Protein Powders for Encapsulation: A Comparison of Spray-Freeze Drying and Spray Drying of Darbepoetin Alfa

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Purpose. To evaluate spray-freeze drying and spray drying processes for fabricating micron-sized particles of darbepoetin alfa (NESP, Aranesp®) with uniform size distribution and retention of protein integrity, requirements for encapsulation.

Methods. Darbepoetin alfa was spray-freeze dried using ultrasonic atomization at 120 kHz and 25 kHz and spray dried at bench-top and pilot scales. Reconstituted powders were evaluated by size exclusion chromatography and UV/VIS spectroscopy. Powder physical properties were also characterized.

Results. Spray-freeze drying resulted in aggregation of darbepoetin alfa. Aggregates (primarily insoluble) formed on drying and/or reconstitution. Particle size distributions were broad (span $\geq$ 3.6) at both nozzle frequencies. Annealing before drying reduced aggregate levels slightly but increased particle size over 5-fold. Spray drying at inlet temperatures up to 135°C (and outlet temperatures up to 95°C) showed little impact on integrity. Integrity at bench-top and pilot scales was identical, with 0.2% dimer and no high molecular weight or insoluble aggregates detected. Particle size was small ($\leq$ 2.3 μm) with uniform distribution (span $\leq$ 1.4) at both process scales.

Conclusions. Under the conditions tested spray drying, conducted at bench-top and pilot scales with commercially available equipment, was superior to spray-freeze drying for the fabrication of darbepoetin alfa particles for encapsulation.

KEY WORDS: NESP; spray lyophilization; protein stability; protein encapsulation.

INTRODUCTION

The improvement of protein pharmaceuticals with controlled release formulations remains an important goal in drug delivery (1). Protein and peptide therapeutics have been encapsulated by various techniques including double emulsion (w/o/w) and suspension atomization, used to manufacture Lupron Depot® (2) and Nutropin Depot™ (3), respectively. In general, proteins better withstand process stresses, including organic solvent exposure and temperature extremes, when encapsulated as solid dispersions (4). Options for making protein powders (5) include lyophilization, spray-freeze drying, precipitation, and spray drying. For therapeutics, protein integrity must be maintained through encapsulation and storage; often this requires minimizing water content and including stabilizing excipients such as sugars. Drug particles need to be small relative to the microsphere diameter to ensure uniform distribution in the polymer matrix and to minimize "burst," or immediate release, of surface-associated drug. Process costs and yields must be amenable to commercialization. Spray-freeze drying (6) has been applied to human growth hormone (hGH; Ref. 7), recombinant human erythropoietin (rHuEPO; Ref. 8), anti-HER2 recombinant human monoclonal antibody (rHuMAb; Ref. 9), insulin-like growth factor-1 (IGF-1; Ref. 10), and nerve growth factor (11).

Spray drying offers a number of potential benefits for preparing protein particles for encapsulation. Although spray-freeze drying requires cryogenic atomization followed by lyophilization cycles of several days, spray drying is an established pharmaceutical process well suited to rapid production of micron-sized particles for encapsulation. The availability of commercial equipment at both bench-top and pilot scales creates the opportunity to use the same process throughout development while avoiding investment in custom cryogenic equipment. Several groups have used spray drying at a bench-top scale to fabricate particles of protein therapeutics with good aerosol properties for pulmonary delivery, or as an alternative to lyophilization. Atomization and high drying temperatures required by spray drying can compromise protein integrity via shear, interfacial, and thermal stresses. Process optimization and/or formulation approaches minimized these problems with tissue plasminogen activator (12), hGH (13), deoxyribonuclease (rhDNase; Ref. 14), and anti-IgE rHuMAb (15).

Darbepoetin alfa (NESP, Aranesp®; Ref. 16) is a 37-kd sialoglycoprotein with five N-linked glycosylation sites, two more than rHuEPO. Compared with rHuEPO, darbepoetin alfa exhibits an increased terminal half-life allowing for reduced dosing frequency, resulting in less disruption to patients' lives. A polymeric microsphere formulation of darbepoetin alfa may offer the additional advantage of controllably extending duration of action from a single injection. The objective of the present study was to evaluate spray-freeze drying as a process for preparing darbepoetin alfa particles for encapsulation, and to compare the results with spray drying at bench-top and pilot scales.

MATERIALS AND METHODS

Materials

Darbepoetin alfa was from Amgen (Thousand Oaks, CA, USA). α,α-Trehalose dihydrate (high purity, low endotoxin) was from Pfanstiehl (Waukegan, IL, USA). Poly(lactide-co-glycolide) 50:50 (RG504H, inherent viscosity 0.49 dL/g in chloroform) was from Boehringer Ingelheim (Ingelheim, Germany) and dichloromethane (USP-NF grade) from J.T. Baker (Phillipsburg, NJ, USA). Industrial-grade nitrogen (99.998% purity, with <8 ppm residual oxygen and <5 ppm residual moisture) and compressed air were from Praxair (Torrance, CA, USA). All other chemicals were of analytical grade or purer and were from commercial suppliers.

