Effect of Spray Drying and Subsequent Processing Conditions on Residual Moisture Content and Physical/Biochemical Stability of Protein Inhalation Powders

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Purpose. To understand the effect of spray drying and powder processing environments on the residual moisture content and aerosol performance of inhalation protein powders. Also, the long-term effect of storage conditions on the powder's physical and biochemical stability was presented.

Methods. Excipient-free as well as mannitol-formulated powders of a humanized monoclonal antibody (anti-IgE) and recombinant human deoxyribonuclease (rhDNase) were prepared using a Buchi 190 model spray dryer. Residual moisture content and moisture uptake behavior of the powder were measured using thermal gravimetric analysis and gravimetric moisture sorption isotherm, respectively. Protein aggregation, the primary degradation product observed upon storage, was determined by size-exclusion HPLC. Aerosol performance of the dry powders was evaluated after blending with lactose carriers using a multi-stage liquid impinger (MSLI).

Results. Spray-dried powders with a moisture level (~ 3%) equivalent to the freeze-dried materials could only be achieved using high-temperature spray-drying conditions, which were not favorable to large-male manufacturing, or subsequent vacuum drying. These dry powders would equilibrate with the subsequent processing and storage environments regardless of the manufacturing condition. As long as the relative humidity of air during processing and storage was lower than 50%, powders maintained their aerosol performance (fine particle fraction). However, powders stored under drier conditions exhibited better long-term protein biochemical stability.

Conclusions. Manufacturing, powder processing, and storage environments affected powder's residual moisture level in a reversible fashion. Therefore, the storage condition determined powder's overall stability, but residual moisture had a greater impact on protein chemical stability than on powder physical stability.

KEY WORDS: spray drying; residual moisture; equilibrium moisture content; relative humidity; gravimetric moisture sorption isotherm; protein aggregation.

INTRODUCTION

The impact of moisture and temperature on solid-state protein stability is well documented (1–3). In general, a protein's chemical stability decreases with increasing moisture in the solid due to changes either in dynamic activity or conformational stability of the protein, or due to water serving as a reactant and/or as a medium for mobilization of reactants (3). As far as protein inhalation powders are concerned, their physical stability (particle size and surface morphology) is equally important as chemical stability (4). Generally the level of moisture in the powder affects particle size and excipient crystallization significantly during long-term storage, thereby deteriorating the dispersion performance of the powder (5–6). Therefore, in the development of a protein inhalation powder product, it is essential to understand how process conditions affect the powder's residual moisture content and subsequently the product's chemical and physical stability.

Preparation of protein inhalation powders requires drying to form solid product particles from a formulated aqueous solution. It is well-known that the driving force for dehydration is the difference of partial water vapor pressure (or relative humidity) between the solid surface and the environment. When there is enough water initially to keep the surface saturated, the surface relative humidity is 100%. This surface vapor pressure decreases as the sub-surface can no longer supply sufficient water for surface saturation due either to slowed down diffusion or to the reduced moisture level within the protein solid. However, drying will continue until the driving force decreases to zero. Therefore, the final moisture level of the protein solid is determined by two factors, the nature of the material and the humidity of the drying conditions. The former is due to the interactions of water molecules with protein and/or formulation excipient molecules (7). The number and distribution of strong and weak binding sites in protein and excipient molecules are among the intrinsic properties determining this interaction. Therefore, the moisture level in the protein solid can be primarily controlled by the humidity of the environment where the powder is manufactured, processed, and stored, due to the equilibration between the powder's residual moisture and environmental humidity. We employed moisture sorption isotherms to confirm the presence of such equilibration.

In this study we applied recombinant human deoxyribonuclease (rhDNase) and recombinant human anti-IgE monoclonal antibody (anti-IgE) as the model proteins. Spray drying was the dehydration method of preparing the anti-IgE and rhDNase powders from their mannitol and trehalose formulations. The scope of the study was focused on studying 1) the effect of spray-drying conditions and the humidity environment on the residual moisture level of the final powder product and 2) the consequent impact of residual moisture on the product's physical and biochemical stability. However, this paper is not intended to develop protein aerosol formulations with optimized stability, and the authors have no intention to either discuss the mechanisms governing stability properties or generalize the conclusions of this study to other protein/sugar systems.

Overall, this study allows us to understand the relative importance of manufacturing, powder processing, and storage environments on the powder's stability and to determine an optimal process condition and environment humidity for producing the anti-IgE and rhDNase inhalation powders.

MATERIALS AND METHODS

Materials

Proteins

Recombinant-derived humanized anti-IgE monoclonal antibody (146 kDa molecular weight) and recombinant human

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deoxyribonuclease (rhDNase) (32.74 kDa) were produced at Genentech, Inc. Both recombinant proteins are glycoproteins produced in Chinese hamster ovary cell lines. Excipient-free anti-IgE antibody and rhDNase solutions were prepared by ultrafiltration (UF) and diafiltration (DF) into a concentration of 50 g/L, and then appropriate amounts of a sugar excipient were added to prepare a desired formulation. All protein solutions were filtered with a 0.22 μm filter before use.

