Dry Powders for Pulmonary Delivery of Peptides and Proteins†

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Abstract

Because proteins and peptides are poorly absorbed through the gastrointestinal tract, the lungs are a promising organ for administering these substances because of their large inner surface area, thin epithelium, and relatively low protease activity. Pulmonary absorption rate constants are inversely related to molecular weights. Adding an absorption enhancer is a promising method to increase systemic bioavailability of inhaled peptides and proteins. The local lung toxicity of soluble powder seems to be minimal. Spray drying is a useful and widely applied technique to prepare powders for inhalation, and supercritical fluids have recently been used for producing such powders. Selecting operating conditions and adding proper additives such as sugars yields hollow porous particles, which are easily dispersed and avoid phagocytic clearance in the lungs, with maximized chemical and physical stability.

1. Introduction

Recent advances in biotechnology have made it possible to use macromolecules such as peptides and proteins as therapeutic agents. At this time intravenous, intramuscular, and subcutaneous injections are the practical routes for administering such macromolecules. One would expect peroral administration to be the most convenient for patients, but the peroral bioavailability of macromolecules is extremely low due to their large molecular size and high susceptibility to enzymes in the gastrointestinal tract. Meanwhile, it has been suggested that the lungs are useful for administering macromolecules, which are poorly absorbed from the intestines.

So far, inhalation therapy has been used with low-molecular-weight drugs to treat local lung diseases such as asthma and infections. Recombinant human deoxyribonuclease (rhDNase) is the first protein approved for inhalation therapy (1). Patients with cystic fibrosis are given 2.5 ml of a 1 mg/ml solution of rhDNase by nebulization (2).

In the area of systemic therapy, insulin is the peptide that is expected to be the first approved for inhalation therapy. Exubera® is a rapid-acting dry powder insulin being developed through a collaboration between Pfizer Inc. and Aventis Pharma. The six-month Phase III studies involving 328 patients with type 1 diabetes and 309 patients with type 2 diabetes were completed in 2002 (3, 4). The AERx® Pulmonary Drug Delivery System is a broadly applicable technology platform that converts large or small molecules into fine-particle aerosols. AERx®iDMS (insulin Diabetes Management System) was developed through a collaboration between Novo Nordisk and Aradigm Co. A 12-week-long Phase II study including 107 non-smoking patients with type 2 diabetes has been completed (5).

The three main delivery systems used for aerosol inhalation in humans are pressurized metered-dose inhalers (MDI), nebulizers, and dry powder inhalers (DPI) (6). DPIs appear to be the most promising of these for future use because the device is small and relatively inexpensive, no propellants are used, and breath actuation can be used successfully by many patients with poor MDI technique (6, 7).

This review will focus on the dry powder peptides and proteins for inhalation. The success of inhalation therapy with dry powders is determined by the active ingredient's biological aspects, by the physicochemical aspects of formulation, and by inhaler performance (**Fig. 1**). Here we briefly review some of the

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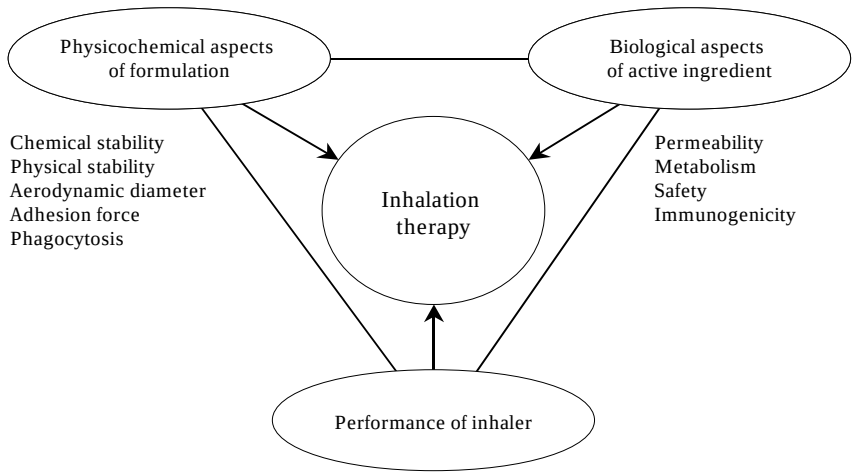

Fig. 1 Factors determining successful inhalation therapy with dry powders.

biological and physicochemical aspects of inhalation therapy with dry powder peptides and proteins. Extensive reviews on inhalers are available elsewhere (8, 9).

