AEROSOLIZABLE PARTICLES RESISTANT TO HYGROSCOPIC GROWTH

Field of the Invention

The present invention is related to the improved delivery of a dry powder active agent formulation to the deep lung. More particularly, the invention is directed to aerosolizable dry powder particles, which, upon inhalation, are resistant to hygroscopic growth. This feature of the powder (i.e., hygroscopic growth resistance) permits a greater proportion of the inhaled particles to reach the deep lung, thereby increasing the bioavailability of an active agent that is delivered to the lung.

Background of the Invention

Pulmonary delivery of active agents has been shown to be an effective route of administration for both local and systemic drug applications. Pulmonary active agent formulations are designed to be delivered via inhalation by the patient of a drug dispersion so that the active agent within the dispersion can reach the lung. It has been found that certain drugs delivered to the lung are readily absorbed through the alveolar region directly into the blood circulation. However, the percentage of inhaled drug that actually reaches the deep lung is quite small. For pulmonary delivery, drug losses average about 30% to the device, and about 35% to the oropharanx (upper airways). Out of the remaining 35%, about 20% drug is lost to the conducting airways, while about 15% is absorbed in the alveolar region. As pointed out by Gonda *et al, Critical Reviews in Therapeutic Drug Carrier Systems*, Volume 6, Issue 4 (1990) pages 273-313, absorption of drug in the distal airways and alveoli is expected to be faster than in the upper airways since the diffusion barriers are thinner and the surface area is greater in those regions. However, since only a small fraction of inhaled drug actually reaches the alveolar surface, new approaches are needed for increasing the amount of drug that ultimately reaches the systemic circulation.

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In one approach to solving this problem. Backstrom *et al*, U.S. Patent No. 5,506,203 describes the use of permeation enhancers to increase absorption through the layer of epithelial cells in the lower respiratory tract, thereby ultimately increasing the amount of drug reaching the systemic circulation. The inhaled compounds of Backstrom are delivered in the form of particles with a diameter of less than 10 microns. Permeation enhancers employed include surfactants, salts of fatty acids, bile salts and their derivatives, and others. Wong *et al*, U.S. Patent No. 5,451,569 similarly describes the use of lung surfactant to enhance the pulmonary absorption of proteins and peptides.

In an effort to eliminate the need for permeation enhancers, International Publication WO 96/32149, assigned to Inhale Therapeutic Systems, describes the pulmonary delivery of aerosolized medicaments that are in dry powder form and are dispersible. Such medicaments are readily absorbed in the lungs without the need to employ permeation enhancers. Similar efforts to increase the bioavailability of inhaled drugs are described in International Publication WO 97/44013, assigned to MIT and Penn State. In this publication, aerodynamically light particles (having densities less than 0.4 grams/cm^3 with a large mean diameter greater than 5 microns) are used for enhanced delivery of a therapeutic or diagnostic agent to the alveolar region of the lung. To further enhance drug bioavailability, International Publication WO 98//31346, also assigned to MIT and Penn State, discloses the incorporation of surfactant into the aerodynamically light particles for promoting absorption of the agent and for increasing its bioavailability.

Besides the problem of low absorption of pulmonarily delivered active agents through the epithelial cells in the lower respiratory tract, another factor contributing to the small quantities of inhaled drug reaching the deep lung is hygroscopic growth. Due to their water-soluble nature, most aerosols bring about the increased deposition of particles in the upper respiratory tract as a result of hygroscopic growth (Hickey, *et al, Journal of Pharmaceutical Sciences*, Volume 79, Number 11, pages 1009-1014). To investigate the growth rate of powders in humid environments, powders of disodium fluorescein coated with a fatty acid were prepared by an adsorption coacervation technique. The coated powders possessed MMAD's between about 4

and 7 microns and showed a reduced growth rate when compared to uncoated powders.

In spite of many of the above approaches, the percentage of drug generally reaching the alveolar surface of the lung upon inhalation is still quite low. Thus, new and improved efforts are needed for increasing the amount of inhaled drug deposited in the deep lung, to thereby increase the bioavailability of inhaled active agents.

Summary of the Invention

It is not only the size and density of the particles that are important parameters for increased bioavailability of drugs delivered to the alveolar region of the lung, but also their ability to absorb water as they travel through the lung to the alveoli. We have discovered that merely coating a particle is not sufficient to minimize absorption of water in the lung, rather the entire particle must contain hygroscopic growth inhibiting properties in order to maintain an appropriate particle size distribution in the aerosol as it travels through the lung, to enable its passage, without prior deposition in the upper lung regions, to the alveolar surface.

Accordingly, in one aspect, the invention is directed to particles for delivery of an active agent to the alveoli of a human patient. The particles comprise the active agent and at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins. The hygroscopic growth inhibitor is incorporated within the particles and the particles maintain an aerosol particle size distribution below 3 microns MMAD when delivered to the alveoli.

In another aspect, the invention is directed to particles containing an active agent and a hygroscopic growth inhibitor, where the particles are highly dispersible, and exhibit a drop in emitted dose under simulated lung conditions of no more than about 25%.

According to yet another aspect, the invention is directed to particles having low moisture absorptivities. The particles contain an active agent and at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin,

hydroxyethylstarch, dextranomer, and maltodextrins incorporated within the particles, and are further characterized by a sorption index of less than about 6.5.

In another aspect, the present invention is directed to a method for preparing particles for delivering an active agent to the alveoli of a human patient. The method comprises preparing a mixture of at least about 40% by weight (solids) of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins, an active agent and a solvent. The mixture is then spray dried to obtain homogeneous particles of the hygroscopic growth inhibitor and the active agent. The particle size distribution of the resulting particles remains less than 3 microns MMAD when delivered via inhalation to the deep lung. Alternatively, the resulting particles are characterized by exhibiting a drop in emitted dose of no more than 25% under simulated lung conditions. In yet another alternative, the spray-dried particles are characterized by a moisture sorption index of less than about 6.5.

In yet another aspect, the invention is directed to a method for delivery of an active agent to the lungs of a human patient, where aerosolized particles having the above described features are administered by inhalation to a human patient.

Another aspect of the invention is directed to a method for increasing the quantity of an inhaled active agent deposited in the deep lung. The method involves incorporating into active agent-containing dry powder particles for inhalation, at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins, such that, upon aerosolization and inhalation of the particles, at least 20% of the nominal dose is deposited in the deep lung.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples.

Brief Description of the Figures

Fig. 1 shows moisture sorption profiles of various spray-dried powder formulations, with moisture uptake (% by weight) on the vertical axis and % relative humidity on the horizontal axis. (*Circles*: 20% insulin, 59% sodium citrate, 18% mannitol, 2.6 glycine; *Squares*: 100% dextran (10 K); *Diamonds*: 100% hydroxypropylmethylcellulose; *X*: 100% hydroxypropyl-β-cyclodextrin, and +; 100% low molecular weight hydroxyethylstarch);

Fig. 2 shows moisture sorption profiles for 3 different spray-dried powder formulations. (*Circles*: 20% insulin, 59% sodium citrate, 18% mannitol, 2.6 glycine; *Squares*: 20% insulin, 2.6% glycine, 40% hydroxyethylstarch, 18% mannitol, 19% sodium citrate; and *Diamonds*: 100% hydroxyethylstarch). The addition of one or more HGIs to a particular formulation reduces its moisture sorption.