Methods

Fabrication of Protein Powders

Spray-Freeze Drying. Protein powder, with a nominal composition of 45% darbepoetin alfa, 25% trehalose, and 30% sodium phosphate (wt%), was prepared for encapsulation by spray-freeze drying a 0.2-μm filtered solution of dar-

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bepoetin alfa (3.8 mg/mL) and trehalose (2.1 mg/mL) in 20 mM sodium phosphate, pH 6.0. Formulated darbepoetin alfa solution at ambient temperature was pumped through either a 120-kHz or 25-kHz ultrasonic atomization probe (Sono-Tek, Milton, NY, USA) at 2.0 W into 950 mL of liquid nitrogen (in a 9.7-cm diameter, 13-cm high stainless-steel cylindrical container) at 3.0 mL/min. The frozen slurry was transferred in an aluminum pan to a Virtis 25EL lyophilizer with shelves at –70°C. Vacuum (100 mT) was applied after one hour, and the shelf temperature increased to –35°C over 2 h and held for 40 h. The shelf temperature was then increased to 20°C over 4 h and held for ≥20 h. The batch size was 1 g.

Spray Drying at Bench-Top Scale. Darbepoetin alfa powder was prepared by spray drying at bench-top scale using a Buchi 190 Mini Spray Dryer (Brinkman, Westbury, NY, USA) or equivalent equipped with a 0.7-mm diameter two-fluid nozzle. A custom cyclone (17), used to improve collection yields, was manufactured by Adams and Chittenden Scientific Glass (Berkeley, CA, USA). Industrial-grade nitrogen or dehumidified (Model A40 dehumidifer, Arrow Pneumatic Inc., Lake Zurich, IL, USA) compressed air (80–120 psig), used for drying and atomization, was filtered with 0.2-μm cartridge (Pall, East Hills, NY, USA) and/or Ballston AR-0795-371H canister filters (Haverhill, MA, USA) before use. Darbepoetin alfa solution was prepared as above and pumped to the nozzle using a Watson Marlow (Wilmington, MA, USA) peristaltic pump (Model 505DI) equipped with 1/16-inch internal diameter Tygon tubing. The conditions were as follows: feed temperature, 4°C; feed flow rate, 2.0 mL/min; drying gas flow rate, 800 standard liters per min (SLPM); atomization gas flow rate, 21 SLPM. The inlet temperature, T_{in}, was set as described in the text. The collection cyclone was cooled to 2°C. Batch sizes ranged from 0.4–1.8 gm.

Spray Drying at Pilot Scale. Darbepoetin alfa powder was prepared by spray drying at pilot scale using a Niro Mobile Minor® (Columbia, MD, USA) equipped with a 0.11-m^3 drying chamber extension and custom cyclone (Adams & Chittenden Scientific Glass; Ref. 17). Industrial grade nitrogen, filtered as above, was used for atomization and drying. Darbepoetin alfa solution (240 mL, corresponding to a 2-g batch size) was prepared and pumped as above and spray dried using a two-fluid nozzle (Niro Model 48838, recommended for obtaining micron-sized particles) with single point collection under the following conditions: feed temperature, 4°C; feed flow rate, 8.0 mL/min; drying gas flow rate,730 SLPM; atomization gas flow rate, 120 SLPM (selected as described; Ref. 18); T_{in}, 205°C; cyclone collection jar temperature, 15°C.

Characterization of Protein Integrity

Ion-Exchange–Size-Exclusion Chromatography (IEC-SEC). Darbepoetin alfa was analyzed for soluble aggregate content by anion exchange chromatography in series with size exclusion chromatography (IEC-SEC), using a Hewlett Packard Model 1100 with mobile phases A (10 mM sodium phosphate, 40 mM sodium chloride, pH 7.0) and B (20 mM sodium phosphate, 450 mM sodium chloride, pH 6.0). Before analysis powders were rehydrated in 20 mM phosphate buffer, pH 6.0, to a concentration of ≈1 mg/mL peptide content based on the theoretical powder protein content. Samples (10 μg) were injected after equilibration with 100% A, under which conditions darbepoetin alfa species bind to the IEC column (Pharmacia Resource Q 1 mL). After a 5-min adsorption step (100% A), darbepoetin alfa species were desorbed and separated by size exclusion (40 min, 100% B) using a TSK G3000SW XL silica gel column (TOSO Haas). Protein was detected by monitoring optical density (OD) at 220 nm. The dimer and high molecular weight aggregate detection limits were 0.1%.

