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Spray-coating of rhDNase on lactose: effect of system design, operational parameters and protein formulation

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Abstract

The feasibility of spray-coating fine lactose powders with recombinant human deoxyribonuclease (rhDNase) using a bench-top fluid-bed processor (STREA-1) was studied. The effect of operating parameters, system design and protein formulation on coating performance was evaluated and compared with a laboratory-scale, Würster processor (GPCG-1), as reported previously (Maa and Hsu, 1996a). Protein denaturation occurred during spray-coating in both processors, though to a lesser degree in STREA-1 than in GPCG-1. The cause of protein denaturation during coating was determined to be thermally induced and most likely occurred during drying. The combined effect of shear and heat on protein aggregation during atomization was found to be insignificant. GPCG-1 outperformed STREA-1 in terms of particle agglomeration and product yield. Particle agglomeration in the latter could be reduced by increasing the atomizing pressure and decreasing the liquid feed rate. Overall, this report demonstrates that it is feasible to use the bench-top fluid-bed processor for protein spray-coating, but the application on fine carriers ($< 100\ \mu m$) is limited.

Keywords: Aggregation; Agglomeration; rhDNase; Fluid-bed; Spray coating; STREA-1; Würster process

1. Introduction

Spray-coating is a useful alternative in producing pharmaceutical protein dosage forms for pulmonary, oral, or controlled delivery. A previous study has shown that it is feasible to spray-coat recombinant human deoxyribonuclease (rhDNase) onto a lactose microcarrier as small as 50 μm using a laboratory-scale spray-coater (Glatt GPCG-1) (Maa and Hsu, 1996a). Despite its robust mechanical integrity, spray-coated rhDNase suffered serious chemical denaturation (i.e. aggregation), which was attributed to thermal degradation. To closely examine the spray-coating process, we explored a smaller scale, bench-top spray-coater, STREA-1 (Niro, Columbia, MD).

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STREA-1 has been widely used in the pharmaceutical industry to perform fluid-bed drying, granulation and coating of solid low-molecular-weight drug powders (Olsen, 1989a,b). This coater came with two spraying designs, top-spray and bottom-spray. Both systems were evaluated in this study. The bottom-spray system was based on a Würster process, which has been known for spray-coating powders as small as 30–40 μm (Fukumori et al., 1988) using GPCG-1. As far as the production scale is concerned, STREA-1 can process 0.1–2 l of carriers, compared 0.5–5 l handled by GPCG-1.

Two major problems in protein spray-coating are particle agglomeration and protein denaturation. Thus, it is important to understand these issues and ultimately overcome them, in order to make protein spray-coating commercially feasible. Particle agglomeration is the most serious problem encountered in the spray-coating process (Fukumori et al., 1987, 1988, 1991a,b, 1992). Many factors play a role in particle agglomeration, including the nature and concentration of the coating material, as well as atomized liquid droplet size and size distribution (Fukumori et al., 1992). Fukumori et al. (1991a) found that the inertia of particle flow is crucial, particles larger than 100 μm possessing sufficient inertia to overcome the cohesive force amongst particles themselves and the adhesive force with the chamber wall. As such, fluidization performance can affect the particle flow pattern, thus affecting particle agglomeration.

Another important issue during spray-coating is product denaturation. It has been reported that rhDNase coating prepared by GPCG-1 suffered serious aggregation (20–30%), which was attributed to thermal degradation based on the hypothesis that the coating had been exposed to high temperatures (Maa and Hsu, 1996a). However, it was not shown whether this rhDNase denaturation occurred during atomization and/or during drying. Therefore, another objective of the present study was to further examine the cause of such aggregation and to understand how formulations affect product quality. Upon two-fluid atomization, the high-speed air generates shear stresses causing liquid dispersed into fine droplets with a large air–liquid interfacial area. The protein solution might also have encountered heat when passing through the nozzle head. Therefore, thermal stress, shear stress and air–liquid interface might all affect the protein during atomization. During drying, thin coating is first dried to a partially wet, viscous membrane (early-stage drying) and then to a dried solid layer (late-stage drying). In this study, experiments were performed to understand how atomization and drying affect rhDNase aggregation during spray-coating. The effect of protein formulation on coating performance was also discussed.

2. Materials and methods

2.1. Materials

2.1.1. rhDNase

Recombinant human deoxyribonuclease (rhDNase), a glycosylated protein with a molecular mass of 32.74 kDa, was produced at Genentech (South San Francisco, CA), using Chinese hamster ovary cell line. The concentrated rhDNase solution was prepared by ultrafiltration/diafiltration of an 11 mg/ml rhDNase bulk (containing 150 mM sodium chloride and 1 mM calcium chloride) using tangential flow filtration. This increased the rhDNase concentration to approximately 28 mg/ml and reduced salt concentration to approximately 10 mM. Lower rhDNase concentrations were prepared by dilution of the bulk with purified water. All solutions were filtered with a 0.22 μm filter prior to the experiments.

2.1.2. Lactose

The lactose powder (Pharmatose 100 M, DMV International, Fraser, New York) was sieved with US standard sieves (ASTM E-11) of 53, 125 and 250 μm on a sieve shaker (CSC Scientific) at 200 rpm for 1 h. Each load of sieving was 250–300 g. Only the fractions of 53–125 and 125–250 μm were used for the study. The yield of each fraction was approximately 40%.

2.1.3. Excipients

Calcium chloride ($CaCl_2$) was obtained from J.T. Baker in pellet form. Trehalose was obtained from Sigma. Polysorbate (Tween) 20 (Karshamns) was prepared into a 20% aqueous solution before being added to the protein solution. All materials were used as supplied.