2. Biological aspects of the pulmonary absorption of peptides and proteins

This section summarizes the histological features of the lungs, drug permeability through the lung epithelium, the metabolism of proteins and peptides in lung tissue, and the safety of peptides and proteins administered through the lungs. It also reviews how chemicals and enzyme inhibitors enhance the pulmonary absorption of peptides and proteins.

2.1 Histological features of the lungs

The respiratory tract can be divided into upper airways (the nose, mouth, larynx, and pharynx) and lower airways (from the trachea to the alveoli) (10). The average weight of human lungs is 0.6 kg. Because the lungs receive the entire cardiac output, their blood flow is as high as 5,700 ml/min, more than five times that of the portal system (1,125 ml/min), including the stomach and the small and large intestines (11).

Airway diameter decreases and surface area increases according to the successive branching of the airways. The cross sectional area of the trachea is about 2.5 cm^2, while that of the respiratory zone is much wider (10). The total cross sectional area of the alveoli is about 10^4 cm^2. The total surface are of the airway tubes also increases and that of alveoli is more than 100 m^2 as large as that of the small intestine.

The epithelial layer of the trachea is composed mainly of columnar ciliated cells. The thickness from the airway surface to blood vessels is on the order of 30 to 40 μm (12). Particulates deposited in the upper airways are rapidly carried away by mucociliary transport, resulting in a short of residence time (13).

The alveolar surface is populated by two major epithelial cell types: the terminally differentiated type I cell and its progenitor type II cell (14). The alveolar epithelium is quite thin. In the alveoli drugs have to travel only 0.5 to 1.0 μm to enter the blood stream. Total fluid volume in the human lungs is approximately 10 ml (15). Lung pH at the site of drug absorption has been estimated at about 6.6 using pulmonary absorption data for several weak electrolytes in rats (16). The alveolar surface is lined by a surface-active material called the lung surfactant, which is a mixture of lipids, proteins, and carbohydrates (17). Phospholipids account for 75-80% of the total weight, and dipalmitoyl phosphatidylcholine accounts for nearly half of that. The lung surfactant reduces alveolar surface activity and stabilizes alveolar structure.

Lavage of a normal adult lung yields a cell count that is 93% macrophages, 7% lymphocytes, and less than 1% neutrophils, eosinophils, or basophils (18). Alveolar macrophages interact with microorganisms or particulates, act as effector and accessory cells in inflammatory and immune reactions, and protect alveolar structures to form a protease attack (18).

2.2 Drug absorption through the lungs

The pulmonary absorption of small molecules basically obeys pH-partition theory, i.e., drugs are likely absorbed by diffusion across a lipid membrane (19,

20). In vivo rat lung absorption data for saccharides of various molecular weights (122 to 75,000) showed that the absorption rate constants were inversely related to molecular weight and directly related to the diffusion coefficients of the compounds (21). The transport of dextrans (4 to 150 kDa) across cultured rat alveolar epithelial cell monolayers suggested that macromolecules with radii under 5 μm traverse the alveolar epithelial barrier via paracellular pathways, while macromolecules with radii of 6 μm or larger cross the barrier via other pathways such as pinocytosis (22). The penetration of hydrophilic compounds through excised rabbit trachea sacs also inversely correlates to molecular weight (23). When several hydrophilic and lipophilic drugs were administered through rat trachea as aerosols, the absorption rates were roughly twice as rapid as when administered by the intratracheal injection of drug solutions. These results suggest that drug absorption is more rapid in the alveolar region than in the tracheobronchial region of the lungs (24). The distal or deep lung is the optimal site for the high absorption of proteins. Inhaler systems should be designed to maximize deposition in this region (12).

It is known that peptides or proteins could be absorbed through the lungs (25, 26). The pulmonary bioavailability of peptides can be easily evaluated with small animals by the method proposed by Enna and Schanker (21). The pulmonary bioavailability of insulin administered as a pH 7.0 solution was 13 to 14% better than subcutaneous administration in rats (27).

2.3 Metabolism in the lungs

In general, the metabolic activity of the lungs is much lower than that of the intestinal wall and liver. In the lungs there is no first-pass conjugation of salbutamol, which undergoes extensive first-pass conjugation in the intestinal wall and liver. The systemic bioavailability of orally administered budesonide is 11%, whereas that when inhaled is 73%. Fluticason propionate's hepatic first-pass metabolism is 99%, but it is zero in the lungs (28).