Excipients

Excipients used in this study included mannitol and trehalose (ACS reagents). They were obtained from Sigma Chemical Company (Saint Louis, Missouri) and were used as supplied. Both are common sugar excipients used in liquid and lyophilized protein formulations serving as protein stabilizers. The weight ratios of protein to sugar were selected, for example, those in Table 1, with no purpose of optimizing physical and biochemical stability.

Methods

Protein Powder Preparation

Protein powders were prepared from the formulations described above by spray drying. Spray drying was performed using a Model 190 Buchi mini spray dryer (Brinkmann). Using compressed air from an in-house supply (~80 psi), a two-fluid nozzle (0.5 mm) atomized the protein solution. The air was filtered through a 0.22 μm Milidisk filter (Millipore) before entering the nozzle, and the flow rate was controlled by a variable area flow meter (Cole Parmer, 150 mm). A peristaltic pump (1-100 rpm, Masterflex, Cole Parmer) pumped liquid protein feed to the nozzle using silicone tubing (3 mm ID). Cooling water was circulated through a jacket around the nozzle. The standard operating condition was: T_{inlet} (inlet air temperature) of 105°C, Q_{DA} (drying air flow rate) of 1000 L/min, Q_{AA} (atomizing air flow rate) of 1050 L/hr, and Q_{LF} (liquid feed rate) of 15 mL/min. This condition resulted in an T_{outlet} (outlet air temperature) of 50–55°C. The batch size (solution volume) of each spray drying experiment was around 30 mL, so each experiment lasted approximately two minutes.

In addition to the built-in thermocouples for measuring the inlet and outlet air temperatures, the temperature and relative humidity distribution inside the drying chamber and the receiver was measured by digital thermo-hygrometers (Daigger Scientific). Measurements were taken at two locations along the drying chamber, 15-cm and 33-cm below the nozzle by taping the devices directly on the chamber wall. The hygrometer was placed inside the receiver without taping. Readings were recorded when steady-state values were reached.

In the case where protein powders of a low residual moisture content were needed, dry powders were further dried in a vacuum oven (Model 5831, Napco) where the vacuum was pulled by a pump (Trivac, Model D2A, Leybold-Heraeus Vacuum Products). The temperature was monitored by a thermometer and the degree of vacuum was monitored by measuring the relative humidity inside the oven using a digital thermohygrometer (Daigger Scientific).

Freeze-dried and spray freeze-dried powders were used in the moisture sorption isotherm experiments. The freeze-dried solid was prepared in a 50 cc vial (a fill volume of 20 mL) using a freeze-drying cycle of −50°C freezing (one-hour hold), primary drying at −25°C (two-hour ramp and 40-hour hold), and secondary drying at 20°C (four-hour ramp and 20-hour hold). The spray freeze-dried powder was prepared based on the same atomization as spray drying. Atomized droplets were immediately frozen in liquid nitrogen and freeze dried using the freeze-drying cycle mentioned above.

Table 1. Residual Moistures for Powders Prepared by Freeze Drying, Spray Drying, Spray Freeze Drying, and Vacuum Drying

Formulation	Moisture content (%)		
	Freeze drying	Spray drying	Vacuum drying[a]
Pure rhDNase	5.5	7.8	—
60:40 rhDNase:Trehalose	2.8	7.0	—
50:50 rhDNase:Mannitol	2.9	7.7	—
80:20 Anti-IgE antibody:Mannitol	3.0	7.2	2.8

[a] Spray-dried powder was further vacuum dried at 25 °C and 6% RH for 72 hours.

Protein and Powder Characterizations

Native Size-Exclusion Chromatography (SEC-HPLC). SEC-HPLC for anti-IgE antibody was carried out on a Bio-Select SEC 250-5 column (Bio-Rad). The column was equilibrated and run in phosphate buffered saline at a flow rate of 0.5 mL/min using a 1090L HPLC (Hewlett Packard) equipped with a diode array detector. Molecular weight standards (Bio-Rad) consisting of thyroglobulin (670 kd), gamma-globulin (158 kd), ovalbumin (44 kd), and cyanocobalamin (1.35 kd) were used to calibrate the column. The sample load was 25 μg and protein was detected by monitoring the UV adsorption at 214 nm.

For rhDNase, chromatography was carried out on a silica-based Tosoh TSK 2000SW XL column (7.8-mm I.D. x 30-cm L; particle size, 5 μm). Each sample was diluted to 1 mg/mL with a placebo solution, and 100 μL was injected into the column. The mobile phase for rhDNase 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 minutes. Protein concentration was measured by optical absorption at 280 nm.