UV/VIS Spectroscopy. Darbepoetin alfa powders were rehydrated as above and analyzed for insoluble aggregate content by UV/VIS spectroscopy using a Beckman DU7400 photodiode array spectrophotometer. The OD at 280 nm, the absorption maximum, was determined against a buffer blank before and after filtration (0.2 μm, low-protein binding Aerodisk HT) and centrifugation (5 min; 7200 g) and insoluble aggregate level calculated as described (19). The value reported represents the mean of four determinations. Samples showing no significant change in OD_{280} compared with that observed for an insoluble aggregate-free control were reported as having no detectable insoluble aggregates (corresponding to a detection limit of approximately 1%). Clarity was determined by comparing the mean OD from 360 to 340 nm to that of European Pharmacopoeia reference suspensions as described (20).

Gel Electrophoresis. Covalent soluble aggregate content was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in Tris-glycine using Novex pre-cast 14% gels run under constant current conditions (45 mA). Protein was detected using the Wako II Silver Stain kit.

Physical Characterization of Protein Powders

Protein powder water content was determined in duplicate (20 mg per sample) using a Mettler DL37 Karl Fischer Coulometer (Highstown, NJ, USA) with the Aqua Star Coulomat single solution reagent (EM Science, Darmstadt, Germany). Powder prepared by spray drying at pilot scale was assayed using an Aquastar C2000 (EM Science). Variability (relative standard deviation) was <1% of the value reported.

Particle size was determined in triplicate by laser light scattering using a Malvern Mastersizer X. Protein powders were suspended in a 7% solution of poly(lactide-co-glycolide) (PLGA) in methylene chloride and sonicated prior to analysis. Spray dried particles were bath sonicated (Branson Model 2210) for 30 s, whereas spray-freeze dried particles were probe sonicated (Vibracell VC 50, Sonics and Materials, Danbury, CT, USA) for 4 min. Particle size was reported as volume median diameter. Variability was <6% of the value reported. Span, a measure of polydispersity, was defined as $(D_{90} - D_{10})/D_{50}$ where D_i is the volume diameter at i cumulative volume percent. Variability was <3%.

Particle morphology was characterized by scanning electron microscopy (SEM) using a Philips XL30ESEM (EDAX, Inc., Mahwah, NJ, USA). Samples were mounted on a carbon adhesive tab and sputter coated (10–15 nm) with gold palladium (60/40 alloy). Specific surface area was calculated by fitting the nitrogen sorption isotherm, determined with a Micromeritics Gemini II 2370 (Norcross, GA, USA), to the Brunauer, Emmett, and Teller equation, using software provided by the instrument manufacturer. Nitrogen sorption of desiccated samples was determined in duplicate at 77 K from relative pressure 0.05 to 0.2. Powder true density was determined in triplicate by helium pycnometry using an AccuPyc 1330 (Micromeritics); variability was <3%.

RESULTS AND DISCUSSION

Spray-Freeze Drying at Bench Scale

Spray-freeze drying was evaluated using ultrasonic atomization with nozzle frequencies of 120 kHz and 25 kHz. The expected volume mean droplet diameters for these nozzles are approximately 30 μm and 100 μm, respectively, per the manufacturer. Fluffy powders were obtained, with yields of approximately 80%. Integrity results appear in Table I. Both samples were slightly opalescent on reconstitution, and contained significant insoluble aggregates as determined by UV/VIS spectroscopy. The soluble aggregate content increased less than 2% compared to the starting material.

The physical properties of darbepoetin alfa powders prepared at the two nozzle frequencies were evaluated and the results compared (Table II). Powders prepared with the two nozzles exhibited differing degrees of friability, or susceptibility to size reduction, when subjected to probe sonication. Particles prepared with the high frequency nozzle were reduced to 5.2 μm, whereas low frequency nozzle material was reduced to 3.2 μm. In both cases particle size distributions were broad with spans of 4.0 and 3.6, respectively. Particle morphology was evaluated by SEM. Discrete, porous particles were visible in the powder prepared at 120 kHz, but not in that prepared at 25 kHz where porous flakes were observed (Fig. 1A and B). A trend ($p = 0.15$) toward higher specific surface area was observed at 120 kHz (29 m^2/g) compared to 25 kHz (24 m^2/g). Moisture levels of spray-freeze dried powders were 13% for both samples (Table II), suggesting drying was incomplete.

Stresses imposed by spray-freeze drying (21–23) correlate with those of more thoroughly characterized processes such as spray drying and lyophilization. Droplet formation by ultrasonic atomization can result in denaturation at the air-liquid interface, as well as thermal denaturation. Rapid freezing on atomization into liquid nitrogen results in a high total ice surface area; surface denaturation at the ice-liquid interface can result. Crystallization of the dibasic form of sodium phosphate (the buffer used here) during freezing, and consequent acid-induced denaturation, can occur but are not expected with rapid freezing. These possible causes of darbepoetin alfa aggregation were investigated by collecting spray-frozen material prior to lyophilization. Material prepared with each nozzle was thawed, assayed for aggregates by both UV/VIS spectroscopy and IEC-SEC, and found not to differ from the formulated starting material. Denaturation occurring on atomization or freezing, if any, was reversible on thawing.