However, it is known that peptides such as insulin are subjected to enzymatic degradation in the lungs (29-31). The degradation of insulin in lung cytosol from diabetic rats was significantly less than in that from normal rats (30). An experiment with synthesized model peptides suggested that the lung has the ability to metabolize peptides through pathways not observed in the rat intestine. However, avoiding the hepatic first-pass effect through the pulmonary route would eliminate the disadvantage of pulmonary metabolism (32). Type II cells have higher metabolic activity than type I cells. The degradation rate constant of luteinizing hormone releasing hormone (LHRH) in type II cells was higher than that of type I cells but lower than that of nasal and rectal epithelial membranes. The transformation of type II cells into type I cells resulted in a more than 10-fold decrease in LHRH proteolytic activities (14).

2.4 Safety of inhaled proteins

Researchers have investigated the systemic toxicity of therapeutic peptides and proteins following subcutaneous administration. When discussing the safety aspect of inhaled proteins, interest focuses on local toxicity to the lungs and on adverse immune reactions. Assaying bronchoalveolar lavage is useful in screening lung injuries from inhaled substances (33). An increase in the extracellular activity of lactate dehydrogenase (LDH), a cytosol enzyme, indicates cell lysis or cell membrane damage. An increase in the number of phagocytic cells suggests an inflammation reaction in the lungs. When a suspension of superfine silica was intratracheally instillated in rats, the LDH activity and number of cells recovered in the lavage increased at 4 hr and reached a maximum at 24 hr. The LDH activity and number of cells declined thereafter for a week, then gradually increased over the succeeding two months (34, 35).

However, the local lung toxicity of soluble powder seems to be minimal. When insulin dry powder formulated with mannitol was intratracheally administered in rats, the LDH level (36) and number of cells (unpublished data) in the lavage did not increase over 24 hr. Clinical studies for inhaled DNase, insulin, interferon α, interferon γ, leuprolide acetate, and α-1-antitrypsin showed virtually no adverse lung reactions (12).

2.5 Additives for improving bioavailability of inhaled peptides and proteins

One of the reasons of the low bioavailability of large molecules relates to low diffusivity through the epithelial barrier. To overcome this, several chemicals and enzyme inhibitors were examined as pulmonary absorption enhancers. In the 1990s there were many reports on the enhancement of the pulmonary absorption of peptides and proteins. These reports examined bile acids, surfactants, fatty acids, citric acid, and protease inhibitors (**Tables 1** and **2**).

Glycocholate and bacitracin had higher enhancing activity for the pulmonary absorption of peptide so-

Table 1 Effect of absorption enhancers on pulmonary absorption of peptides and proteins

Peptide/Protein	Enhancer	DF[a]	ER[b]	Ref.
insulin	50 mM glycocholate	SL	5.1	27, 37
insulin	10 mM glycocholate	SL	4.2	38
eel calcitonin	10 mM glycocholate	SL	4.0	38
eel calcitonin	20 mM glycocholate	SL	3.1	39
TSH	50 mM glycocholate	SL	6.3	37
FSH	50 mM glycocholate	SL	5.9	37
HCG	50 mM glycocholate	SL	20	37
salmon calcitonin	250 μg/dose taurocholic acid	SL	1.4	40
salmon calcitonin	250 μg/dose taurocholic acid	DP	1.9	40
insulin	1% Span 85	SL	3.1	27
insulin	1% Span 85	SL	7.2	36
insulin	160 μg/dose Span 85	DP	0.7	36
insulin	10 mM MM[c]	SL	2.5	38
eel calcitonin	10 mM MM	SL	4.0	38
salmon calcitonin	250 μg/dose dimethyl-β-cyclodextrin	SL	2.4	40
salmon calcitonin	250 μg/dose dimethyl-β-cyclodextrin	DP	2.1	40
salmon calcitonin	250 μg/dose lecithin	SL	1.5	40
salmon calcitonin	250 μg/dose lecithin	DP	1.8	40
salmon calcitonin	250 μg/dose octyl-β-glucoside	SL	1.4	40
salmon calcitonin	250 μg/dose octyl-β-glucoside	DP	1.6	40
insulin	5 mM LM[d]	SL	7.1	38
eel calcitonin	5 mM LM	SL	5.7	38
salmon calcitonin	0.5% palmitoleic acid	SL	2.5	41
salmon calcitonin	0.5% linoleic acid	SL	2.4	41
salmon calcitonin	0.5% oleic acid	SL	2.2	41
salmon calcitonin	250 μg/dose oleic acid	SL	1.6	40
salmon calcitonin	250 μg/dose oleic acid	DP	2.8	40
insulin	100 mM EDTA	SL	0.6	27
eel calcitonin	10 mM EDTA	SL	1.8	38
insulin	100 mM salicylate	SL	0.5	27
insulin	citrate (pH5.0)	SL	3.4	36
insulin	citrate (pH3.0)	SL	4.5	36
insulin	36 μg/dose citric acid	DP	2.1	36
insulin	citrate (pH 3.0)	SL	3.2	37
insulin	0.5 mg/dose citrate	DP	2.7	37
TSH	citrate (pH 3.0)	SL	3.2	37
FSH	citrate (pH 3.0)	SL	3.9	37
HCG	citrate (pH 3.0)	SL	26	37
salmon calcitonin	250 μg/dose citric acid	SL	1.5	40
salmon calcitonin	250 μg/dose citric acid	DP	2.2	40

