

EXUBERA®: Pharmaceutical Development of a Novel Product for Pulmonary Delivery of Insulin

STEVEN WHITE, B.S.,^{1,*} DAVID B. BENNETT, Ph.D.,¹ SCOT CHEU, B.S.,¹
PATRICK W. CONLEY, B.S.,¹ DONALD B. GUZEK, M.A.,¹ STEVEN GRAY, M.S.,¹
JOHN HOWARD, M.S.,¹ RICHARD MALCOLMSON, Ph.D.,¹ JOANN M. PARKER, M.S.,¹
PHIL ROBERTS, M.S.,¹ NEGAR SADRZADEH, Ph.D.,¹
JACQUELINE D. SCHUMACHER, B.A.,¹ SANGITA SESHADRI, Ph.D.,¹
GREGORY W. SLUGGETT, Ph.D.,¹ CYNTHIA L. STEVENSON, Ph.D.,¹
and NANCY J. HARPER, Ph.D.²

ABSTRACT

Development of a product for pulmonary delivery of insulin presented significant technology challenges for this first-in-class pharmaceutical product. These included developing (a) a chemically stabilized protein, (b) a dry powder formulation exhibiting required aerosol physical characteristics, (c) low-dose powder filling and packaging technology, and (d) a mechanical device for powder dispersal and reliable dosing to the patient. The insulin drug is formulated using a novel excipient combination to create a powder with a high glass transition temperature (T_g). The high T_g minimizes insulin mobility (thus reactivity), enabling ambient storage conditions. The formulation composition results in minimal hygroscopicity, where customized packaging produced product ruggedness to humidity. The formulated insulin powder is manufactured by spray-drying. This technology was further engineered to produce the desired reproducible powder characteristics with tight control over particle size and moisture content. A solution step prior to drying assures homogeneity and minimizes dependence on the physical form of the components. Novel low-dose filling and packaging technology reproducibly meters milligram quantities of microfine powder to meet stringent quality requirements for dose control. The technology for accurate, uniform, high-throughput metering of drug powders allows for automation and is scaleable for commercial operations. Finally, the mechanical device design provides powder deagglomeration and dispersion processes in a reusable dry powder inhaler with unique characteristics. The device was designed to rely on patient-generated compressed air as the energy source. A sonic discharge of air through the novel TransJector reproducibly extracts, deagglomerates, and disperses the inhalation powder. A clear holding (spacer-type) chamber allows for patient feedback via dose visualization, and separates powder dispersal from the inspiratory effort. The EXUBERA® [Pfizer (New York, NY) and sanofi-aventis (Paris, France)] product provides insulin into the bloodstream with similar reproducibly and effectiveness as subcutaneous injections.

¹Nektar Therapeutics, San Carlos, California.

²Pfizer Global R&D, Groton, Connecticut.

*Present address: Epic Therapeutics, Inc., Norwood, Massachusetts.

INTRODUCTION

INSULIN IS THE PRIMARY THERAPY for type 1 diabetes mellitus, and is also an integral component for the treatment of type 2 diabetes. Insulin was once considered the therapy of last resort for patients with type 2 diabetes, but physicians are now utilizing it earlier in the treatment of the disease state. Insulin therapy has a number of advantages by providing patients with insulin who have none (type 1), or supplementing the body's natural hormone (type 2), which is deficient or used inefficiently. Furthermore, insulin is an antihyperglycemic agent with a proven long-term safety profile.¹

Many patients with type 2 diabetes have poor glucose control, where their glycosylated hemoglobin (HbA_{1c}) level is greater than 7%.² Patients on insulin, or insulin with an oral therapy regimen, can restore normal levels of glycemia.³ Currently, the use of comparable intensive insulin regimens is limited by their lack of flexibility, needle phobia, and patient motivation. Specifically, anxiety associated with insulin needles may affect patient compliance.⁴ Improvements in insulin delivery provide solutions to insulin therapy and its effectiveness in clinical practice.⁵

The pulmonary route of administration was targeted to increase the compliance and life quality for patients with diabetes, who may otherwise endure multiple daily subcutaneous injections of insulin. Increased compliance is important for decreasing the severe and debilitating long-term effects of poorly controlled diabetes.⁶ The advantages of pulmonary delivery of macromolecules include a well-established delivery route, large alveolar surface for absorption ($\sim 100 \text{ m}^2$), rapid absorption across alveolar membranes, with no absorption enhancers, and ultimately delivery as aerosols.⁷⁻¹⁰

For aerosol drug delivery, it is generally recognized that absorption into the systemic circulation is higher where the layers between air and capillary blood are thin: the alveoli, alveolar ducts, and respiratory bronchioles collectively termed the deep lung.¹¹ Pulmonary delivery of insulin is designed to optimize alveolar deposition of the drug while minimizing deposition in the mouth, throat, and upper airways. This is accomplished through the

use of a holding chamber that encourages a slow deep inspiration of an aerosol cloud that contains drug particles with the appropriate size for alveolar depositions (mean aerodynamic particle diameter of $\sim 3 \mu\text{m}$).¹²

In order to provide a pharmaceutical alternative to rapid-acting subcutaneous insulin injections, dose flexibility was required to accommodate the varying insulin needs of patients with diabetes. To enable dosing flexibility, a pre-metered unit-dose concept was developed, utilizing two product strengths (1 mg and 3 mg). Combinations of these product strengths provide flexibility in dose titration.

