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WO 1998/051278 A2 (Inex Pharmaceuticals Corporation), 19 November 1998
WO 1996/040963 A1 (Megabios Corporation), 19 December 1996
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WO 02/053190 A3

(54) Title: PARTICLES FOR INHALATION HAVING SUSTAINED RELEASE PROPERTIES

(57) Abstract: The invention generally relates to a method for pulmonary delivery of therapeutic, prophylactic and diagnostic agents to a patient wherein the agent is released in a sustained fashion, and to particles suitable for use in the method. In particular, the invention relates to a method for the pulmonary delivery of a therapeutic, prophylactic or diagnostic agent comprising administering to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising a therapeutic, prophylactic or diagnostic agent or any combination thereof in association with a charged lipid, wherein the charged lipid has an overall net charge which is opposite to that of the agent upon association with the agent. Release of the agent from the administered particles occurs in a sustained fashion.

PARTICLES FOR INHALATION HAVING SUSTAINED RELEASE
PROPERTIES

BACKGROUND OF THE INVENTION

Pulmonary delivery of bioactive agents, for example, therapeutic, diagnostic and prophylactic agents provides an attractive alternative to, for example, oral, transdermal and parenteral administration. That is, pulmonary administration can typically be completed without the need for medical intervention (self - administration), the pain often associated with injection therapy is avoided, and the amount of enzymatic and pH mediated degradation of the bioactive agent, frequently encountered with oral therapies, can be significantly reduced. In addition, the lungs provide a large mucosal surface for drug absorption and there is no first-pass liver effect of absorbed drugs. Further, it has been shown that high bioavailability of many molecules, for example, macromolecules, can be achieved via pulmonary delivery or inhalation. Typically, the deep lung, or alveoli, is the primary target of inhaled bioactive agents, particularly for agents requiring systemic delivery.

The release kinetics or release profile of a bioactive agent into the local and/or systemic circulation is a key consideration in most therapies, including those employing pulmonary delivery. That is, many illnesses or conditions require administration of a constant or sustained levels of a bioactive agent to provide an effective therapy. Typically, this can be accomplished through a multiple dosing regimen or by employing a system that releases the medicament in a sustained fashion.

However, delivery of bioactive agents to the pulmonary system typically results in rapid release of the agent following administration. For example, U.S. Patent No. 5,997,848 to Patton *et al.* describes the rapid absorption of insulin following administration of a dry powder formulation via pulmonary delivery. The peak insulin level was reached in about 30 minutes for primates and in about 20 minutes for human subjects. Further, Heinemann, Traut and Heise teach in *Diabetic Medicine* 14:63-72 (1997) that the onset of action, assessed by glucose infusion rate,

in healthy volunteers after inhalation was rapid with the half-maximal action reached in about 30 minutes.

As such, a need exists for formulations suitable for inhalation comprising bioactive agents and wherein the bioactive agent of the formulation is released in a 5 sustained fashion into the systemic and/or local circulation.

SUMMARY OF THE INVENTION

This invention is based upon the unexpected discovery that combining a charged agent with a lipid carrying an opposite charge results in a sustained release profile of the agent.

10 The invention generally relates to a method for pulmonary delivery of therapeutic, prophylactic and diagnostic agents to a patient wherein the agent is released in a sustained fashion, and to particles suitable for use in the method. In particular, the invention relates to a method for the pulmonary delivery of a therapeutic, prophylactic or diagnostic agent comprising administering to the 15 respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising a therapeutic, prophylactic or diagnostic agent or any combination thereof in association with a charged lipid, wherein the charged lipid has an overall net charge which is opposite to that of the agent upon association with the agent. Release of the agent from the administered particles 20 occurs in a sustained fashion.

In one embodiment, the association of the therapeutic, prophylactic or diagnostic agent and the oppositely charged lipid can result from ionic 25 complexation. In another embodiment, association of the therapeutic, prophylactic or diagnostic agent and the oppositely charged lipid can result from hydrogen bonding.

In yet a further embodiment, the association of the therapeutic, prophylactic or diagnostic agent and the oppositely charged lipid can result from a combination of ionic complexation and hydrogen bonding.

30 The particles suitable for use in the method can comprise a therapeutic, prophylactic or diagnostic agent in association with a charged lipid having a charge

opposite to that of the agent. The charges are opposite upon association, prior to administration. In a preferred embodiment, the charges of the agent and lipid upon association, prior to administration, are those which the agent and lipid possess at pulmonary pH.

5 For example, the particles suitable for pulmonary delivery can comprise a therapeutic, prophylactic or diagnostic agent which possesses an overall net negative charge, in association with a lipid which possesses an overall net positive charge. For example, the agent can be insulin which has an overall net charge which is negative and the lipid can be 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphatidylcholine
10 (DPePC).

Alternatively, the particles suitable for pulmonary delivery can comprise a therapeutic, prophylactic or diagnostic agent which possesses an overall net positive charge in association with a lipid which possesses an overall net negative charge. For example, the agent can be albuterol which possesses an overall positive charge
15 and the lipid can be 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)](DPPG) which possesses an overall net negative charge.

Further, the particles suitable for pulmonary delivery can comprise a therapeutic, prophylactic or diagnostic agent which has an overall net charge which can be modified by adjusting the pH of a solution of the agent, prior to association
20 with the lipid. For example, at a pH of about 7.4 insulin has an overall net charge which is negative. Therefore, insulin and a positively charged lipid can be associated at this pH prior to administration to prepare a particle having an agent in association with a charged lipid wherein the charged lipid has a charge opposite to that of the agent. However, the charges on insulin can also be modified, when in
25 solution, to possess an overall net charge which is positive by modifying the pH of the solution to be less than the pI of insulin (pI=5.5). As such, when insulin is in solution at a pH of about 4, for example, it will possess an overall net charge which is positive. As this is the case, the positively charged insulin can be associated with a negatively charged lipid, for example, 1,2-distearoyl-*sn*-glycero-3-[phospho-*rac*-
30 (1-glycerol)] (DSPG).

Modification of the charge of the therapeutic, prophylactic or diagnostic agent prior to association with the charged lipid, can be accomplished with many agents, particularly, proteins. For example, charges on proteins can be modulated by spray drying feed solutions below or above the isoelectric points (pI) of the protein.

5 Charge modulation can also be accomplished for small molecules by spray drying feed solutions below or above the pKa of the molecule.

In a particular embodiment, the particles of the invention comprise more than one lipid, more than one bioactive agent or both. Also charged lipids can be combined with lipids without a net charge.

10 The particles, can further comprise a carboxylic acid which is distinct from the bioactive agent and lipid. In one embodiment, the carboxylic acid includes at least two carboxyl groups. Carboxylic acids, include the salts thereof as well as combinations of two or more carboxylic acids and/or salts thereof. In a preferred embodiment, the carboxylic acid is a hydrophilic carboxylic acid or salt thereof.

15 Citric acid and citrates, such as, for example sodium citrate, are preferred. Combinations or mixtures of carboxylic acids and/or their salts also can be employed.

20 The particles suitable for use in the invention can further comprise a multivalent salt or its ionic components. In a preferred embodiment, the salt is a divalent salt. In another preferred embodiment, the salt is a salt of an alkaline-earth metal, such as, for example, calcium chloride. The particles of the invention can also include mixtures or combinations of salts and/or their ionic components.

The particles suitable for use in the invention can further comprise an amino acid. In a preferred embodiment the amino acid is hydrophobic.

25 The particles, also referred to herein as powder, can be in the form of a dry powder suitable for inhalation. The particles can have a tap density of less than about 0.4 g/cm³, preferably less than about 0.1 g/cm³. Further, the particles suitable for use in the invention can have a median geometric diameter of from about 5 micrometers to about 30 micrometers. In yet another embodiment, the particles 30 suitable for use in the invention have an aerodynamic diameter of from about 1 to about 5 micrometers.

The invention has numerous advantages. For example, particles suitable for inhalation can be designed to possess a sustained release profile. This sustained released profile provides for prolonged residence of the administered bioactive agent in the lung and increases the amount of time in which therapeutic levels of the agent 5 are present in the local environment or systemic circulation. The sustained release of agent provides a desirable alternative to injection therapy currently used for many therapeutic, diagnostic and prophylactic agent requiring sustained release of agent, such as insulin for the treatment of diabetes. In addition, the invention provides a method of delivery to the pulmonary system wherein the high initial release of agent 10 typically seen in inhalation therapy is reduced. Consequently, patient compliance and comfort can be increased by not only reducing frequency of dosing, but by providing a therapy which is more amenable to patients.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the *in vivo* release profile of dry powder 15 formulations comprising insulin and either a lipid (DPPC) with no overall net a charge or lipid having an overall net charge opposite to that of insulin (DSePC and DPePC).

FIG. 2 is a graph of the *in vivo* release profile of dry powder formulations comprising insulin in combination with a lipid with no overall net charge (DPPC) or 20 insulin in combination with a charged lipid having an overall negative charge (DPPG) and spray dried with the active agent at either pH 4 or 7.4.

FIG. 3 is a graph showing the *in vivo* release profile of dry powder formulation comprising estrone sulfate (-) and either a lipid with no overall net charge (DPPC) or a lipid having an overall charge opposite (DPePC, +) to that of the 25 estrone sulfate.

FIG. 4 is a graph of percent of PenH above baseline versus time following administration of dry powder formulations of albuterol sulfate and lipid in animals which have been challenged repeatedly over time with methacholine given by nebulization.

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention follows.

Therapeutic, prophylactic or diagnostic agents, can also be referred to herein as "bioactive agents", "medicaments" or "drugs".

10 The invention relates to a method for the pulmonary delivery of therapeutic, prophylactic and diagnostic agents comprising administering to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising a therapeutic, prophylactic or diagnostic agent or any combination thereof in association with a charged lipid, wherein the charged lipid 15 has an overall net charge which is opposite to that of the agent. The agent is released from the administered particles in a sustained fashion.

The particles of the invention release bioactive agent in a sustained fashion. As such, the particles possess sustained release properties. "Sustained release", as that term is used herein, refers to a release of active agent in which the period of 20 release of an effective level of agent is longer than that seen with the same bioactive agent which is not associated with an oppositely charged lipid, prior to administration. In addition, a sustained release also refers to a reduction in the burst of agent typically seen in first two hours following administration, and more preferably in the first hour, often referred to as the initial burst. In a preferred 25 embodiment, the sustained release is characterized by both the period of release being longer in addition to a decreased burst. For example, a sustained release of insulin can be a release showing elevated levels out to at least 4 hours post administration, such as about 6 hours or more.

"Pulmonary delivery", as that term is used herein refers to delivery to the 30 respiratory tract. The "respiratory tract", as defined herein, encompasses the upper

airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by bifurcations into the bronchi and bronchioli (e.g., terminal and respiratory). The upper and lower airways are called the conducting airways. The terminal bronchioli then divide into respiratory bronchioli which then 5 lead to the ultimate respiratory zone, namely, the alveoli, or deep lung. The deep lung, or alveoli, are typically the desired the target of inhaled therapeutic formulations for systemic drug delivery.

In one embodiment, the therapeutic, prophylactic or diagnostic agent and the oppositely charged lipid can be in association primarily as a result of ionic bonding, 10 for example, ionic complexation. In another embodiment, the therapeutic, prophylactic or diagnostic agent and the oppositely charged lipid can be in association primarily as a result of hydrogen bonding. It is understood that a combination of ionic and hydrogen bonding can contribute to the association of the bioactive and charged lipid.

15 Ionic bonding is bonding which occurs via charge/charge interactions between atoms or groups of atoms. Since opposite charges attract, the atoms in an ionic compound are held together by this attraction.

Hydrogen bonding refers to bonding wherein a hydrogen atom is shared 20 between two molecules. For example, a hydrogen atom covalently attached to an electronegative atom such as nitrogen, oxygen, sulfur or phosphorous shares its partial positive charge with a second electronegative atom, for example, nitrogen, oxygen, sulfur or phosphorous.

The particles suitable for use in the method can comprise a therapeutic, prophylactic or diagnostic agent in association with a charged lipid having a charge 25 opposite to that of the agent upon association, prior to administration. In a preferred embodiment, the charges possessed by the agent and lipid, upon association, are the same as the charges which the agent and lipid possess at pulmonary pH following administration.