[a] Dosage form. SL=solution and DP=dry powder.
[b] Enhancement ratio. Ratio of AUC or biological response between a dosage form with absorption enhancer and that without absorption enhancer.
[c] Mixed micelles of linoleic acid and HCO60 at a molar ratio of 30:4 in phosphate buffered saline.
[d] *N*-Lauryl-β-D-maltopyranoside.

lutions. Yamamoto et al. intravenously injected Evans Blue and examined the leakage of Evans Blue in the lung. Although increasing calcitonin activity, 5 mM *N*-Lauryl-β-D-maltopyranoside increased Evans Blue leakage, suggesting lung toxicity. On the other hand, 1 mM *N*-Lauryl-β-D-maltopyranoside, 10 mM glycocholate, and 10 mM mixed micelles of linoleic acid and HCO60 were safe and effective enhancers (42). Citrate is a potent pulmonary absorption enhancer for peptides formulated as dry powders. When insulin dry powder containing 0.036 mg/dose of citric acid was administered to rat lungs, bronchoalveolar lavage was as low as that for saline administration, suggesting that citric acid is a safe additive (36). Adding an

Table 2 Effect of enzyme inhibitors on pulmonary absorption of peptides and proteins

Peptide/Protein	Enzyme inhibitor	DF[a]	ER[b]	Ref.
insulin	1 mM bacitracin	SL	0.9	27
insulin	20 mM bacitracin	SL	6.8	38
eel calcitonin	20 mM bacitracin	SL	19	39
salmon calcitonin	0.2 mM bacitracin	SL	2.2	41
insulin	10 mM bacitracin	SL	7.0	36
insulin	420 μg/dose bacitracin	DP	1.0	36
insulin	13 mM nafamostat	SL	2.1	27
eel calcitonin	20 mM nafamostat	SL	3.9	39
insulin	10 mM surfactin	SL	6.1	27
insulin	10 mg/mL aprotinin	SL	2.0	38
insulin	10 mg/mL STI	SL	2.5	38
salmon calcitonin	0.2 mM chymostatin	SL	2.1	41

[a] Dosage form. SL=solution and DP=dry powder.
[b] Enhancement ratio. Ratio of AUC or biological response between a dosage form with absorption enhancer and that without absorption enhancer.

absorption enhancer is a promising method for increasing the systemic bioavailability of inhaled peptides and proteins, but long-term safety should be examined for application to humans.

It should be noted that the effect of absorption enhancers depends on their formulation. Bacitracin and Span 85 increased pulmonary insulin absorption from solutions in rats, but were not effective when formulated as dry powders with insulin (36).

In research on protease inhibitors, a relatively favorable correlation was observed between the calcitonin absorption-enhancing activity and membrane enzyme inhibition activity of 18 protease inhibitors (41).

3. Physical aspects of dry powder peptides and proteins

Spray drying is a useful and widely applied technique to prepare powders for inhalation. Supercritical fluids have recently been applied for producing powders for inhalation. In this section, we briefly review these techniques, stability of the produced dry powder peptides and proteins, and aerodynamic diameter being one of the most critical factors to determine the success of inhalation therapy.