2.1.4. STREA-1 spray-coater

STREA-1 (Fig. 1) is a bench-top laboratory fluid-bed dryer (Niro, Columbia, MD). It consists of a control unit, a drying chamber and a two-fluid nozzle. The control unit allows the operating time, the drying air flow, the air inlet temperature, the atomization pressure and the blow-out pressure to be controlled. The pressure drop across the filter (on the top of the spray chamber to retain particles) and across the air distributor (on the bottom of the spray column) has to be monitored in order to provide appropriate drying air flow to fluidize the carriers. The chamber associated with top-spray (Fig. 1b) is available in both polyacrylate (Plexiglas) and stainless steel, but only stainless steel in the bottom-spray design. Three vertical side-ports in the top-spray Plexiglas chamber are used for nozzle insertion. The bottom-spray chamber (Fig. 1a) comes with a vertical partition tube sitting on the top of a dish-shaped air distributor. The nozzle is located in the lower center of the partition tube. The atomization nozzle has an orifice diameter of 0.7 mm, where air and liquid are externally mixed.

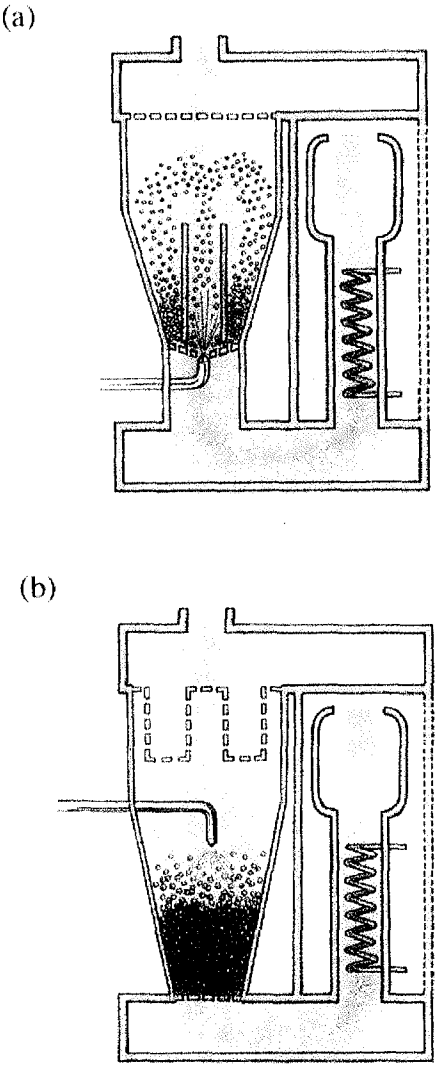


Fig. 1. The configuration of the bottom-spray (a) and the top-spray (b) STREA-1 systems.

2.1.5. GPCG-1 spray-coater

This system has been previously described (Maa and Hsu, 1996a). This device is a microprocess-controlled laboratory unit (Glatt Air Techniques, Ramsey, NJ). Generally, its loading capacity is 0.5–5 l depending upon the powder density. In the current study, atomization was performed using a 0.7 mm two-fluid nozzle and operated in a bottom-spray Würster mode.

2.2. Methods

2.2.1. Spray coating

Lactose powder was loaded into the fluidization chamber and fluidized with a drying air at a desired flow rate and temperature; then rhDNase solution was fed to the nozzle using a Masterflux pump (Console Drive, Cole Parmer). Fig. 1a shows a bottom-spray Würster system with a dished air-distributor plate and Fig. 1b shows a top-spray coater. When introduced upward through the plate, the high-velocity air lifts the powder in the chamber to a height depending on the air velocity, particle size and powder density.

In the bottom spray coater, the Würster chamber is partitioned by a draft tube which allows air to be moved faster inside than outside the tube. When particles enter the high-velocity spout, they are uniformly accelerated and physically separated from each other, facilitating uniform coating inside the tube. The process air that moves the particles also serves to dry the coating. When the airstream and particles clear the top of the partition, the air spreads out to fill the expansion chamber, and the particles settle out to the bottom of the bed outside the partition because of the lower air velocity. The settled particles then re-enter the partition to receive additional coating. The coating process continued like this for many cycles. The base operating conditions in this study were: 200 g of lactose powder and 400 ml of liquid, drying air flow rate (Q_D) of 40–50 m^3/h, atomizing air pressure of 17.4 psi, liquid feed rate (Q_L) of 6.7 ml/min, and drying air inlet temperature (T_{inlet}) of 90°C.

2.2.2. Spraying and spray-drying

The top-spray STREA-1 system was used without fluidization of microcarriers for the spray-drying and the spraying study. The spraying study was to investigate the combined effect of shear stress and air–water interface on the protein. Spray-drying was to examine how thermal stress combined with shear stress and an air–liquid interface affects the protein. In both cases, protein solution was sprayed into a Plexiglas chamber. For spraying, the atomized droplets were collected in a beaker sitting under the chamber. For spray-drying, the sprayed droplets were dried on the wall of the chamber and were then redissolved into water for analysis. To examine the thermal effect, similar spraying experiments were conducted using protein solutions preheated to 60°C in a jacked glass vessel circulated with 50/50 water/ethylene glycol.

A mass flow meter (Model 820, Sierra Instrument) was installed right before the nozzle to measure the volumetric flow rate of the atomizing air. The air flow speed was calculated based on the air flow rate and the opening area between the nozzle orifice and the nozzle cap. The outside diameter of the orifice and the diameter of the opening in the nozzle cap were measured to be 2.0 and 2.7 mm, respectively. Accordingly, the annular area of this nozzle-tip opening was calculated to be 0.022 cm^2.

2.2.3. Shear experiment by rotor/stator homogenizer

A Virtishear homogenizer (Virtis, Templest IQ) consisting of a digital display microprocessor control, an overhead drive and a homogenizing shaft (1 cm diameter) was used. The shaft tip is a rotor/stator assembly. The configuration of the assembly has been detailed previously (Maa and Hsu, 1996d). When the rotor rotates, the assembly draws the liquid in from the bottom of the shaft and sends it out through four 0.4 cm openings equally spaced above the rectangular slots for circulation. The rotational speed can be steadily controlled between a range of 5000 and 25 000 rpm. The emulsification vessel is a jacketed glass container with an inner cylinder 6 cm high and 2.5 cm in diameter. The inner tube holds approximately 50 ml of aqueous solution.