For example, the particles suitable for pulmonary delivery can comprise a 30 therapeutic, prophylactic or diagnostic agent which possesses an overall net negative charge in association with a lipid which possesses an overall net positive charge.

For example, the agent can be insulin and the lipid can be an ethylphosphatidyl-choline, such as 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphatidylcholine (DPePC).

Alternatively, the particles suitable for pulmonary delivery can comprise a therapeutic, prophylactic or diagnostic agent which possesses an overall net positive 5 charge in association with a lipid which possesses an overall net negative charge, preferably in the pulmonary pH range. For example, the agent can be albuterol sulfate which possesses an overall positive charge and the lipid can be 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DPPG) which possesses an overall net negative charge.

10 Further, the particles suitable for pulmonary delivery can comprise a therapeutic, prophylactic or diagnostic agent which has an overall net charge which can be modified by adjusting the pH of a solution of the agent prior to association with the charged lipid. For example, at a pH of about 7.4 insulin has an overall net charge which is negative. Therefore, insulin and a positively charged lipid can be 15 associated at this pH, prior to administration, to prepare a particle having an bioactive agent in association with a charged lipid wherein the charged lipid has a charge opposite to that of the agent upon association. However, insulin can also be modified when in solution to possess an overall net charge which is positive by modifying the pH of the solution to be less than the pI of insulin (pI=5.5). As such, 20 when insulin is in solution at a pH of 4, for example, it will possess an overall net charge which is positive. As this is the case, the positively charged insulin can be associated with a negatively charged lipid, for example, 1,2-distearoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DSPG). Modification of the charge of the therapeutic, prophylactic or diagnostic agent is applicable to many agents, particularly, proteins.

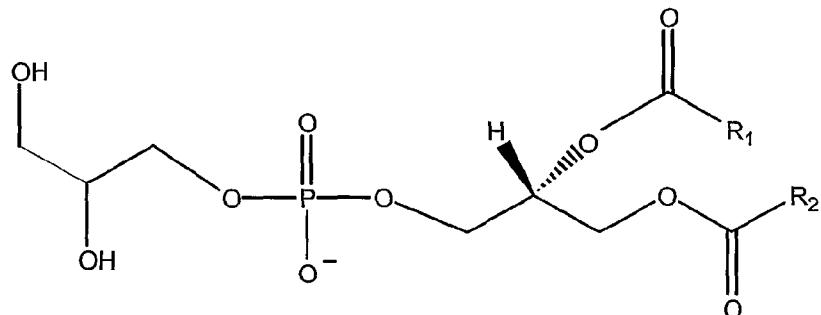
25 "Pulmonary pH range", as that term is used herein, refers to the pH range which can be encountered in the lung of a patient. Typically, in humans, this range of pH is from about 6.4 to about 7.0, such as from 6.4 to about 6.7. pH values of the airway lining fluid (ALF) have been reported in "Comparative Biology of the Normal Lung", CRC Press, (1991) by R.A. Parent and range from 6.44 to 6.74)

30 "Charged lipid" as that term is used herein, refers to lipids which are capable of possessing an overall net charge. The charge on the lipid can be negative or

positive. The lipid can be chosen to have a charge opposite to that of the active agent when the lipid and active agent are associated. In a preferred embodiment the charged lipid is a charged phospholipid. Preferably, the phospholipid is endogenous to the lung or can be metabolized upon administration to a lung endogenous 5 phospholipid. Combinations of charged lipids can be used. The combination of charged lipid also has an overall net charge opposite to that of the bioactive agent upon association.

10 The charged phospholipid can be a negatively charged lipid such as, a 1,2-diacyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] and a 1,2-diacyl-*sn*-glycerol-3-phosphate.

The 1,2-diacyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] phospholipids can be represented by the Formula I:



15 wherein R₁ and R₂ are independently aliphatic groups having from about 3 to about 24 carbon atoms, preferably from about 10 to about 20 carbon atoms.

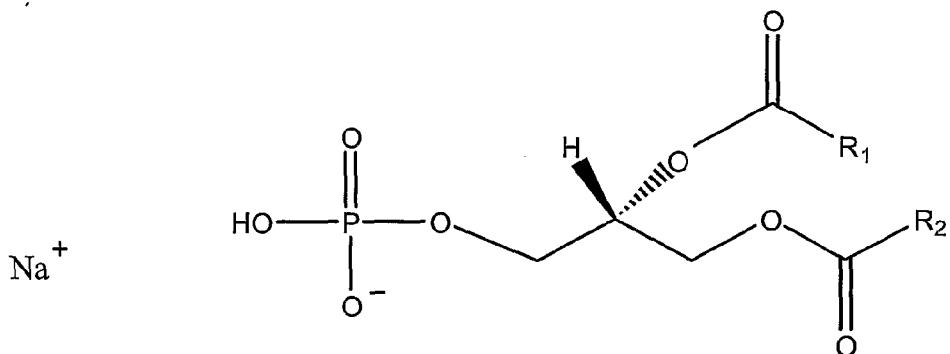
20 Aliphatic group as that term is used herein in Formulas I-VI refers to substituted or unsubstituted straight chained, branched or cyclic C₁-C₂₄ hydrocarbons which can be completely saturated, which can contain one or more heteroatoms such as nitrogen, oxygen or sulfur and/or which can contain one or more units of unsaturation.

Suitable substituents on an aliphatic group include -OH, halogen (-Br, -Cl, -I and -F) -O(aliphatic, substituted), -CN, -NO₂, -COOH, -NH₂, -NH(aliphatic group, substituted aliphatic), -N(aliphatic group, substituted aliphatic group)₂, -COO

(aliphatic group, substituted aliphatic group), -CONH₂, -CONH(aliphatic, substituted aliphatic group), -SH, -S(aliphatic, substituted aliphatic group) and -NH-C(=NH)-NH₂. A substituted aliphatic group can also have a benzyl, substituted benzyl, aryl (e.g., phenyl, naphthyl or pyridyl) or substituted aryl group as a substituent. A substituted aliphatic can have one or more substituents.

5 Specific examples of this type of negatively charged phospholipid include, but are not limited to, 1,2-distearoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DSPG), 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DMPG), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)] (DPPG), 1,2-dilauroyl-*sn*-10 glycero-3-[phospho-*rac*-(1-glycerol)] (DLPG), and 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DOPG).

The 1,2-diacyl-*sn*-glycerol-3-phosphate phospholipids can be represented by the Formula II:



15 R₁ and R₂ are independently an aliphatic group having from about 3 to about 24 carbon atoms, preferably from about 10 to about 20 carbon atoms.

Specific examples of this type of phospholipid include, but are not limited to, 1,2-dimyristoyl-*sn*-glycero-3-phosphate (DMPA), 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA), 1,2-dioleoyl-*sn*-glycero-3-phosphate (DOPA), 1,2-distearoyl-*sn*-glycero-3-phosphate (DSPA), and 1,2-dilauroyl-*sn*-glycero-3-phosphate (DLPA).

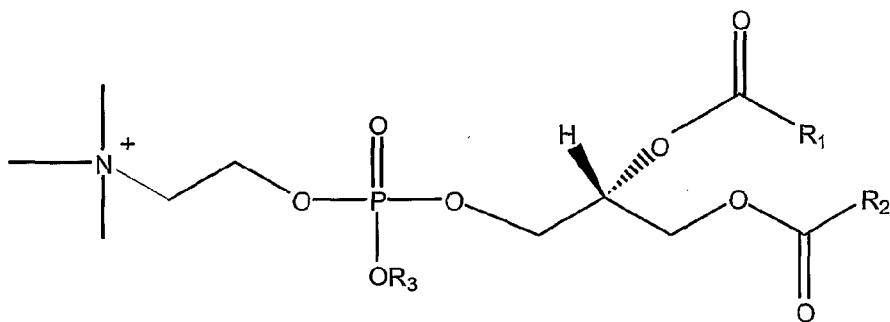
The charged lipid can be a positively charged lipid such as a 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine, such as 1,2-diacyl-*sn*-glycero-3-ethylphospho-

-11-

choline and a 1,2-diacyl-*sn*-glycero-3-alkylphosphoalkanolamine, such as 1,2-diacyl-*sn*-glycero-3-alkylphosphoethanolamine.

The 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine phospholipids can be represented by the Formula III:

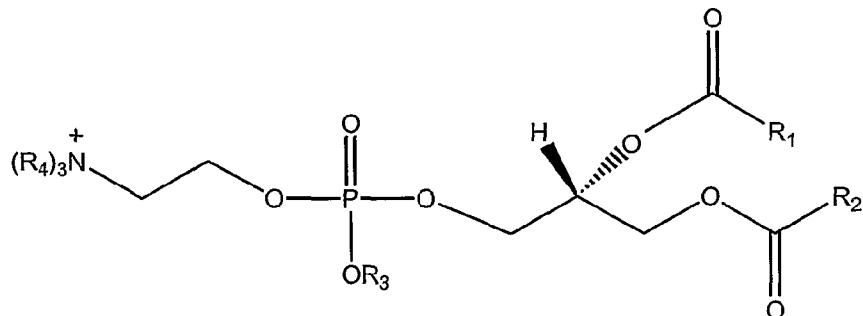
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wherein R₁ and R₂ are independently an aliphatic group having from about 3 to about 24 carbon atoms, preferably from about 10 to about 20 carbon atoms. R₃ is an aliphatic group having from about 1 to about 24 carbons, for example, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl and the like.

10 Specific examples of this type of positively charged phospholipid include, but are not limited to, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine(DPePC), 1,2-dimyristoyl-*sn*-glycero-3-ethylphosphocholine(DMePC), 1,2-distearoyl-*sn*-glycero-3-ethylphosphocholine(DSePC), 1,2-dilauroyl-*sn*-glycero-3-ethylphosphocholine (DLePC), and 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine(DOePC).

15 The 1,2-diacyl-*sn*-glycero-3-alkylphosphoalkanolamine phospholipids can be represented by the Formula IV:



-12-

wherein R₁ and R₂ are independently an aliphatic group having from about 3 to about 24 carbon atoms, preferably from about 10 to about 20 carbon atoms. R₃ is an aliphatic group having from about 1 to about 24 carbons, for example, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl and the like. R₄ is independently hydrogen, 5 or an aliphatic group having from about 1 to about 6 carbon atoms.

Specific examples of this type of positively charged phospholipid include, but are not limited to, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphospholipid (DPePE), 1,2-dimyristoyl-*sn*-glycero-3-ethylphospholipid (DMePE), 1,2-distearoyl-*sn*-glycero-3-ethylphospholipid (DSePE), 1,2-dilauroyl-*sn*-glycero-3-ethylphospholipid (DLePE), and 1,2-dioleoyl-*sn*-glycero-3-ethylphospholipid (DOePE).

Other charged lipids suitable for use in the invention include those described in U.S. Patent No. 5,466,841 to Horrobin *et al.* issued on November 14, 1995, U. S. Patent Nos. 5,698,721 and 5,902,802 to Heath issued December 16, 1997 and May 15 11, 1999, respectively, and U.S. Patent No. 4,480,041 to Myles *et al.* issued October 30, 1984, the entire contents of all of which are incorporated herein by reference.

The charged lipid and the therapeutic, prophylactic or diagnostic agent can be present in the particles of the invention at a charge ratio of lipid to active of from about 0.25:1 or more, preferably from about 0.25:1 to about 1:0.25, for example, 20 about 0.5:1 to about 1:0.5. Preferably the charge ratio is about 1:1. When an excess of charge is present, it is preferred that the excess charge is contributed by the lipid.

A suitable charge ratio can be determined as follows. First, the number of charges present on both the bioactive agent and lipid, at the conditions under which association of the two will occur, prior to administration, should be determined. 25 Next, the equivalent weight of both the bioactive agent and lipid should be determined. This can be carried out following the example below employing insulin as the bioactive agent and DPePC as the charged lipid at a pH of about 7.4.

	Molecular Weight of Insulin:	5,800 g/mole
	Number of Negative Charges on Insulin:	6 equivalent
30	Equivalent Weight Per Charge:	5,800 x 1/6 = 967 g

Molecular Weight of DPePC:	763 g/mole
Number of Negative Charges on DPePC:	1 equivalent
Equivalent Weight Per Charge:	$763 \times 1/1 = 763$ g

Therefore, to obtain for example, a 1:1 charge ratio of DPePC to insulin

5 763 g DPePC is associated with 967 g insulin

OR

1 g DPePC is associated with 1.27 ($967/763 = 1.27$) g insulin.