Table II. Properties of Darbepoetin Alfa Powders Prepared by Spray-Freeze Dry and Spray Dry Processes

Property	Spray-freeze dry		Spray dry	
	120 kHz	25 kHz	Bench-top[a]	Pilot
Particle size				
Volume median diameter, μm	5.2	3.2	2.0	2.3
Span	4.0	3.6	1.4	1.3
Specific surface area, m^2/g	29	24	8	10
Water content, %	13	13	7.6	5.9
Density, g/cm^3			1.83	

[a] Results for batch prepared with $T_{in} = 135°C$.

The possibility of a precipitation-induced pH shift was evaluated further. Increasing the sugar:buffer and protein:buffer weight ratios can inhibit buffer crystallization. In addition dibasic sodium phosphate precipitation is dependent on initial concentration and pH. On rapid cooling of 10 mM dibasic sodium phosphate, precipitation was not evidenced by SEM (24). Slow (0.62°C/min) cooling of 8 mM sodium phosphate at pH 5.7, close to the pH 6.0 conditions herein, resulted in a modest shift of approximately 0.5 pH units (25). The sodium phosphate concentration in the present investigation was halved, to 10 mM, with darbepoetin alfa and trehalose concentrations remaining the same. After spray-freeze drying at 25 kHz, aggregate levels (soluble and insoluble) were found to be identical to those in powder prepared from 20 mM sodium phosphate, indicating aggregation was not related to buffer crystallization on freezing.

High residual moisture is linked with loss of protein integrity. A longer secondary drying cycle resulted in lower moisture levels in spray-freeze dried bovine serum albumin (BSA; Ref. 21) and γ–interferon (rhIFN-γ; Ref. 23). Darbepoetin alfa was spray-freeze dried at 25 kHz with a decreased shelf ramp rate (0.06°C/min), higher final shelf temperature (25°C), and increased secondary drying time (108 h). Although the powder moisture level was reduced to 7.8%, soluble high molecular weight aggregates and dimer increased to 5.0% and 0.3%, respectively, and insoluble aggregates to 26%. This result indicated aggregation in the original samples was not a consequence of incomplete drying.

Darbepoetin alfa was concluded to have aggregated dur-

Table I. Integrity of Reconstituted Darbepoetin Alfa Powders Prepared by Spray-Freeze Dry and Spray Dry Processes

Property	Spray-freeze dry		Spray dry	
	120 kHz	25 kHz	Bench-top	Pilot
Clarity	Slightly opalescent	Slightly opalescent	Clear	Clear
Integrity:				
Insoluble aggregates, % ± SD	9 ± 2	9 ± 2	ND[a]	ND
Soluble aggregates (IEC-SEC), %[b]				
Monomer	98.2	98.7	99.8	99.8
Dimer	0.1	0.1	0.2	0.2
High MW	1.7	1.1	ND	ND

[a] ND, not detected.

[b] Darbepoetin alfa bulk control was ≥99.7% monomer, ≤0.3% dimer, with no high molecular weight aggregates detected by IEC–SEC.