3.1 Spray dry for preparation of dry powder peptides and proteins

Spray drying is a useful and widely applied technique for one-step preparation of powders for inhalation with a drug solution or suspension. The independent variables of spray drying processes are liquid feed rate, atomizing air flow rate, drying air flow rate, and inlet air temperature. Outlet temperature linearly depends on each of these variables (43), suggesting that it can be estimated if the regression lines between outlet temperature and the independent variables are available for a spray drier. The inlet temperature is usually several tens of degrees higher than the outlet temperature. Determining the temperature variation within a drying chamber revealed that the temperature at 5 cm below the nozzle was much closer to the outlet temperature than the inlet temperature, and that the temperature at 17 cm below the nozzle, midway between the nozzle and outlet, was approximately the same as the outlet temperature (43). This means that during spray drying, droplets in the drying chamber were exposed to the temperature swayed by the outlet temperature.

It is likely that proteins are susceptible to degradation upon spray drying due to the relatively high temperatures (44). **Table 3** summarizes the effect of inlet and outlet temperatures on spray-dried peptides and proteins. Spray drying of a 5 mg/ml aqueous insulin solution caused minor degradation of insulin at outlet temperatures below 120℃. However, degradation of high-molecular-weight proteins, A-21 desamido insulin, and other insulin-related compounds increased with outlet temperature above 120℃ (47). β-galactosidase activity is susceptible to spray drying temperature, and only half of its activity remained after spray drying without additives at an outlet temperature of 50℃. When 6% β-galactosidase was spray-dried with 5% mannitol, no activity was lost at outlet temperatures below 50℃, but it deceased above 50℃. Replacing mannitol with trehalose stabilized the spray-dried β-galactosidase, and its activity was maintained at

Table 3 Effect of inlet and outlet temperatures on spray-dried peptides and proteins

Peptide/Protein	Temperature (℃)		Activity (%)	Degradation (%)	Moisture (%)	D[a] (μm)	Ref.
	Inlet	Outlet					
β-galactosidase		50	100				45
		70	80				
		90	30				
oxyhemoglobin	50			2.0			46
	80			2.9			
	120			5.0			
rhDNase	91	53			8.3	2.0	43
	120	72			7.6	2.2	
	150	90			6.1	2.3	
insulin	100	50		0.36[b]	5.1	3.2[b]	47
	100	74		0.44	3.1	3.1	
	160	100		0.49	3.1	2.2	
	220	120		0.77	3.3	2.1	
	220	150		3.32	1.9	4.2	

[a] Geometric or aerodynamic diameter.
[b] High-molecular-weight protein.

100% at an outlet temperature of 100℃ (45).

Surface denaturation at the air-liquid interface of sprayed droplets may play a significant role in protein degradation. Spray drying of mannitol-formulated human growth hormone (hGH) at room temperature resulted in increased protein degradation by increasing the atomizing air rate, which suggested degradation at the air-liquid interface during spray drying (48). Adding polysorbate-20 into the liquid feed significantly reduced the formation of insoluble hGH aggregates, and adding divalent metal zinc ions effectively suppressed the formation of soluble hGH aggregates (49).

3.2 Application of supercritical fluids to preparation of dry powder proteins

Fluids at temperatures and pressures above critical values are called supercritical fluids (SCFs). SCF densities of similar to those of liquids, while their viscosities and diffusivities are in the range of gases (50). The application of SCFs to particle design has recently emerged as a promising techniques for producing powders for inhalation. Carbon dioxide is the most widely used supercritical solvent because it is cheap and nontoxic, and because of its easily accessible critical parameters (Tc=31.1℃ and Pc=73.8 bar) (50).

When a substrate has a reasonable solubility in a SCF, dry powders are obtained by depressurizing the SCF solution through an adequate nozzle. This process is called the rapid expansion of supercritical solutions (RESS). However, the solubility of many peptides and proteins in SCFs is relatively low. When a SCF is a poor solvent for the substrate, it can be used as an anti-solvent to precipitate the substrate dissolved in a good solvent (51).

Dimethylsulfoxide (DMSO) is a good solvent for lysozyme. When CO_2 is put into lysozyme dissolved in DMSO, and the CO_2 mole fraction reaches a critical value, the solution becomes saturated and causes the catastrophic precipitation of lysozyme (52). This process is called the gas-antisolvent (GAS) precipitation process.