Alternatively,

967 g insulin is associated with 763 g DPePC

10 OR

1 g insulin is associated with 0.79 ($763/967 = 0.79$) g DPePC.

In molar terms,

1 mole DPePC is associated with 1/6 mole insulin

OR

15 1 mole insulin is associated with 6 moles DPePC .

This analysis can be used to determine the amount of lipid and active agent needed for any ratio desired and any combination of bioactive agent and lipid.

16 The charged lipid can be present in the particles in an amount ranging from about 1 to about 99 % by weight. Preferably, the charged lipid is present in the particles in an amount ranging from about 10% to about 90% by weight.

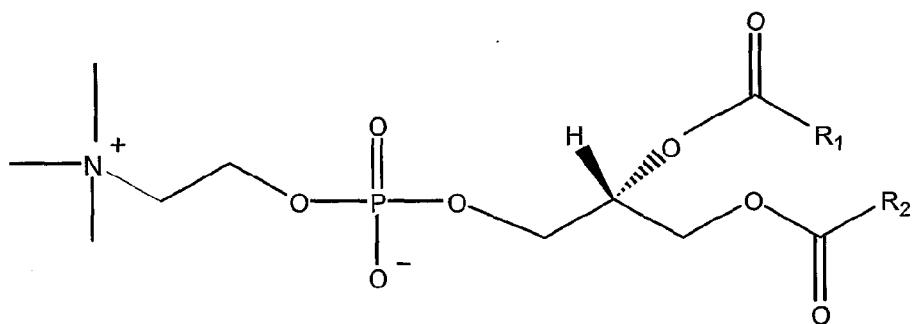
17 The particles of the invention can also comprise phospholipids, which are zwitterionic and therefore do not possess an overall net charge. Such lipids, can assist in providing particles with the proper characteristics for inhalation. Such phospholipids suitable for use in the invention include, but are not limited to, a 1,2-diacyl-*sn*-glycero-3-phosphocholine and a 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine. These lipids can preferably be present in the particles in an amount ranging from about 10% to about 90% by weight. Preferably, these lipids

-14-

can be present in the particles in an amount ranging from about 50% to about 80% by weight.

The 1,2-diacyl-*sn*-glycero-3-phosphocholine phospholipids can be represented by Formula V:

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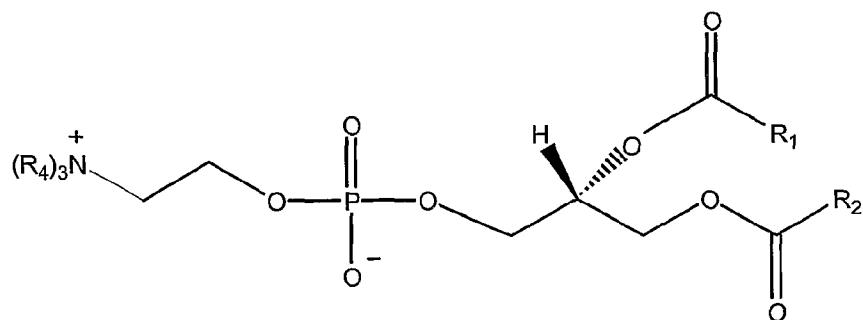


R₁ and R₂ are independently an aliphatic group having from about 3 to about 24 carbon atoms, preferably from about 10 to about 20 carbon atoms.

Specific examples of 1,2-diacyl-*sn*-glycero-3-phosphocholine phospholipids include, but are not limited to, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dilauroyl-*sn*-3-glycero-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC),

The 1,2-diacyl-*sn*-glycero-3-phosphoalkanolamine phospholipids can be represented by Formula VI:

15



wherein R₁ and R₂ are independently an aliphatic group having from about 3 to about 24 carbon atoms, preferably, from about 10 to about 20 carbon atoms and R₄ is independently hydrogen or an aliphatic group having from about 1 to about 6 carbon atoms.

5 Specific examples of this type of phospholipid include, but are not limited to, 1,2-dipalmitoyl-*sn*-glycero-3-ethanolamine(DPPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine(DMPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine(DSPE), 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).

10 Therapeutic, prophylactic or diagnostic agents, can also be referred to herein as "bioactive agents", "medicaments" or "drugs". It is understood that one or more bioactive agents can be present in the particles of the invention. Hydrophilic as well as hydrophobic agents can be used. The agent must be capable of possessing an overall net charge. The amount of bioactive agent present in the particles of the 15 invention can be from about 0.1 weight % to about 95 weight %, for example, from about 5 to about 75%, such as from about 10 to about 50%. Particles in which the drug is distributed throughout a particle are preferred.

20 Suitable bioactive agents include agents which can act locally, systemically or a combination thereof. The term "bioactive agent," as used herein, is an agent, or its pharmaceutically acceptable salt, which when released *in vivo*, possesses the desired biological activity, for example therapeutic, diagnostic and/or prophylactic properties *in vivo*.

25 Examples of bioactive agent include, but are not limited to, synthetic inorganic and organic compounds, proteins and peptides, polysaccharides and other sugars, lipids, and DNA and RNA nucleic acid sequences having therapeutic, prophylactic or diagnostic activities. Agents with a wide range of molecular weight can be used, for example, between 100 and 500,000 grams or more per mole.

30 The agents can have a variety of biological activities, such as vasoactive agents, neuroactive agents, hormones, anticoagulants, immunomodulating agents, cytotoxic agents, prophylactic agents, antibiotics, antivirals, antisense, antigens, antineoplastic agents and antibodies.

Proteins, include complete proteins, muteins and active fragments thereof, such as insulin, immunoglobulins, antibodies, cytokines (e.g., lymphokines, monokines, chemokines), interleukins, interferons (β -IFN, α -IFN and γ -IFN), erythropoietin, nucleases, tumor necrosis factor, colony stimulating factors, enzymes 5 (e.g. superoxide dismutase, tissue plasminogen activator), tumor suppressors, blood proteins, hormones and hormone analogs (e.g., growth hormone, adrenocorticotropic hormone and luteinizing hormone releasing hormone (LHRH)), vaccines (e.g., tumoral, bacterial and viral antigens), antigens, blood coagulation factors; growth factors; granulocyte colony-stimulating factor ("G-CSF"); peptides include protein 10 inhibitors, protein antagonists, and protein agonists, calcitonin; nucleic acids include, for example, antisense molecules, oligonucleotides, and ribozymes. Polysaccharides, such as heparin, can also be administered.

Bioactive agent for local delivery within the lung, include such as agents as those for the treatment of asthma, chronic obstructive pulmonary disease (COPD), 15 emphysema, or cystic fibrosis. For example, genes for the treatment of diseases such as cystic fibrosis can be administered, as can beta agonists steroids, anticholinergics, and leukotriene modifiers for asthma.

Other specific bioactive agents include, estrone sulfate, albuterol sulfate, parathyroid hormone-related peptide, somatostatin, nicotine, clonidine, salicylate, 20 cromolyn sodium, salmeterol, formeterol, L-dopa, Carbidopa or a combination thereof, gabapentin, clorazepate, carbamazepine and diazepam.

Nucleic acid sequences include genes, antisense molecules which can, for instance, bind to complementary DNA to inhibit transcription, and ribozymes.

The particles can include any of a variety of diagnostic agents to locally or 25 systemically deliver the agents following administration to a patient. For example, imaging agents which include commercially available agents used in positron emission tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI) can be employed.

30 Examples of suitable materials for use as contrast agents in MRI include the gadolinium chelates currently available, such as diethylene triamine pentacetic acid

(DTPA) and gadopentotate dimeglumine, as well as iron, magnesium, manganese, copper and chromium.

Examples of materials useful for CAT and x-rays include iodine based materials for intravenous administration, such as ionic monomers typified by 5 diatrizoate and iothalamate and ionic dimers, for example, ioxagalte.

Diagnostic agents can be detected using standard techniques available in the art and commercially available equipment.

The particles can further comprise a carboxylic acid which is distinct from the agent and lipid. In one embodiment, the carboxylic acid includes at least two 10 carboxyl groups. Carboxylic acids, include the salts thereof as well as combinations of two or more carboxylic acids and/or salts thereof. In a preferred embodiment, the carboxylic acid is a hydrophilic carboxylic acid or salt thereof. Suitable carboxylic acids include but are not limited to hydroxydicarboxylic acids, hydroxytricarboxylic acids and the like. Citric acid and citrates, such as, for example sodium citrate, are 15 preferred. Combinations or mixtures of carboxylic acids and/or their salts also can be employed.

The carboxylic acid can be present in the particles in an amount ranging from about 0 to about 80% weight. Preferably, the carboxylic acid can be present in the particles in an amount of about 10 to about 20%.

20 The particles suitable for use in the invention can further comprise a multivalent salt or its ionic components. As used herein, a "multivalent" salt refers to salts having a ionic component with a valency greater than one. For example, divalent salts. In a preferred embodiment, the salt is a divalent salt. In another preferred embodiment, the salt is a salt of an alkaline-earth metal, such as, for 25 example, calcium chloride. The particles of the invention can also include mixtures or combinations of salts and/or their ionic components.

The salt or its ionic components are present in the particles in an amount ranging from about 0 to about 40% weight.

30 The particles suitable for use in the invention can further comprise an amino acid. In a preferred embodiment the amino acid is hydrophobic. Suitable naturally occurring hydrophobic amino acids, include but are not limited to, leucine,

isoleucine, alanine, valine, phenylalanine, glycine and tryptophan. Combinations of hydrophobic amino acids can also be employed. Non-naturally occurring amino acids include, for example, beta-amino acids. Both D, L configurations and racemic mixtures of hydrophobic amino acids can be employed. Suitable hydrophobic amino acids can also include amino acid derivatives or analogs. As used herein, an amino acid analog includes the D or L configuration of an amino acid having the following formula: -NH-CHR-CO-, wherein R is an aliphatic group, a substituted aliphatic group, a benzyl group, a substituted benzyl group, an aromatic group or a substituted aromatic group and wherein R does not correspond to the side chain of a naturally-occurring amino acid. As used herein, aliphatic groups include straight chained, branched or cyclic C1-C8 hydrocarbons which are completely saturated, which contain one or two heteroatoms such as nitrogen, oxygen or sulfur and/or which contain one or more units of unsaturation. Aromatic or aryl groups include carbocyclic aromatic groups such as phenyl and naphthyl and heterocyclic aromatic groups such as imidazolyl, indolyl, thienyl, furanyl, pyridyl, pyranyl, oxazolyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl and acridinyl.

Suitable substituents on an aliphatic, aromatic or benzyl group include -OH, halogen (-Br, -Cl, -I and -F) -O(aliphatic, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), -CN, -NO₂, -COOH, -NH₂, -NH(aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), -N(aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group)₂, -COO(aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), -CONH₂, -CONH(aliphatic, substituted aliphatic group, benzyl, substituted benzyl, aryl or substituted aryl group)), -SH, -S(aliphatic, substituted aliphatic, benzyl, substituted benzyl, aromatic or substituted aromatic group) and -NH-C(=NH)-NH₂. A substituted benzylic or aromatic group can also have an aliphatic or substituted aliphatic group as a substituent. A substituted aliphatic group can also have a benzyl, substituted benzyl, aryl or substituted aryl group as a substituent. A substituted aliphatic, substituted aromatic or substituted benzyl group can have one or more substituents. Modifying

an amino acid substituent can increase, for example, the hydrophilicity or hydrophobicity of natural amino acids which are hydrophilic.

A number of the suitable amino acids, amino acids analogs and salts thereof can be obtained commercially. Others can be synthesized by methods known in the art. Synthetic techniques are described, for example, in Green and Wuts, *"Protecting Groups in Organic Synthesis"*, John Wiley and Sons, Chapters 5 and 7, 1991.

Hydrophobicity is generally defined with respect to the partition of an amino acid between a nonpolar solvent and water. Hydrophobic amino acids are those 10 acids which show a preference for the nonpolar solvent. Relative hydrophobicity of amino acids can be expressed on a hydrophobicity scale on which glycine has the value 0.5. On such a scale, amino acids which have a preference for water have values below 0.5 and those that have a preference for nonpolar solvents have a value above 0.5. As used herein, the term hydrophobic amino acid refers to an amino acid 15 that, on the hydrophobicity scale has a value greater or equal to 0.5, in other words, has a tendency to partition in the nonpolar acid which is at least equal to that of glycine.