Development of a product for pulmonary delivery of insulin presented significant technology challenges for this first-in-class pharmaceutical product. The efforts were focused on formulation development, powder production, powder filling technology, and device development, where specific advances were made developing (a) insulin stabilization in a dry powder to provide room temperature storage, (b) a dry powder formulation exhibiting the required physical characteristics for pulmonary delivery and manufacturing processes providing particles suitable for aerosolization, (c) low-dose powder filling and packaging technology for delivery of very small doses of powder, and (d) a rugged, reusable mechanical device for powder dispersal and reliable dosing to the patient. These advances led to the development of the EXUBERA[®] [Pfizer (New York, NY) and sanofi-aventis (Paris, France)] system for pulmonary delivery of insulin.¹³⁻¹⁵

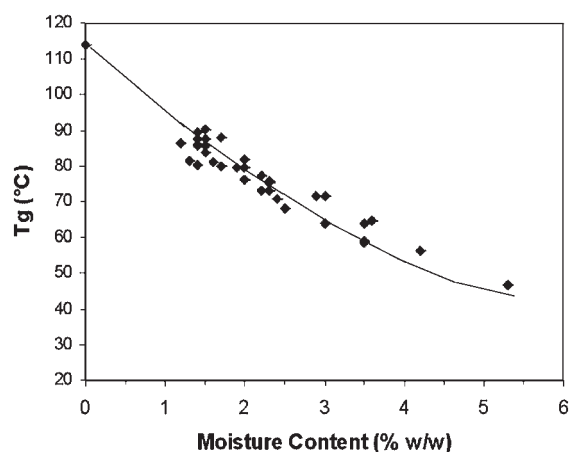


FIG. 1. T_g at 25°C as a function of moisture content.

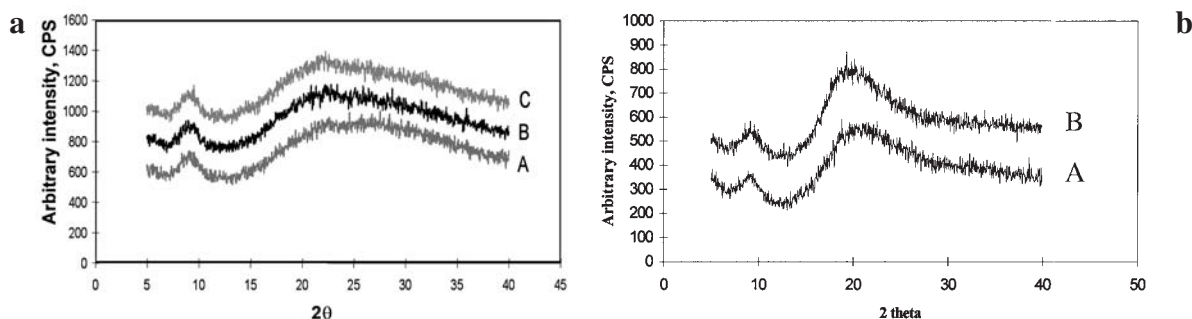


FIG. 2. X-ray powder diffraction patterns of insulin spray-dried powder for inhalation (a) when exposed to relative humidity between 55% and 88% after 22.5 (curve A), 26 (curve B), and 28 h (curve C) at 25°C and (b) before (curve A) and after (curve B) exposure to 150°C at ambient relative humidity for 15 min. CPS, cycles per second.

FORMULATION DEVELOPMENT

Protein stabilization

A dry powder product was selected, and had significant advantages over aqueous dosage forms. These advantages included stability and storage, high mass per inhalation, low microbial growth, and the avoidance of preservatives. Compared with aqueous liquid formulations and propellant-based systems, dry powder formulations offer better opportunities to optimize the physical and chemical stability of insulin in order to allow room temperature storage stability.¹⁶

Formulation development utilized excipients producing stable rugged powder. Insulin powder for inhalation is manufactured as homogeneous powder from an aqueous solution. This provides a unique advantage over conventional dry powder pulmonary products, which are typically non-homogeneous blends.

The amorphous insulin powder contains 60% insulin in a buffered, sugar-based matrix.

Amorphous pharmaceutical solids have been shown to be markedly more soluble than their crystalline counterparts.¹⁷ Amorphous solids, termed glasses, do not melt but exhibit a glass transition temperature (T_g) that depends on the formation conditions, processing history, chemical composition, and the time scale of observation. The T_g is a temperature at which a transition begins from a mechanical solid phase to a viscoelastic, supercooled rubber phase.

When amorphous solids are stored well below their T_g , molecular motions and chemical reactivity are slowed. Molecular mobility becomes insignificant when the storage temperature is significantly below the T_g .¹⁸ Labile proteins exhibit kinetic stability and acceptable shelf life when stabilized in an amorphous glass stored below the T_g .¹⁹ The T_g for insulin powder was assessed, by modulated differen-

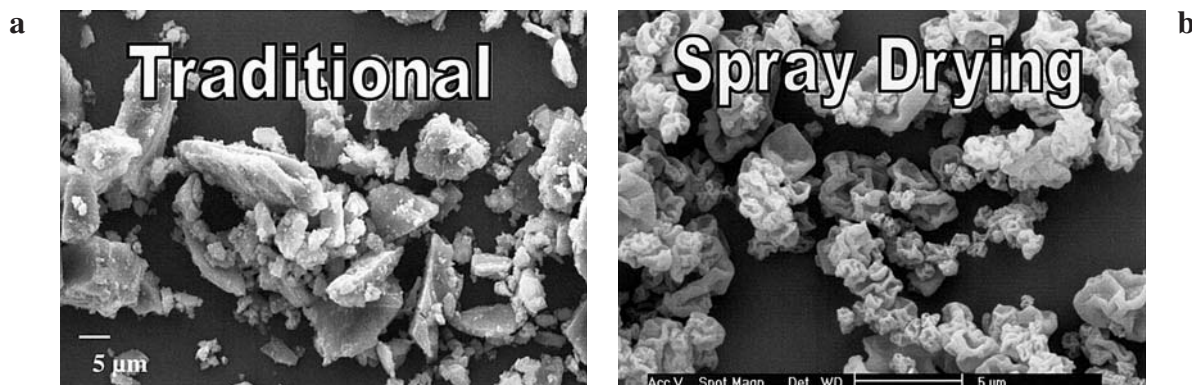


FIG. 3. Scanning electron micrographs of (a) traditional jet-milled powder and (b) spray-dried insulin powder for inhalation.