Other techniques to produce powders with SCFs as anti-solvents are the aerosol solvent extraction system (ASES) (53, 54), precipitation with a compressed fluid antisolvent (PCA) (55, 56), the supercritical antisolvent technique (SAS) (57-60), and solution enhanced dispersion by supercritical fluids (SEDS) (61, 62). These techniques introduce protein solutions through a nozzle at a relatively low flow rate into the flow of a SCF in a vessel. The SCF removes the solvent of the protein solution and precipitates the protein in the vessel.

Yeo et al. applied the SAS technique to a 5 mg/ml insulin solution in DMSO. The slow CO_2 injection rate favored the growth of larger particles (57). A significant increase in β-sheet content and a corresponding decrease in α-helix content were observed for the precipitated insulin relative to a commercial powder. However, the precipitated insulin solution in 0.01 M HCl yielded a solution structure similar to that of the dissolved commercial powder (58). Intravenous administration to rats revealed that the processed insulin maintained its biological activity (57). The increase in β-sheet content and the concomitant de-

crease in α-helix content, which were reversible upon reconstitution, were also observed in lysozyme and trypsin precipitated by the SAS technique (60).

When the solubility of the solvent (for instance, water) in the SCF (for instance, CO_2) is very low, a modifier (for instance, ethanol) is employed in the system. Powders of lysozyme, albumin, insulin, and recombinant human deoxyribonuclease (rhDNase) were prepared by the ASES process. An aqueous solution of a protein was put into a precipitation chamber through the inside tube of a coaxial nozzle. Supercritical CO_2 modified by ethanol was fed through the outside tube of the coaxial nozzle. The mole fraction of ethanol in CO_2 was 0.2 and the volumetric flow rate ratio of the aqueous solution to CO_2 was 0.4/12. The ASES process at 35 or 45℃ and at 80 to 90 bar produced lysozyme powder without activity loss. Some aggregation was observed for insulin and albumin powders. rhDNase was substantially denatured during the processing (63).

A new supercritical CO_2-assisted aerosolization coupled with bubble drying was recently reported (64). This process involves mixing a stream of drug solution and a SCF stream inside a low dead-volume tee. The emulsion is allowed to expand out of a capillary restrictor, resulting in the aerosolization of the drug solution.

3.3 Stability of peptides and proteins

A crystalline solid of small molecule drugs is generally less prone to chemical decomposition than the amorphous form. In some cases, however, the crystalline state may not be more stable for protein and peptide formulations (65). The primary degradation pathways of biosynthetic human insulin involve deamidation at the Asn^{A21} site and covalent dimer formation. When storing at 25℃ and 40℃ at relative humidities between 0 and 75%, amorphous insulin was far more stable than crystalline insulin under all conditions (66).

The hydration state of proteins affects their solid-state stability. The aggregation of humanized monoclonal antibodies and rhDNase in mannitol-formulated spray-dried powders increased as storage humidity rose (67). Deamidation at the Asn^{A21} site of crystalline insulin increased sharply as moisture content increased, while that of amorphous insulin was almost independent of moisture (66).

It is known that the chemical stability of proteins in the solid state is enhanced by the presence of certain amorphous sugars. Two hypotheses have been proposed for the mechanism of protein stabilization by an amorphous sugar (68). The water substitution hypothesis supposes that sugar molecules form hydrogen bonds with dried proteins in place of water molecules to maintain higher-order protein structure. The glassy state theory supposes that the high viscosity of an amorphous sugar prevents proteins from degrading physically or chemically by retarding molecular movement. **Table 4** summarizes the effect of sugars on spray-dried peptides and proteins.

Izutsu et al. examined the stabilizing effect of mannitol during the freeze-drying of L-lactate dehydrogenase, β-galactosidase, and L-asparaginase. The activities of the freeze-dried enzymes depended on the content of amorphous mannitol in the cake. The stabilizing effect of mannitol decreased as mannitol crystallinity increased, suggesting that amorphous mannitol protected proteins against degradation (72).

The physical state of sugars used as an excipient for protein powder plays a role not only in maintaining protein stability but also in providing suitable aerosol performance. When recombinant humanized anti-IgE monoclonal antibodies (rhuMAbE25) were spray-dried with 10, 20, and 30% mannitol, the spray-dried powders with 10 and 20% mannitol remained amorphous during storage, while the powder with 30% mannitol crystallized. The fine-particle fraction (FPF) for the powder with 10 and 20% mannitol was maintained at 30-40% during storage for 36 weeks at 30℃. However, the fraction of the powder with 30% mannitol exhibited a dramatic decrease upon storage due to mannitol crystallization and an increase in particle size (44).