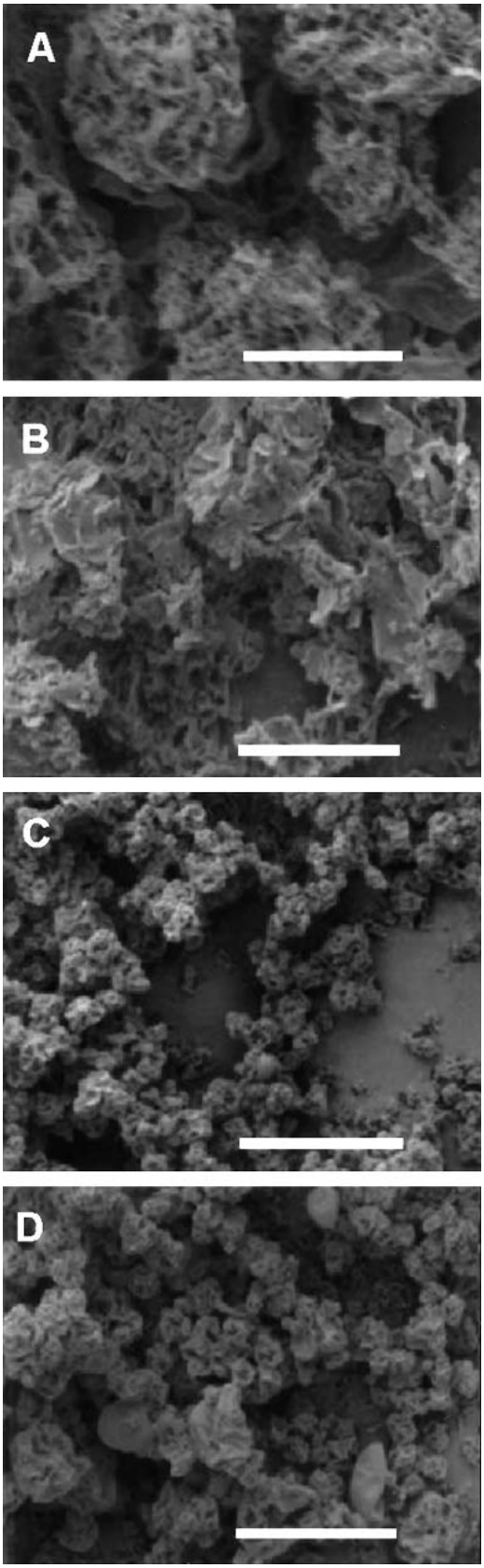

Fig. 1. Scanning electron microscopy of darbepoetin alfa powders prepared by bench-top spray-freeze drying with 120 kHz (A) and 25 kHz (B) nozzles, or by spray drying at bench-top (C) and pilot (D) scales. Bar equals 10 μm.

ing drying or upon reconstitution. The latter possibility was investigated by reconstituting the original spray-freeze dried powder prepared at 25 kHz with phosphate buffer containing a surfactant, a strategy used to minimize aggregation in reconstituted lyophilized protein powders (26). Addition of 0.005% polysorbate 80 (above its CMC of 0.0014%) to the reconstitution medium resulted in a 2-fold reduction in total soluble aggregates (to 0.6%) but the insoluble aggregate content was unchanged (9 ± 3%). While further optimization of the reconstitution buffer might have an effect, any benefits realized might not be representative of hydration of encapsulated drug particles, which likely occurs over hours or days (27).

Trehalose prevents both freezing- and drying-induced protein degradation. Increased sugar weight ratios correlate with protection on drying, while freezing damage depends on the bulk sugar concentration. The trehalose concentration in the formulated darbepoetin alfa solution was increased 5-fold to 1% w/v, corresponding to a 5.5:1 sugar:peptide mass ratio (and a reduction in buffer salt content in the powder from 30 to 16%). The solution was spray-freeze dried at 25 kHz using the modified drying cycle. No reduction in aggregate levels (soluble or insoluble) was observed. In contrast when the original formulation was filled in vials and frozen on the lyophilizer shelf at a moderate (0.4°C/min) cooling rate and dried concurrently, soluble aggregates dropped to <0.5% and insoluble to 3%. Water contents were 5.6% and 5.8% for spray-freeze dried and in-vial lyophilized powders, respectively. That a trehalose level sufficient to protect against aggregation on conventional lyophilization was ineffective on spray-freeze drying drew attention to stresses unique to, or heightened in, the latter process as possible causes of aggregation.

The impact of spray-freeze drying on protein integrity has been the subject of few previous studies. Insoluble aggregate contents were not reported in reconstituted spray-freeze dried hGH (which was zinc-complexed; Ref. 7), rHuEPO (8), or IGF-1 (10). Insoluble aggregates formed in the atomization step on spray-freeze drying of rhIFN-γ (23); levels increased following freezing and drying. Adsorption at air-liquid and solid-air interfaces was the predominant cause. Maa and Prestrelski (5) reported aggregation on spray-freeze drying of anti-IgE rHuMAb and rhDNase with a number of formulations. Annealing prior to primary drying reduced powder specific surface area, with a parallel reduction in aggregation. Costantino *et al.* (21) followed the same approach to reduce soluble aggregate levels in spray-freeze dried BSA. However the decrease in specific surface area resulting from annealing coincided with reduced particle friability. The effect of a 2 h annealing step at –5°C before primary drying of darbepoetin alfa prepared by spray-freeze drying at 25 kHz was assessed using the original drying cycle. Soluble aggregate levels dropped to <1% (0.5% high molecular weight aggregates plus 0.2% dimer) and insoluble aggregates to 7%, a slight reduction. (Although moisture in this sample was not determined, a subsequent study showed annealing resulted in a modest decrease of 0.8% in powder moisture content.) Annealing caused a significant ($p < 0.001$) decrease in specific surface area, to 6 m^2/g, and a dramatic reduction in friability. The median particle size following sonication was 17 μm, with a span of 2.3.