Examples of amino acids which can be employed include, but are not limited to: glycine, proline, alanine, cysteine, methionine, valine, leucine, tyrosine, 20 isoleucine, phenylalanine, tryptophan. Preferred hydrophobic amino acids include leucine, isoleucine, alanine, valine, phenylalanine, glycine and tryptophan. Combinations of hydrophobic amino acids can also be employed. Furthermore, 25 combinations of hydrophobic and hydrophilic (preferentially partitioning in water) amino acids, where the overall combination is hydrophobic, can also be employed.

Combinations of one or more amino acids can also be employed.

The amino acid can be present in the particles of the invention in an amount from about 0% to about 60 weight %. Preferably, the amino acid can be present in the particles in an amount ranging from about 5 to about 30 weight %. The salt of a hydrophobic amino acid can be present in the particles of the invention in an amount 30 of from about 0% to about 60 weight %. Preferably, the amino acid salt is present in the particles in an amount ranging from about 5 to about 30 weight %. Methods of

forming and delivering particles which include an amino acid are described in U.S. Patent Application No 09/382,959, filed on August 25, 1999, entitled Use of Simple Amino Acids to Form Porous Particles During Spray Drying the entire teaching of which is incorporated herein by reference.

5 In a further embodiment, the particles can also include other materials such as, for example, buffer salts, dextran, polysaccharides, lactose, trehalose, cyclodextrins, proteins, peptides, polypeptides, fatty acids, fatty acid esters, inorganic compounds, phosphates.

10 In one embodiment of the invention, the particles can further comprise polymers. The use of polymers can further prolong release. Biocompatible or biodegradable polymers are preferred. Such polymers are described, for example, in U.S. Patent No. 5,874,064, issued on February 23, 1999 to Edwards *et al.*, the teachings of which are incorporated herein by reference in their entirety.

15 In yet another embodiment, the particles include a surfactant other than one of the charged lipids described above. As used herein, the term "surfactant" refers to any agent which preferentially absorbs to an interface between two immiscible phases, such as the interface between water and an organic polymer solution, a water/air interface or organic solvent/air interface. Surfactants generally possess a hydrophilic moiety and a lipophilic moiety, such that, upon absorbing to 20 microparticles, they tend to present moieties to the external environment that do not attract similarly-coated particles, thus reducing particle agglomeration. Surfactants may also promote absorption of a therapeutic or diagnostic agent and increase bioavailability of the agent.

25 Suitable surfactants which can be employed in fabricating the particles of the invention include but are not limited to hexadecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; glycocholate; surfactin; a poloxamer; a sorbitan fatty acid ester such as sorbitan trioleate (Span 85); and tyloxapol.

30 The surfactant can be present in the particles in an amount ranging from about 0 to about 60 weight %. Preferably, it can be present in the particles in an amount ranging from about 5 to about 50 weight %.

It is understood that when the particles includes a carboxylic acid, a multivalent salt, an amino acid, a surfactant or any combination thereof that interaction between these components of the particle and the charged lipid can occur.

5 The particles, also referred to herein as powder, can be in the form of a dry powder suitable for inhalation. In a particular embodiment, the particles can have a tap density of less than about 0.4 g/cm³. Particles which have a tap density of less than about 0.4 g/cm³ are referred to herein as "aerodynamically light particles". More preferred are particles having a tap density less than about 0.1 g/cm³.

10 Aerodynamically light particles have a preferred size, e.g., a volume median geometric diameter (VMGD) of at least about 5 microns (μm). In one embodiment, the VMGD is from about 5 μm to about 30 μm . In another embodiment of the invention, the particles have a VMGD ranging from about 9 μm to about 30 μm . In other embodiments, the particles have a median diameter, mass median diameter 15 (MMD), a mass median envelope diameter (MMED) or a mass median geometric diameter (MMGD) of at least 5 μm , for example from about 5 μm to about 30 μm .

Aerodynamically light particles preferably have "mass median aerodynamic diameter" (MMAD), also referred to herein as "aerodynamic diameter", between about 1 μm and about 5 μm . In one embodiment of the invention, the MMAD is 20 between about 1 μm and about 3 μm . In another embodiment, the MMAD is between about 3 μm and about 5 μm .

In another embodiment of the invention, the particles have an envelope mass density, also referred to herein as "mass density" of less than about 0.4 g/cm³. The envelope mass density of an isotropic particle is defined as the mass of the particle 25 divided by the minimum sphere envelope volume within which it can be enclosed.

Tap density can be measured by using instruments known to those skilled in the art such as the Dual Platform Microprocessor Controlled Tap Density Tester (Vankel, NC) or a GeoPyc™ instrument (Micrometrics Instrument Corp., Norcross, GA 30093). Tap density is a standard measure of the envelope mass density. Tap 30 density can be determined using the method of USP Bulk Density and Tapped Density, United States Pharmacopia convention, Rockville, MD, 10th Supplement,

4950-4951, 1999. Features which can contribute to low tap density include irregular surface texture and porous structure.

The diameter of the particles, for example, their VMGD, can be measured using an electrical zone sensing instrument such as a Multisizer IIe, (Coulter 5 Electronic, Luton, Beds, England), or a laser diffraction instrument (for example Helos, manufactured by Sympatec, Princeton, NJ). Other instruments for measuring particle diameter are well known in the art. The diameter of particles in a sample will range depending upon factors such as particle composition and methods of synthesis. The distribution of size of particles in a sample can be selected to permit 10 optimal deposition within targeted sites within the respiratory tract.

Experimentally, aerodynamic diameter can be determined by employing a gravitational settling method, whereby the time for an ensemble of particles to settle a certain distance is used to infer directly the aerodynamic diameter of the particles. An indirect method for measuring the mass median aerodynamic diameter (MMAD) 15 is the multi-stage liquid impinger (MSLI).

The aerodynamic diameter, d_{aer} , can be calculated from the equation:

$$d_{aer} = d_g \sqrt{\rho_{tap}}$$

where d_g is the geometric diameter, for example the MMGD and ρ is the powder density.

20 Particles which have a tap density less than about 0.4 g/cm³, median diameters of at least about 5 μ m, and an aerodynamic diameter of between about 1 μ m and about 5 μ m, preferably between about 1 μ m and about 3 μ m, are more capable of escaping inertial and gravitational deposition in the oropharyngeal region, and are targeted to the airways or the deep lung. The use of larger, more porous 25 particles is advantageous since they are able to aerosolize more efficiently than smaller, denser aerosol particles such as those currently used for inhalation therapies.

In comparison to smaller particles the larger aerodynamically light particles, preferably having a VMGD of at least about 5 μ m, also can potentially more successfully avoid phagocytic engulfment by alveolar macrophages and clearance

from the lungs, due to size exclusion of the particles from the phagocytes' cytosolic space. Phagocytosis of particles by alveolar macrophages diminishes precipitously as particle diameter increases beyond about 3 μm . Kawaguchi, H., *et al.*, *Biomaterials* 7: 61-66 (1986); Krenis, L.J. and Strauss, B., *Proc. Soc. Exp. Med.*, 5 *107*: 748-750 (1961); and Rudt, S. and Muller, R.H., *J. Contr. Rel.*, 22: 263-272 (1992). For particles of statistically isotropic shape, such as spheres with rough surfaces, the particle envelope volume is approximately equivalent to the volume of cytosolic space required within a macrophage for complete particle phagocytosis.

The particles may be fabricated with the appropriate material, surface 10 roughness, diameter and tap density for localized delivery to selected regions of the respiratory tract such as the deep lung or upper or central airways. For example, higher density or larger particles may be used for upper airway delivery, or a mixture of varying sized particles in a sample, provided with the same or different therapeutic agent may be administered to target different regions of the lung in one 15 administration. Particles having an aerodynamic diameter ranging from about 3 to about 5 μm are preferred for delivery to the central and upper airways. Particles having an aerodynamic diameter ranging from about 1 to about 3 μm are preferred for delivery to the deep lung.

Inertial impaction and gravitational settling of aerosols are predominant 20 deposition mechanisms in the airways and acini of the lungs during normal breathing conditions. Edwards, D.A., *J. Aerosol Sci.*, 26: 293-317 (1995). The importance of both deposition mechanisms increases in proportion to the mass of aerosols and not to particle (or envelope) volume. Since the site of aerosol deposition in the lungs is determined by the mass of the aerosol (at least for particles of mean aerodynamic 25 diameter greater than approximately 1 μm), diminishing the tap density by increasing particle surface irregularities and particle porosity permits the delivery of larger particle envelope volumes into the lungs, all other physical parameters being equal.

The low tap density particles have a small aerodynamic diameter in 30 comparison to the actual envelope sphere diameter. The aerodynamic diameter, d_{aer} , is related to the envelope sphere diameter, d (Gonda, I., "Physico-chemical

principles in aerosol delivery," in *Topics in Pharmaceutical Sciences 1991* (eds. D.J.A. Crommelin and K.K. Midha), pp. 95-117, Stuttgart: Medpharm Scientific Publishers, 1992), by the formula:

$$d_{\text{aer}} = d\sqrt{\rho}$$

5 where the envelope mass ρ is in units of g/cm³. Maximal deposition of monodispersed aerosol particles in the alveolar region of the human lung (~60%) occurs for an aerodynamic diameter of approximately $d_{\text{aer}} = 3 \mu\text{m}$. Heyder, J. *et al.*, *J. Aerosol Sci.*, 17: 811-825 (1986). Due to their small envelope mass density, the actual diameter d of aerodynamically light particles comprising a monodisperse 10 inhaled powder that will exhibit maximum deep-lung deposition is:

$$d = 3/\sqrt{\rho} \mu\text{m} \text{ (where } \rho < 1 \text{ g/cm}^3\text{);}$$

where d is always greater than 3 μm . For example, aerodynamically light particles that display an envelope mass density, $\rho = 0.1 \text{ g/cm}^3$, will exhibit a maximum 15 deposition for particles having envelope diameters as large as 9.5 μm . The increased particle size diminishes interparticle adhesion forces. Visser, J., *Powder Technology*, 58: 1-10. Thus, large particle size increases efficiency of aerosolization to the deep lung for particles of low envelope mass density, in addition to contributing to lower phagocytic losses.

20 The aerodynamic diameter can be calculated to provide for maximum deposition within the lungs, previously achieved by the use of very small particles of less than about five microns in diameter, preferably between about one and about three microns, which are then subject to phagocytosis. Selection of particles which have a larger diameter, but which are sufficiently light (hence the characterization 25 "aerodynamically light"), results in an equivalent delivery to the lungs, but the larger size particles are not phagocytosed. Improved delivery can be obtained by using particles with a rough or uneven surface relative to those with a smooth surface.

Suitable particles can be fabricated or separated, for example by filtration or centrifugation, to provide a particle sample with a preselected size distribution. For example, greater than about 30%, 50%, 70%, or 80% of the particles in a sample can have a diameter within a selected range of at least about 5 μm . The selected range 5 within which a certain percentage of the particles must fall may be for example, between about 5 and about 30 μm , or optimally between about 5 and about 15 μm . In one preferred embodiment, at least a portion of the particles have a diameter between about 9 and about 11 μm . Optionally, the particle sample also can be fabricated wherein at least about 90%, or optionally about 95% or about 99%, have a 10 diameter within the selected range. The presence of the higher proportion of the aerodynamically light, larger diameter particles in the particle sample enhances the delivery of therapeutic or diagnostic agents incorporated therein to the deep lung. Large diameter particles generally mean particles having a median geometric diameter of at least about 5 μm .

15 The particles can be prepared by spray drying. For example, a spray drying mixture, also referred to herein as "feed solution" or "feed mixture", which includes the bioactive agent and one or more charged lipids having a charge opposite to that of the active agent upon association are fed to a spray dryer.