2.2.4. Incubation experiments

Thermal stress on the protein was investigated by incubating 20 ml of protein solution in a jacketed glass vessel circulated with 50/50 water/ethylene glycol at a pre-set temperature. Protein aggregation was analyzed after each incubation period.

2.2.5. Protein and powder characterizations

2.2.5.1. Size exclusion chromatography-high pressure liquid chromatography (SEC-HPLC). Samples of rhDNase were diluted to 1 mg/ml using water and 100 μl was injected into a silica-based Tosoh TSK 2000SW XL column (7.8 mm internal diameter × 30 cm length; particle size 5 μm). The mobile phase was a mixture of 5 mM HEPES, 150 mM NaCl and 1 mM $CaCl_2$ at pH 7.0, and was pumped at a flow rate of 1 ml/min. The run time was 15 min. Protein concentration was measured by optical absorption at 280 nm.

2.2.5.2. UV spectrophotometry. The amount of insoluble rhDNase aggregates was determined by measuring the difference in protein concentration (Kontron Uvikon 860) of the reconstituted sample

before and after 0.22 μm filtration (Millipore, GSVP) at a wavelength of 280 nm. Insoluble aggregates less than 0.22 μm in size were not necessarily removed by filtration. All measurements were made referenced against pure water.

2.2.5.3. Product yield and rhDNase loading determination. The yield of the process was determined based on the ratio of the amount of the powder recovered after spray-coating to that initially loaded by weight. The amount of rhDNase loaded on lactose was determined by analyzing protein concentration in each reconstituted solution.

2.2.5.4. Particle agglomeration. Each pre-weighed powder sample was sieved in an autosieve (GilSonic, Gilson Company) at 60 rpm for 10 min (ramping both up and down in 1 min). Particles of sizes ranging from 53 to 125 μm were sieved into three size groups: greater than 125 μm, 45–125 μm, and smaller than 45 μm. Particles of sizes ranging from 125 to 250 μm were also sieved into three size groups: greater than 250 μm, 125–250 μm and smaller than 125 μm. The fraction of the powder in each size range was determined by weight.

2.2.5.5. Scanning electron microscopy (SEM). The surface morphology of coated powder was examined using a Philip SEM system (Model 525M). Powder samples were mounted to a sample stub, and coated under a high vacuum (< 0.05 mtorr) with a layer of 10 nm gold–platinum. All samples were scanned at a voltage of 4.0 kV.

3. Results and discussion

Spray coating is a complex process consisting of three major operations: fluidization, atomization and drying. Table 1 summarizes all the parameters involved in the spray-coating process. These parameters are either operating variables, system design variables, or properties associated with the powder or the coating liquid. In all steps, the volumetric flow rates of drying air (Q_D), atomizing air (Q_A), and the liquid feed (Q_L), in addition to the drying air inlet temperature (T_{inlet}), are the most basic operating variables. Spray design includes the top-spray and the bottom-spray systems. The drying chamber associated with top-spraying was made of either polyacrylate or stainless steel. Only the stainless steel chamber was available for the bottom-spraying system. Powders used in this study were lactose with two size fractions: 53–125 and 125–250 μm. The liquid feeds used were pure water, aqueous lactose solution and aqueous rhDNase. Surface tension, viscosity and density of the aqueous lactose and rhDNase solutions were determined to be not significantly different from those of pure water (data not shown).

Coater performance was evaluated based on three criteria: product quality, product yield and production time. An optimum operation will produce a quality product with minimal particle agglomeration and protein denaturation at a high yield in a short production time. GPCG-1 met

Table 1
Parameters involved in regular spray-coating

Fluidization	Drying air flow rate[a] Spray design[b] (top-spray vs. bottom-spray) Chamber material[b] (stainless steel, glass, polymer, etc.) Powder size and density[d] (lactose, 53–125 and 125–250 μm) Particle surface and charge[d] Coating material[c] (rhDNase, lactose)
Atomization	Atomizing air flow rate[a] Liquid feed rate[a] Nozzle design[b] (external vs. internal mixing) Liquid viscosity[c] Liquid surface tension[c] Liquid density[c]
Drying	Drying air inlet temperature[a] Drying air flow rate[a] Liquid feed rate[a] Atomizing air flow rate[a] Liquid concentration[c]

[a] Operating variables.
[b] System design.
[c] Liquid properties.
[d] Particle properties.

these criteria, except that the protein in the coating was highly aggregated (Maa and Hsu, 1996a). Therefore, the coating performance of STREA-1 was evaluated based upon available design options using operating variables comparable to those used in GPCG-1 operation.

3.1. Fluidization performance

The lactose powder of 125–250 μm in size was fluidized using a top-spray polyacrylate chamber (Plexiglas) at the base conditions. The drawback of this system is that lactose particles tended to stick to the wall of the chamber due to electrostatic force, making fluidization impossible. When replaced by a stainless steel chamber, the problem of powder sticking to the wall was much alleviated. A voltmeter (Fluke, 8060 A) was used to measure the voltage difference (ΔV) between the chamber and the electrically-ground metal stand below the chamber. The value of ΔV was determined to be 64 mV for the polyacrylate chamber versus 4 mV for the stainless steel chamber, consistent with the finding above. Like the electrically-ground chamber ($\Delta V = 0$), the stainless steel chamber (4 mV) did not affect the fluidization pattern because steel conducts excessive static charge to the air. Another approach to reducing the electrostatic force was to incorporate an air ionization cartridge (Model 6110a, Ion Systems) before the nozzle. This air ionizer was designed to neutralize static charge on surfaces with clean, dry compressed air sources. The effect of air ionization on particle agglomeration after coating will be discussed later.