The improvement in integrity on annealing suggested

surface denaturation at the liquid-ice interface as a possible source of darbepoetin alfa aggregation. Even if reversible on thawing, such conformational alterations could lead to aggregation on drying and/or reconstitution. Surfactants were effective at inhibiting liquid-ice surface denaturation during lyophilization of IL1-ra (28) and spray-freeze drying of BSA (22). Polysorbate 80 (0.01% w/v) was added to the original darbepoetin alfa formulation and, following spray-freeze drying with the modified cycle, integrity assessed; soluble aggregate levels decreased to 1.4% (from 5.3%), but insoluble aggregates increased to 30%; the reconstituted powder was very opalescent. (The powder water content was 9.0%.) Although surface denaturation may play a role in darbepoetin alfa aggregation on spray-freeze drying, it is likely not the only factor. Shear associated with phase separation is an alternative mechanism of denaturation during spray-freezing (5).

Although various ultrasonic nozzles have been used in literature reports of spray-freeze drying, the effect of nozzle frequency has not been evaluated. Recent work has shown that droplet size influences freezing rate, as well as specific surface area, friability and drug integrity (5,21). In the present study insoluble aggregate levels were the same, and soluble aggregate levels <2%, at both nozzle frequencies (Table I). Morphology and particle size following probe sonication varied slightly with nozzle frequency, a possible consequence of differences in freezing rate.

Spray Drying at Bench-Top and Pilot Scales

Spray drying was considered as an alternative process for fabricating micron-sized darbepoetin alfa particles with good retention of integrity and a uniform size distribution. Initial studies focused on identifying process conditions. Thermal denaturation is a primary concern in spray drying proteins; in practice the operating temperature should be minimized to reduce thermal stress. The impact of inlet temperature, T_{in}, and consequent outlet temperature, T_{out}, on product integrity and water content were evaluated using a bench-top spray drier. These studies used the initial trehalose formulation describe above, which contained a 1:1 sugar:peptide weight ratio, comparable to that used by several investigators (15,29) to stabilize proteins against spray drying. Free-flowing powders were obtained, with yields of 70-90%. Integrity results appear in Table III. Minor levels (0.2%) of soluble dimer formed at $T_{in} \leq 135°C$. At $T_{in} = 165°C$ and 200°C dimer formation increased; in addition, soluble high molecular weight aggregates were detected. Insoluble aggregates were not detected in any of the preparations and each of the reconstituted solutions was clear. The increase in soluble aggregates with operating temperature suggests they result from thermal degradation, as opposed to shear or surface denaturation.

Powder moisture content ranged from 6.8 to 8.4% (Table III) with no significant trend with T_{in}. This result, consistent with that seen with anti-IgE rHuMAb and rhDNase (29), is attributable to the dryer residence time, which does not allow the powder to reach its equilibrium moisture level due to diffusional limitations (30). Secondary drying (for example, under vacuum) would be necessary to further reduce powder residual moisture. Residual moisture can impact storage stability, which was not evaluated, as well as ability to withstand organic solvent exposure (4). The powder prepared at $T_{in} = 135°C$ was suspended in a methylene chloride/PLGA solution, recovered by centrifugation after 30 min at room temperature, dried under vacuum, and the integrity of the reconstituted powder assessed. No increase in aggregate content (soluble or insoluble) was observed relative to an unexposed control. Therefore secondary drying was not implemented.

The thermal history of the dried product begins with the droplet surface reaching the wet-bulb temperature at a given T_{in} (12,18). Assuming negligible effect of dissolved solids on the saturation water vapor pressure of the darbepoetin alfa formulation, one can approximate the initial droplet surface temperature from the psychrometric chart for a pure water-air system. Initial droplet surface temperatures were estimated to range from 29 to 44°C for the conditions in Table III, below the point where darbepoetin alfa thermal unfolding occurs (determined to be 46°C by calorimetry; results not shown).

Particle temperature increases as drying progresses. T_{out} represents the upper limit of in-process particle temperature and the actual maximum is often assumed to be well below that value (12,18). Although for the bench-top spray drier used in this study the mean air residence time in the drying chamber is only a few seconds or less, pilot scale spray driers such as the Mobile Minor® have residence times of 20–40 s. Product can remain in the drying chamber longer than this due to recirculating airflow and air velocity heterogeneity. Therefore the thermostability of spray-dried darbepoetin alfa powder was evaluated to assess the possible impact of ex-

Table III. Effect of Inlet Air Temperature on Darbepoetin Alfa Integrity and Water Content After Bench-Top Spray Drying

Spray drying conditions		Integrity of reconstituted powder				
			Aggregate content, %			
				Soluble		
T_{in}, °C	T_{out}, °C	Clarity	Insoluble	Dimer	HMW[a]	Water content, %
Control	—	Clear	ND[b]	ND	ND	—
90	62	Clear	ND	0.2	ND	8.4
120	81	Clear	ND	0.2	ND	6.8
135	95	Clear	ND	0.2	ND	7.6
165	110	Clear	ND	0.3	0.1	8.0
200	136	Clear	ND	0.7	0.8	7.8

[a] HMW, high molecular weight.
[b] ND, Not detected.

tended exposure of dried particles to 100°C, arbitrarily selected as representative of a high T_{out}. (Note that little impact on integrity was observed at bench-top scale with T_{out} = 95°C; Table III.) The results, shown in Fig. 2, indicate the powder is remarkably thermostable. The primary degradation product is soluble dimer, found to be covalent and non-reducible by SDS-PAGE analysis (results not shown). Importantly, powder exposed for 5 min resulted in < 1% soluble dimer. The thermostability of the darbepoetin alfa powder indicated significant degradation was unlikely to result, despite increased residence time, upon spray drying at larger scale.