Gravimetric Moisture Sorption Isotherm. The moisture sorption and desorption were determined with a vacuum moisture balance (MB-300G, VTI Corp., Hialeah, FL.). The microbalance was calibrated internally before each measurement, and the accuracy of the percent relative humidity was periodically examined by determining the amount of moisture absorbed by Providone K90 at 80% RH and 25°C. Typical operating parameters include drying the sample at 25°C and ~0.3 mmHg before determining the moisture sorption isotherms in 10% RH intervals up to 80% RH also at 25°C. The equilibrium criteria used for all weight change measurements was ≤ 5 μg (for a sample weight of approximately 10 mg) over 5 min.

Moisture Content. Moisture content of the protein powder was measured using a thermogravimetric analyzer (TGA 7,

Perkin-Elmer) linked to a data station (Model 7700, Perkin Elmer). Samples (~5 mg) were loaded in aluminum pans and heated at 4°C/min under 30 mL/min N_2 gas purge. The moisture content was based on the loss in weight between room temperature and 150°C.

The concern about moisture adsorption/desorption during sample preparation and processing prior to and during sample analysis in TGA has been addressed in this study. The ideal situation will be to carry out all the preparation work in a relative humidity environment which matches the condition that the powder sees before leaving the receiving vessel. However, this procedure is in general not feasible and will cause tremendous inconvenience. To minimize the error that might be associated with sample handling of this nature, we tried to deliver and prepare the TGA samples as soon as possible to minimize sample exposure (3–5 minutes) to the ambient environment. This short exposure has been demonstrated to minimize moisture content change (unpublished results).

Specific Surface Area. The specific surface area per unit weight of the powder samples was determined by the multipoint BET method from the adsorption of nitrogen gas at 77°K (Autosorb-3 Gas Sorption Analyzer, Quantachrome Corp.). All samples were outgased by vacuum at 25°C for 16 hrs.

Particle Size Analysis. The Malvern laser defraction analyzer (Mastersizer-X) used the diffraction pattern produced by the presence of particles or droplets in a monochromatic coherent laser beam to deduce their size distribution. The instrument determines their diffraction pattern using an annular detector with 32 concentric detector rings and then inverts the diffraction pattern using a light scattering theory based on a model independence analysis. The diffraction data was inverted into the particle size distribution assuming a relative refractive index of 1.33 and zero optical adsorption. Several milligrams of powder were suspended in 5–10 mL of isopropyl alcohol containing a drop of 1% v/v polysorbate-20. Each suspension was sonicated (Model 08849-00 Ultrasonic Cleaner, Cole Parmer) for about one minute before being loaded into a stirred sample cell. During analysis a laser beam was passed perpendicularly through the sample cell and then through a lens with a focal length of 100 mm. Intensity of the scattered light was measured at different angles to calculate the volume median diameter and the distribution span. Volume median diameter was the diameter at the 50% point of the entire volume distribution. The span was defined as [D(v,90)-D(v,10)]/D(v,50), where D(v,90), D(v,10), and D(v,50) were the respective diameters at 90, 10, and 50% cumulative volume.

Preparation of Blends. Before powder dispersion measurement, each powder was blended with a lactose carrier (200M Pharmatose, DMV International) at a 10:1 (carrier:powder) weight ratio by mixing using a tumbling mixer (Turbula, Glen Mill) and sieving using a stainless steel sieve (250 μm). The blend was first mixed for 5 min and then sieved by tapping. Some clumps were gently pressed through the sieve to deagglomerate the particles. The same mixing and sieving procedures were repeated for the second time.

Powder Dispersion. The dispersibility of each powder/carrier blend was assessed using a multiple-stage liquid impinger with a glass throat (MLSI, Astra, Copley Instrument, Nottingham, UK) through a dry powder inhaler (Dryhaler, Dura Pharmaceuticals). Ten doses (10–20 mg each) of the blended sample were weighed and loaded individually directly into the dose chamber of the device as described previously (8). The powder was dispersed at an air flow rate of 60 L/min. The amount of protein deposited on the throat, impinger stages, and the filter, as well as the amount retained in the device were assayed by quantitative wash recovery by measuring the UV absorbance at 280 nm using an absorptivity of 1.6 $cm^{-1}(mg/mL)^{-1}$ for both proteins. The percentage of the total dose collected on the third and the fourth stages and on the filter, representing the particles with the aerodynamic diameter ≤ 6.4 μm, was defined as the fine particle fraction. This fraction of powder has the potential to penetrate and deposit in the deep lung region.

Biochemical Stability. Soluble aggregation of spray-dried powders of anti-IgE antibody was measured following storage at 11% and 38% RH and temperatures of 2–8, 30, and 40°C. A relative humidity of 11% and 38% was maintained using saturated lithium chloride and calcium chloride (9,10). For rhDNase, the powder samples were stored at 25 °C and a relative humidity of 11%, 57%, and 84%. These relative humidities were maintained using the saturated salt of lithium chloride, sodium bromide, and potassium chloride, respectively (9,10). Prior to analysis, spray dried protein powder was reconstituted with water to a final protein concentration of 10 mg/mL.