For example, when employing a protein active agent, the agent may be 20 dissolved in a buffer system above or below the pI of the agent. Specifically, insulin for example may be dissolved in an aqueous buffer system (e.g., citrate, phosphate, acetate, etc.) or in 0.01 N HCl. The pH of the resultant solution then can be adjusted to a desired value using an appropriate base solution (e.g., 1 N NaOH). In one preferred embodiment, the pH may be adjusted to about pH 7.4. At this pH 25 insulin molecules have a net negative charge (pI = 5.5). In another embodiment, the pH may be adjusted to about pH 4.0. At this pH insulin molecules have a net positive charge (pI = 5.5). Typically the cationic phospholipid is dissolved in an organic solvent or combination of solvents. The two solutions are then mixed together and the resulting mixture is spray dried.

30 For a small molecule active agent, the agent may be dissolved in a buffer system above or below the pKa of the ionizable group(s). Specifically, albuterol

sulfate or estrone sulfate, for example, can be dissolved in an aqueous buffer system (e.g., citrate, phosphate, acetate, etc.) or in sterile water for irrigation. The pH of the resultant solution then can be adjusted to a desired value using an appropriate acid or base solution. If the pH is adjusted to about pH 3 to about pH 8 range, estrone sulfate will possess one negative charge per molecule and albuterol sulfate will possess one positive charge per molecule. Therefore, charge interaction can be engineered by the choice of an appropriate phospholipid. Typically the negatively charged or the positively charged phospholipid is dissolved in an organic solvent or combination of solvents and the two solutions are then mixed together and the resulting mixture is spray dried.

Suitable organic solvents that can be present in the mixture being spray dried include, but are not limited to, alcohols for example, ethanol, methanol, propanol, isopropanol, butanols, and others. Other organic solvents include, but are not limited to, perfluorocarbons, dichloromethane, chloroform, ether, ethyl acetate, methyl tert-butyl ether and others. Aqueous solvents that can be present in the feed mixture include water and buffered solutions. Both organic and aqueous solvents can be present in the spray-drying mixture fed to the spray dryer. In one embodiment, an ethanol water solvent is preferred with the ethanol:water ratio ranging from about 50:50 to about 90:10. The mixture can have a , acidic or alkaline pH. Optionally, a pH buffer can be included. Preferably, the pH can range from about 3 to about 10.

The total amount of solvent or solvents being employed in the mixture being spray dried generally is greater than 99 weight percent. The amount of solids (drug, charged lipid and other ingredients) present in the mixture being spray dried generally is less than about 1.0 weight percent. Preferably, the amount of solids in the mixture being spray dried ranges from about 0.05% to about 0.5% by weight.

Using a mixture which includes an organic and an aqueous solvent in the spray drying process allows for the combination of hydrophilic and hydrophobic components, while not requiring the formation of liposomes or other structures or complexes to facilitate solubilization of the combination of such components within the particles.

Suitable spray-drying techniques are described, for example, by K. Masters in "Spray Drying Handbook", John Wiley & Sons, New York, 1984. Generally, during spray-drying, heat from a hot gas such as heated air or nitrogen is used to evaporate the solvent from droplets formed by atomizing a continuous liquid feed.

5 Other spray-drying techniques are well known to those skilled in the art. In a preferred embodiment, a rotary atomizer is employed. An example of a suitable spray dryer using rotary atomization includes the Mobile Minor spray dryer, manufactured by Niro, Denmark. The hot gas can be, for example, air, nitrogen or argon.

10 Preferably, the particles of the invention are obtained by spray drying using an inlet temperature between about 100° C and about 400° C and an outlet temperature between about 50°C and about 130°C.

15 The spray dried particles can be fabricated with a rough surface texture to reduce particle agglomeration and improve flowability of the powder. The spray-dried particle can be fabricated with features which enhance aerosolization via dry powder inhaler devices, and lead to lower deposition in the mouth, throat and inhaler device.

20 The particles of the invention can be employed in compositions suitable for drug delivery via the pulmonary system. For example, such compositions can include the particles and a pharmaceutically acceptable carrier for administration to a patient, preferably for administration via inhalation. The particles can be co-delivered with larger carrier particles, not including a therapeutic agent, the latter possessing mass median diameters for example in the range between about 50 μm and about 100 μm . The particles can be administered alone or in any appropriate 25 pharmaceutically acceptable carrier, such as a liquid, for example saline, or a powder, for administration to the respiratory system.

30 Particles including a medicament, for example one or more of the drugs listed above, are administered to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis. Administration of particles to the respiratory system can be by means such as known in the art. For example, particles are delivered from an inhalation device. In a preferred embodiment, particles are

administered via a dry powder inhaler (DPI). Metered-dose-inhalers (MDI), nebulizers or instillation techniques also can be employed.

Various suitable devices and methods of inhalation which can be used to administer particles to a patient's respiratory tract are known in the art. For example, suitable inhalers are described in U.S. Patent No. 4,069,819, issued August 5, 1976 to Valentini, *et al.*, U.S. Patent No. 4,995,385 issued February 26, 1991 to Valentini, *et al.*, and U.S. Patent No. 5,997,848 issued December 7, 1999 to Patton, *et al.* Various suitable devices and methods of inhalation which can be used to administer particles to a patient's respiratory tract are known in the art. For example, suitable inhalers are described in U.S. Patent Nos. 4,995,385, and 4,069,819 issued to Valentini, *et al.*, U.S. Patent No. 5,997,848 issued to Patton. Other examples include, but are not limited to, the Spinhaler® (Fisons, Loughborough, U.K.), Rotahaler® (Glaxo-Wellcome, Research Triangle Technology Park, North Carolina), FlowCaps® (Hovione, Loures, Portugal), Inhalator® (Boehringer-Ingelheim, Germany), and the Aerolizer® (Novartis, Switzerland), the diskhaler (Glaxo-Wellcome, RTP, NC) and others, such as known to those skilled in the art. Preferably, the particles are administered as a dry powder via a dry powder inhaler.

Preferably, particles administered to the respiratory tract travel through the upper airways (oropharynx and larynx), the lower airways which include the trachea followed by bifurcations into the bronchi and bronchioli and through the terminal bronchioli which in turn divide into respiratory bronchioli leading then to the ultimate respiratory zone, the alveoli or the deep lung. In a preferred embodiment of the invention, most of the mass of particles deposits in the deep lung. In another embodiment of the invention, delivery is primarily to the central airways. Delivery to the upper airways can also be obtained.

In one embodiment of the invention, delivery to the pulmonary system of particles is in a single, breath-actuated step, as described in U.S. Patent Application, High Efficient Delivery of a Large Therapeutic Mass Aerosol, Application No. 09/591,307, filed June 9, 2000, which is incorporated herein by reference in its entirety. In another embodiment of the invention, at least 50% of the mass of the

particles stored in the inhaler receptacle is delivered to a subject's respiratory system in a single, breath-activated step. In a further embodiment, at least 5 milligrams and preferably at least 10 milligrams of a medicament is delivered by administering, in a single breath, to a subject's respiratory tract particles enclosed in the receptacle.

5 Amounts as high as 15, 20, 25, 30, 35, 40 and 50 milligrams can be delivered.

As used herein, the term "effective amount" means the amount needed to achieve the desired therapeutic or diagnostic effect or efficacy. The actual effective amounts of drug can vary according to the specific drug or combination thereof being utilized, the particular composition formulated, the mode of administration, 10 and the age, weight, condition of the patient, and severity of the symptoms or condition being treated. Dosages for a particular patient can be determined by one of ordinary skill in the art using conventional considerations, (e.g. by means of an appropriate, conventional pharmacological protocol). For example, effective amounts of albuterol sulfate range from about 100 micrograms (μ g) to about 10 15 milligrams (mg).

Aerosol dosage, formulations and delivery systems also may be selected for a particular therapeutic application, as described, for example, in Gonda, I. "Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract," in *Critical Reviews in Therapeutic Drug Carrier Systems*, 6: 273-313, 1990; and in Moren, 20 "Aerosol dosage forms and formulations," in: *Aerosols in Medicine. Principles, Diagnosis and Therapy*, Moren, *et al.*, Eds, Elsevier, Amsterdam, 1985.

Drug release rates can be described in terms of release constants. The first order release constant can be expressed using the following equations:

$$M_{(t)} = M_{(\infty)} * (1 - e^{-kt}) \quad (1)$$

25 Where k is the first order release constant. $M_{(\infty)}$ is the total mass of drug in the drug delivery system, e.g. the dry powder, and $M_{(t)}$ is the amount of drug mass released from dry powders at time t .

Equations (1) may be expressed either in amount (i.e., mass) of drug released or concentration of drug released in a specified volume of release medium.

-30-

For example, Equation (1) may be expressed as:

$$C_{(t)} = C_{(\infty)} * (1 - e^{-k*t}) \quad \text{or} \quad \text{Release}_{(t)} = \text{Release}_{(\infty)} * (1 - e^{-k*t}) \quad (2)$$

Where k is the first order release constant. $C_{(\infty)}$ is the maximum theoretical concentration of drug in the release medium, and $C_{(t)}$ is the concentration of drug

5 being released from dry powders to the release medium at time t .

Drug release rates in terms of first order release constant can be calculated using the following equations:

$$k = -\ln (M_{(\infty)} - M_{(t)}) / M_{(\infty)} / t \quad (3)$$

The release constants presented in Tables 4 and 8 employ equation (2).

10 As used herein, the term "a" or "an" refers to one or more.

The term "nominal dose" as used herein, refers to the total mass of bioactive agent which is present in the mass of particles targeted for administration and represents the maximum amount of bioactive agent available for administration.

EXEMPLIFICATION

15 MATERIALS

Humulin L (human insulin zinc suspension) was obtained from Lilly (100U/mL)

MASS MEDIAN AERODYNAMIC DIAMETER-MMAD (μm)

The mass median aerodynamic diameter was determined using an Aerosizer/Aerodisperser (Amherst Process Instrument, Amherst, MA).

20 Approximately 2 mg of powder formulation was introduced into the Aerodisperser and the aerodynamic size was determined by time of flight measurements.

VOLUME MEDIAN GEOMETRIC DIAMETER-VMGD (μm)

The volume median geometric diameter was measured using a RODOS dry powder disperser (Sympatec, Princeton, NJ) in conjunction with a HELOS laser diffractometer (Sympatec). Powder was introduced into the RODOS inlet and

aerosolized by shear forces generated by a compressed air stream regulated at 2 bar. The aerosol cloud was subsequently drawn into the measuring zone of the HELOS, where it scattered light from a laser beam and produced a fraunhofer diffraction pattern used to infer the particle size distribution and determine the median value.

5 Where noted, the volume median geometric diameter was determined using a Coulter Multisizer II. Approximately 5-10 mg powder formulation was added to 50 mL isoton II solution until the coincidence of particles was between 5 and 8%.

DETERMINATION OF PLASMA INSULIN LEVELS

10 Quantification of insulin in rat plasma was performed using a human insulin specific RIA kit (Linco Research, Inc., St. Charles, MO, catalog #HI-14K). The assay shows less than 0.1% cross reactivity with rat insulin. The assay kit procedure was modified to accommodate the low plasma volumes obtained from rats, and had a sensitivity of approximately 5 μ U/mL.

DETERMINATION OF ESTRONE -SULFATE PLASMA LEVELS

15 Quantification of estrone-sulfate in rat plasma was performed using an estrone-sulfate RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX, catalog #DSL-C5400). The assay kit procedure was modified to accommodate the low plasma volumes obtained from rats and to correct for influence of the human serum standard matrix, and had a sensitivity of approximately 0.025ng/mL.

20 PREPARATION OF INSULIN FORMULATIONS

The powder formulations listed in Table 1 were prepared as follows. Pre-spray drying solutions were prepared by dissolving the lipid in ethanol and the insulin, leucine, and/or sodium citrate in water. The ethanol solution was then mixed with the water solution at a ratio of 60/40 ethanol water. Final total solute concentration of the solution used for spray drying varied from 1 g/L to 3 g/L. As an example, the DPPC/citrate/insulin (60/10/30) spray drying solution was prepared by dissolving 600 mg DPPC in 600 mL of ethanol, dissolving 100 mg of sodium citrate and 300 mg of insulin in 400 mL of water and then mixing the two solutions to yield

one liter of cosolvent with a total solute concentration of 1 g/L (w/v). Higher solute concentrations of 3 g/L (w/v) were prepared by dissolving three times more of each solute in the same volumes of ethanol and water.