Fig. 3 shows the TAM (thermal activity monitor) results for various insulin dry powder formulations, illustrating the efficiency of two exemplary hygroscopic growth inhibiting agents in significantly reducing the hydration properties of these powders;

Fig. 4 is a moisture sorption plot for three spray-dried formulations, illustrating the effectiveness of hygroscopic growth inhibiting agent-containing formulations in decreasing both the rate and overall extent of water uptake. (*Circles*: 20% insulin, 59% sodium citrate, 18% mannitol, 2.6 glycine; *Squares*: 100% spray-dried hydroxypropyl-β-cyclodextrin, and *Diamonds*: 20% insulin, 20% leucine, 50% β-cyclodextrin sulfonylbutyl ether, 10% sodium citrate; and

Fig. 5 is a moisture sorption plot comparing 5 different spray-dried formulations. The plot further illustrates the ability of HGI-containing formulations to significantly reduce the rate and extent of water uptake when compared to non-HGI containing formulations. (*Circles*: 20% insulin, 20% leucine, 50% hydroxyethylstarch, 10% sodium citrate; *Squares*: 20% insulin, 5% leucine, 50% hydroxyethylstarch, 25% sodium citrate: *Diamonds*: 100% hydroxyethylstarch; *X*: : 20% insulin, 59% sodium citrate, 18% mannitol, 2.6 glycine; +: 20% leucine, 50% hydroxyethylstarch, 30% sodium citrate).

Detailed Description of the Invention

The present invention provides a particulate composition and method for the pulmonary delivery of particles composed of an active agent and a hygroscopic growth inhibiting agent, where the particle size distribution of the particles is less than 3 microns MMAD when delivered to the alveoli. The invention is surprising in that it provides for increased bioavailability of the active agent over active agent particles absent the hygroscopic growth inhibiting agent or having the hygroscopic growth inhibiting agent solely adsorbed to their surface. It is thought that by having the

hygroscopic growth inhibiting agent distributed throughout the particles rather than present just as a coating on the surface, as the surface of the particles are eroded or dissolved during their passage through the airways, new internal layers of the hygroscopic growth inhibiting agent are exposed, thus providing to the particles a new layer of hygroscopic growth inhibiting capability.

I. Definitions

The following terms as used herein have the meanings indicated.

"Active agent" as described herein includes an agent, drug, compound, composition of matter or mixture which provides some pharmacologic, often beneficial, effect that can be demonstrated *in-vivo* or *in vitro*. This includes foods, food supplements, nutrients, drugs, vaccines, vitamins, and other beneficial agents. As used herein, the terms further include any physiologically or pharmacologically active substance that produces a localized or systemic effect in a patient.

"Dry powder" refers to a powder composition that contains finely dispersed solid particles that are free flowing and capable of (i) being readily dispersed in an inhalation device and (ii) inhaled by a subject so that a portion of the particles reach the lungs to permit penetration into the alveoli. Such a powder is considered to be "respirable" or suitable for pulmonary delivery. A dry powder typically contains less than about 10% moisture, preferably less than 5% moisture, and more preferably contains less than about 3% moisture.

"Hygroscopic growth inhibitor, (HGI)", means any material that, when incorporated into the particles of the invention, reduces the rate and/or extent of water uptake. Materials suitable for use as a hygroscopic growth inhibitor are effective, when incorporated into the particles of the invention at a suitable concentration, to inhibit the hygroscopic growth of the particles under conditions typically found in the lung by at least 5%, preferably by at least 10%, and more preferably by at least 15%, when compared to particles having the same relative amounts of particle components, absent the HGI.

The hygroscopic growth of the particles is generally described in terms of a hygroscopic growth ratio, that is, the ratio of the MMAD of the particles under

conditions typically found in the lung to the MMAD of the dry particles prior to inhalation. As an illustration, a particle having a hygroscopic growth ratio of 1 does not change size upon inhalation and exposure to the environmental conditions of the lung. The hygroscopic growth of particles is determined experimentally by treating the powders in an environmental chamber simulating the conditions of the lung, i.e., 32-37°C and 95-99.5% relative humidity. More specifically, a dose of the particles is aerosolized in a growth chamber as described above. The aerosol is then passed into a cascade impactor, to determine the mass median aerodynamic diameter of the particles.

Alternatively, one can calculate the MMAD of a particular powder composition under simulated lung conditions to determine the equilibrium growth ratio. The MMAD of an aerosolized powder particle in the lung is determined by calculating the solids concentration (powder to water ratio) at which an aqueous solution of the powder becomes isotonic, i.e., the concentration at which a liquid droplet reaches equilibrium in the lung, which then allows calculation of the MMAD of the isotonic droplet. The MMAD of the isotonic droplet is then divided by the experimentally determined MMAD of the powder under ambient conditions to obtain the hygroscopic growth ratio.

Particles incorporating a HGI and having an MMAD below 3 microns under simulated lung conditions as described above are encompassed by the present invention.

"Simulated lung conditions" are 32-37°C and 95-99.5% relative humidity.

"Sorption Index" or "SI" is the sum of the percent weight gain of a dry powder of the invention determined at 10%, 20%, 30% and 40% relative humidity (25 °C), divided by four. The sorption index is determined using a gravimetric sorption analyzer, such as the DVS-1000, manufactured by Moisture Measurements System (London, UK) or moisture balance, manufactured by VTI Corporation (Hialeah, FL).

"Particles of active agent" means the active agent as defined above in the form of particles that are suitable for pulmonary delivery. The particles form a dry powder. It is to be understood that more than one active agent may be incorporated

into the aerosolized active agent formulation and that the use of the term "agent" in no way excludes the use of two or more such agents.

Particles having a hygroscopic growth inhibitor "incorporated " within are those particles having the HGI distributed throughout the particle, rather than present solely as a coating on the surface.

By "in-lung pulmonary bioavailability" is meant the amount of active agent which, after deposition in the lungs, is absorbed and becomes available in the systemic circulation of a mammal relative to the amount that is absorbed into the blood from a subcutaneous injection site (% absorbed/ % deposited) relative to subcutaneous). Representative model systems for determining in-lung bioavailibilitics include rat, dog, and non-human primates. Relative in-lung pulmonary bioavailibilities may be based upon direct intratracheal administration or by inhalation.

"Emitted Dose" or "ED" provides an indication of the distribution of dry powder within a suitable inhaler device after a firing or dispersion event. ED is defined as the ratio of the emitted dose to the nominal dose (*i.e.*, the mass of powder per unit dose placed into a suitable inhaler device prior to firing). The ED is an experimentally-determined parameter, and is typically determined using an *in-vitro* device set up which mimics patient-dosing. To determine an ED value, a nominal dose of dry powder is placed into a suitable dry powder inhaler, which is then actuated, dispersing the powder. The resulting aerosol cloud is then drawn by vacuum from the device, where it is captured on a tared filter attached to the device mouthpiece. The amount of powder that reaches the filter constitutes the emitted dose. For example, for a 5 mg, dry powder-containing dosage form placed into an inhalation device, if dispersion of the powder results in the recovery of 4 mg of powder on a tared filter as described above, then the emitted dose for the dry powder composition is: 4 mg (emitted dose)/5 mg (nominal dose) x 100 = 80%. For non-homogenous powders, ED values provide an indication of the distribution of drug within an inhaler device after firing rather than of dry powder, and are based on drug weight rather than on total powder weight.

"Drop in emitted dose under simulated lung conditions" means the ED value under ambient conditions (%) minus the ED value at 32-37 °C and 95-99.5% relative humidity.

A "dispersible" powder is one having a ED value of at least about 30%, more preferably 40-50%, and even more preferably at least about 50-60%.

"Mass median diameter" or "MMD" is a measure of mean particle size, since the powders of the invention are generally polydisperse (*i.e.*, consist of a range of particle sizes). MMD values as reported herein are determined by centrifugal sedimentation, although any number of commonly employed techniques can be used for measuring mean particle size (e.g., electron microscopy, light scattering, laser diffraction).

"Mass median acrodynamic diameter" or "MMAD" is a measure of the aerodynamic size of a dispersed particle. The aerodynamic diameter is used to describe an aerosolized powder in terms of its settling behavior, and is the diameter of a unit density sphere having the same settling velocity, in air, as the particle. The aerodynamic diameter encompasses particle shape, density and physical size of a particle. As used herein, MMAD refers to the midpoint or median of the aerodynamic particle size distribution of an aerosolized powder determined by cascade impaction, unless otherwise indicated.