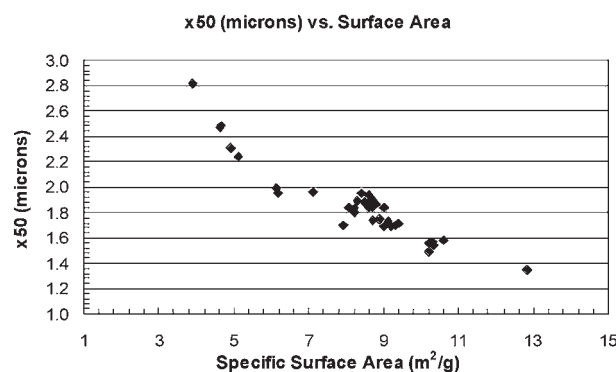


FIG. 4. Median particle size versus specific surface area for bulk insulin powder, where $\times 50$ (or 50% cumulative undersize diameter) was found to be most predictive.

tial scanning calorimetry, as the extrapolated onset temperature of the C_p transitions. The T_g for spray-dried powder is between 78°C and 95°C at target moisture content, and decreases with residual moisture (Fig. 1).²⁰ Insulin powder exhibits a single T_g , indicative of a single amorphous phase. No evidence of component crystallization or melting is detected in the heat flow signal from 0°C to 150°C. Thus, even with exposure to temperature conditions favorable for crystallization ($T \gg T_g$) insulin powder exhibits no signs of crystallization.

This is further demonstrated by the absence of sharp diffraction peaks, by x-ray diffraction, revealing an absence of crystallinity, where the particle morphology of the insulin powder for inhalation does not change with moisture or temperature (Fig. 2).

The spray-dried insulin formulation is a strong glass, with a temperature range of 20°C between the extrapolated onset and end point of the glass transition.²¹ A strong glass exhibits mobility that obeys an Arrhenius relationship over a wide temperature range, and has a weaker temperature dependence relative to fragile glasses of molecular mobility above the onset of the glass transition. In contrast, fragile glasses deviate from Arrhenius behavior and exhibit rapid increases in molecular mobility above the T_g .^{22,23}

Studies demonstrated that moisture content in the insulin powder impact the T_g and insulin stability. Water acts as a plasticizer and lowers the T_g of amorphous solids.²⁴ Increasing the moisture content of the formulation decreases the T_g and increases the rate of insulin degra-

dation. When the T_g remains above pharmaceutical room temperature storage conditions, then adequate shelf stability is obtained (Fig. 1). In order to maintain an appropriate difference between T_g and storage temperatures, a moisture content of less than 5% is essential to ensure chemical stability. A consistent T_g of $\sim 80^\circ\text{C}$ was observed for spray-dried powders with water content of approximately 2% (wt/wt). Moisture sorption curves at 25°C also show no signs of water-induced crystallization.²⁰

POWDER PRODUCTION

Spray-drying and physical characterization

The application of spray-drying technology to produce particles for pulmonary use requires process conditions outside those normally utilized for pharmaceutical products. Generally, spray-drying is used to produce particles much larger than that required for aerosol delivery: food applications range from 50 to 100 μm , and typical pharmaceutical applications range from 10 to 50 μm .

Spray-drying was selected over other particle processing technologies for its ability to produce homogeneous particles within the desired small particle size range ($<5 \mu\text{m}$), low moisture content, and high drug purity. Furthermore, spray-drying is a continuous production method, scalable for commercial production volumes. Solution spray-drying ensures compositional homogeneity of the insulin powder, since the insulin and the excipients are dissolved prior to spray-drying. This also eliminates the need for control of the specific particle morphology of the ingoing components.

The spray-drying process, though only taking milliseconds to produce a given particle, is

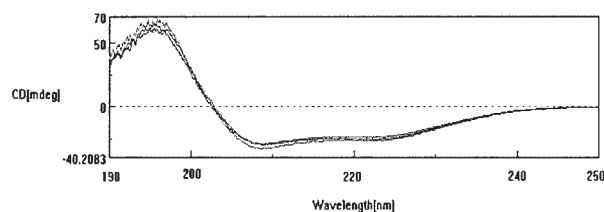


FIG. 5. Circular dichroism (CD) spectra of insulin drug substance (red), formulated solution prior to spray-drying (blue), and bulk insulin powder for inhalation (green).

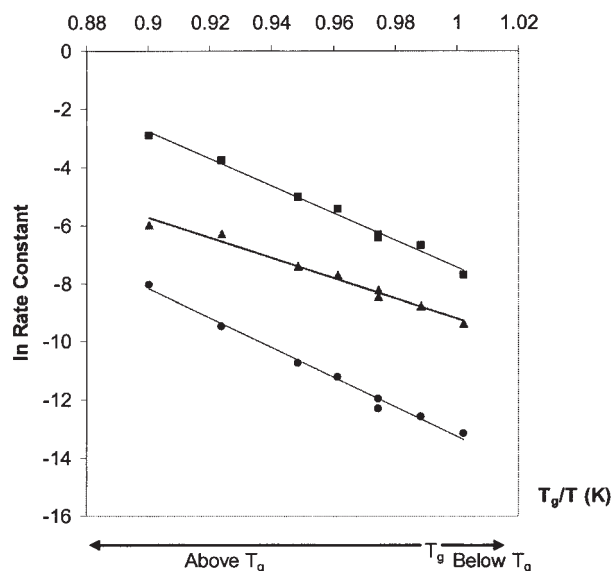


FIG. 6. Insulin degradation Arrhenius plot depicting insulin degradation products: deamidated insulin (triangles), insulin-related substances (squares), and high-molecular-weight protein (circles).

composed of several stages. Insulin solution is first atomized to form droplets. As these droplets cool, moisture is rapidly removed from the system, creating a particle skin, and shrinkage to form rugose, raisin-shaped particles [5% (wt/vol) solids].^{25,26} These particles are collected with a cyclone and further dried to remove residual moisture.²⁷

The typical size reduction technique for aerosol applications is jet milling, which does not result in a uniform particle size distribution and shape by scanning electron microscopy (Fig. 3a). In contrast, spray-drying of the formulated insulin solution results in uniform particle morphology with a raisin-like appearance (Fig. 3b). The resulting insulin powder for inhalation has also proven to be stable to increased temperature and humidity (75% relative humidity, 25°C for 36 h).

