shells. Fig. 5 shows the effect of polysorbate 20 concentration on the FPD of SD rhGH powders. It was demonstrated that by decreasing polysorbate 20 concentration from 0.05% (F_2 and F_3) to 0.005% (F_5) the FPD was increased from about 0.6 mg up to about 4.0 mg. Spray drying of rhGH without polysorbate and in the presence of other excipients (F_4), showed the highest FPD value of 4.5 mg. As previously reported (Chew and Chan, 2001), the surface asperities of the solid, nonporous, corrugated particles could have significantly reduced

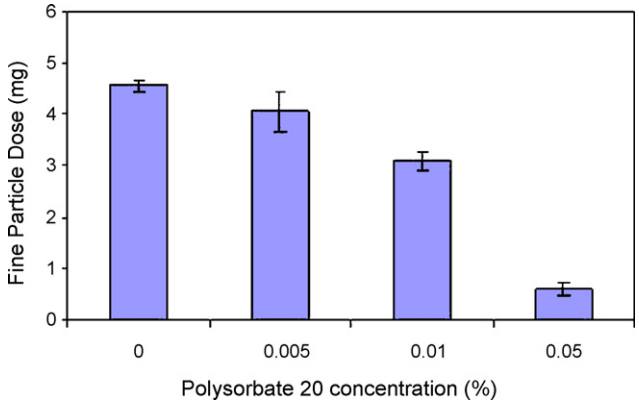

Fig. 5. Fine particle dose of rhGH as a function of polysorbate 20 concentration in the initial feeds. All formulations obtained in the presence of the same amounts of lactose (by lactose:rhGH weight ratio of 2:1) and Zn^{2+} (by Zn^{2+}:rhGH molar ratio of 2:1) in the initial feeds.

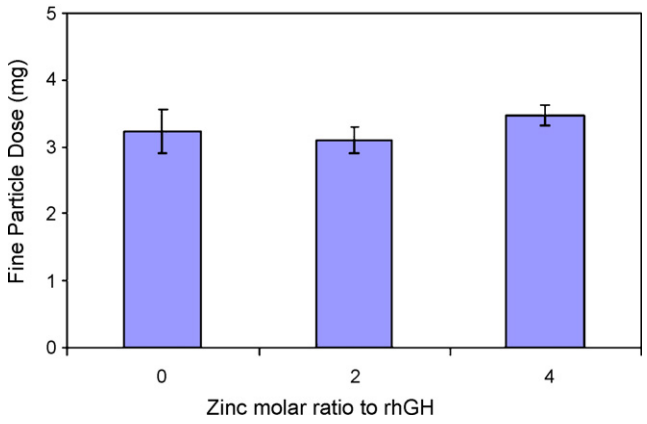

Fig. 6. Fine particle dose of rhGH as a function of zinc concentration in the initial feeds. All formulations obtained in the presence of the same amounts of lactose (by lactose:rhGH weight ratio of 2:1) and polysorbate 20 (by polysorbate 20 concentration of 0.01) in the initial feeds.

the true area of contact between particles, and thus lowered the cohesion (Ranade, 1987). The reduced point-to-point contact would reduce the influence of van der Waal's forces of cohesion by increasing the average distance between particles (French et al., 1996). This can explain why these particles showed enhanced aerosol performance over the smooth spherical particles.

The influence of Zn^{2+} on the FPD of the SD rhGH powders is also demonstrated in Fig. 6. It was found negligible differences among FPDs of rhGH aerosolized from F_6, F_7 and F_8 samples. Thus, the presence and concentration of Zn^{2+} has not affected the aerosol performance of rhGH spray dried samples.

4. Conclusion

Co-spray drying of rhGH with polysorbate 20 at a concentration of about 0.05% (w/w) could successfully protect rhGH against aggregation and preserved its structural stability upon spray drying process by excluding protein molecules from exposure to the air–liquid interface at the surface of droplets. Although, using of Zn^{2+} as divalent cation in formulation of the SD rhGH did not show any noticeable effect on the aggregation results, the CD analysis revealed that incorporation of Zn^{2+} in 2:1 molar ratio to the rhGH in formulation caused more structural stability of the protein molecules upon exposure to spray drying process stresses.

The evaluation of aerosol performance of various SD rhGH powders showed that by increasing the concentration of polysorbate 20 in the formulations the aerosolization efficiency would decrease. Consequently, using 0.01% (w/w) concentration of polysorbate 20, 2:1 molar ratio of Zn^{2+} to rhGH and 2:1 weight ratio of lactose to rhGH in the preparation of the feed for spray drying of rhGH, we achieved to the optimized formulation (F_6) which satisfied both stability and aerosol performance criteria.

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