Moisture mediates the crystal growth of sugars in dry protein powders. Spray drying rhDNase with lactose produced spherical powders with noncrystalline substances. However, α-lactose monohydrate crystals were identified in the powders stored at high humidities (73).

3.4 Mass median aerodynamic diameter

Particle size and its distribution affect particle retention in the lungs (70, 74). Particles larger than 20 μm likely fail to go beyond the terminal bronchioles. Those larger than 6 μm fail to reach the alveolar ducts. The optimum particle size to reach and be deposited in the alveolar region seems to be 1 to 5 μm (70, 71, 74, 75). Submicrometer-size particles are exhaled, deposited, or both by random Brownian motion in distal regions. It should be noted that the particle size referred to in this context is not geometric diameter but aerodynamic diameter.

The theoretical aerodynamic diameter, d_{aer}, of indi-

Table 4 Effect of sugars on spray-dried peptides and proteins

Peptide/Protein	Sugar[a]	Activity (%)	Degradation (%)	FPF (%)	Moisture (%)	D[b] (μm)	Ref.
β-galactosidase	none[c]	42			4.5	3.9	45
	10% mannitol	81			1.5	4.3	
	10% sucrose	102			2.8	4.3	
	10% trehalose	109			4.0	4.0	
catalase	none	197[d]				5.8	69
	50% lactose	153				6.4	
	50% mannitol	107				4.4	
insulin	none	18[d]				2.9	69
	50% lactose	24				3.9	
	67% mannitol	18				5.0	
oxyhemoglobin	none[e]		50				46
	0.01M sucrose		40				
	0.10M sucrose		10				
	0.20M sucrose		2				
Albumin/DPPC (20/60)	20% sucrose			53		4.8[c]	70
	20% mannitol			11.3		10.4	
Albumin/DPPC (20/60)	20% lactose[b]			38		1.2	71
	20% trehalose			34		1.1	
	20% mannitol			11		1.5	
rhDNase	none			46		3.4	63
	50% mannitol			14		6.1	
	20% trehalose			29		2.9	
	40% trehalose			36		2.6	
	60% trehalose			20		3.2	
	40% sucrose			27		2.8	
anti-IgE MAb	none			27		3.3	63
	10% mannitol			25		3.8	
	20% mannitol			29		4.0	
	40% trehalose			31		3.3	
rhuMAbE25	none			27	2.23[f]		44
	10% mannitol			35	1.31		
	20% mannitol			28	0.86		
	30% mannitol			27	1.00		
	40% mannitol			8.5	1.11		

[a] Percentage stands for the sugar content in dry powder except for β-galactosidase and oxyhemoglobin.
[b] Geometric or aerodynamic diameter.
[c] Concentration in feed solution. β-Galactosidase concentration was 6%.
[d] Activity relative to that of raw material.
[e] Concentration in feed solution. Oxyhemoglobin concentration was 10%.
[f] Pseudo first-order degradation rate constant at 30℃.

vidual particles is calculated according to the following definition (76):

$$d_{aer}=(\rho/F)^{0.5}d \quad (1)$$

Where:
d is mass median particle diameter as measured by light microscopy,
ρ is particle density, and
F is the dynamic shape correction factor.

The F values for spheres and cubes are 1.00 and 1.08, respectively (76). The tap density could be an estimate of the particle density, ρ, although it remains an approximately 20% systemic underestimate of ρ (15).

The experimental mass median aerodynamic diameter (MMAD) of the particles is obtained as follows with an Andersen cascade impactor (70, 71). The cumulative mass of powder less than the stated size of each stage of the impactor is calculated and plotted on a log probability scale, as the percent of total mass recovered in the impactor against the effective cut-off diameter. The MMAD of the particles is defined from this graph as the particle size at which the line crosses the 50% mark. The geometric standard deviation (GSD) is calculated as $GSD=(X/Y)^{0.5}$, where X and Y are the particle sizes at which the line crosses the 84.13% mark and the 15.87% mark, respectively.