Long-Term Physical Stability. The dispersibility of spray-dried anti-IgE antibody powders was evaluated following storage at the relative humidities of 10%, 20%, 30%, 40%, and 50% and temperatures of 30 °C for 5 and 15 weeks. These relative humidities (10%–50%) were maintained using the saturated salt of lithium chloride, potassium acetate, magnesium chloride, calcium chloride, and magnesium nitrate, respectively (9,10). For rhDNase powder samples, they were stored at 25 °C and two relative humidities (11% and 38%).

RESULTS AND DISCUSSION

Effect of Powder Formation/drying on Residual Moisture

To understand how the drying process affects powder's residual moisture level, we compared protein powders prepared by spray drying and freeze drying because the latter is the most commonly used drying technique for protein products. Under the conditions of this study, regardless of protein formulations (Table 1), powders prepared by freeze drying were consistently drier than those prepared by spray drying (T_{inlet} = 105 °C, T_{outlet} = 53 °C), 3% vs. 7%. However, after the spray-dried powder underwent vacuum drying (6% RH and 25°C), its moisture level decreased to 3%, reaching the same level as the freeze-dried material. This implies that the powder's residual moisture was determined by the state of process power input between the protein solid and the drying environment.

As far as freeze-drying principles are concerned, the majority of water (>99%) was removed through sublimation during primary drying, but the final residual moisture was determined by secondary drying. Secondary drying was generally a vacuum drying process performed at near the room temperature; there-

fore, a long drying time was normally used to ensure that the solid is dried to its equilibrium moisture content, i.e. in equilibration with the environment.

A common representation of the equilibrium moisture content of the powder in relation to its environmental condition is the use of a computer-controlled vacuum moisture microbalance (gravimetric moisture sorption/desorption isotherm). Relative humidity in the isothermal sample chamber is controlled by pulling different degrees of vacuum. When given sufficient time, sample weight reaches a constant value (e.g. equilibrium moisture content) corresponding to each relative humidity. The sorption and desorption isotherms (Figure 1a) for the spray-dried anti-IgE powder (anti-IgE antibody:mannitol = 4:1) overlapped, i.e. in the absence of hysteresis, suggesting that water removal and uptake was reversible. Figure 1b shows the sorption isotherms for the same anti-IgE powder at three different temperatures, 5, 25, and 40 °C. The overlapping of the three isotherms suggests that the equilibrium moisture content was determined by the environmental relative humidity instead of the absolute humidity which increased with increasing temperature at the same relative humidity. Figure 1c shows the sorption isotherms for excipient-free rhDNase powders prepared by freeze drying, spray drying, and spray freeze drying. The three isotherms again overlapped, suggesting that the equilibrium moisture content is not simply a surface phenomenon in view of the fact that the specific surface areas of the three powders were very different: 2.8 m^2/g for spray-dried powder (2.8 μm in median particle diameter); 1.2 m^2/g for freeze-dried powder; 81.4 m^2/g for the spray freezed-dried powder (6.8 μm in median particle diameter). Water molecules might penetrate deep into the particles during moisture sorption. All these results suggest that for the protein formulations tested in this study water sorption and desorption is reversible and might be dominated by the environmental relative humidity during powder preparation and processing. Please note that the conclusion above will not hold if proteins or sugar excipients undergo a physical state change such as crystalline vs. amorphous, which did not happen in our cases based on unpublished X-ray powder diffraction analysis.