The solution was then used to produce dry powders. A Nitro Atomizer
 5 Portable Spray Dryer (Niro, Inc., Columbus, MD) was used. Compressed air with variable pressure (1 to 5 bar) ran a rotary atomizer (2,000 to 30,000 rpm) located above the dryer. Liquid feed with varying rate (20 to 66 mL/min) was pumped continuously by an electronic metering pump (LMI, Model #A151-192s) to the atomizer. Both the inlet and outlet temperatures were measured. The inlet
 10 temperature was controlled manually; it could be varied between 100°C and 400°C and was established at 100, 110, 150, 175 or 200°C, with a limit of control of 5°C. The outlet temperature was determined by the inlet temperature and such factors as the gas and liquid feed rates (it varied between 50°C and 130°C). A container was tightly attached to the cyclone for collecting the powder product.

15 TABLE 1

POWDER FORMULATION NUMBER	COMPOSITION (%)						
	DPePC	DSePC	DPPG	DPPC	Leucine	Citrate	Insulin
1†				70	10		20
2		70			20		10
3		70			10		20
4	50						50
5‡			40			10	50
6	70				10		20
7	50						50
8	54.5						45.5
9	50				10		40
10	70				10		2
11	70				8	2	20

		COMPOSITION (%)						
POWDER FORMULATION NUMBER		DPePC	DSePC	DPPG	DPPC	Leucine	Citrate	Insulin
5	12 †				40		10	50
	13†				60		10	30
	13A†				60		10	30
	14‡				70		20	
	15†				70	20		10

† Lots # 4-xxx-201002 (#1), 4-XXX-201 065 (#12), 04-00024 (#13), 4-xxx-114068C

(#13A) and 4-xxx-167113 (#15), which contain the lipid DPPC, serve as negative controls.

10 10 ‡Powder formulation #5 was spray dried at pH=4.0.

‡Powder formulation #14 was spray dried at pH=7.4.

The physical characteristic of the insulin containing powders is set forth in Table 2. The MMAD and VMGD were determined as detailed above.

TABLE 2

15	Formulations	Compositions (% weight basis)	MMAD (μ m) §	VMGD (μ m) ¶	Density (g/cc) ‡
	Humulin R	--	--	--	--
	Humulin L	--	--	--	--
	Humulin U	--	--	--	--
20	1	DPPC/Leu/Insulin (Sigma) = 70/10/20	2.6	13.4	0.038
	2	DSePC (Avanti)/Leu/Insulin (Sigma) = 70/10/20	3.3	10.0	0.109
	3	DSePC (Avanti)/Leu/Insulin (Sigma) = 70/10/20	3.4	13.6	0.063
	4	DPePC (Avanti)/Insulin (Sigma) = 50/50	3.2	15.3	0.044
	5	DPPG/Sodium Citrate/Insulin = 40/10/50	3.9	11.6	0.113
	6	DPePC (Genzyme)/Leu/Insulin (BioBras) = 70/10/20	2.6	9.1	0.082
	7	DPePC (Avanti)/Insulin (BioBras)=50/50	2.8	11.4	0.060
	8	DPePC (Genzyme)/Insulin (BioBras) = 54.5/45.5	2.8	12.6	0.049

Formulations	Compositions (% weight basis)	MMAD (μm) §	VMGD (μm) ¶	Density (g/cc) ‡
9	DPePC (Genzyme)/Leu/Insulin (BioBras) = 50/10/40	2.2	8.4	0.069
10	DPePC (Avanti)/Leu/Insulin (BioBras) = 70/10/20	3.7	15.5	0.057
11	DPePC (Avanti)/Leu/Sodium Citrate/Insulin (BioBras) = 70/8/2/20	2.6	15.3	0.029
12	DPPC/Sodium Citrate/Insulin = 40/10/50	3.5	11.6	0.091
5 13	DPPC/Insulin/Sodium Citrate = 60/30/10	1.9	8.0	0.056

† Used as a control formulation for comparison in either *in vitro* or *in vivo* studies.

§ *Mass median aerodynamic diameter*

¶ *Volumetric median geometric diameter at 2 bar pressure*

‡ Determined using $d_{\text{aer}} = d_g \sqrt{\rho}$

10 The data presented in Table 2 showing the physical characteristics of the formulations comprising insulin are predictive of the respirability of the formulations. That is, as discussed above the large geometric diameters, small aerodynamic diameters and low densities possessed by the powder prepared as described herein render the particles respirable.

15 IN VIVO INSULIN EXPERIMENTS

The following experiment was performed to determine the rate and extent of insulin absorption into the blood stream of rats following pulmonary administration of dry powder formulations comprising insulin to rats.

20 The nominal insulin dose administered was 100 μg per rat. To achieve the nominal doses, the total weight of powder administered per rat ranged from 0.2 mg to 1 mg, depending on percent composition of each powder. Male Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY). At the time of use, the animals weighed 386 g in average (± 5 g S.E.M.). The animals were allowed free access to food and water.

25 The powders were delivered to the lungs using an insufflator device for rats (PennCentury, Philadelphia, PA). The powder amount was transferred into the insufflator sample chamber. The delivery tube of the insufflator was then inserted through the mouth into the trachea and advanced until the tip of the tube was about a

centimeter from the carina (first bifurcation). The volume of air used to deliver the powder from the insufflator sample chamber was 3 mL, delivered from a 10 mL syringe. In order to maximize powder delivery to the rat, the syringe was recharged and discharged two more times for a total of three air discharges per powder dose.

5 The injectable insulin formulation Humulin L was administered via subcutaneous injection, with an injection volume of 7.2 μ L for a nominal dose of 25 μ g insulin. Catheters were placed into the jugular veins of the rats the day prior to dosing. At sampling times, blood samples were drawn from the jugular vein catheters and immediately transferred to EDTA coated tubes. Sampling times were
10 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hrs. after powder administration. In some cases an additional sampling time (12 hrs.) was included, and/or the 24 hr. time point omitted. After centrifugation, plasma was collected from the blood samples.
15 Plasma samples were stored at 4°C if analysis was performed within 24 hours or at -75°C if analysis would occur later than 24 hours after collection. The plasma insulin concentration was determined as described above.

Table 3 contains the insulin plasma levels quantified using the assay described above.

TABLE 3

20	Time (hrs)	PLASMA INSULIN CONCENTRATION (μ U/mL) \pm S.E.M.										
		1	2	3	4	5	6	13A	14	Hum- lin L	15	
0	5.0 \pm 0.0	5.2 \pm 0.2	5.0 \pm 0.0	5.0 \pm 0.0	5.3 \pm 0.2	5.7 \pm 0.7	5.0 \pm 0.0	5.0 \pm 0.0	5.0 \pm 0.0	5.0 \pm 0.0	\pm 5.0 \pm 0.0	
0.25	1256.4 \pm 144.3	61.6 \pm 22.5	98.5 \pm 25.3	518.2 \pm 179.2	240.8 \pm 67.6	206.8 \pm 35.1	1097.7 \pm 247.5	933.9 \pm 259.7	269.1 \pm 82.8	1101.9 \pm 258.9		
0.5	1335.8 \pm 81.9	85.2 \pm 21.7	136.7 \pm 37.6	516.8 \pm 190.9	326.2 \pm 166.9	177.3 \pm 7.8	893.5 \pm 177.0	544.9 \pm 221.1	459.9 \pm 91.6	1005.4 \pm 263.9		
1	859.0 \pm 199.4	85.4 \pm 17.6	173.0 \pm 28.8	497.0 \pm 93.9	157.3 \pm 52.5	170.5 \pm 32.9	582.5 \pm 286.3	229.6 \pm 74.4	764.7 \pm 178.8	387.5 \pm 143.9		

		PLASMA INSULIN CONCENTRATION (μ U/mL) \pm S.E.M.										
		Time (hrs)	1	2	3	4	5	6	13A	14	Hum-Lin L	15
5	2	648.6 \pm 171.1	94.8 \pm 25.0	158.3 \pm 39.1	496.5 \pm 104.9	167.7 \pm 70.5	182.2 \pm 75.0	208.5 \pm 78.3	129.8 \pm 45.7	204.4 \pm 36.7	343.8 \pm 95.3	
	4	277.6 \pm 86.8	52.5 \pm 9.1	98.0 \pm 24.3	343.8 \pm 66.7	144.8 \pm 43.8	170.2 \pm 56.3	34.9 \pm 5.4	41.9 \pm 28.7	32.1 \pm 22.6	170.6 \pm 79.9	
	6	104.0 \pm 43.1	33.0 \pm 10.7	58.7 \pm 4.1	251.2 \pm 68.4	95.7 \pm 27.3	159.5 \pm 43.4	12.3 \pm 2.4	9.0 \pm 2.9	11.1 \pm 7.5	15.4 \pm 4.5	
	8	54.4 \pm 34.7	30.2 \pm 8.1	42.5 \pm 17.8	63.2 \pm 16.5	52.5 \pm 13.7	94.8 \pm 23.5	5.2 \pm 0.1	5.0 \pm 0.0	5.5 \pm 2.1	6.5 \pm 0.6	
	12				17.2 \pm 6.5							
	24				5.0 \pm 0.0	5.5 \pm 0.3						
	n	5	5	6	6	6	6			8		

The *in vivo* release data of Table 3 show that powder formulations comprising insulin and positively charged lipids (DPePC and DSePC) have significantly lower initial burst of insulin than that seen with powder formulations comprising insulin and the lipid DPPC (Formulations 1 and 13) and sustained elevated levels at 6 to 8 hours. Figure 1 sets forth the release profile for insulin from Formulations 2, 3, 6 and 15.

In addition, the use of charged lipids having a charge which is the same of the active at neutral pH, can also be employed provided that the preparation of the spray dried formulation is conducted at a pH where the lipid and active agent possess overall charges which are opposite and are therefore capable of charge interaction. See, for example, Formulation 5 which employs the negatively charged lipid DPPG. Formulation 5 was prepared and spray dried at a pH of about 4.0. At this pH, DPPG is negatively charged and insulin becomes positively charged (pI=5.5) thereby providing for a charge interaction to occur. However, when the

DPPG and insulin are prepared and spray dried at pH=7.4 where both the DPPG and insulin possess an overall negative charge, Formulation 14, the proper environment for charge interaction to occur is not provided. It is noted that Formulation 5 showed a significantly lower initial burst of insulin ($240.8 \pm 67.6 \mu\text{U}/\text{mL}$) as compared to Formulation 14 ($933.9 \pm 259.7 \mu\text{U}/\text{mL}$) with higher sustained levels at 6 to 8 hours post treatment. Figure 2 shows a comparison of the *in vivo* release profile for Formulations 5, 14 and 13A (lipid, DPPC).

IN VITRO ANALYSIS OF INSULIN-CONTAINING FORMULATIONS

The *in vitro* release of insulin containing dry powder formulations was performed as described by Gietz *et al.* in *Eur. J. Pharm. Biopharm.*, 45:259-264 (1998), with several modifications. Briefly, in 20 mL screw-capped glass scintillation vials about 10 mg of each dry powder formulation was mixed with 4 mL of warm (37°C) 1% agarose solution using polystyrene stir bars. The resulting mixture was then distributed in 1 mL aliquots to a set of five fresh 20 mL glass scintillation vials. The dispersion of dry powder in agarose was cooled in an ambient temperature dessicator box protected from light to allow gelling. Release studies were conducted on an orbital shaker at about 37°C . At predetermined time points, previous release medium (1.5 mL) was removed and fresh release medium (1.5 mL) was added to each vial. Typical time points for these studies were 5 minutes, 1, 2, 4, 6 and 24 hours. The release medium used consisted of 20 mM 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES), 138 mM NaCl, 0.5% Pluronic (Synperonic PE/F68; to prevent insulin fibrillation in the release medium); pH 7.4. A Pierce (Rockford, IL) protein assay kit (See *Anal Biochem*, 150:76-85 (1985)) using known concentrations of insulin standard was used to monitor insulin concentrations in the release medium.

Table 4 summarizes the *in vitro* release data and first order release constants for powder formulations of Table 1 comprising insulin.