The EXUBERA spray-dried bulk insulin powder was also characterized for true density ($\sim 1.48 \text{ g/cm}^3$ by helium pycnometry) and bulk density ($\sim 0.2 \text{ g/cm}^3$).²² These powder characteristics have been shown to be typical for fine powders of a size suitable for inhalation applications. Process robustness was confirmed by correlating specific surface area (nitrogen adsorption/BET) and primary particle

size (Sympatec) (Fig. 4).²² At standard processing conditions, powder-specific surface area is consistent at approximately $9 \text{ m}^2/\text{g}$. Specific surface area varies ($\sim 4\text{--}13 \text{ m}^2/\text{g}$) as a function of processing conditions (varied outside standard conditions). Specific surface area shows a strong correlation with particle size distribution.²² Therefore, control of surface area is achieved by controlling primary particle size.

Chemical and physical characteristics

The spray-drying process does not affect the secondary structure of insulin, assuring biological activity. Circular dichroism and Fourier transform infrared spectroscopy of drug substance and drug product are essentially unchanged after spray-drying and temperature-accelerated storage. Circular dichroism spectra obtained before and after spray drying indicate similar α -helical conformation (Fig. 5).²⁸ By Fourier transform infrared spectroscopy, the structure of insulin is dominated by α -helix, as indicated by the prominent band at approximately 1658 cm^{-1} . Smaller intensity bands at approximately 1682 cm^{-1} and 1648 cm^{-1} are

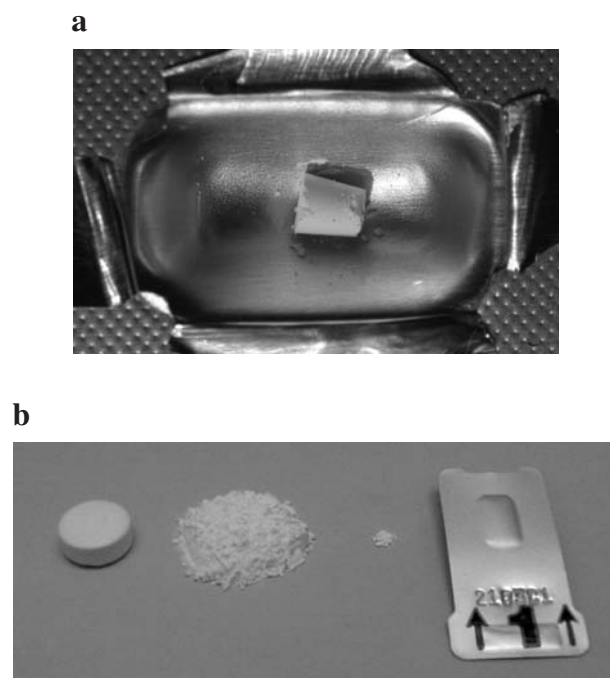


FIG. 7. Metered dose powder filling, with (a) insulin powder in a unit-dose blister and (b) relative proportion of insulin blister to standard pharmaceutical tablets.

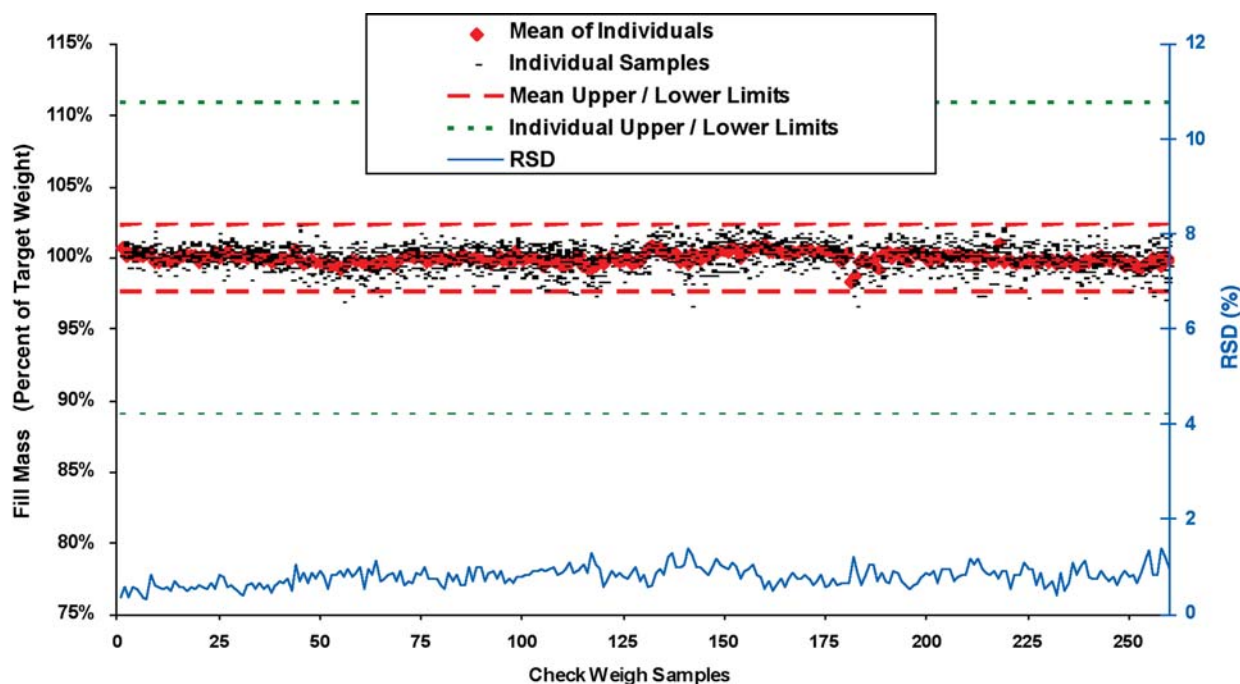


FIG. 8. Fill weight process control chart. RSD, relative standard deviation.

attributed to turn and unordered structures, respectively.²⁹ Similarly, two bands at 1693 cm^{-1} and 1613 cm^{-1} are attributed to β -sheet structures.