"Pharmaceutically acceptable excipient or carrier" refers to an excipient that may be included in the particles of the invention and taken into the lungs in association with the particles with no significant adverse toxicological effects to the subject, and particularly to the lungs of the subject.

"Pharmacologically effective amount" or "physiologically effective amount of a bioactive agent" is the amount of an active agent present in a particulate dry powder composition as described herein that is needed to provide a desired level of bioactive agent in the bloodstream of a subject to be treated to give an anticipated physiological response when such composition is administered pulmonarily. The precise amount will depend upon numerous factors, e.g., the bioactive agent, the specific activity of the composition, the delivery device employed, physical characteristics of the powder, its

intended use, and patient considerations, and can readily be determined by one skilled in the art, based upon the information provided herein.

II. Components of the Inhalable Powder

The particles of the present invention are designed to resist the hygroscopic growth which normally occurs upon pulmonary administration of dry powder formulations, to thereby enable a greater proportion of the inhaled particles to reach the deep lung. This feature of the particles, i.e., resistance to hygroscopic growth, is achieved by the incorporation of a hygroscopic growth inhibiting agent, i.e., an agent whose presence within the particles is effective to reduce the rate and/or extent of water uptake by the particles, particularly when exposed to the environmental conditions of the lung.

A. Active Agent

The active agent for incorporation in the particulate compositions described herein include antibiotics, antiviral agents, anepileptics, analgesics, anti-inflammatory agents and bronchodilators. The active agent may be an inorganic or organic compound, including, without limitation, drugs which act on the peripheral nerves, adrenergic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulatory system, synoptic sites, neuroeffector junctional sites, endocrine and hormone systems, the immunological system, the reproductive system, the skeletal system, autacoid systems, the alimentary and excretory systems, the histamine system the central nervous system. Suitable agents may be selected from, for example, polysaccharides, steroids, hypnotics and sedatives, psychic energizers, tranquilizers, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, muscle contractants, antimicrobials, antimalarials, hormonal agents including contraceptives, sympathomimetics, polypeptides, and proteins capable of eliciting physiological effects, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, neoplastics, antineoplastics, hypoglycemics, nutritional agents and supplements, growth supplements, fats, antienteritis agents, electrolytes, vaccines and diagnostic agents.

Examples of active agents suitable for use in this invention include but are not limited to calcitonin, erythropoietin (EPO), Factor VIII, Factor IX, ceredase, cerezyme, cyclosporin, granulocyte colony stimulating factor (GCSF), alpha-1 proteinase inhibitor, elcatonin, granulocyte macrophage colony stimulating factor (GMCSF), growth hormone, human growth hormone (HGH), growth hormone releasing hormone (GHRH), heparin, low molecular weight heparin (LMWH), interferon alpha, interferon beta, interferon gamma, interleukin-2, luteinizing hormone releasing hormone (LHRH) , insulin, somatostatin, somatostatin analogs including octreotide, vasopressin analog, follicle stimulating hormone (FSH), insulin-like growth factor, insulintropin, interleukin-1 receptor antagonist, interleukin-3, interleukin-4, interleukin-6, macrophage colony stimulating factor (M-CSF), nerve growth factor, parathyroid hormone (PTH), thymosin alpha 1, IIb/IIIa inhibitor, alpha-1 antitrypsin, VLA-4, respiratory syncytial virus antibody, cystic fibrosis transmembrane regulator (CFTR) gene, deoxyreibonuclease (Dnase), bactericidal/permeability increasing protein (BPI), anti-CMV antibody, interleukin-1 receptor, 13-cis retinoic acid, pentamidine isethiouate, albuterol sulfate, metaproterenol sulfate, beclomethasone diprepionate, triamcinolone acetamide, budesonide acetonide, fluticasone, ipratropium bromide, flunisolide, cromolyn sodium, ergotamine tartrate and the analogues, agonists and antagonists of the above. Active agents may further comprise nucleic acids, present as bare nucleic acid molecules, viral vectors, associated viral particles, plasmid DNA or RNA or other nucleic acid constructions of a type suitable for transfection or transformation of cells, particularly cells of the alveolar region of the lungs. The active agents may be in various forms, such as water soluble or insoluble, charged or uncharged molecules, components of molecular complexes or pharmacologically acceptable salts. The active agents may be naturally occurring molecules or they may be recombinantly produced, or they may be analogs of the naturally occurring or recombinantly produced proteins with one or more amino acids added or deleted. Further, the active agent may comprise live attenuated or killed viruses suitable for use as vaccines.

The amount of active agent in the aerosolized particles will be that amount necessary to deliver a therapeutically effective amount of the active agent per unit

dose to achieve the desired result. In practice, this will vary widely depending upon the particular agent, its bioactivity, the severity of the condition to be treated, the patient population, dosing requirements, and the desired therapeutic effect. The particles will generally contain anywhere from 1% by weight to about 99% by weight active agent, typically from about 2% to about 95% by weight active agent, and more typically from about 5% to 85% by weight active agent. However, the particles are particularly useful for active agents that must be delivered in doses of from 0.001 mg/day to 100 mg/day, preferably 0.01 mg/day to 50 mg/day.

B. Hygroscopic Growth Inhibitor

An essential feature of the particles is the hygroscopic growth inhibitor. The hygroscopic growth inhibitor (HGI) is effective to reduce the rate and/or extent to which moisture is absorbed by the particles upon inhalation, so that the particles maintain an MMAD of less than 3 microns upon delivery to the alveoli.

A material suitable for use as an HGI is first identified by a preliminary screening to determine its moisture absorptivity profile after spray-drying; low absorptive materials are those preferred for use in the present invention, such as those materials in Fig. 1. Those HGI materials are then further tested for suitability by preparing particles containing an appropriate amount of the HGI (typically greater than about 5 to 10 percent by weight of the composition). In some cases, the HGI may, in addition to being present as part of the bulk powder, also form an additional coating on the surface of the particles. Moisture isotherms are then determined for active agent particles containing the HGI and for control particles having the same relative amounts of components absent the HGI, to determine whether the presence of the HGI is effective to reduce either the extent or rate of water absorption by the powder. Typically, both high and low concentrations of HGI are tested, to determine the useful ranges for incorporation into the powders of the invention.

Materials found to be useful as hygroscopic growth inhibitors include, but are not limited to the following: double chain phospholipids, cyclodextrins and their derivatives, hydroxyethylstarch (HES), dextran, dextranomer, maltodextrins, starches, hydroxypropylmethylcellulose (HPMC), cellulose ethyl hydroxyethyl ether, and other

cellulose derivatives, such as those described in "Cellulosics : Chemical, Biochemical and Material Aspects" (Ellis Horwood Series in Polymer Science and Technology) by J.F., B.Sc. Kennedy, G.O., B.Sc. Phillips, P.A. Williams (Editor), and in "Comprehensive Cellulose Chemistry" by D. Klemm (Editor), Bertram Philipp, T. Heinze (1998). In some instances, the active agent may also function as a hygroscopic growth inhibiting agent. Active agents which tend to act as HGIs include insulin, salmon calcitonin, and PTH.

Double chain phospholipids for use in the present invention include phosphatidylcholines such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)- 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), and the like. Also suitable for use as a hygroscopic growth inhibitor are phophatidylethanolamines such as 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE), 1,2-dialmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and similarly derivatized phosphatidylglycerols and phosphatidic acids.