Darbepoetin alfa was spray dried at pilot scale using T_{in} = 205°C (with a resulting T_{out} of about 98°C), with yields of 39–56%. The product was compared with that prepared at bench-top scale. Particle size and specific surface area were comparable for both process scales, while water content was slightly lower at pilot scale (Table II). Spray drying produced raisin-like particles at both scales (Fig. 1C and D), indicative of rapid drying (29). Aggregate content of the reconstituted particles was identical to that observed at bench-top scale (Table I). Pilot scale powder was evaluated by SDS-PAGE and compared to bulk darbepoetin alfa solution (Fig. 3). A trace level of non-reducible dimer was attributed to thermal degradation. No increase in clips was detected. Further characterization of the pilot scale darbepoetin alfa powder demonstrated no oxidation, deamidation, or change in glycosylation compared to bulk (results not shown).

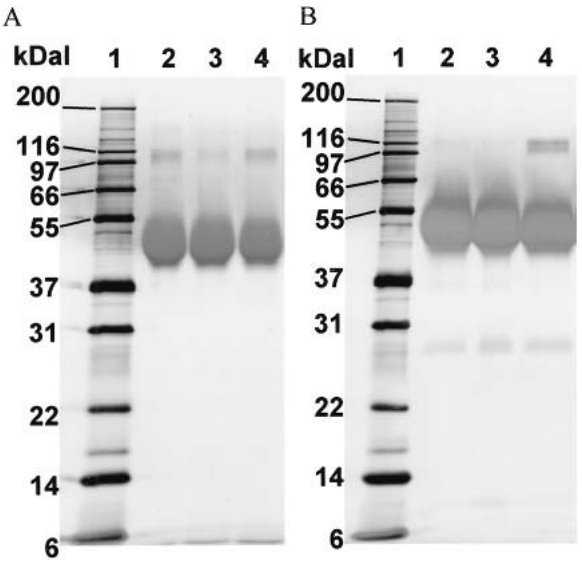

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of darbepoetin alfa samples under non-reduced (A) or reduced (B) conditions. Lane 1, molecular weight standards. Lane 2, filtered bulk darbepoetin alfa solution. Lane 3, bulk darbepoetin alfa solution. Lane 4, reconstituted darbepoetin alfa powder prepared by spray drying at pilot scale. As a result of its sialic-acid containing carbohydrate content, darbepoetin alfa monomer runs near 50 kd, and dimer near 100 kd.

Comparison of Particle Size Distribution

An important benefit of spray drying over spray-freeze drying under the conditions tested becomes apparent on closer evaluation of the particle size data as depicted by cumulative particle size distributions for each of the darbepoetin alfa powders (Fig. 4). Regardless of scale, spray drying resulted in a much narrower particle size distribution. The uniformity of particle size distribution impacts drug distribution in the fabricated microsphere. Particle settling in the polymer solution can result in heterogeneity in drug load within the microsphere population, or in lower total load. Particle sedimentation rate is proportional to the square of the radius, according to Stokes' relation for a sphere. Visible settling of darbepoetin alfa powders prepared by spray-freeze drying was observed within 5 min of suspension in methylene chloride/PLGA solutions while no settling occurred with powders prepared by spray drying over that time frame. While settling might be minimized by incorporation of a con-

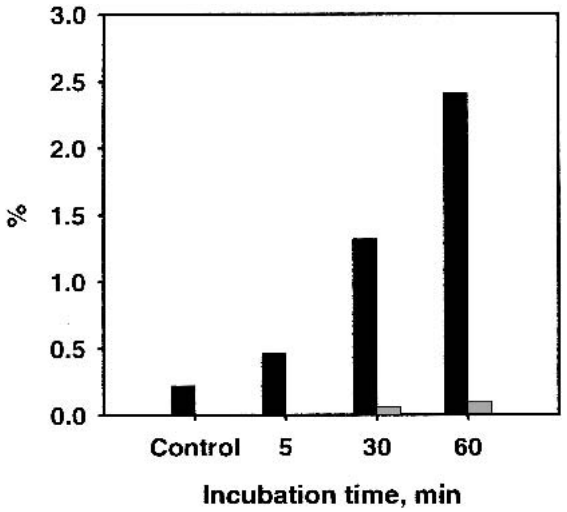

Fig. 2. Soluble dimer (black bar) and high molecular weight (gray bar) aggregate content of darbepoetin alfa powders spray dried at bench-top scale following incubation at 100°C. Powder prepared with T_{in} = 135°C was placed in sealed vials and incubated in a 100°C water bath and aggregate formation assessed as described. No insoluble aggregates were detected in any of the samples.