The insulin powder was further characterized by reversed-phase high-performance liquid chromatography and size exclusion chromatography, for its chemical stability under temperature-accelerated conditions.^{30,31} The rate of insulin degradation was characterized and was found to fit to Arrhenius kinetics over a wide temperature range, encompassing the T_g (Fig. 6).³⁰ These data also confirm that spray-dried insulin is a strong glass.²¹

Powder filling and packaging

In order for the insulin unit dose to effectively interact with the delivery system, a unit-dose blister technology was selected. This involves a volumetric dose metering system to fill insulin powder reproducibly into blisters. Because of the low fill masses required to achieve the insulin dosage forms, novel filling technology was developed. Specific design challenges included low density, cohesive powders, fill weights $<5\text{ mg}$, high-throughput filling ($>1,500\text{ fills/min}$), and stringent requirements for accuracy, precision, and consis-

tency. Novel processes and equipment were designed to induce controllable insulin powder flow and volumetric metering as loose compacts into unit-dose blisters. The relative proportions of a standard pharmaceutical dosage form (tablet/capsule) are contrasted with the insulin unit dose (Fig. 7).

The powder filling dose metering system was integrated with packaging equipment that formed the aluminum foil blisters on-line and sealed the filled blisters. The packaging design is composed of a compact package to facilitate patient handling, provide compatibility at the device interface, and assure insulin powder stability.¹⁶ Achievement of these manufacturing goals (powder processing, filling and packaging) is demonstrated in tight fill weight control with $<2\%$ relative standard deviation (Fig. 8).

PULMONARY INHALER DEVELOPMENT

Inhaler design

Typical limitations of traditional inhalation devices include low efficiency, variable dosing, poor moisture barriers, low drug content per inhalation, inapplicability to macromolecules, and sensitivity to the breathing maneuver.¹⁶ In

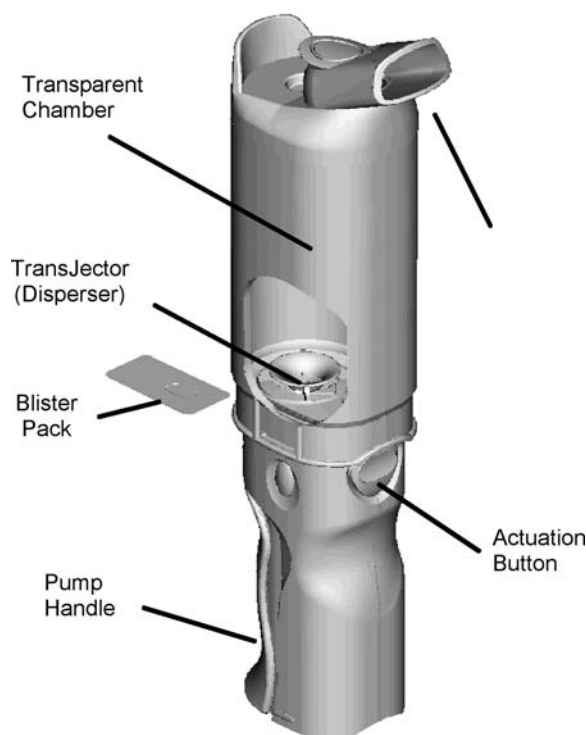


FIG. 9. Pulmonary delivery system.

comparison, the EXUBERA inhaler is a novel pulmonary delivery system developed by Nektar Therapeutics (San Carlos, CA) that solves many of these challenges.

The pulmonary delivery system is a reusable dry powder inhaler that has been designed to deliver insulin to the small airways and alveoli for systemic insulin absorption (Fig. 9).^{32,33} The inhaler was designed to provide reproducible powder extraction, deagglomeration, and dispersion, capable of aerosolizing relatively small amounts of cohesive powder (1–10 mg). It is solely mechanical, and requires only modest effort by the patient to operate. Unlike typical high-resistance inhaler systems, aerosolization of insulin powder is independent of patient inspiratory effort, providing opportunities for improved performance and product consistency.^{16,34}

The inhaler consists of three subsystems: base, TransJector, and chamber/mouthpiece. The inhaler performs four key functions: puncture of the insulin powder-containing blister after loading into the device, extraction of the powder from the blister, dispersal of the powder into the chamber, and facilitation of in-

halation delivery of the powder cloud to the patient. Specifically, the base contains an air pump and valves that generate, store, and release compressed air. The patient opens and closes the pump handle once before actuation to draw in and compress ambient air. Upon actuation, an individual blister is punctured, and compressed air is released through a jet structure in the TransJector and then vented into the chamber. The sudden sonic discharge of this mass of air through the small jets in the TransJector results in extraction and dispersion of insulin powder (Fig. 10). The rapidly moving air