Cyclodextrins are another class of compounds found to be useful as hygroscopic growth inhibitors. Cyclodextrins, cyclic oligosacchrides shaped like a truncated cone and having a hydrophobic cavity in center, are composed of more than six D-glucose residues. Cyclodextrins for use in the present invention include alpha-cyclodextrin (six glucose residues), beta-cyclodextrin, (seven glucose residues), and gamma-cyclodextrin (eight glucose residues) according to the number of glucose residues, respectively, as well as derivatives, such as 2-hydroxypropyl-β-cyclodextrin (2-HPβC) and β-cyclodextrin sulfonylbutyl ether. 2-HPβC is a particularly preferred excipient, as illustrated by its moisture sorption profile (Fig. 1). At a target relative humidity of 80%, 2-HPβC exhibited a change of mass of only about 16% due to water uptake, over a course of about 8 hours. Cyclodextrin exhibits a similar profile. The beneficial moisture sorption properties of an exemplary formulation containing sulfobutylether-β-cyclodextrin (2-SBEbC) are shown in Fig. 4. Thus, these materials

(i) are quite resistant to water uptake. and (ii) exhibit a slow rate of water uptake, making them suitable materials for incorporation in the powders of the invention.

Also useful as hygroscopic growth inhibiting agents are dextrans, which are polysaccharides containing glucose monomers. Dextrans for use in the present invention possess a molecular weight ranging between about 10,000-100,000. Preferred are dextran 10, dextran 40, dextran 70, and dextran 75. Dextran derivatives such as dextranomer (dextran 2,3-dihydroxypropyl 2-hydroxy-1,3-propanediyl eithers), maltodextran and dextran sulfate sodium may also be used. Dextran's resistance to moisture uptake was illustrated in a moisture sorption balance experiment, where it was shown that at 70% relative humidity, spray-dried dextran absorbed only 19% water, while at 80% relative humidity, dextran exhibited a water sorption of 24% by weight, as illustrated in Example 3 and shown in Fig. 1.

Derivatized celluloses such as hydroxypropyl methylcellulose (HPMC), cellulose ethyl hydroxyethyl ether and hydroxypropyl cellulose, with molecular weights ranging from 10,000 to 400,000, are also useful as hygroscopic growth inhibitors.

Derivatized starches may also be employed as hygroscopic growth inhibitors. One particularly preferred hygroscopic growth inhibitor is hydroxyethylstarch (HES) having a molecular weight range from about 70,000 to about 400,000 (see, e.g., Fig. 2). A review of HES can be found in *Intensive Care Med* (1999) *25*:258-268.

Also suitable for use as a hygroscopic growth inhibitor is maltodextrin, a hydrolyzed starch, and its commercially available derivatives. Preferred is Maltodextrin 40, having an average molecular weight of about 3600.

An HGI useful in the particles and methods of the invention will combine effective minimization of hygroscopic growth with (1) lack of toxicity in the concentrations used and (2) good powder properties, i.e., lack of a sticky or waxy consistency in the solid state. Toxicity of a given substance can be tested by standard means, such as an MTT assay, as for example described in *Int. J.Pharm.* 65 (1990), p. 249-259.

The hygroscopic growth inhibitor is present in the particles in an amount sufficient to minimize or prevent hygroscopic growth of the particles, such that the particles maintain a size below 3 microns upon aerosolized delivery to the alveoli. The optimal ratio of active agent to HGI can be ascertained for any given HGI by testing various proportions in an *in vitro* model such as described herein. For example, an active agent is typically combined with an HGI, such as hydroxyethylstarch, in the following w/w proportions: 10/90, 25/75, 50/50, 75/25, and 90/10, to determine which ratios give a significant reduction in the extent or rate of water uptake in the powders. From these data, an optimal concentration of HGI can be determined. Different HGIs, in combination with different active agents, and optionally additional excipients, will have different optimal concentrations, so that each HGI must be separately tested.

Generally, the particles contain at least about 5 to about 20 percent by weight HGI, preferably at least about 20 to 40 percent HGI, and even more preferably at least about 40 to about 60 weight percent or more HGI. The amount of HGI necessary to reduce the moisture absorbing properties of the powder will be less in situations where the active agent is a protein or polypeptide, since proteins and polypeptides also act to inhibit hygroscopic growth. In instances where the active agent is not a protein or polypeptide, the particles will preferably contain at least about 40% HGI, with the amount of HGI in the particles ranging from about 40% to 99% by weight. The presence of the HGI in the particles maximizes deposition of the aerosolized particles in the lower respiratory tract, in particular upon the alveolar surface, as opposed to the mouth, throat, and upper airways, thereby increasing the bioavailability of an active agent delivered to the lung.

C. Other Excipients

In addition to the hygroscopic growth inhibitor, the active agent powders of the present invention may optionally be combined with pharmaceutical carriers or excipients which are suitable for respiratory and pulmonary administration. Such carriers may serve simply as bulking agents when it is desired to reduce the active agent concentration in the powder which is being delivered to a patient. However, the

carriers may also serve to further improve the dispersibility of the powder within a powder dispersion device, functioning to provide more efficient and reproducible delivery of the active agent and to improve handling characteristics of the active agent, (e.g., flowability and consistency) to facilitate manufacturing and powder filling. In particular, the excipient materials can often function to improve the physical and chemical stability of the particles, to further minimize the residual moisture content and hinder moisture uptake, and to enhance particle size, degree of aggregation, surface properties (*i.e.*, rugosity), ease of inhalation, and targeting of the resultant particles to the deep lung.

These excipients, if present, are generally present in the composition in amounts ranging from about 1 % to about 50 percent by weight, and include but are not limited to proteins, peptides, amino acids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which may be present singly or in combination. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/polypeptide components, which may also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, di- and tripeptides such as trileucine, and the like. Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like.

The compositions may also include a buffer or a pH adjusting agent. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Additionally, the compositions of the invention may include additional excipients/additives, such as

Ficolls (a polymeric sugar), flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), and chelating agents (e.g., EDTA). Other pharmaceutical excipients and/or additives suitable for use in the matrix compositions described herein are listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are herein incorporated by reference. Preferred excipients for use in the present formulations include mannitol, raffinose, and sodium citrate, leucine, isoleucine, valine, sucrose, raffinose, tri-leucine, and mannitol,

III. Preparing the Powder Formulation

Dry powder active agent formulations are preferably prepared by spray drying under conditions which result in a substantially amorphous powder. Spray drying of the formulations is carried out, for example, as described generally in the "Spray Drying Handbook", 5th ed., K. Masters, John Wiley & Sons, Inc., NY, NY (1991), and in Platz, R., *et al.*, International Patent Publication No. WO 97/41833 (1997), the contents of which are incorporated herein by reference.

Solutions, emulsions, or suspensions containing the active agent, hygroscopic growth inhibitor, and optionally other excipients, are prepared. Solutions or suspensions for spray drying will typically contain from about 0.1 to 10 weight percent per volume solids. The pH range of the solutions is generally maintained between about 3 and 9, and will depend upon the impact of pH on the stability of the active agent. Near neutral pHs are preferred, since such pHs may aid in maintaining the physiological compatibility of the powder after dissolution of powder within the lung. The pre-spray-dried formulation may optionally contain additional water-miscible solvents, such as alcohols or acetone. Representative alcohols are lower alcohols such as methanol, ethanol, propanol, isopropanol, and the like. The resultant solutions will generally contain active agent at a concentration from 0.01% (weight/volume) to about 2% (weight/volume), usually from 0.1% to 1% (weight/volume).

The solutions are then spray dried in a conventional spray drier, such as those available from commercial suppliers such as Niro A/S (Denmark), Buchi (Switzerland) and the like, resulting in a stable, dry powder. Optimal conditions for spray drying the formulations will vary depending upon the formulation components, and are generally determined experimentally. The gas used to spray dry the material is typically air, although inert gases such as nitrogen or argon are also suitable. Moreover, the temperature of both the inlet and outlet of the gas used to dry the sprayed material is such that it does not cause deactivation/decompositions of the active agent in the spray dried material. Such temperatures are typically determined experimentally, although generally, the inlet temperature will range from about 50° C to about 200° C while the outlet temperature will range from about 30° C to about 150° C.