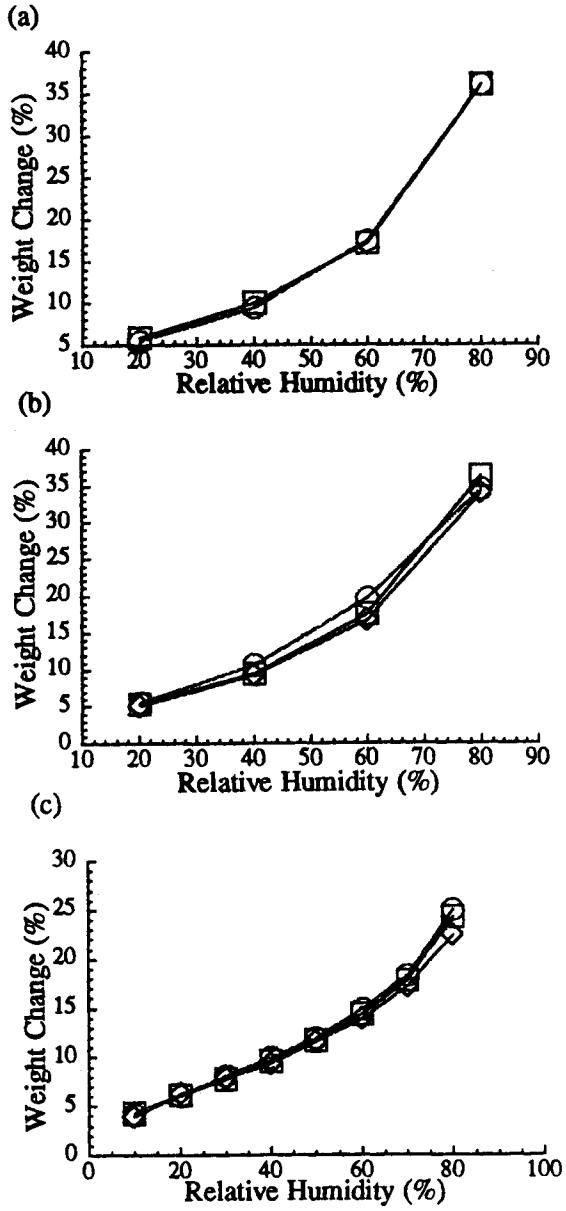

Fig. 1. (a) Moisture sorption (○) and desorption (□) isotherms for the spray-dried powder (anti-IgE antibody:mannitol = 80:20). (b). Moisture sorption isotherms for the spray-dried powder (anti-IgE antibody:mannitol = 80:20) at 5 (○), 25 (□), and 40 (◇) °C. (c) Moisture sorption isotherms for the spray-dried powder (excipient-free rhDNase) prepared by freeze drying (○), spray drying (□), and spray freeze drying (◇).

Water Removal upon Spray Drying

Spray drying took place in the drying chamber where sprayed droplets were in contact with the drying air. Atomized droplets were dried almost instantaneously (less than 1 sec for the bench-top dryer) when encountering the drying air in the drying chamber. Compared to the long drying time (tens of hours) in the freeze drying process, particles might not have sufficient time to reach the equilibrium moisture content in the spray-drying process. This might be why the spray-dried powder contained a higher moisture level than the freeze-dried solid. The next question is how the drying condition affected the residual moisture of the spray-dried powder, i.e. can spray drying produce drier powders?

The driving force for spray drying is the difference in vapor pressure between the drying air (P_{DA}) and the droplet surface ($P_{droplet}$), i.e. $P_{droplet} - P_{DA}$. P_{DA} was determined by the absolute humidity (AH) of the air before entering the chamber and by the inlet air temperature (T_{inlet}). Drying air with higher T_{inlet} and lower AH resulted in a lower RH, thereby a greater driving force for water removal. The temperature and RH of the drying air along the drying chamber were measured to find a correlation with the powder's final residual moisture content.

Our earlier study (11) found that the drying air temperature within the chamber reached the outlet temperature (T_{outlet}) right outside the nozzle, suggesting that the majority of water was removed early on at the top of the chamber. This appears to resemble the primary drying in the lyophilization process. As spray drying proceeded, the powder's moisture level was determined by the RH of the air occupying the rest of the dryer, resembling the secondary drying of the freeze drying operation. Since the powder was collected in the receiving vessel, the

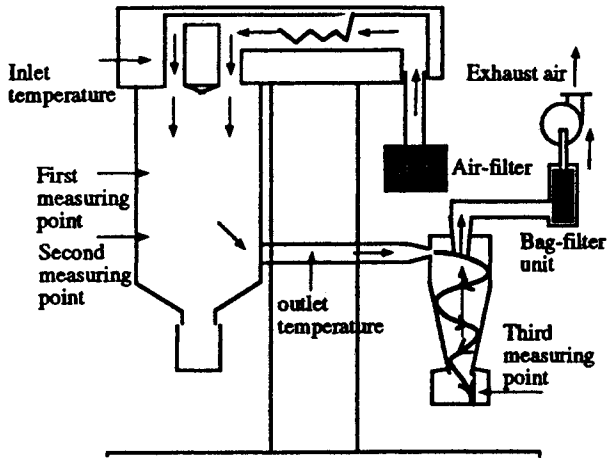

Fig. 2. Schematic representation of the spray-drying system along with the measured relative humidities and temperatures inside the drying chamber and the receiving vessel corresponding to different spray-drying conditions.

air condition inside the vessel should play an important role. Therefore, we attempted various spray-drying conditions for achieving different RHs to understand its effect on the powder's moisture level.