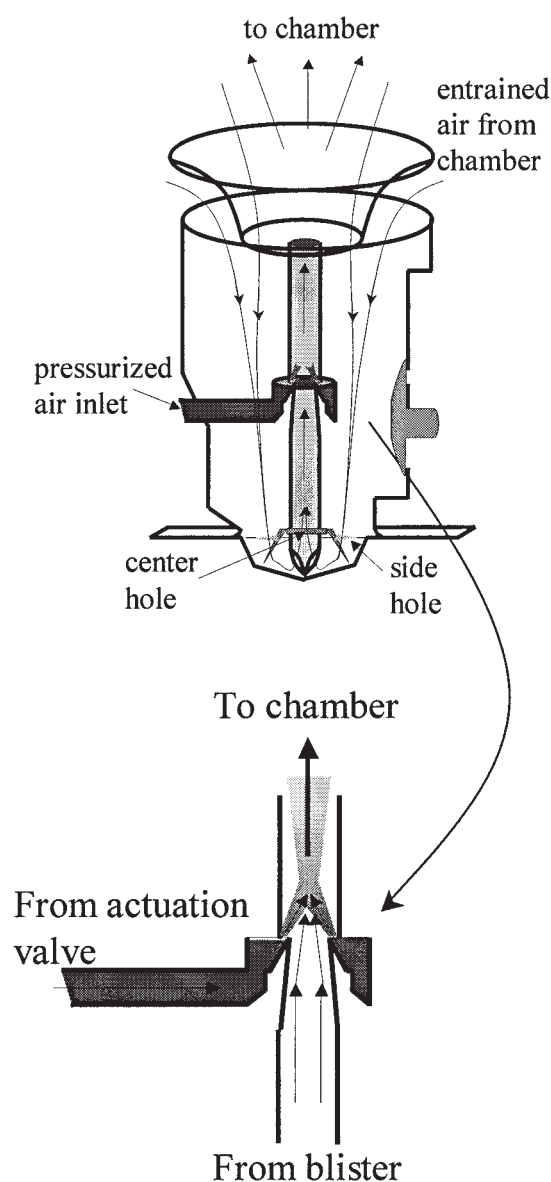


FIG. 10. TransJector airflow during actuation.

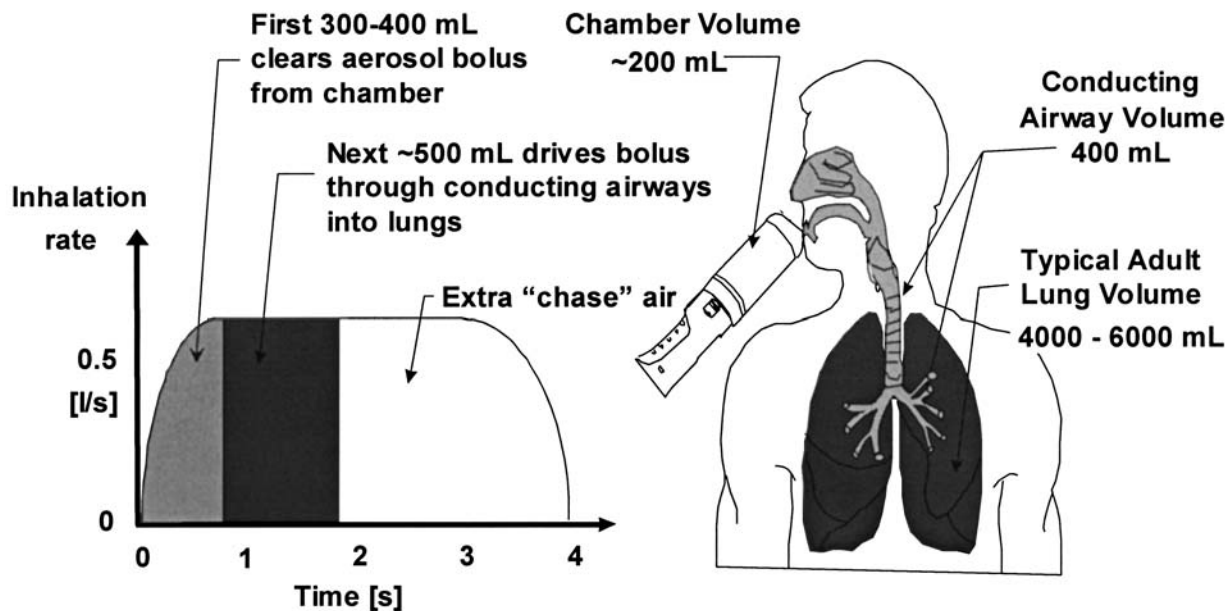


FIG. 11. Aerosol inhalation ex-pulmonary delivery system.

creates a vacuum in the TransJector and blister, causing the insulin powder to be drawn into the chamber and dispersed into a cloud. The energy generated is sufficient to disperse the powder into particles less than $5\ \mu\text{m}$ in aerodynamic diameter. After the aerosol cloud has formed in the chamber, the patient rotates the mouthpiece 180° into the open position and inhales the dose.

The design and function of the EXUBERA inhaler eliminate the need for exact coordination of inhaler actuation and inhalation by the patient. The emitted dose, particle size distribution, and fine particle dose of the aerosol are controlled primarily by characteristics of the insulin powder and the inhaler, and are relatively independent of variables that may be introduced by the patient. The inhaler was specifically designed such that patients can use the device with a simple, slow, deep inhalation.

Inhaler performance

Insulin powder is delivered into the deep lung (Fig. 11). Dosing occurs during emptying of the chamber volume ($\sim 200\ \text{mL}$), specifically within the first 300–400 mL of the inhalation breath. The following 500 mL of inhalation volume moves the dose

through the conducting airways into the deep lung.

Particle size distribution, via actuation through the inhaler, is predicted by properties of the spray-dried powder (Fig. 12). Spray-dried powder primary particle size distribution is measured by laser light scattering following high-energy pneumatic dispersion, and the corresponding aerosolized powder (ex-device) is measured by cascade impaction. This minor

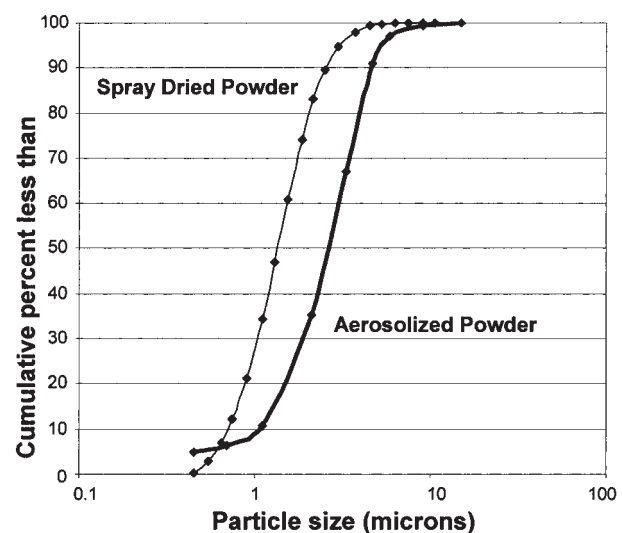


FIG. 12. Spray-dried primary particle to ex-device aerosol particle size distribution.

shift in particle size distribution is consistent with the powder being predominately dispersed to primary particles in the inhaler-aerosolized cloud.