Alternatively, the dry powders may be prepared by lyophilization, vacuum drying, spray freeze drying, super critical fluid processing, or other forms of evaporative drying. In some instances, it may be desirable to provide the dry powder formulation in a form that possesses improved handling/processing characteristics, e.g., reduced static, better flowability, low caking, and the like, by preparing compositions composed of fine particle aggregates, that is, aggregates or agglomerates of the above-described matrix dry powder particles, where the aggregates are readily broken back down to the fine powder components for pulmonary delivery, as described, e.g., Johnson, K., *et al.*, U.S. Patent No. 5,654,007, 1997, incorporated herein by reference. Alternatively, the powders may be prepared by agglomerating the powder components, sieving the materials to obtain the agglomerates, spheronizing to provide a more spherical agglomerate, and sizing to obtain a uniformly-sized product, as described, e.g., in Ahlneck, C.; *et al.*, International PCT Publication No. WO 95/09616,1995, incorporated herein by reference. Dry powders may also be prepared by blending, grinding, sieving or jet milling formulation components in dry powder form. The resulting powder is generally substantially amorphous in form.

Dry powders are preferably maintained under dry (*i.e.*, relatively low humidity) conditions during manufacture, processing, and storage.

IV. Characteristics of the Powder

The powder particles of the invention have the capability of maintaining an aerodynamic diameter of less than 3 μ when delivered to the alveoli. As can be seen from Example 1, powders lacking a hygroscopic growth inhibitor and having an initial MMAD of 3.5 microns behaved like powders having an MMAD from 5-6 microns. Calculations further indicated that, at equilibrium in the lung, the powder would grow to 9 microns MMAD. From this data, it was discovered that the incorporation of a hygroscopic growth inhibitor into the powder formulations described herein was effective to notably decrease the rate and/or extent of hygroscopic growth of dry powder particles, thereby increasing not only the bioavailability of an active agent contained in the powder particles, but increasing the dispersibility of such formulations as well.

For the powders of the invention, the MMAD of the particles in most cases will be less than about 3 μ prior to pulmonary administration. Typically, the particles will grow to some degree upon pulmonary administration, although to a degree less than they would in the absence of the hygroscopic growth inhibitor, and will typically exhibit hygroscopic growth ratios of less than about 2.5, preferably less than about 2.0, even more preferably from about 1.5 to 2.0, and most preferably less than 1.5. Hygroscopic growth ratios can be determined experimentally, by comparing the MMAD of the powder determined under ambient conditions versus under the MMAD determined under simulated lung conditions in an environmental chamber ($MMAD_{lung}/MMAD_{ambient}$). Alternatively, the MMAD of a particle under lung conditions can be calculated as follows. First, using the molecular weights of all of the constituents of the particles, the isotonicity for each of the components is determined. These isotonicities are then added, to determine the isotonicity of the particle. From this value, the volume of solution required to reach isotonicity is calculated; this volume is then taken to be a volume of a sphere. From this sphere volume, the diameter of the sphere is then calculated, and represents the calculated MMAD of the particles under conditions found in the lung. This calculated MMAD can then be used to determined the hygroscopic growth ratio as described above.

The moisture uptake characteristics of the particles are typically determined by moisture sorption experiments. Moisture sorption data for powders can be determined by a number of techniques, such as moisture sorption balance or thermal activity monitor (TAM). Moisture sorption balances are determined by measuring the weight loss or gain as a function of increasing or decreasing relative humidities at a constant temperature. A carrier gas introduced at a known RH is created by mixing a wet and dry stream of gas. This gas is then exposed to the sample located in a non-hygroscopic sample cup attached to a microbalance. Depending on the morphology of the sample, it may absorb, adsorb or desorb moisture. This sorption is detected by the microbalance as a weight increase or decrease. A computer program is used to collect data point (generally time, temperature, relative humidity and weight) throughout the experiment and at user defined equilibrium points.

The powders of the invention may also be characterized by a sorption index, SI, i.e., the sum of the percent weight gain of the powder determined at 10%, 20%, 30% and 40% relative humidity, divided by four. The sorption index is determined using a gravimetric sorption analyzer, such as the DVS-1000, manufactured by Surface Measurements Systems (London, U.K.), or by moisture balance, using an instrument such as the MB 300G, manufactured by VTI Corporation (Hialeah, FL). Powders of the invention will typically have sorption indices less than about 7.5, preferably less than about 7.0, more preferably less than about 6.5, and most preferably below 6.0. Powders exhibiting such SI values are shown in Example 2.

Powders preferred in the present invention are those which take up water slowly, i.e., at a rate of less than about 0.75% moisture as a function of relative humidity, preferably less than about 0.50% moisture as a function of relative humidity, and more preferably less than about 0.35% moisture as a function of relative humidity, and most preferably less than about 0.25% moisture as a function of relative humidity (e.g., see Fig. 1). As another measure, the particles absorb less than about 60% moisture (wt), preferably less than 30% moisture, more preferably less than 25% moisture, even more preferably less than 20% moisture, and most preferably between about 10 to 20% by weight water under humid conditions, 80% relative humidity. Figs. 1 and 2 illustrate how powders containing a hygroscopic growth

inhibiting agent, when compared to powder formulations lacking an HGI, exhibit both a decreased rate of water uptake (indicated by smaller slopes in comparison to the control formulation) and a lower overall extent of moisture uptake.

In looking at Fig. 1, under conditions of 80% relative humidity, the spray-dried control powder containing 20% insulin, 59% sodium citrate, 18% mannitol and 2.6% glycine absorbed 60% moisture by weight, while under the same conditions, spray-dried dextran, hydroxypropylmethylcellulose, hydroxypropyl-β-cyclodextrin, and hydroxyethylstarch, absorbed 24%, 16%, 16%, and 24% moisture, respectively, thus illustrating the superior hygroscopic growth inhibiting properties of these materials. Similarly, in looking at Fig. 2, under conditions of 80% relative humidity, while the control absorbed 60% moisture, under the same conditions, spray dried powders containing 20% insulin, 40% hydroxyethylstarch, 2.6% glycine, 18% mannitol, 19% sodium citrate, and 100% hydroxyethylstarch, absorbed 50% and 24% moisture, respectively. Moreover, in both figures, the rate of water uptake was substantially reduced for the HGI materials, when compared to the controls.

The powders of the invention can also be characterized by maintaining good dispersibilities when exposed to the hot and humid conditions such as those found in the environment of the lung. The powders of the invention will generally exhibit a drop in emitted dose (ED) at 32°C and 95% relative humidity (when compared to the ED under ambient conditions) of no more than about 30% (meaning $ED_{ambient}$ minus ED_{humid} equals 30 or less), preferably no more than about from about 20 to 25%, more preferably no more than 15%, and most preferably no more than about 10%.

As an illustration, Example 2 shows powder formulations whose EDs, when evaluated in an environmental chamber, decrease only from about 10-15% (60% maltodextrin formulations). Also exhibiting good EDs under lung conditions were powder formulations containing 60% hydroxyethylstarch. As can be seen from the data in Table 1, increasing the amount of hydroxyethylstarch from 40% to 60% (samples 2/3 versus 4/5) was effective to reduce the environmental chamber ED drop. On average, these formulations showed a drop of about 20% in ED under lung conditions, while maintaining ED values of about 55%.

The emitted dose (ED) of the HGI-containing powders, under ambient conditions, is greater than 30% and usually greater than 40%. More preferably, the emitted dose of the powders of the invention is greater than 50%, and is often greater than 60%. The powders of the invention typically contain a large proportion of small aerosol particle sizes and are thus extremely effective when delivered in aerosolized form, in (i) reaching the alveolar region of the lung, (ii) diffusion to the interstitium, and (iii) subsequent passage into the bloodstream through the endothelium.