Another problem with fluidization is the control on drying air and atomizing air flow. Increased air flow resulted in increased air filter resistance (due to fouling by small particles), which in turn limited the overall air flow and fluidization performance. The maximum filter resistance measured was approximately 300 mm water (0.43 psi). This limit was often exceeded when lactose powders of less than 250 μm were fluidized. Because of the bag filter fouling problem, STREA-1 is less suitable for spray-coating of fine powders than GPCG-1.

3.2. Coating performance between GPCG-1 and STREA-1

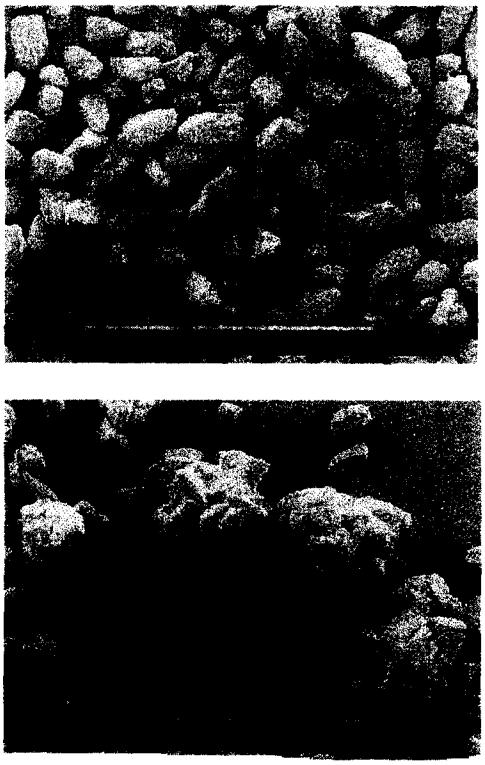
Table 2 summarizes the conditions and the results for spray-coating of lactose powder (125–250 μm) using GPCG-1 and STREA-1. The conditions between the two preparations were matched. STREA-1 resulted in less rhDNase aggregation than GPCG-1, especially in the formation of insoluble aggregates. The cause for protein denaturation was previously attributed to the thermal effect (Maa and Hsu, 1996a). The more detrimental effect by GPCG-1 is probably because each coating/drying cycle is longer as a result of a longer path of particle flow in a larger fluidization chamber.

Particle agglomeration was a serious concern for STREA-1. Fig. 2a is the SEM indicating that, prior to spray-coating, lactose particles were discrete. Upon coating using the conditions listed in Table 2, some of these particles became agglomerated (Fig. 2b). Agglomeration occurred due to coating adhesion between particles, overcoming the dispersion force by the inertia of particle movement in the chamber. Table 3 summarizes the production yield and the wt.% of agglomerated lactose coated using GPCG-1 and STREA-1. Of four batches prepared by GPCG-1, coarse lactose (125–250 μm) had lower agglomeration

Table 2
Comparison of spray-coating using GPCG-1 and STREA-1

	GPCG-1	STREA-1
Conditions		
Batch size (kg)	0.5	0.2
DNase conc. (mg/ml)	28	20
Lactose powder (μm)	125–250	125–250
Inlet temp. (°C)	75	75
Outlet temp. (°C)	35	40
Drying air volume (m^3/h)	46	45
Feed (g/min)	10	6.8
Atomizing pressure (psi)	43.5	43
Coating time (min)	97	90
Results		
Theoretical loading (%)	5	6
Actual loading (%)	5.58	5.52
Soluble aggregate (%)	10.6	4.2
Insoluble aggregate (%)	24.4	0

(a)



(b)

Fig. 2. SEM for lactose powder (125–250 μm) before spray-coating (a) and after spray-coating (b) using the condition for STREA-1 listed in Table 2.

and higher yield than fine lactose (53–125 μm). In general, GPCG-1 produced powders of lower agglomeration and higher yield than STREA-1. Coating materials affected agglomeration significantly. Powder coated with pure rhDNase is less sticky than that coated with lactose-containing rhDNase or lactose itself. The addition of either Tween 20 or $CaCl_2$ made rhDNase-coated powder stickier. In view of the fact that pure water (without any coating material) still caused agglomeration, it suggests that the inertia of particle movement played a role. The larger the fluidization chamber, the greater the inertia of particle flow. Therefore, larger coaters produce less agglomerated particles.

Table 4 suggests that system design (top-spray versus bottom-spray) affects agglomeration and production yield significantly. Top-spraying resulted in higher agglomeration and lower production yield than bottom-spraying. The bottom-spray coating (Fig. 1a) was based on a Würster process where the liquid was atomized into a partition tube. When pure water was sprayed, the yield by top-spraying was significantly lower (27% versus 87% in bottom-spraying), because of particle attachment to the wall of the chamber. Particle build-up on the chamber wall was significant for top-spraying regardless of coating conditions, suggesting that this design is not suitable for microcarriers of less than 250 μm. Agglomeration was found to be higher in the fine powder (53–125 μm) than in the coarse one (125–250 μm). It is because large particles possess higher inertia and have a better chance to overcome the adhesive force during coating than small particles, suggesting it is difficult to coat fine powders using small coaters. The use of an air ionization cartridge could not alleviate agglomeration, suggesting that particle agglomeration was not dominated by the electrostatic force between lactose particles.

The approach to reducing agglomeration was to increase the atomization pressure (atomizing air speed) and to decrease the liquid feed rate (Table 4). Increasing the atomizing air speed not only decreased the droplet size of the spray but also increased the inertia of particles flowing through the partition tube. Fukumori et al. (1992) concluded that even a small amount of coarse droplets could lead to significant agglomeration. Upon spraying the rhDNase/lactose solution using a high atomizing pressure (17.4 psi) and a low liquid feed rate (2.5 ml/min), the small lactose powder agglomerated (35.8%) more significantly than the large powder (10.4%), suggesting that the relative size between sprayed droplets and fluidized particles was important. The higher the particle/droplet ratio, the lower the agglomeration. However, using a high air pressure resulted in more particle deposition on the filter, this in turn increased the filter resistance and limited the fluidization performance. Although resulting in a decrease in agglomeration, the low feed rate increased the processing time and made the process become impractical.