The schematic representation of the drying system is shown in Figure 2. The associated drying conditions as well as measured RH and temperature of the drying air are listed in Table 2. By changing the inlet air temperature and liquid feed rate, the RHs of the air at three locations (the center and the bottom of the chamber as well as inside the receiver) varied from 3% to 56% with the RH increasing and the temperature decreasing along the chamber. Relative humidities higher than 60% were not included in this study because water condensation occurred in the drying chamber. As expected, the temperature measured at the bottom of the chamber (the second measuring point) was close to T_{outlet}. The RH inside the receiving vessel ($RH_{receiver}$) was the highest because air inside the receiver had the lowest temperature in the whole dryer. Since we have previously demonstrated that T_{outlet} was the single most important parameter that determined the water drying rate (11), based on Table 2 we established a linear relationship between $RH_{receiver}$ and T_{outlet}, where $RH_{receiver} = 104 - 1.2 \times T_{outlet}$, R = 0.97. Figure 3 shows the relationship between the moisture content (MC) of the spray-dried powder (anti-IgE antibody:mannitol = 80:20) and $RH_{receiver}$ as, $MC = 13.7 - 0.11 \times T_{outlet}$, R = 0.992. The MC varied from 4.1% to 9%, corresponding to $RH_{receiver}$ from 6% to 56%. When compared with the moisture sorption isotherm (equilibrium MC) of the same powder (also in Figure 3), the two curves intercepted at around 20% RH. At RH < 20% (T_{outlet} > 70°C), the powder's MC is higher than the equilibrium MC, whereas it was lower than the equilibrium MC at RH > 20% (T_{outlet} < 70°C). This may be related to non-equilibrium drying which is different from the moisture sorption/desorption isotherm condition. Note that although the powder product sat in the receiver for the longest time, the equilibration with the air inside the receiver would not be reached considering the short experiment duration (approximately 2 minutes). Based on the result in Figure 3, the driest powder that the bench-top spray dryer could produce contained approximately 3–4% residual moisture by using a T_{outlet} higher than 80°C. To achieve such a high T_{outlet}, a high T_{inlet} and/or a low liquid feed rate would be needed. This represents an undesirable manufacturing condition which slows down the production rate and may impose potential adverse effects on protein denaturation (12,13).

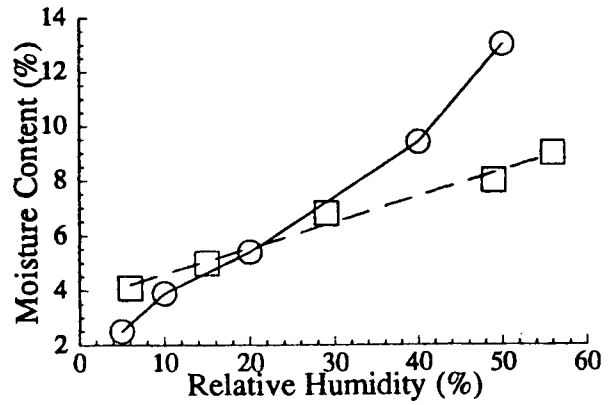

Fig. 3. The relationship between spray-dried powder's moisture content (anti-IgE antibody:mannitol = 80:20) and the measured relative humidities of the drying air inside the receiving vessel (○), with the broken line representing a linear regression of $MC = 13.7 - 0.11 \times T_{outlet}$, in comparison with the moisture sorption isotherm of the same powder (□).

Table 2. Conditions[a] for the Spray Drying of Water for Determining the Relative Humidities and Temperatures of the Air at Three Different Locations Inside the Dryer shown in Figure 2

T_{inlet} (°C)	RH(Temp)[b] at 1st MP [%(°C)]	RH(Temp) at 2nd MP [%(°C)]	RH(Temp) at 3rd MP [%(°C)]	T_{outlet} (°C)	Liquid feed rate (mL/min)
107	— (88)	3 (83)	6 (80)	84	5
103	6 (80)	10 (75)	15 (59)	78	8
103	15 (58)	19 (55)	29 (48)	57	15
90	25 (55)	40 (48)	49 (45)	50	15
101	41 (44)	52 (42)	56 (39)	43	19.5

[a] The drying and atomizing air flow rate was kept at 1100 L/min and 1140 L/hr.
[b] MP: Measureing Point; RH: Relative Humidity; Temp: Temperature.

Effect of Dehumidification

Since mass transfer was affected by the driving force, $P_{droplet} - P_{DA}$, another approach of improving moisture removal was to decrease P_{DA} by dehumidifying the air prior to entering the chamber. A dehumidifier (HonyCombe, Cargocaire) was used to reduce the relative humidity of ambient air down to approximately 5%. Unfortunately, this additional dehumidification step did not dry the spray-dried powder further. This can be explained using the psychrometric chart based on the non-dehumidified air (50% RH and 20°C) and dehumidified air (5% RH). After drying, the condition of the non-dehumidified air coming out of the dryer was determined to be at 30% RH and 50°C. Based on mass balance, water removal rate = $Q_{DA} \times \rho_{air} \times (AH_{out} - AH_{in})$, where ρ_{air} is the air density and AH is