Product performance was characterized by emitted dose, particle size distribution, and fine particle dose. Aerosol delivery across a range of flow rates (5–60 L/min) and flow volumes (400–1,400 mL) do not materially affect aerosol performance metrics for either the 1-mg or the 3-mg blisters.³⁴ Emitted dose and particle size distribution show negligible dependence on priming as well as device orientation for both 1 mg and 3 mg. All device orientations (0°, 24°, 90°, 180°, 270°) give mean emitted dose and fine particle dose values within 2–3% of the controls.

Furthermore, robustness performance (in vitro dose delivery and mechanical integrity) is demonstrated over a range of environmental conditions (temperature, humidity, and atmospheric pressure). Long-term in-use performance consistency (aerosol and mechanical) has been demonstrated through in vitro use-life simulations and through testing of inhalers retrieved from clinical trials following up to 2 years of usage. These studies have resulted in the demonstration of improved performance over standard dry powder inhalers.

CONCLUSIONS

Pulmonary delivery of insulin is not new. In 1925, Gänsslen³⁵ predicted the potential of insulin inhalation therapy when he studied its use with a nebulizer. Although blood glucose levels were reduced, the bioavailability, variability, and usability of the system made the application impractical. Only recently have the technical limitations associated with delivering insulin efficiently to the deep (alveolar) lung been fully realized and developed as a viable treatment option. The EXUBERA insulin powder for inhalation system required a novel technology solution and the integration of four key technologies to provide this first-in-class product. Other companies are also currently developing alternative formulations and delivery systems for the treatment of diabetes, includ-

ing Eli Lilly & Company (Indianapolis, IN) and Novo Nordisk (Bagsvaerd, Denmark).^{36–38} However, the development of EXUBERA provides a unique combination of insulin dry powder formulation and a delivery system, resulting in effective clinical treatment of diabetes.^{3,39–41}

ACKNOWLEDGMENTS

The authors would like to thank the EXUBERA® team members within Pfizer, Nektar, aano-fi-aventis, and Bespak for their inspiration and teamwork. The authors are also indebted to the creativeness and invention of John Patton, Adrian Smith, Bob Platz, and Linda Foster.

REFERENCES

1. Home PD, Boulton AJM, Jimenez J, Landgraf R, Osterbrink B, Christiansen JS: Issues relating to the early or earlier use of insulin in Type 2 diabetes. *Pract Diabetes Int* 2003;20:63–71.
2. American Diabetes Association: Test of glycemia in diabetes. *Diabetes Care* 2002;25(Suppl):S97–S99.
3. Rosenstock J; the EXUBERA® Phase III Study Group: Mealtime rapid-acting inhale insulin (EXUBERA®) improves glycemic control in patients with Type 2 diabetes failing combination oral agents: a 3-month, randomized, comparative trial [abstract]. *Diabetes* 2002;51:A132.
4. Zambanini A, Newson RB, Maisey M, Feher MD: Injection related anxiety in insulin-treated diabetes. *Diabetes Res Clin Pract* 1999;46:239–246.
5. Home PD: Intensive insulin therapy in clinical practice. *Diabetologia* 1997;40(Suppl):S83–S87.
6. Cefalu WT: Rationale for and strategies to achieve glycemic control. In: Leahy JL, Cefalu WT, eds. *Insulin Therapy*. New York: Marcel Dekker, 2002:1–11.
7. Patton JS: Mechanisms of macromolecule absorption by the lungs. *Adv Drug Deliv Rev* 1996;19:3–36.
8. Smith SJ, Bernstein JA: Therapeutic uses of lung aerosols. In: Hickey AJ, ed. *Inhalation Aerosols: Physical and Biological Basis for Therapy*. New York: Marcel Dekker, 1996:233–269.
9. Patton JS, Bukar J, Nagarajan S: Inhaled insulin. *Adv Drug Deliv Rev* 1999;35:235–247.
10. Patton JS, Bukar JG, Eldon MA: Clinical pharmacokinetics and pharmacodynamics of inhaled insulin. *Clin Pharmacokinet* 2004;43:781–801.
11. Patton JS, Platz RM: Routes of delivery: case studies.