Table 3
Spray-coating[a] of various aqueous solutions on lactose using GPCG-1[b] and STREA-1

Coater	Lactose (μm)	Coating solution (mg/ml)	Agglomeration (%)	Yield (%)
GPCG (bottom)	53–125	DNase (28)	7.6 (>125 μm)	92
GPCG (bottom)	125–250	DNase (28)	1.5 (>250 μm)	100
GPCG (bottom)	53–125	DNase (14)	2.8 (>125 μm)	86
GPCG (bottom)	125–250	DNase (14)	0.3 (>250 μm)	100
STREA (bottom)	125–250	Pure water	13.3 (>250 μm)	89
STREA (bottom)	125–250	Lactose (20)	85.4 (>250 μm)	91
STREA (bottom)	125–250	rhDNase (20)	35.7 (>250 μm)	86
STREA (bottom)	125–250	DNase (7.5)/lactose (3.2)	87.6 (>250 μm)	87
STREA (bottom)	125–250	DNase (8.8)/Tween (1)	99.1 (>250 μm)	93
STREA (bottom)	125–250	DNase (20)/$CaCl_2$ (20 mM)	94.8 (>250 μm)	85

[a] Spray-coating condition: $Q_L = 6.8$ ml/min, atomizing pressure = 8.7 psi, $Q_D = 40–50$ m^3/h, $T_{in} = 90$°C, 200 g of lactose powder and 400 ml of solution.
[b] Data from Maa and Hsu (1996a).

3.3. Causes for rhDNase denaturation

Spray-coating and spray-drying share the same atomization principle. Both use a two-fluid nozzle to atomize liquid into fine droplets due to high shear stress generated by high-speed atomizing air. These fine droplets possess a large air–liquid interfacial area and are exposed to a high temperature upon contacting the hot air. In addition, protein solution might have been subjected to thermal stress when flowing through the hot nozzle head. Therefore, heat, shear stress and air–liquid interface may all affect the protein during atomization. Although spray-coating and spray-drying share a similar drying principle, their thermal stress might be different. During the early stage of these operations, the coating (as the result of the spray-coating process) or the droplet (as the result of the spray-drying process) is first dried to a partially wet, viscous liquid and is further dried to a solid film or particle, respectively, during late-stage drying. Upon coating, the drying film continues to be exposed to the hot air prior to the next coating cycle (referred to as dry heating herein). In addition, the wet coating and droplet have experienced a longer exposure to a higher average temperature than the droplet during spray-drying (Maa and Hsu, 1996a). Therefore, drying and atomization might be the two major causes for protein denaturation and will be discussed in the following.

3.4. Effect of dry heating

To examine the thermal effect on a dried coating, i.e. the dry heating stage, rhDNase-coated (12% loading) lactose powder (125–250 μm) prepared by GPCG-1 (Maa and Hsu, 1996a) was further fluidized and dried in STREA-1 for 1 h using a drying air of 90°C at a flow rate of 45 m^3/h. No additional aggregation was found after this extended dry heating, suggesting that the protein is stable as long as it is dry.

3.5. Thermal denaturation of rhDNase

It was previously determined using scanning microcalorimetry (Maa and Hsu, 1996a) that rhDNase (in the liquid state) thermally denatured with a peak onset temperature at 52°C and a maximum peak temperature at 63°C. Also, $CaCl_2$ was found to thermally stabilize rhDNase, upshifting both temperatures by approximately 10°C. The effect of high temperatures on rhDNase aggregation is shown in Fig. 3a, where the protein solutions (20 mg/ml) with and without Ca^{+2} were incubated for 10 min in a preheated jacketed glass vessel. When the incubation temperature was lower than the peak onset temperature, e.g. 50°C, rhDNase remained intact. Above the onset temperature, the degree of rhDNase aggregation increased with increasing temperatures and reached almost 100% denaturation at

Table 4
Comparison of top-spray and bottom-spray configurations on spray-coating of various aqueous solutions on lactose[a]

STREA-1	Lactose (μm)	Coating solution (mg/ml)	Agglomeration (%)	Yield (%)
Top	125–250	Pure water	11.0 (>250 μm)	27
Top[b]	125–250	Pure water	33.9 (>250 μm)	50
Bottom	125–250	Pure water	13.3 (>250 μm)	87
Bottom[b]	125–250	Pure water	0.0 (>250 μm)	80
Bottom[c]	125–250	Pure water	0.1 (>250 μm)	83
Bottom	125–250	DNase (7.5)/lactose (3.2)	87.6 (>250 μm)	87
Bottom[b]	125–250	DNase (7.5)/lactose (3.2)	10.4 (>250 μm)	83
Bottom[d]	125–250	Pure water	60.8 (>250 μm)	95
Bottom	53–125	Pure water	60.5 (>125 μm)	88
Bottom[b]	53–125	Pure water	0.5 (>125 μm)	74
Bottom[b]	53–125	DNase (7.5)/lactose (3.2)	35.8 (>125 μm)	85

[a] Unless otherwise stated, the spray-coating conditions are: $Q_L = 6.8$ ml/min, atomizing pressure = 8.7 psi, $Q_D = 40–50$ m^3/h, $T_{in} = 90$°C, 200 g of lactose powder and 400 ml of solution.
[b] $Q_L = 2.5$ ml/min and atomizing pressure = 17.4 psi.
[c] $Q_L = 6.8$ ml/min and atomizing pressure = 17.4 psi.
[d] Air ionization cartridge in use.

70°C. In the case of protein containing Ca^{2+} (20 mM), rhDNase remained stable at 65°C. The effect of incubation time on aggregation of rhDNase containing no Ca^{2+} at 60 and 65°C is also shown in Fig. 3b, indicating that protein denaturation increased with longer exposure to high temperatures.