Table 3. The Moisture Content of the Spray-dried Powder (anti-IgE antibody:mannitol = 80:20) with and Without Vacuum Drying at Different Storage Conditions

Sample	SD/VD[a]	Time exposed to ambient conditions[b] (hrs)	Storage condition	Moisture content (%)
1	SD	0	No	7.2
1	SD	0	Sealed in vial for 3 days	6.9
1	SD	2	No	8.0
1	SD	18	No	9.3
2	SD+VD	0	No	2.4
2	SD+VD	2	No	6.9
2	SD+VD	2	Sealed in vial for 3 days	7.4
3	SD+VD	0	Sealed in vial and opened for 3 time	4.3
3	SD+VD	1	No	8.2
3	SD+VD	2	No	9.0
3	SD+VD	6	No	9.3

[a] SD: Spray dried under the standard condition; VD: Vacuum dried at 25 °C and 6% RH for 72 hrs.
[b] The ambient condition: 40%—55% RH and 20—22 °C.

the absolute humidity, the dehumidified drying air would exit the chamber at 50°C and 23% RH. This small difference, 23% vs. 30%, would have no significant effect on powder's MC (Figure 3).

Effect of Vacuum Drying

Due to the short drying time, it is difficult to produce spray-dried powders with a MC lower than 3%. To achieve a lower MC, the best approach was to further dry the powder (secondary drying) with vacuum drying. Table 3 summarizes the variation of the MC of a spray-dried powder (anti-IgE antibody:mannitol = 80:20) with or without a subsequent vacuum drying followed by exposure to an ambient environment (20°C and 40–55% RH) and different storage conditions. The MC of the powder (Sample 1) was 7.2% after spray drying, and the moisture level maintained for three days if the powder was sealed immediately after spray drying, but the powder gained moisture up to 9.3% after being exposed to the ambient environment for 18 hours. Vacuum drying (20°C and 6% RH) for 3 days could effectively dry the spray-dried powder (Sample 2) down to a MC of 2.4%. However, after two-hour exposure to the ambient air, the MC of this powder increased to 6.9% and maintained the same level after the powder was then sealed and stored for 3 days. In a separate vacuum and spray-dried powder (Sample 3) which had been sealed and opened three times, the MC increased to 4.3% though each exposure duration was short. The same powder absorbed moisture to 8.2% after being exposed to the ambient air for one hour, to 9.0% for two hours, and to 9.3% for six hours. All these results suggest that the moisture level of the powder product was highly dependent on the processing environment regardless of the powder's manufacturing conditions. Although an additional manufacturing step, vacuum drying, could help reduce the MC of the spray-dried powder to the level of freeze-dried powder, the powder would adsorb moisture quickly if it was not processed in the RH environment drier than the powder's manufacturing environment.

Physical and Biochemical Stability

How does the powder's equilibrium moisture content affect the short-term and long-term aerosol performance as well as the long-term biochemical stability? For long-term stability, samples of protein raw powders and blends were stored in contained environments at a fixed RH and temperature and were analyzed at specific time-points. To understand the short-term effect of residual moisture on a powder's aerosol performance, a spray-dried powder (a moisture level of 7.5%, anti-IgE antibody:mannitol = 80:20) blended with a lactose carrier in the ambient environment was aerosolized to compare a spray-dried and vacuum-dried powder (with a low moisture level of 3%) blended in a controlled environment (20% RH, nitrogen-purged glove box). Their respective fine powder fractions (< 6.4 μm) were determined to be 29±3% and 27±3%, showing no significant difference. Aerosol performance of the same powder after long-term exposure to different relative humidities, 10–50%, at 30°C for up to 15 weeks, is presented in Figure 4.

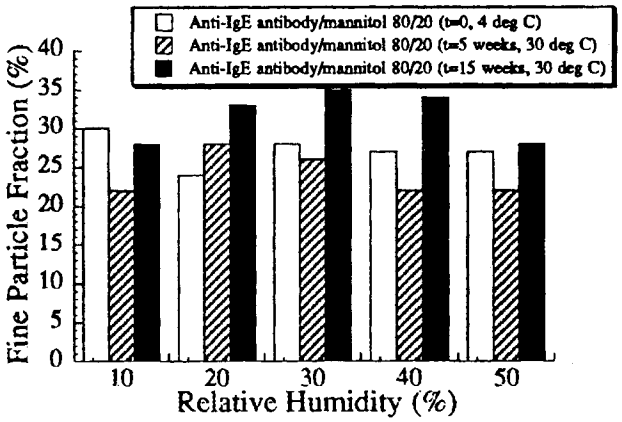

Fig. 4. Powder's aerosol performance (physical stability) for the spray-dried powder of anti-IgE antibody:mannitol = 80:20 upon long-term exposure to a controlled RH environment at 30°C at t=0 (○), 5=5 weeks (□), and t= 15 weeks (◇).