The dry powders of the invention will generally have an overall moisture content under ambient conditions below about 10% by weight, usually below about 5% by weight, and preferably below about 3% by weight. Such low moisture-containing solids tend to exhibit a greater stability than the corresponding "high moisture" solids.

V. Pulmonary Administration of the Powder

The HGI-containing dry powder formulations described herein are preferably delivered using any suitable dry powder inhaler (DPI), *i.e.*, an inhaler device that utilizes the patient's inhaled breath as a vehicle to transport the dry powder drug to the lungs. Preferred are Inhale Therapeutic Systems' dry powder inhalation devices as described in Patton, J.S., et al., U.S. Patent No. 5,458,135 (1995); Smith, A., *et al.*, U.S. Patent No. 5,740,794, (1998); and Smith, A., *et al.*, U.S. Patent No., 5,785,049, (1998).

When administered using a device of this type, the dry powder is contained in a receptacle having a puncturable lid or other access surface, preferably a blister package or cartridge, where the receptacle may contain a single dosage unit or multiple dosage units. Convenient methods for filling large numbers of cavities with metered doses of dry powder medicament are described, e.g., in Parks, D.J., *et al.*, International Patent Publication WO 97/41031, (1997).

Also suitable for delivering the dry powders described herein are dry powder inhalers of the type described, for example, in Cocozza, S., U.S. Patent No. 3,906,950, (1974), and Cocozza, S., U.S. Patent No. 4,013,075, (1977), wherein a premeasured dose of dry powder for delivery to a subject is contained within a hard gelatin capsule.

Other dry powder dispersion devices for pulmonarily administering dry powders include those described, for example, in Newell, R.E., *et al.*, European Patent No. EP 129985, (1988); in Hodson, P.D., *et al.*, European Patent No. EP 472598, (1996); in Cocozza, S., *et al.*, European Patent No. EP 467172, (1994), and in Lloyd, L.J. *et al.*, U.S. Patent No. 5,522,385, (1996). Also suitable for delivering the matrix dry powders of the invention are inhalation devices such as the Astra-Draco "TURBUHALER". This type of device is described in detail in Virtanen, R., U.S. Patent No. 4,668,218, (1987); in Wetterlin, K., *et al*, U.S. Patent No. 4,667,668, issued May 26, (1987); and in Wetterlin, K., *et al.*, U.S. Patent No. 4,805,811, (1989). Also suitable are devices which employ the use of a piston to provide air for either entraining powdered medicament, lifting medicament from a carrier screen by passing air through the screen, or mixing air with powder medicament in a mixing chamber with subsequent introduction of the powder to the patient through the mouthpiece of the device, such as described in Mulhauser, P., *et al,* U.S. Patent No. 5,388,572, (1997).

The HGI-containing dry powders may also be delivered using a pressurized, metered dose inhaler (MDI) containing a solution or suspension of drug in a pharmaceutically inert liquid propellant, e.g., a chlorofluorocarbon or fluorocarbon, as described in Laube, *et al.*, U.S. Patent No. 5,320,094, (1994), and in Rubsamen, R.M., *et al*, U.S. Patent No. 5,672,581 (1994).

Prior to use, the HGI-containing dry powders are generally stored under ambient conditions, and preferably are stored at temperatures at or below about 25°C, and relative humidities (RH) ranging from about 30 to 60%. More preferred relative humidity conditions, e.g., less than about 30%, may be achieved by the incorporation of a desiccating agent in the secondary packaging of the dosage form. The respirable dry powders of the invention are characterized not only by good aerosol performance, but by good stability, as well.

When aerosolized for direct delivery to the lung, an active agent contained in the dry powder formulations described herein will exhibit an increased in-lung bioavailability, due to the presence of the HGI within the powder particles, which allows a greater percentage of the inhaled particles to reach the deep lung without

prior deposition in the upper airways due to hygroscopic growth. Such HGI-containing formulations thus allow for the administration of smaller quantities of drug per unit dose, and may even eliminate the need for multiple inhalations per day. Moreover, the presence of the HGI provides enhanced stability to the powder formulation by reducing or preventing moisture uptake, thereby enhancing the shelf life and shipping stability of the dry powder formulations.

The disclosure of each publication, patent or patent application mentioned in this specification is incorporated by reference herein to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be incorporated by reference.

The following examples illustrate, but in no way are intended to limit the scope of the present invention.

Examples

Materials and Methods

Salmon calcitonin (sCalcitonin) was purchased from Bachem (Torrance, CA).

Human Serum Albumin (HSA) was purchased from Miles Inc. (Kankakee, IL). Sodium citrate dihydrate was obtained from J.T. Baker (Phillipsburg, NJ). L,α-dipalmitoylphosphatidylcholine (DPPC) is obtained from Avanti Polar Lipids, Alabama.

Example 1

The following active agent containing particles were prepared to investigate moisture uptake and hygroscopic growth properties.

A. Powder Production

Salmon Calcitonin powders were prepared as follows. Bulk sCalcitonin was dissolved in sodium citrate buffer containing mannitol and HSA to give an aqueous solution having a final solids concentration of 7.5 mg/ml and a pH of 6.7±0.3. The solution was then filtered through a 0.22 μm filter, followed by spray drying using a Buchi 190 mini spray dryer (Buchi Labortechnik AG, Meierseggstrasse, Switzerland). The spray dryer was operated at an inlet temperature between 110°C and 120°C, a liquid feed rate of 5 ml/min, and an outlet temperature between 70°C and 80°C, resulting in the collection of a fine white amorphous powder.

Powders containing the following hygroscopic growth inhibitors (HGIs): DPPC, cyclodextrin, hydroxyethylstarch, dextran, dextranomer, maltodextran, hydroxypropylcellulose, hydroxypropylmethylcellulose, and cellulose ethyl hydroxyethyl ether are similarly prepared.

The composition of the powder absent the HGI was 5% sCalcitonin/6.25% HSA/ 73.75% mannitol/15% citrate by weight. The powder incorporating the HGI possesses the same relative amounts of sCalcitonin/HSA/mannitol/citrate, but also contains from about 10% to 90% by weight of the hygroscopic growth inhibitor.

The resulting powders are stored in tightly capped containers in a dry environment (<10% RH) prior to hygroscopic growth analysis.

B. Powder Analysis

The particle size distribution of the sCalcitonin powder was measured by liquid centrifugal sedimentation in a Horiba CAPA-700 Particle Size Analyzer following dispersion of the powders in Sedisperse A-11 (Micrometrics, Norcross, GA).

The moisture content of the sCalcitonin powder was measured by the Karl Fischer technique using a Mitsubishi CA-06 Moisture Meter.

The aerosol particle size distribution was determined using a cascade impactor (Graseby Andersen, Smyrna, GA) at a flow rate of 28 l/min, ignoring powder loss on inlet manifold and stage 0 jet plate.

Emitted doses (ED) were evaluated using an Inhale Therapeutic Systems' aerosol device, similar to that described in WO96/09085. The emitted dose is defined as the percentage of the nominal dose contained within a blister package that exits the mouthpiece of the aerosol device and is captured on a glass fiber filter (Gelman, 47 mm diameter) through which a vacuum was drawn (30L/min) for 2.5 seconds following device actuation. ED is calculated by dividing the mass of the powder collected on the filter by the mass of the powder in the blister pack.

The ED of 5% sCalcitonin powders ranged between 52.6 and 53.9%.

The MMAD of the 5%s Calcitonin powders was approximately 3.5-3.6 microns.

These analyses are similarly performed on the DPPC-containing sCalcitonin powders.

C. Particle Growth

The following study was undertaken to explore the bioavailability of sCalcitonin formulations delivered to the lung as a dry powder aerosol.