TABLE 4

Powder Formulation Number	Cumulative % Insulin Released at 6 hr	Cumulative % Insulin Released at 24 hr	Maximum ‡ Release at 24 hr (Cumulative %)	First Order ‡ Release Constants (hr ⁻¹)
5	Humulin R	92.67 ± 0.36	94.88 ± 0.22	1.0105 ± 0.2602
	Humulin L	19.43 ± 0.41	29.71 ± 0.28	0.0924 ± 0.0183
	Humulin U	5.17 ± 0.18	12.65 ± 0.43	0.0158 ± 0.0127
	2	31.50 ± 0.33	47.52 ± 0.43	0.1749 ± 0.0038
	3	26.34 ± 0.71	37.49 ± 0.27	0.1837 ± 0.0079
	4	24.66 ± 0.20	31.58 ± 0.33	0.2457 ± 0.0214
	5	29.75 ± 0.17	35.28 ± 0.19	0.4130 ± 0.0878
	6	17.04 ± 0.71	24.71 ± 0.81	0.1767 ± 0.0083
	7	13.53 ± 0.19	19.12 ± 0.40	0.1788 ± 0.0101
	8	13.97 ± 0.27	17.81 ± 0.46	0.2419 ± 0.0178
	9	17.47 ± 0.38	22.17 ± 0.22	0.2734 ± 0.0196
	10	25.96 ± 0.31	34.94 ± 0.31	0.2051 ± 0.0120
	11	34.33 ± 0.51	47.21 ± 0.47	0.1994 ± 0.0082
	12	61.78 ± 0.33	68.56 ± 0.23	0.5759 ± 0.0988
	13	78.47 ± 0.40	85.75 ± 0.63	0.5232 ± 0.0861

20 ‡ Release_(t) = Release_(inf) * (1 - e^{-kt})

† Used as a control formulation.

25 The data presented in Table 4 show that for insulin containing powder formulations employing the positively charged lipid DPePC (Formulations 4 and 6-11) and DSePC (Formulations 2 and 3), first order release constants similar to that observed with the slow release injectable insulin formulation, Humulin L, can be

achieved. Further, the first order release constants of these same formulations is significantly lower than that observed with the fast release injectable insulin formulation, Humulin R. As such, sustained release dry powder insulin formulations having varying compositions of positively charged lipid can be 5 formulated.

PREPARATION OF ESTRONE SULFATE-CONTAINING POWDER FORMULATIONS

The estrone sulfate powder formulations listed in Table were prepared as 10 follows. Pre-spray drying solutions were prepared by dissolving the lipin in ethanol and estrone sulfate and leucine in water. The ethanol solution was then mixed with the water solution at a ration 70/30 ethanol/water. Final total solute concentration of the solution used for spray drying varied from 1 g/L to 3 g/L. As an example, the DPePC/leucine/estrone sulfate (76/20/4) spray drying solution was prepared by 15 dissolving 760 mg of DPePC in 700 mL of ethanol, dissolving 200 mg of leucine and 40 mg of estrone sulfate in 300 mL of water and then mixing the two solutions to yield one liter of cosolvent with a total solute concentration of 1 g/L (w/v). Higher solute concentrations of, for example, 3 g/L (w/v) were prepared by dissolving three times more of each solute in the same volumes of ethanol and water

20 The mixture was spray dried following the procedure described above for the insulin containing powder formulation. During spray drying, the feed rate was about 50 mL/min, the inlet temperature ranged from about 110°C to about 120°C, and the outlet temperature was about 52°C.

25 The physical characteristic of the estrone sulfate containing powders is set forth in Table 5. The MMAD and VMGD were determined as detailed above.

TABLE 5

POWDER FORMULATION NUMBER	COMPOSITIONS (% WEIGHT BASIS)	MMAD (μ m) §	VMGD (μ m) ¶	DENSITY (g/cc) ‡
5	16 DPePC (Avanti)/Leucine/ Estrone Sulfate (sodium salt) = 76/20/4	5.9	16.0	0.136
	17 DPPC/Leucine/ Estrone Sulfate (sodium salt) = 76/20/4	3.7	12.7 #	0.085

†Used as a control for comparison for *in vivo* studies

§Mass median aerodynamic diameter

¶Volumetric median geometric diameter at 2 bar pressure

10 #Measured using Coulter Multisizer

‡Determined using $d_{aer} = d_g \sqrt{\rho}$

The data presented in Table 5 showing the physical characteristics of the formulations comprising estrone sulfate are predictive of the respirability of the formulations. That is, as discussed above the large geometric diameters, small aerodynamic diameters and low densities possessed by the powder prepared as described herein render the particles respirable.

IN VIVO EXPERIMENTS-ESTRONE SULFATE CONTAINING POWDERS

The following experiment was performed to determine the rate and extent of estrone sulfate absorption into the blood stream of rats following pulmonary 20 administration of dry powder formulations comprising estrone sulfate.

The nominal estrone-sulfate dose administered was 40 μ g per rat, in 1mg of powder. Male Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY). At the time of use, the animals weighed an average of 415 g (\pm 10 g S.E.M.). The animals were allowed free access to food and water.

25 The powders were delivered to the lungs using an insufflator device for rats (PennCentury, Philadelphia, PA). The powder amount was transferred into the insufflator sample chamber. The delivery tube of the insufflator was then inserted

through the mouth into the trachea and advanced until the tip of the tube was about a centimeter from the carina (first bifurcation). The volume of air used to deliver the powder from the insufflator sample chamber was 3 mL, delivered from a 10 mL syringe. In order to maximize powder delivery to the rat, the syringe was recharged 5 and discharged two more times for a total of three air discharges per powder dose.

Catheters were placed into the jugular veins of the rats the day prior to dosing. At sampling times, blood samples were drawn from the jugular vein catheters and immediately transferred to EDTA coated tubes. Sampling times were 0, 0.25, 0.5, 1, 2, 4, and 6 hours after powder administration. After centrifugation, 10 plasma was collected from the blood samples. Plasma samples were stored at 4°C if analysis was performed within 24 hours or at -75°C if analysis would occur later than 24 hours after collection.

Table 6 contains the estrone sulfate plasma levels quantified using the assay described above.

15 TABLE 6

PLASMA ESTRONE-SULFATE CONCENTRATION (ng/mL) ± S.E.M.		
TIME (HRS)	FORMULATION 16	FORMULATION 17
0	0.07 ± 0.02	0.08 ± 0.05
0.25	12.07 ± 1.96	22.26 ± 8.96
0.5	18.88 ± 2.21	23.39 ± 12.72
1	12.20 ± 3.31	10.59 ± 0.61
2	4.65 ± 0.77	3.45 ± 0.63
4	4.02 ± 1.42	0.86 ± 0.10
6	1.49 ± 0.48	0.33 ± 0.12
n	4	3

The results presented in Table 6 and depicted graphically in Figure 3, show that the formulation comprising DPePC (overall positive charge) and estrone sulfate (negative charge) exhibited sustained release of estrone sulfate when compared to the formulation employing the lipid DPPC (no overall net charge) and estrone sulfate. Specifically, at six hours post administration, the plasma level of estrone sulfate for the DPePC containing formulation was 1.49 ± 0.48 ng/mL as compared to 0.33 ± 0.12 ng/mL for the DPPC containing formulation.

PREPARATION OF ALBUTEROL-CONTAINING POWDER FORMULATIONS

The albuterol sulfate powder formulations listed in Table 7, were prepared as follows. Pre-spray drying solutions were prepared by dissolving the lipid in ethanol and albuterol sulfate and leucine in water. The ethanol solution was then mixed with the water solution at a ratio of 70/30 ethanol/water. Final total solute concentration of the solution used for spray drying varied from 1 g/L to 3 g/L. As an example, the DPPC/leucine/albuterol sulfate (76/16/8) spray drying solution was prepared by dissolving 760 mg of DPPC in 700 mL of ethanol, dissolving 160 mg leucine and 870 mg of albuterol sulfate in 300 mL water and then mixing the two solutions to yield one liter of cosolvent with a total solute concentration of 1 g/L (w/v). Higher solute concentrations of 3 g/L (w/v) were prepared by dissolving three times more of each solute in the same volumes of ethanol and water. The solution was spray-dried as described above for the insulin containing formulation. Specifically, the inlet temperature was from about 110°C to about 140°C, and the outlet temperature ranged from about 45-57°C.

The physical characteristics of the albuterol sulfate containing powders is set forth in Table 7. The MMAD and VMGD were determined as detailed above.

TABLE 7

POWDER FORMULATION NUMBER	COMPOSITIONS (% WEIGHT BASIS)	MMAD (μ m) §	VMGD (μ m) ¶	DENSITY (g/cc) ‡
5	18 DSPC/Leucine/ Albuterol Sulfate = 76/16/8	3.3	6.1	0.293
	19 DSPG/Leucine/ Albuterol Sulfate = 76/16/8	4.1	6.4	0.410
	20 DPPC/Leucine/ Albuterol Sulfate = 76/23/1	2.8	12.0	0.054
	21 DPPG/Leucine/ Albuterol Sulfate = 76/16/8	3.3	7.1	0.216

10 †Used as a control for comparison in either *in vitro* or *in vivo* studies

§Mass median aerodynamic diameter

¶Volumetric median geometric diameter at 2 bar pressure

‡Determined using $d_{aer} = d_g \sqrt{\rho}$

15 The data presented in Table 7 showing the physical characteristics of the formulations comprising albuterol sulfate are predictive of the respirability of the formulations. That is, as discussed above the large geometric diameters, small aerodynamic diameters and low densities possessed by the formulations prepared as described herein render the formulations respirable.

IN VIVO TESTING OF ALBUTEROL SULFATE FORMULATIONS

20 A whole-body plethysmography method for evaluating pulmonary function in guinea pigs was used to assess the sustained effects of the albuterol sulfate formulations listed in Table 7.

The system used was the BUXCO whole-body unrestrained plethysmograph

system with BUXCO XA pulmonary function software (BUXCO Electronics, Inc., Sharon, CT). The method was conducted as described by Silbaugh S.A. and Mauderly, J.L., in American Physiological Society, Vol. 84:1666-1669 (1984) and Chang, B.T., *et al* in Journal of Pharmacological and Toxicological Methods, Vol. 39(3):163-168 (1998). This method allows individual animals to be challenged repeatedly over time with methacholine given by nebulization. A calculated measurement of airway resistance based on flow parameters, the enhanced pause PenH was used as a marker for protection from methacholine-induced bronchoconstriction. Baseline pulmonary function (airway hyperresponsiveness) values were measured prior to any experimental treatment. Airway hyperresponsiveness was then assessed in response to saline and methacholine at various timepoints (2-3, 16 and 24 hours) following administration of albuterol-sulfate formulations. Average PenH is calculated from data collected between 4 and 9 minutes following challenge with saline or methacholine. The percent of baseline PenH at each timepoint is calculated for each experimental animal. Values from animals that received the same albuterol sulfate formulation were subsequently averaged to determine the mean group response (\pm standard error) at each timepoint.

The nominal dose of albuterol-sulfate administered was 50 μ g for the DPPG-based formulation (#21) and 25 μ g for the DPPC-based formulation (#20). To achieve those nominal doses, the total weights of powder administered were 0.625 mg and 2.5 mg, respectively.

Male Hartley guinea pigs were obtained from Elm Hill Breeding Labs (Chelmsford, MA). At the time of use, the animals weighed an average of 363 g (\pm 5 g S.E.M.). The animals were allowed free access to food and water. The powder amount was transferred into the insufflator sample chamber (insufflation device for guinea pigs, Penn Century, Philadelphia, PA). The delivery tube of the insufflator was inserted through the mouth into the trachea and advanced until the tip of the tube was about a centimeter from the carina (first bifurcation). The volume of the air used to deliver the powder from the insufflator sample chamber was 3 mL, delivered from a 10 mL syringe. In order to maximize powder delivery to the guinea pig, the syringe was recharged and discharged two more times for a total of three air

-45-

discharges per powder dose. Methacholine challenges were performed at time points 2-3, 16 and 24 hours after administration.

Figure 4 shows that the formulation comprising DPPG (overall negative charge) and albuterol sulfate (overall positive charge) provided sustained protection 5 against methacholine-induced bronchoconstriction when compared to the formulation comprising DPPC (no overall net charge) and albuterol sulfate for at least 24 hours following administration.

In another experiment, as much as 200 μ g of albuterol sulfate in a DPPC-based formulation did not provide prolonged protection against induced 10 bronchoconstriction.