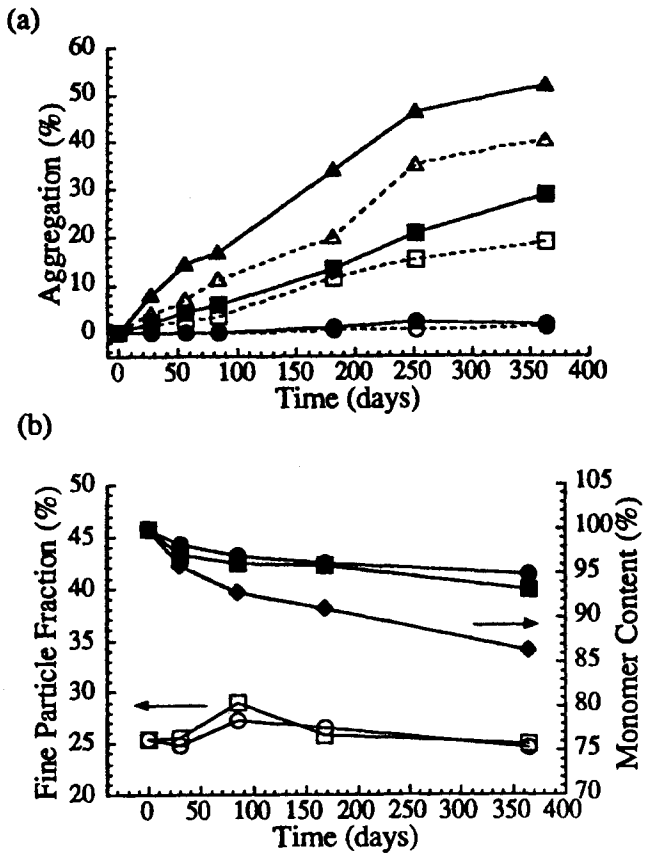

Fig. 5. (a) Powder's (anti-IgE antibody:mannitol = 80:20) aggregation upon long-term exposure to a controlled environment: 2–8°C and 11% RH (○), 2–8°C and 38% RH (●), 30°C and 11% RH (□), 30°C and 38% (■), 40°C and 11% (△), and 40°C and 38% (▲). (b). Powder's (excipient-free rhDNase) aerosol performance (physical stability) upon long-term exposure to 11% (○) and 38% RH (□) at 25°C. The same powder's aggregation upon long-term exposure to 11% (●), 57% RH ■), and 84% (◆) RH at 25°C.

Considering a standard deviation of 3% with this analysis based on unpublished results, the dispersibility of the powder did not vary with storage humidity. In view of the equilibrium moisture content under 50% RH which was approximately 11%, this suggests that anti-IgE antibody powders maintained their aerosol performance as long as they were manufactured, processed, and stored under conditions where the environmental RH was within 50%. However, the storage environment affects the protein's biochemical stability. The primary degradation product determined upon storage of spray-dried anti-IgE antibody powders was an increase in soluble protein aggregate measured by size-exclusion chromatography. Aggregation of a spray-dried powder (anti-IgE antibody:mannitol = 80:20) following storage at 2–8, 30, and 40°C for up to 1 year is shown in Figure 5(a). The amount of aggregate increased with increasing temperature and increasing relative humidity.

In the case of spray-dried excipient-free rhDNase/100M lactose blended powders, the long-term physical and chemical stability corresponding to different relative humidities at 25°C are presented in Figure 5(b). Similarly, the effect of RH (11% and 38%) on powder's aerosol performance is not as obvious as that on protein's biochemical stability.

The above conclusion may not hold true for humidity-sensitive proteins such as Galactosidase (5) and for protein formulations containing other sugar excipients (4). For those moisture-sensitive formulations, aerosol powders should be manufactured, processed, and stored under the driest environment that is achievable in the current manufacturing facility.

CONCLUSIONS

The residual moisture content of a dry protein powder was determined by the manufacturing environment (relative humidity and texture), normally in equilibration with the drying medium if sufficient drying time was given. Spray drying was an exception because the drying time was very short and the drying environment was changing. Spray drying conditions that allowed drier powders (~3%) to be manufactured were not favorable to large-scale manufacturing. Vacuum drying of spray-dried powders could effectively reduce the moisture content. However, the processing and storage environments following powder manufacturing affected the powder's moisture level significantly because the powder's equilibrium moisture content was reversible regardless of the manufacturing condition. For each protein formulation investigated in this study, the residual moisture in the powder had no significant effect on the powder's aerosol performance during storage at RH < 50%. However, humid storage environments or high moisture contents reduced the protein's long-term biochemical stability. Therefore, for the benefit of long-term biochemical stability, the protein powder should be manufacturing and processed under the driest environment that a manufacturing facility allows (< 20% RH) even though the environmental control does not have to be so stringent for the sake of the powder's physical stability.

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