3.6. Atomization effect

The effect of atomization on rhDNase denaturation was evaluated based on the combined influence of shear (defined as the product of shear rate and the time subjected to this shear rate), air–liquid interface, and heat. It was previously determined that the shear effect alone (Maa and Hsu, 1996b) and the combined effect of shear and air–liquid interface (Maa and Hsu, 1996c) on rhDNase aggregation was not significant. Those two studies were modeled based on a rotor/stator homogenization system. In spray-coating, the protein might encounter heat during atomization (flowing through a hot nozzle head). Therefore, the effect of elevated temperature, in combination with shear and an air–liquid interface, was evaluated using the rotor/stator homogenization model to simulate the atomization process.

Fig. 4 shows rhDNase aggregation as a function of shear using 20 ml of rhDNase solution homogenized at 24 000 rpm at 50 and 60°C in the presence of air–liquid interface and at 60°C under the air-free condition. The rhDNase solution containing 20 mM $CaCl_2$ was homogenized at 60°C in the presence of air. To determine the extent of denaturation contributed by shear alone, all the data collected at 60°C during homogenization was adjusted by subtracting the aggregation contributed by thermal incubation at 60°C (data from Fig. 3). Highly-sheared rhDNase remained intact at 50°C. However, sheared rhDNase became susceptible to aggregation at 60°C with or without air–liquid interface, suggesting that shear stress facilitated aggregation when the rhDNase was thermally unstable and that the air–liquid interface alone played only a minor role. The data show that above the onset peak temperature, rhDNase was susceptible to aggregation unless $CaCl_2$ was present. This result confirms that, when rhDNase was thermally stable, the effect of shear stress and air–liquid interface on rhDNase integrity was not significant.

To consider the possible influence of shear stress, air–liquid interface and heat on rhDNase aggregation during atomization, rhDNase solu-

tion was preheated to 60°C and was atomized at different atomizing pressures into a Plexiglas chamber. During two-fluid atomization, high shear rates were induced by the relative velocity between the liquid feed and the atomizing air (Masters, 1991). The results for the atomizing pressure, the volumetric flow rate of the atomizing air and the air flow speed were determined experimentally and are listed in Table 5. The mean Sauter diameter of the droplet, defined by $\Sigma n_i d_i^3 / \Sigma n_i d_i^2$, was calculated (listed in Table 5) according to the equation (Katta and Gauvin, 1975):

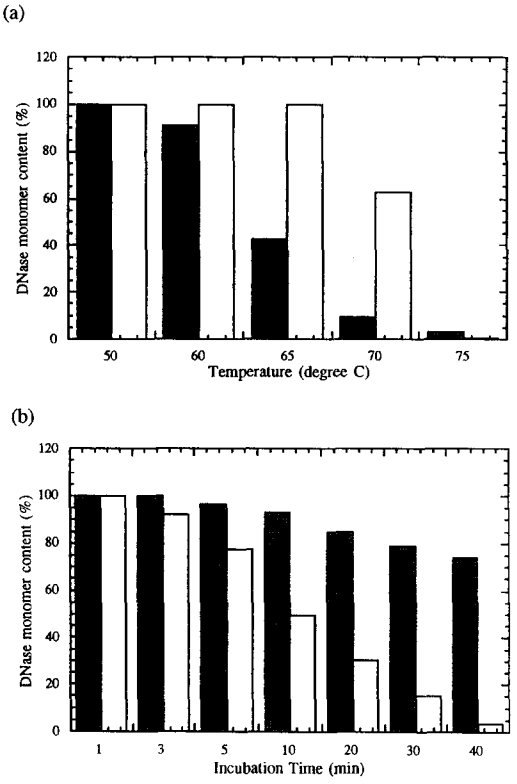


Fig. 3. Aggregation of rhDNase as a function of incubation temperature (a) and time (b). In (a), rhDNase solution (1 ml at 20 mg/ml) (shaded bar) and the same solution containing 20 mM of $CaCl_2$ (empty bar) were incubated in a Lauda circulator at different temperatures for 10 min. In (b), rhDNase solutions (20 ml) were incubated in a jacketed glass vessel at 60°C (shaded bar) and 65°C (empty bar) as a function of incubation time.

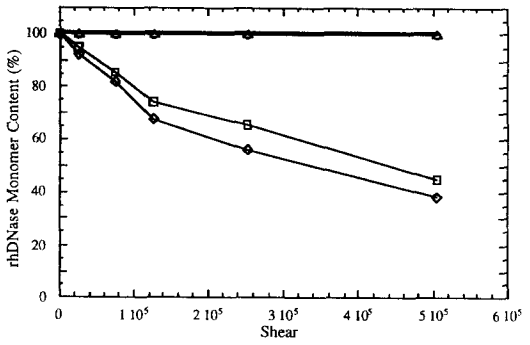


Fig. 4. The combined effect of shear, air–liquid interface and heat on rhDNase aggregation. The rhDNase solution (20 ml at 8 mg/ml) was preheated to 50°C (○) and 60°C (◇) for 10 min in a jacketed glass vessel for homogenization at 24 000 rpm. The same solution heated to 60°C was homogenized without the air–liquid interface (□). Protein solution containing 20 mM of $CaCl_2$ was preheated to 60°C, and then homogenized under the same condition (△).