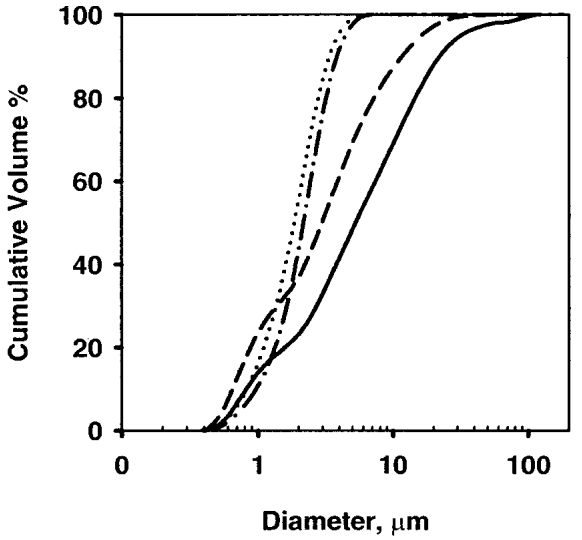

Fig. 4. Cumulative particle size distributions for darbepoetin alfa powders prepared by spray-freeze drying using 120 kHz (solid line) and 25 kHz (dashed line) atomization nozzles, or by spray drying at bench-top (dotted line) and pilot (dash-dotted line) scales.

tinuous mixing step during encapsulation, or by increasing the polymer solution concentration, the largest particles could contribute directly to initial drug release depending on microsphere diameter (21).

In spray drying, size distribution is a direct consequence of atomization. In the absence of agglomeration and under optimized drying conditions, each particle originates from a single dried droplet. The pneumatic nozzles used here produce homogeneous droplet size distributions. In practice the distribution of the dried particles is often narrower than that of the wet spray (18). The median droplet diameter at the bench-top scale was calculated to be 12 μm by assuming a spherical geometry, and using the known dried particle density (1.83 g/mL; Table II) and solids concentration of the formulated darbepoetin alfa solution (8.4 mg/ml). This represents nearly a 3-fold reduction in diameter compared to the 120 kHz ultrasonic nozzle used in spray-freeze drying. However this does not account for the dramatic variability in particle size distribution with process (Fig. 4). Spray-freeze drying results in porous, high specific surface area particles of large diameter. The median diameter of the powder prepared at 120 kHz was determined to be 33 μm by light scattering prior to probe sonication. (Note that particles prepared at 25 kHz appear to have disintegrated following drying; Fig. 1B.) The ultimate size distribution, that is, that relevant to encapsulation, results from the subsequent fragmentation step. As shown in Fig. 4, 15–30% of the cumulative volume of spray-freeze dried darbepoetin alfa derived from particles >10 μm, indicating incomplete fragmentation. Alternatives to probe sonication (or alternative atomizer designs and/or conditions), which might provide a more favorable particle size distribution, were not evaluated. Recent studies (21) of spray-freeze atomization with two-fluid nozzles, which provide a wider range of droplet sizes than explored here (18), showed that high air/liquid mass flow ratios resulted in submicron particles following probe sonication. Friability increased with porosity and specific surface area. However, high interfacial area was linked with protein denaturation (21), consistent with the results reported above, and therefore this approach was not evaluated.

CONCLUSIONS

Spray-freeze drying has been to date the most commonly used process for preparing protein powders for encapsulation. The process produces high specific surface area solids that are more friable than solids produced by other techniques. However, spray-freeze drying was considered unsuited to fabrication of darbepoetin alfa particles for encapsulation because of aggregate formation. Aggregation, concluded to occur on drying or on powder reconstitution, was comparable for the two ultrasonic nozzle frequencies tested. Fragmentation by probe sonication resulted in broad particle size distributions. A longer drying cycle, used to reduce moisture levels in the final powder, resulted in a substantial increase in high molecular weight and insoluble aggregates. Annealing prior to drying reduced, but did not eliminate, aggregate levels, and was accompanied by a decrease in powder specific surface area and a dramatic increase in particle size. These problems, which might possibly be solved with additional formulation and/or process optimization, were not observed with spray drying, which at both bench-top and pilot scales had little impact on darbepoetin alfa integrity and resulted in a superior particle size distribution. Spray drying is faster, uses commercially available equipment, and circumvents the use of cryogenic gasses as well as an appendant particle fragmentation step. Based on the conditions tested, spray drying is concluded to be a superior process for producing darbepoetin alfa particles for encapsulation.

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