Fine particle fraction (FPF) is defined as the mass

fraction of particles smaller than a certain aerodynamic diameter (for instance, 5 μm). The twin impinger is often used to estimate FPF values and is valuable for the routine quality assessment of aerosols during product development, stability testing, and for quality assurance and comparison of products (77). The particles captured in stage 2 are considered to be the FPF. The aerodynamic cutoff diameter between stages 1 and 2 of the twin impinger with an air stream of 60 l/h is 6.4 μm (78). The cutoff size of the impinger stage can easily be changed because it is inversely proportional to the square root of the air flow (79).

3.5 Hollow porous particles

Equation 1 predicts that a large and light particle may have the same aerodynamic diameter as a small and heavy particle. Edwards et al. showed in 1997 that the FPF for large porous particles was much higher than that for small nonporous particles, even though the aerodynamic diameters were nearly identical. Large porous particles also increased systemic bioavailability of insulin in rats (80). The advantage of large porous particles can be attributed to the smaller surface-to-volume ratio for the porous particles, which results in less particle aggregation.

Another benefit to the use of large particles is that they can avoid phagocytic clearance from the lungs. Radiolabeled polystyrene microspheres of 3, 9, and 15 μm in diameter administered into rat lungs were cleared with biphasic patterns. The half lives for the late phases of the 3 and 9 μm microspheres were 69 and 580 days, respectively, while that for the 15 μm microsphere was found not measurable during the 106-day study (81).

Spray drying produced hollow porous powders consisting of albumin, DPPC, and lactose. The solution feed rate and pressure of the compressed air had little impact on powder properties. Increasing the inlet temperature tended to make the powders heavier (15). The lower the bulk powder tap density, the higher the FPF. Removing albumin or DPPC from the composition led to denser and smaller particles. Replacing lactose with mannitol resulted in a poor FPF value. The FPF was maximized at albumin/DPPC/lactose=10/60/30 (71).

Large porous particles composed of albuterol sulfate (4%), a short-acting bronchodilator, and human serum albumin (18%), lactose (18%), and DPPC (60%) were prepared by spray drying. Inhalation of the albuterol particles obtained produced a significant inhibition of carbachol-induced bronchoconstriction for at least 16 hr in guinea pigs, while small nonporous albuterol particles were effective for up to 5 hr. It is possible that the long-lasting action observed in the large porous particles was at least partly due to the slower clearance by phagocytosis (82).

PulmoSphere™ particles are hollow porous particles with geometric diameters between 3 and 5 μm and tap densities of about 0.1 g/cm^3 prepared by a spray-drying method. A submicrometer fluorocarbon-in-water emulsion stabilized by a monolayer of phospholipid at the fluorocarbon-water interface is combined with a second aqueous phase containing the drug and any wall-forming excipients desired. Spray drying the aqueous dispersion produces hollow porous powders. The fluorocarbon serves as a blowing agent or inflation agent during the spray-drying step (83). Deposition of PulmoSphere™ particles of albuterol sulfate in the human respiratory tract delivered by pMDI was double the deposition of a conventional micronized drug pMDI formulation (84).

The spray freeze-drying technique has been proposed as a method to produce light and porous protein particles (63). A protein solution with excipient is sprayed in liquid N_2. After spraying, the whole content of the liquid N_2 was lyophilized to harvest powders. This technique produced powders of DNase and anti-IgE MAb with a high FPF up to 70% (63).

When the GAS process with supercritical carbon dioxide is used to produce protein powders, the operating temperature or rate of CO_2 addition had a minor effect on the morphology and size of the powders. Large porous particles were obtained at a high concentration of proteins, while agglomeration of the precipitated particles occurs at dilute concentrations (52). ASES processing at higher temperatures with higher concentrations of the protein reduced the agglomeration of primary particles due to a higher degree of supersaturation and a higher nucleation rate (85).

4. Conclusion

Although pulmonary absorption of peptides and proteins is much better than that through the gastrointestinal tract, the bioavailability of inhaled peptides and proteins is still below that administered intravenously or subcutaneously. The success of inhalation therapy with dry powders is determined by the biological aspects of active ingredients, the physicochemical aspects of formulation, and inhaler performance. The bioavailability of inhaled peptides and proteins will be improved by considering these

factors. The application of hollow porous particles has opened a new avenue for inhalation therapy in humans. We now expect groundbreaking success in increasing permeability, reducing metabolic degradation, maximizing the FPF, and in other areas that will make inhalation therapy with peptides and proteins more effective and economical.

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