$$d_{vs} = \frac{585}{V_{rel}}\left(\frac{\sigma}{\rho}\right)^{0.5} + 597\left(\frac{\mu}{\sqrt{\sigma\rho}}\right)^{0.45}\left(\frac{1000Q_L}{Q_a}\right)^{1.5} \quad (1)$$

where the drying air flow rate ($Q_a = 40$ m^3/h), the liquid flow rate ($Q_L = 6 \times 10^{-4}$ m^3/h), liquid viscosity ($\mu = 0.01$ P), surface tension ($\sigma = 68$ dyne/cm) and liquid density ($\rho = 1000$ kg/m^3) were used for rhDNase solution (Maa and Hsu, 1996c). Based on the data in Table 5, the relationship between V_{rel} and atomizing pressure is linear, and the relationship between V_{rel} and d_{vs} can be expressed as $d_{vs} = 412 V_{rel}^{-1.13}$ for the aqueous rhDNase system. The power term of V_{rel} is consistent with the coefficient of -1.14 reported by Masters (1991). This relationship is also similar to that established for the rotor/stator homogenization system (Maa and Hsu, 1996d): $d_p \propto V_{homo}^{-1.14}$, where d_p is the size of liquid droplets in a liquid–liquid emulsion and V_{homo} the homogenization intensity. This suggests that the velocity in both systems, V_{homo} and V_{rel}, has a similar effect on the droplet size. Therefore, it is valid to select this homogenization system as a hydrodynamic model to estimate the shear and the shear rate associated with atomization. In this homogenization system, the average shear rate ($\langle\gamma\rangle_{homo}$) is linearly proportional to V_{homo} (Maa and Hsu, 1996b), i.e. $d_p \propto \langle\gamma\rangle_{homo}^{-1.14}$. By analogy, i.e. assuming

Table 5
The relationship between atomizing pressure and experimentally determined air flow rate and air flow speed[a]

Atomizing pressure (psi)	Volumetric flow rate (Q_a) (m^3/h)	Air speed (V_{rel}) (m/s)	Droplet size (d_{vs})[b] (μm)
5.8	0.52	66.2	3.88
11.6	0.71	92.0	2.49
17.6	0.93	109.3	1.88
23.2	1.15	147.5	1.51
29.0	1.26	174.2	1.29

[a] Atomization was achieved using an external-mixing nozzle (0.7 mm orifice).
[b] Mean Sauter diameter, $\Sigma n_i d_i^3 / \Sigma n_i d_i^2$, calculated using Eq. (1) (Katta and Gauvin, 1975).

$V_{rel} \propto \langle \gamma \rangle_{atom}^{-1.13}$, $\langle \gamma \rangle_{atom}$ should be comparable to $\langle \gamma \rangle_{homo}$ to obtain a similar size of droplets. Therefore, the shear rate induced during atomization might be equivalent to that induced by rotor/stator homogenization (in the range 10^4–10^5 s^{-1}). However, the duration of atomization shear was very short (< 0.1 s) (Masters, 1991) compared with the duration in the rotor/stator homogenization experiment .

Based on the information above, the effect of atomization and homogenization on rhDNase aggregation was compared. For atomization, rhDNase solution (8 mg/ml) was preheated to 60°C for 10 min and then was sprayed into a Plexiglas chamber at three different pressures, 8.7, 14.5 and 29 psi. The results (Fig. 5) are compared with the aggregation profile for the protein homogenized at 60°C (Fig. 3). At 52 psi (atomizing air velocity = 312.4 m/s), it resulted in 4% rhDNase aggregation, corresponding to a shear of approximately 1×10^4 on the homogenization curve. This confirms our estimate that the shear experienced by the protein upon atomization is low, even though the shear rate might be high. It also suggests that denaturation due to the combined effect of shear, air–liquid interface and heat during atomization (either in GPCG-1 or STREA-1) was not significant, particularly at low atomizing air flow rates.

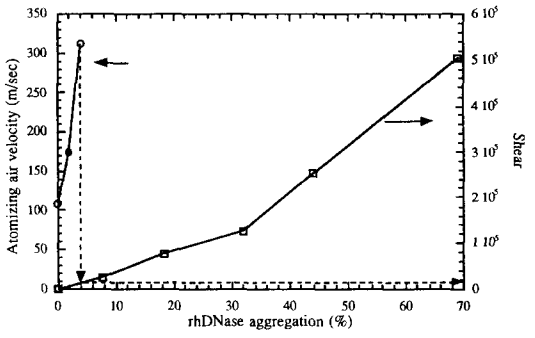


Fig. 5. Aggregation of rhDNase induced by shear during rotor/stator homogenization (□) and induced by the relative air–liquid velocity during atomization (○).

3.7. Formulation effect on thermal denaturation

To get a clearer picture of rhDNase degradation during spray-coating, we conducted a series of experiments to isolate the event potentially responsible. Results of this set of studies are shown in Table 6. In Expt. # 1, protein solution was atomized into ambient temperature. It confirms that stress associated with shear and the air–liquid interface had no effect on the protein. In Expt. # 2, protein solution was preheated to 60°C prior to atomization; the result suggests that the combined stress due to heat, shear and air–liquid interface during atomization on protein aggregation was not significant. Expt. # 3 imitated the spray-drying of a preheated rhDNase solution (60°C) in drying air initially at 90°C. This resulted in 2% aggregation, suggesting that although the protein solution might had been exposed to a temperature beyond its onset point of the denaturation peak during spray-drying, the actual damage to the protein may be less significant compared with spray-coating because of a relatively short period of exposure. In expt. # 4, rhDNase solution (no preheating) was spray-coated onto a lactose powder and produced approximately 6% of aggregates, suggesting that denaturation occurs mostly during drying. As hy-

Table 6
Five experiments for understanding the thermal effect during spray-coating using a STREA-1 processor

Experiment	1	2	3	4	5
DNase conc. (mg/ml)	10	10	10	10	10
Preheating (60°C) for 10 min	No	Yes	Yes	No	No
$CaCl_2$ (mM)	0	0	0	0	20
Atomization	Yes	Yes	Yes	Yes	Yes
Drying	No	No	Yes	Yes	Yes
Inlet temp. (°C)	—	—	90	90	90
Drying air volume (m^3/h)	—	—	40	40	40
Lactose (kg)	No	No	No	0.2	0.2
Soluble aggregate (%)	0	0[a]	2[a]	6	<1

All solutions were atomized at 17.4 psi and delivered at 5 ml/min.
[a] After subtraction of the aggregation due to preheating.

pothesized earlier (Maa and Hsu, 1996a), the inner side of the coating experiences a higher temperature than the outer side of the coating. Therefore, when the coating is partially wet (early-stage drying), protein in the inner side is prone to degradation. Expt. # 5 is the repeat of expt. # 4 except for the protein solution, which contained 20 mM of $CaCl_2$. In this experiment, aggregation was significantly reduced, confirming that rhDNase aggregation is thermally induced and can be prevented by using appropriate excipients to stabilize the protein.