- Pulmonary delivery of peptides and proteins for systemic action. *Adv Drug Deliv Rev* 1992;8:176–196.
12. Byron PB: Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. *J Pharm Sci* 1986;75:433–438.
 13. Gelfand RA, Schwartz SL, Horton M, Law CG, Pun EF: Pharmacological reproducibility of inhaled human insulin dosed pre-meal in patients with type 2 diabetes mellitus [abstract]. *Diabetes* 2002;51:A202.
 14. Quattrin T, Belanger A, Bohannon NJV, Schwartz SL: Efficacy and safety of inhaled insulin (EXUBERA) compared with subcutaneous insulin therapy in patients with Type 1 diabetes. *Diabetes Care* 2004;27:2622–2627.
 15. Hollander PA, Blonde L, Rowe R, Mehta AE, Milburn JL, Hershon KS, Chaisson JL, Levin SR: Efficacy and safety of inhaled insulin in patients with Type 2 diabetes: a 6-month, randomized, comparative trial. *Diabetes Care* 2004;27:2356–2362.
 16. Ashurst I, Malton A, Prime D, Sumby B: Latest advances in the development of dry powder inhalers. *Pharm Sci Technol Today* 2000;3:246–256.
 17. Hancock BC, Parks M: What is the true solubility advantage for amorphous pharmaceuticals? *Pharm Res* 2000;17:397–404.
 18. Hancock BC, Shamblin SL, Zografi G: Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. *Pharm Res* 1995;12:799–806.
 19. Franks F, Hatley RHM, Mathias SF: Materials science and the production of shelf-stable biologicals. *Pharm Technol Int* 1991;3:24–34.
 20. Lechuga-Ballesteros D, Kuo MC, Liang Y, Malcolmson R, Miller DP, Sekulic S, Seshadri S, Stults CLM, Tan T, Joshi V, Zhen C, Williams L, Bennett DB: The physical stability of insulin powder for inhalation. *AAPS J* 2004;6:R6137.
 21. Kajiwarra K, Franks F, Echlin P, Greer AL: Structural and dynamic properties of crystalline and amorphous phases in raffinose-water mixtures. *Pharm Res* 1999;16:1441–1448.
 22. Lechuga-Ballesteros D, Miller DP, Zhang J: Residual water in amorphous solids: measurements and effects on stability. In: Levine H, ed. *Progression Amorphous Food and Pharmaceutical Systems*. London: The Royal Society of Chemistry, 2002:275–316.
 23. Duddu SP, Zhang G, Dal Monte PR: The relationship between protein aggregation and molecular mobility below the glass transition temperature of lyophilized formulations containing a monoclonal antibody. *Pharm Res* 1997;14:596–600.
 24. Hancock BC, Zografi G: The relationship between glass transition temperature and the water content of amorphous pharmaceutical solids. *Pharm Res* 1994;11:471–477.
 25. Malcolmson R, De Moor CP, Miller DP, Liang Y, Zhen C, Kim Y, Merchant J, Bennett D, Mazumder MK, Saracovan I, Sekulic S: Physical properties of bulk insulin powder for inhalation. *AAPS J* 2004;6:R6169.
 26. Stahl K, Claesson M, Lilliehorn P, Linden H, Backstrom K: The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. *Int J Pharm* 2002;233:227–237.
 27. Vehring R, Tep V, Foss WR: Novel experimental method indicates proteins and peptides are protected from high gas temperatures during spray drying. *AAPS PharmSci* 2003;5:M1247.
 28. Pittman I, Tager HS: A spectroscopic investigation of the conformational dynamics of insulin in solution. *Biochemistry* 1995;34:10578–10590.
 29. Dong A, Caughey WS: Infrared methods for study of hemoglobin reactions and structures. *Methods Enzymol* 1994;232:139–175.
 30. Sadrzadeh N, Wang ML, Yu M, Antonino L, Stevenson CL, Bennett DB, Kelly ME: The chemical stability of spray dried insulin powder for inhalation. *AAPS J* 2004;6:M1261.
 31. Strickley RG, Anderson BD: Solid state stability of human insulin. II. Effect of water on reactive intermediate partitioning in lyophiles from pH 2–5 solutions: stabilization against covalent dimer formation. *J Pharm Sci* 1997;86:645–653.
 32. Schuler C, Mao Z, Cameron J: Nektar's dry-powder durable pulmonary drug delivery system: principles of operation. In: *Proceedings of the 31st International Symposium on Controlled Release of Bioactive Materials*. Honolulu, HI: Controlled Release Society, 2004.
 33. Bakshi A, Paboojian S, Rasmussen D, Tuttle D, Snyder H, Clark A, Smith A, Schuler C: Inhale's dry-powder pulmonary drug delivery system: challenges to current modeling of gas-solid flows. Presented at the 3rd ASME/JSME Fluids Engineering Conference. San Francisco: ASME Technical Publishing, 1999:1–14.
 34. Gray S, Parker J, deGroot J, Ozawa L, Chwa T, Harper N: Performance robustness characteristics of insulin powder for inhalation using a pneumatic inhalation device. In: *Drug Delivery to the Lungs 15*. London: The Aerosol Society, 2004:202–205.
 35. Gänsslen G: Über Inhalation von Insulin. *Klin Wochenschr* 1925;4:71.
 36. Henry RR, Mudaliar SR, Howland WC 3rd, Chu N, Kim D, An B, Reinhardt RR: Inhaled insulin using the AERx[®] insulin diabetes management system in healthy and asthmatic subjects. *Diabetes Care* 2003;26:764–769.
 37. Fishman RS, Guinta D, Chambers F, Quintana R, Shapiro DA: Insulin administration via the AeroDose[™] inhaler: comparison to subcutaneously injected insulin [abstract]. *Diabetes* 2000;49:A9.
 38. Rave K, Muchmore D, Gonzales C, Chien J, Ernest CS: Inhaled insulin with an Improves Spiros[®] dry powder inhaler: dose response and time-action profiles [abstract]. *Diabetologia* 2001;44:A211.
 39. Skyler JS; the EXUBERA[®] Phase III Study Group: Efficacy and safety of inhaled insulin (EXUBERA[®]) compared to subcutaneous insulin therapy in an intensive insulin regimen in patients with Type 1 dia-

- betes: results of a 6-month, randomized, comparative trial [abstract]. *Diabetes* 2002;51:A134.
40. Gelfand RA, Schwartz S, Horton M, Gordon LC, Pun EF: Pharmacological reproducibility of inhaled human insulin pre-meal dosing in patients with Type 2 diabetes mellitus (NIDDM) [abstract]. *Diabetes* 1998;47:0388.
41. Balanger A; the EXUBERA® Phase III Study Group: Efficacy and safety of inhaled insulin (EXUBERA®) compared to subcutaneous insulin therapy in an intensive insulin regimen in patients with Type 2 diabetes: results of a 6-month, randomized, comparative trial [abstract]. *Diabetologia* 2002;45:A260.

Address reprint requests to:
Nancy J. Harper, Ph.D.
Pfizer Global R&D
Groton, CT 06340

E-mail: Nancy.J.Harper@pfizer.com