Non-HGI-sCalcitonin particles were administered to 16 healthy volunteers. Each subject received a dry powder aerosol dose. The aerosolized dose contained approximately 2.5mg of powder, containing approximately 750 IU (125μg) of salmon calcitonin, radiolabelled with 10 MBq ^{99m}Tc pertechnitate. The particles were aerosolized using the Inhale Therapeutic Systems' aerosol devices, described above.

The dose of sCalcitonin delivered to the lung and the lung periphery (deep lung) was determined using a modification of standard gamma camera techniques. Quantitation of the sCalcitonin reaching the systemic circulation was achieved by radioimmunoassay of serum samples taken over 6 hours post dose ("Ultra-Sensitive Radioimmunoassay kit for the Quantitative Radioimmunoassay for the Quantitative Determination of salmon calcitonin in serum and Plasma", Diagnostic Systems Laboratories Inc.)

Bioavailability was determined by comparing the dose corrected AUC (area under the curve) estimates using the peripheral (alveolar) dose. Simple trapezoidal integration was used to determine AUCs. Relative bioavailability was determined

relative to subcutaneous injection. Statistical analysis of both the gamma camera deposition data and the relative bioavailability data were performed using a Wilcoxon matched-pairs signed ranks test. The Wilcoxon test is a non-parametric test appropriate for small sample size. A p value of ≤ 0.05 was considered to be significant.

The size distribution of the powder without HGI before and after the radiolabeling process was essentially unchanged. In examining the regional deposition patterns after inhalation, 21.6% of the inhaled dose of powder reached the peripheral lung (45.6% reached the whole lung including the peripheral lung), while the loss to the mouth and oropharynx was 54.4%.

The bioavailability of the salmon calcitonin powder, relative to subcutaneous injection, was 28.0% for the dose delivered to the peripheral lung. Despite the value of 3.5 MMAD obtained for the aerosol size distribution, this powder acted like a powder having an MMAD of between 5 and 6 microns. This can be accounted for as a result of the hygroscopic growth of the particles as they pass through the airways, due to the hygroscopic nature of the formulation.

Support for this mechanism was provided by calculating the aerodynamic equilibrium growth ratio for the formulation, which was 2.53. This ratio indicated that, under airway conditions, the particles grow, and at equilibrium, from a starting MMAD of 3.5 microns, the aerosol particles grow to 9 microns MMAD (i.e, the particles grow to 2.53 times their original aerodynamic diameter). Equilibrium growth ratios are determined by calculating the solids concentration (powder to water ratio) at which an aqueous solution of the powder becomes isotonic, that is, the concentration at which a liquid droplet reaches equilibrium in the lung, which then allows calculation of the MMAD of the isotonic droplet. The growth ratio is:

$$MMAD_{\text{isotonic droplet}}/MMAD_{\text{powder particles ambient}}.$$

Accordingly, sCalcitonin powders which maintain an MMAD of 3.0 microns when delivered to the alveoli are prepared by incorporating one or more HGIs into the particles in concentrations of between about 10-90%, particularly 10, 20, 30, 40, 50, 60, 70, 80 and 90% by weight HGI. The resultant powders are then tested as described above to determine their hygroscopic growth, if any, when exposed to the

environmental conditions of the lung. Powders according to the invention are those which exhibit an inhibition or reduction of hygroscopic growth, and more specifically, maintain that the particle size distribution remains below 3.0 microns MMAD when delivered to the peripheral lung, to thereby increase deposition at the peripheral lung and increase the in-lung bioavailability of an active agent delivered pulmonarily.

Example 2

Powder Measurements in an Environmental Chamber

Spray-dried powders containing one or more hygroscopic growth inhibitors were prepared as described in Example 1. The relative weight percentages of the powder components are provided in Table 1 below.

To assess the hygroscopic behavior of aerosol powders, dry inhalable powders were placed in an environmental chamber (Enviro-Chamber) which simulates the physiological conditions of the human lung (32°C and 95%RH). The chamber was monitored by pre-calibrated humidity and temperature probes (Digi-sense). Pre-collected data by this pre-calibrated probe showed both 95% RH and 32°C were produced consistently by the Enviro-Chamber for a long period of time.

Emitted dose and particle size were measured under standard (24°C and 40%) and humidified (32°C and >95%RH) conditions. Sampling under standard conditions was conducted inside the environmental chamber with the system turned off. The data collected under standard conditions was used as the control baseline. Membrane filters (47-mm) were used for the ED collections and an Andersen cascade impactor for the particle size distribution measurements.

Table 1.

Sample No.	Lot No.	Formulation	ED, n=28 Ambient	ED E-chamber	Sorption Index
1	R98403	60% maltodextrin 20% insulin 2.6% glycine 4.3% mannitol 13.09% citrate 0.013 citric acid, pH 7.3	76.94	67.12	5.3
2	R98403	same	78.51	65.31	5.3
3	R98414	40% HES-hmw 20% insulin 2.6% glycine 10% mannitol 27.4% citrate pH 7.3	72.63	47.52	6.4
4	R98414	same	77.23	49.39	6.4
5	R99041	60% HES-hmw 20% insulin 2.6% glycine 4.3% mannitol 13.09% citrate 0.013% citric acid pH 7.3	75.83	54.55	-
6	R99041	same	74.23	55.48	

*HES-hmw=hydroxyethylstarch

The results in Table 1 illustrate that powders containing a HGI are highly dispersible under ambient conditions, and maintain good dispersibilities under simulated lung conditions. The results also illustrate how formulations can be optimized by adjusting the quantity of HGI, as can be seen for samples 3/4 as compared to samples 5/6, where increasing the percentage of hydroxyethylstarch from 40% to 60% was effective to reduce the drop in ED under high humidity conditions (ED_{ambien}-ED_{lung}). This illustrates the resistance of HGI-containing powders to water uptake, and their ability to maintain flowability, even under extremely humid conditions.

The MMAD of powder R98403 (samples 1 and 2 above) was determined under moderate temperature and humidity conditions (70 °F, and 40% relative humidity). The results are summarized below.

Fill Weight , mg (dosage form)	MMAD, microns	% < 5 microns	% < 3.3 microns
5	2..9	84	59
5	2.9	84	59
2	2.3	96	77
2	2.4	94	74

As evident from the above results, this exemplary powder maintains a low MMAD, even under conditions of elevated temperature and humidity.

Example 3

Spray-dried powders were prepared as described in Example 1. Moisture sorption profiles were determined for these spray-dried HGI-containing powders using a gravimetric sorption analyzer, the DVS-1, manufactured by VTI Corporation (Hialeah, FL). The spray-dried powders had the following compositions (percentages by weight).

A. 20% insulin, 59% sodium citrate, 18% mannitol, 2.6% glycine
B. 100% dextran
C. 100% hydroxypropylmethylcellulose.
D. 100% hydroxypropyl-β-cyclodextrin
E. 100% hydroxyethylstarch (low molecular weight, MW=200,000)
F. 20% insulin, 40% hydroxyethylstarch, 2.6% glycine, 18% mannitol, 19% sodium citrate
G. 20% insulin, 20% leucine, 50% -β-cyclodextrin sulfonylbutyl ether, 10% sodium citrate.

H. 20% insulin, 20% leucine, 50% hydroxyethylstarch, 10% sodium citrate

I. 20% insulin, 5% leucine, 50% hydroxyethylstarch, 25% sodium citrate

J. 20% leucine, 50% hydroxyethylstarch, 30% sodium citrate

Moisture sorption profiles for powders A, B, C, D, and E are presented graphically in Fig. 1.

Moisture sorption profiles for powders A, F, and E are presented in Fig. 2.

Moisture sorption profiles for powders A, D, and G are presented in Fig. 4.

Moisture sorption profiles for powders H, I, E, A, and J are presented in Fig. 5.