Since the performance of STREA-1 was limited due to lack of sufficient inertia force, the formulation effect on coating quality (Table 7) was demonstrated using a GPCG-1 coater which had shown a good coating performance in particle agglomeration and product yield but exerted significant thermal stress. Formulations of rhDNase containing Ca^{+2}, trehalose, or Tween 20 were used. The latter two represent the sugar and the surfactant, which are commonly used to stabilize proteins during long-term storage. As shown earlier, Ca^{+2} reduced rhDNase aggregation (both soluble and insoluble) significantly. Addition of trehalose and Tween 20 could help reduce insoluble aggregation as well. However, the addition of these excipients caused serious particle agglomeration. When trehalose concentration was increased from 14 to 33 mg/ml, particle agglomeration increased from 7.1 to 33.5%. Agglomeration significantly increased to 80% when a small amount of Tween 20 was added (0.2 mg/ml final concentration). It suggests that the selection of formulations is crucial to the protein spray-coating process.

4. Conclusions

It is feasible to spray-coat rhDNase onto a lactose powder using the Würster (bottom-spray) STREA-1 processor because of its unique particle flow pattern. In general, STREA-1 is a more ideal coater for large carriers than small carriers and it can serve as a good model for understanding the protein spray-coating process. STREA-1 yields a coating of improved protein quality, but particle agglomeration is significant compared with the GPCG-1 processor. The degree of particle agglomeration strongly depends on coating materials and operating parameters. The most important operating parameter in the STREA-1 system are the atomizing pressure and the liquid feed rate. Aggregation of rhDNase during coating is thermally induced, mostly during the early-stage drying rather than during atomization, because shear involved in atomization is low. The effect of shear on rhDNase aggregation becomes significant only when rhDNase is heated to beyond its onset point of the thermal denaturation peak. This study also demonstrates that the selection of spray-coating formulation (e.g., sugar and surfactant) is essential to the balance between protein quality and physical properties of the powdered product.

Table 7
Formulation effect on rhDNase aggregation and particle agglomeration during spray-coating of lactose powder (53–125 μm) using a GPCG-1 coater[a]

Formulation	A	B	C	D	E
rhDNase conc. (mg/ml)	14	14	14	14	14
Excipients	No	$CaCl_2$	$CaCl_2$/trehalose	$CaCl_2$/trehalose	$CaCl_2$/trehalose/Tween 20
Conc. (mg/ml)	0	1	1/14	1/33	1/33/0.2
Loading (%)	5	2.5	2.5	2.1	2.1
Agglomeration (%)	2.8	4.5	7.1	33.5	79.2
Soluble aggr. (%)	17.3	2.2	2.5	5.6	5.4
Insoluble aggr. (%)	26.5	5.5	2.0	2.0	<1

[a] Spray-coating conditions: T_{in} at 75°C, Q_L at 10 ml/min, Q_D at 40 m^3/h and Q_A at 1.1 m^3/h.

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References

Fukumori, Y., Fukuda, T., Hanyu, Y., Taheuchi, Y. and Osako, Y., Coating of pharmaceutical powders by fluidized bed process. I. Aqueous enteric coating with methacrylic acid–ethylacrylate copolymer and the dissolution behavior of products. *Chem. Pharm. Bull.*, 35 (1987) 2949–2957.

Fukumori, Y., Yamaoka, Y., Ichikawa, H., Fukuda, T., Taheuchi, Y. and Osako, Y., Coating of pharmaceutical powders by fluidized bed process. II. Microcapsules produced by layering of fine powders on coarse particles and subsequent aqueous enteric coating. *Chem. Pharm. Bull.*, 36 (1988) 1491–1501.

Fukumori, Y., Ichikawa, H., Yamaoka, Y., Akaho, E., Taheuchi, Y., Fukuda, T., Kanamori, R. and Osako, Y., Effect of additives on physical properties of fine ethyl cellulose microcapsules prepared by the Würster process. *Chem. Pharm. Bull.*, 39 (1991a) 164–169.

Fukumori, Y., Ichikawa, H., Yamaoka, Y., Akaho, E., Taheuchi, Y., Fukuda, T., Kanamori, R. and Osako, Y., Microgranulation and encapsulation of pulverized pharmaceutical powders with ethyl cellulose by the Würster process. *Chem. Pharm. Bull.*, 39 (1991b) 1806–1812.

Fukumori, Y., Ichikawa, H., Jono, K., Taheuchi, Y. and Fukuda, T., Computer simulation of agglomeration in the Würster process. *Chem. Pharm. Bull.*, 40 (1992) 2159–2163.

Katta, S. and Gauvin, W.H., Some fundamental aspects of spray drying. *AICHE J.*, 21 (1975) 143–152.

Maa, Y.F. and Hsu, C., Feasibility of protein spray using a fluid-bed Würster processor. *Biotechnol. Bioeng.*, (1996a) in press.

Maa, Y.F. and Hsu, C., Effect of high shear on proteins. *Biotechnol. Bioeng.*, 51 (1996b) 458–465.

Maa, Y.F. and Hsu, C., Protein aggregation by combined effect of shear and air–liquid interface on proteins. *Biotechnol. Bioeng.*, (1996c) in press.

Maa, Y.F. and Hsu, C., Liquid/liquid emulsification by rotor/stator homogenization. *J. Controlled Release*, 38 (1996d) 219–228.

Masters, K. *Spray Drying Handbook*, 5th Edn., Wiley, New York, 1991, pp. 256–257.

Olsen, K.W., Batch fluid-bed processing equipment: a design overview. Part I. *Pharm. Technol.*, 13(1) (1989a) 34–46.

Olsen, K.W., Batch fluid-bed processing equipment: a design overview. Part II. *Pharm. Technol.*, 13(6) (1989b) 39–50.