As can be seen from each of the figures, the addition of a hygroscopic growth inhibiting agent to a particular formulation if effective to noticeably decrease its moisture sorption properties, thereby decreasing the hygroscopic growth of the particles as they travel through the airways to the deep lung.

Example 4

The hydration properties of various spray-dried insulin powder formulations were compared by TAM (thermal activity monitoring) using a Thermal Activity Monitor, Model 2277 (Thermometric, Sweden). Run conditions were as follows: ramp mode using RH perflusion units; ramped from 0% RH to 90% RH at 3%RH/hour at 25 °C with a nitrogen flow of 1.48 SCCM. The dry powder formulations employed were as follows.

A. 20% insulin, 59% sodium citrate, 18% mannitol, 2.6% glycine

B. 60%(wt) insulin, 2.6% glycine, 10% mannitol, 27.4% sodium citrate, 2.6% glycine.

C. 40% HES-hmw, 20% insulin, 2.6% glycine, 10% mannitol, 27.4% citrate

D. 60% maltodextrin, 20% insulin, 2.6% glycine, 4.3% mannitol, 13.09% citrate, 0,13% citric acid.

The TAM results for each of the formulations is presented in Fig. 3. Looking at Fig. 3, it can be seen that the incorporation of an HGI into the 20% insulin formulations resulted in a significant drop in both the extent and rate of moisture absorption when compared to the non-HGI containing 20% insulin formulation, thus indicating the effectiveness of these exemplary HGIs in reducing the extent of hygroscopic growth of these particles.

It is claimed:

1. Particles for delivery of an active agent to the alveoli of a human patient, said particles comprising the active agent and at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins, wherein the hygroscopic growth inhibitor is incorporated within the particles, and wherein the particles exhibit a drop in emitted dose under simulated lung conditions of no more than about 25%.

2. The particles of claim 1, wherein the hygroscopic growth inhibitor is selected from the group consisting of hydroxyethylstarch, dextranomer, and maltodextrins.

3. The particles of claim 1, wherein the hygroscopic growth inhibitor is selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and sulfobutylether β-cyclodextrin.

4. The particles of claim 1, wherein the hygroscopic growth inhibitor is hydroxyethylstarch.

5. The particles of any one of claims 1 to 4, wherein the hygroscopic growth inhibitor is present in the particles in an amount sufficient for the particles to exhibit a rate of moisture uptake of no greater than about 0.50% as a function of relative humidity.

6. The particles of any one of claims 1 to 5, wherein the hygroscopic growth inhibitor is present in the particles in an amount sufficient for the particles to exhibit an overall extent of water uptake of no greater than about 30 weight percent at a relative humidity of 80%.

7. The particles of any one of claims 1 to 6, having an emitted dose under ambient conditions of at least 60%.

8. The particles of any one of claims 1 to 7, containing from about 40 percent to about 99 percent by weight of the hygroscopic growth inhibitor.

9. The particles of claim 8, containing from about 40 percent to about 60 percent by weight of the hygroscopic growth inhibitor.

10. The particles of any one of claims 1 to 9, which when delivered pulmonarily, are deposited in the deep lung to an extent greater than 20% of the nominal dose.

11. The particles of any one of claims 1 to 10, wherein said hygroscopic growth inhibitor is effective to increase the bioavailability of said active agent when delivered to the lung by at least 5 percent, when compared to the bioavailability observed for the active agent contained in the same particles absent said hygroscopic growth inhibitor and delivered to the lung.

12. The particles of any one of claims 1 to 11, wherein the active agent comprises a protein or polypeptide.

13. The particles of claim 12, wherein the active agent comprises insulin.

14. The particles of claim 12, wherein the active agent comprises calcitonin.

15. Spray-dried particles of claim 1.

16. Particles for delivery of an active agent to the alveoli of a human patient, said particles comprising the active agent and at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins incorporated within the particles, wherein the particles have a sorption index of less than about 6.5.

17. The particles of claim 16, wherein the hygroscopic growth inhibitor is selected from the group consisting of hydroxyethylstarch, dextranomer, and maltodextrins.

18. The particles of claim 16, wherein the hygroscopic growth inhibitor is selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and sulfobutylether β-cyclodextrin.

19. The particles of claim 16, wherein the hygroscopic growth inhibitor is hydroxyethylstarch.

20. The particles of any one of claims 16 to 19, having an emitted dose under ambient conditions of at least 60%.

21. The particles of any one of claims 16 to 20, containing from about 40 percent to about 99 percent by weight of the hygroscopic growth inhibitor.

22. The particles of claim 21, containing from about 40 percent to about 60 percent by weight of the hygroscopic growth inhibitor.

23. The particles of any one of claims 16 to 22, which when delivered pulmonarily, are deposited in the deep lung to an extent greater than 20% of the nominal dose.

24. The particles of any one of claims 16 to 23, wherein said hygroscopic growth inhibitor is effective to increase the bioavailability of said active agent when delivered to the lung by at least 5 percent, when compared to the bioavailability observed for the active agent contained in the same particles absent said hygroscopic growth inhibitor and delivered to the lung.

25. The particles of any one of claims 16 to 24, wherein the active agent comprises a protein or polypeptide.

26. The particles of claim 25, wherein the active agent comprises insulin.

27. The particles of claim 25, wherein the active agent comprises calcitonin.

28. Spray-dried particles of claim 16.

29. Particles for delivery of an active agent to the alveoli of a human patient, said particles comprising the active agent and at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins incorporated within the particles, wherein the particles maintain an aerosol particle size distribution below 3 microns MMAD when delivered to the alveoli.

30. A method for preparing particles for delivery of an active agent to the alveoli of a human patient, comprising:

preparing a mixture of (i) at least about 40% by weight (solids) of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins, (ii) an active agent, and (iii) a solvent;

and spray drying the mixture to obtain homogeneous particles of the hygroscopic growth inhibitor and the active agent, wherein the particles exhibit a drop in emitted dose under simulated lung conditions of no more than about 25%.

31. The method of claim 30, wherein the hygroscopic growth inhibitor is selected from the group consisting of hydroxyethylstarch, dextranomer, and maltodextrins.

32. The method of claim 30, wherein the hygroscopic growth inhibitor is selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and sulfobutylether β-cyclodextrin.

33. The method of claim 30, wherein the hygroscopic growth inhibitor is hydroxyethylstarch.

34. The method of any one of claims 30 to 33, wherein the solvent is water.

35. The method of any one of claims 30 to 34, wherein the homogeneous particles contain from about 40 percent to about 99 percent by weight of the hygroscopic growth inhibitor.

36. The method of claim 35, wherein the homogeneous particles contain from about 40 percent to about 60 percent by weight of the hygroscopic growth inhibitor.

37. The method of any one of claims 30 to 36, wherein the active agent comprises a protein or polypeptide.

38. The method of claim 37, wherein the active agent comprises insulin.

39. The method of claim 37, wherein the active agent comprises calcitonin.

40. Particles prepared by the method of claim 30.

41. Particles of any one of claims 1 to 29, in aerosolized form, for use in delivery of an active agent to the lungs of a human patient.

42. Particles of any one of claims 1 to 29, for use in delivery of an active agent to the lungs of a human patient by means of a dry powder inhaler.

43. A method for delivery of an active agent to the lungs of a human patient, said method comprising administering by inhalation the aerosolized active particles of any one of claims 1 to 29.

44. The method of claim 43, wherein said active agent is administered by means of a dry powder inhaler.

45. A method for increasing the quantity of an inhaled active agent deposited in the deep lung, comprising:

incorporating into active agent-containing dry powder particles for inhalation, at least about 40% by weight of a hygroscopic growth inhibitor selected from the group consisting of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether β-cyclodextrin, hydroxyethylstarch, dextranomer, and maltodextrins, such that, upon aerosolization and inhalation of the particles, at least 20% of the nominal dose is deposited in the deep lung.