IN VITRO RELEASE STUDIES-ALBUTEROL SULFATE

Controlled Release Studies of Albuterol Sulfate were conducted using the COSTARTTM Brand Transwell Inserts, With Plates, Sterile. The plates were equipped 15 with 6 wells having an area of 4.7cm². The insert size was 24 mm, the pore size was 3.0 μ m. A predetermined amount of the powder to be tested (approximately 10-15 mg) was placed into a HPMC Size #2 capsule. The capsule was then placed inside an inhaler and the powder was sprayed on the Transwell insert using an in-house vacuum system. Formulations were run in triplicate.

20 After spraying, the insert was placed inside the Transwell plate containing a volume of 1.8 mL of Phosphate Buffered Saline (pH = 7.4) which had previously been equilibrated at 37°C for 30 minutes. The Transwell plate was hermetically sealed in order to prevent evaporation of the buffer during the experiment.

The Transwell Experiment was carried out in an incubator at 37°C on an 25 orbital shaker at a speed of 100 min⁻¹. At specified time-points throughout the experiment, 1.8 mL of phosphate buffered saline was removed from the Transwell plate. The inserts were then placed into a new Transwell plate containing 1.8 mL of fresh phosphate buffered saline. Typical Transwell experiments are conducted for 4 hours. Samples are withdrawn after 5 min., 15, min., 30, min., 1 h, 1.5 h, 2 h, 3 h, 30 and 4 h.

The amount of albuterol sulfate in the PBS buffer sampled at predetermined *in vitro* release time points was quantitated using a RP-HPLC method with Phenomenex Luna 5 μ , C8(2), 250 x 4.6 mm column (Torrance, CA) and UV detection at 275 nm.

5 Table 8 summarizes the *in vitro* release data and first order release constants for the powder formulations of Table 7 comprising albuterol sulfate. The first order release constants for the powder formulation comprising DSPG (negatively charged) and albuterol sulfate is about 4 time slower compared to the powder formulation comprising DSPC (no net overall charge) and albuterol sulfate
10 (positive).

TABLE 8

Powder Formulation Number	Compositions (% weight basis)	Cumulative % Insulin Released at 4 hr	Maximum Release at 4 hr (Cumulative %)‡	First Order Release Constants (hr ⁻¹)‡
15 18	DSPC/Leucine/Albuterol Sulfate (sodium salt) = 76/16/8	106.21 ± 1.73	105.64 ± 0.20	29.7360 ± 0.7504
	DSPG/Leucine/Albuterol Sulfate (sodium salt) = 76/16/8	97.44 ± 0.68	95.13 ± 1.39	7.9334 ± 0.6877

‡ Release_(t) = Release_(inf) * (1 - e^{-kt})

† Used as a control formulation.

20 While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

- 47 -

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form or suggestion that that prior art forms part of the common general knowledge in Australia.

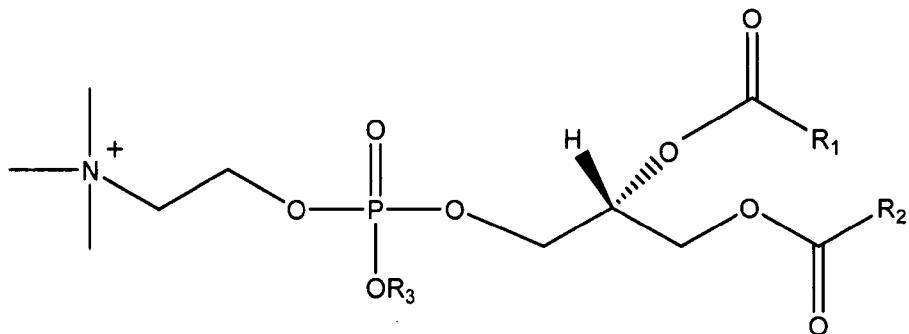
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for delivery via the pulmonary system comprising:
 - 5 administering to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising:
 - 10 a bioactive agent in association with a charged lipid wherein the charged lipid has an overall net positive charge, the agent has an overall net negative charge upon association, the agent is not a nucleic acid and wherein release of the agent is sustained.
 - 15 2. The method of claim 1, wherein association of the agent and charged lipid comprises an ionic complexation.
 3. The method of claim 2, wherein association of the lipid and agent further comprises hydrogen bonding.
 4. The method of any one of claims 1 to 3, wherein the charge ratio of lipid to bioactive agent is from about 0.25:1 to about 1:0.25.
 - 20 5. The method of claim 4, wherein the charge ratio of lipid to bioactive agent is from about 0.5:1 to about 1:0.5.
 6. The method of claim 5, wherein the charge ratio of lipid to bioactive agent is about 1:1.
 - 25 7. The method of any one of claims 1 to 6, wherein the bioactive agent is a protein.
 8. The method of claim 7, wherein the protein is insulin.
 - 30 9. The method of claim 8, wherein the sustained release is at least about 6 hours post administration.

- 49 -

10. The method of claim 1, wherein the lipid is a 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine or a 1,2-diacyl-*sn*-glycero-3-alkylphosphoalkanolamine.

5 11. The method of claim 10, wherein the 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine lipid is represented by Formula III:



wherein,

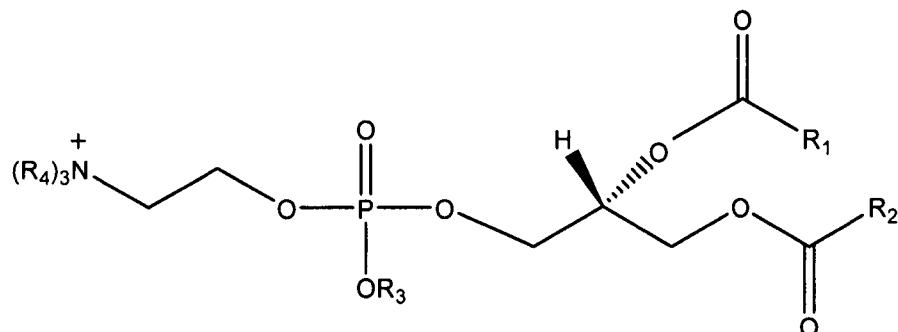
10 R₁ and R₂ are independently an aliphatic group having from 3 to 24 carbons; and

R₃ is an aliphatic group having from 1 to 24 carbons.

12. The method of claim 10, wherein the 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine is 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine(DPePC),
15 1,2-dimyristoyl-*sn*-glycero-3-ethylphosphocholine(DMePC),
1,2-distearoyl-*sn*-glycero-3-ethylphosphocholine(DSePC),
1,2-dilauroyl-*sn*-glycero-3-ethylphosphocholine (DLePC),
1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine(DOePC) or any combination thereof.

13. The method of claim 10, wherein the 1,2-diacyl-*sn*-glycero-3-alkylphospho-alkanolamine is represented by Formula IV:

5



10

wherein,

R₁ and R₂ are independently an aliphatic group having from 3 to 24 carbons;

R₃ is an aliphatic group having from 1 to 24 carbons; and

R₄ is independently hydrogen or an aliphatic group having from 1 to 6 carbons.

15

14. The method of claim 10, wherein the 1,2-diacyl-*sn*-glycero-3-alkylphospho-alkanolamine is 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphoethanolamine (DPePE), 1,2-dimyristoyl-*sn*-glycero-3-ethylphosphoethanolamine (DMePE), 1,2-distearoyl-*sn*-glycero-3-ethylphosphoethanolamine (DSePE),

20

1,2-dilauroyl-*sn*-glycero-3-ethylphosphoethanolamine (DLePE),

1,2-dioleoyl-*sn*-glycero-3-ethylphosphoethanolamine (DOePE) or any combination thereof.

25

15. The method of any one of claims 1 to 14, wherein the particles have a tap density less than about 0.4 g/cm³.

16. The method of claim 15, wherein the particles have a tap density less than about 0.1 g/cm³.

- 51 -

17. The method of any one of claims 1 to 16, wherein the particles have a median geometric diameter of from about 5 micrometers and about 30 micrometers.
18. The method of any one of claims 1 to 17, wherein the particles have an aerodynamic diameter of from about 1 to about 5 microns.
5
19. The method of claim 18, wherein the particles have an aerodynamic diameter of from 1 about to about 3 microns.
- 10 20. The method of claim 18, wherein the particles have an aerodynamic diameter of from about 3 to about 5 microns.
21. The method of any one of claims 1 to 20, wherein delivery to the pulmonary system includes delivery to the deep lung.
15
22. The method of any one of claims 1 to 20, wherein delivery to the pulmonary system includes delivery to the central airways.
23. The method of any one of claims 1 to 20, wherein delivery to the pulmonary system includes delivery to the upper airways.
20
24. The method of any one of claims 1 to 23, wherein the particles further comprise a lipid having no overall net charge.
- 25 25. The method of any one of claims 1 to 24, wherein the particles further comprise a carboxylic acid or salt thereof.
26. The method of claim 25, wherein the carboxylic acid includes at least two carboxyl groups.
30

- 52 -

27. The method of any one of claims 1 to 26, wherein the particles further comprise a multivalent metal salt or ionic components thereof.
28. The method of claim 27, wherein the multivalent salt is a salt of an alkaline earth metal.
29. The method of any one of claims 1 to 28, wherein the particles further comprise an amino acid.
- 10 30. The method of claim 29, wherein the amino acid is hydrophobic.
31. The method of claim 30, wherein the hydrophobic amino acid is leucine, isoleucine, alanine, valine, phenylalanine or any combination thereof.
- 15 32. A method for delivery via the pulmonary system comprising: administering to the respiratory tract of a patient in need of treatment an effective amount of particles comprising: insulin in association with a charged lipid wherein the charged lipid has an overall net positive charge which is opposite to the overall net negative charge of the insulin
20 upon association and wherein release of the insulin is sustained.
33. The method of claim 32, wherein the lipid is a 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine or a 1,2-diacyl-*sn*-glycero-3-alkylphosphoalkanolamine.
- 25 34. The method of claim 33, wherein the 1,2-diacyl-*sn*-glycero-3-alkylphosphocholine is 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine(DPePC), 1,2-dimyristoyl-*sn*-glycero-3-ethylphosphocholine(DMePC),
30 1,2-distearoyl-*sn*-glycero-3-ethylphosphocholine(DSePC), 1,2-dilauroyl-*sn*-glycero-3-ethylphosphocholine (DLePC),

1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (DOePC) or any combination thereof.

35. The method of claim 33, wherein the
5 1,2-diacyl-*sn*-glycero-3-alkylphosphoalkanolamine is
1,2-dipalmitoyl-*sn*-glycero-3-ethylethanolamine (DPePE),
1,2-dimyristoyl-*sn*-glycero-3-ethylphosphoethanolamine (DMePE),
10 1,2-distearoyl-*sn*-glycero-3-ethylphosphoethanolamine (DSePE),
1,2-dilauroyl-*sn*-glycero-3-ethylphosphoethanolamine (DLePE),
1,2-dioleoyl-*sn*-glycero-3-ethylphosphoethanolamine (DOePE) or any combination thereof.
36. The method of claim 32, wherein association of the agent and charged lipid
15 comprises an ionic complexation.
37. The method of claim 36, wherein association of the lipid and agent further
comprises hydrogen bonding.
38. The method of any one of claims 32 to 37, wherein the charge ratio of lipid to
20 bioactive agent is from about 0.25:1 to about 1:0.25.
39. The method of claim 38, wherein the charge ratio of lipid to bioactive agent is from
about 0.5:1 to about 1:0.5.
- 25 40. The method of claim 39, wherein the charge ratio of lipid to bioactive agent is
about 1:1.
41. The method of any one of claims 32 to 40, wherein the particles further comprise a
carboxylic acid.

- 54 -

42. The method of any one of claims 32 to 41, wherein the particles further comprise an amino acid.
43. The method of any one of claims 32 to 42, wherein the particles further comprise a 5 lipid with no overall net charge.
44. The method of any one of claims 32 to 43, wherein the particles further comprise a multivalent metal salt or ionic components thereof.
- 10 45. The method of any one of claims 32 to 44, wherein delivery to the pulmonary system includes delivery to the deep lung.
46. The method of any one of claims 32 to 44, wherein delivery to the pulmonary system includes delivery to the central airways.
- 15 47. The method of any one of claims 32 to 44, wherein delivery to the pulmonary system includes delivery to the upper airways.
48. A method according to any one of claims 1 to 47 substantially as hereinbefore 20 described.

DATED this 21st day of December, 2005

Advanced Inhalation Research, Inc.

25 by DAVIES COLLISON CAVE
Patent Attorneys for the Applicant

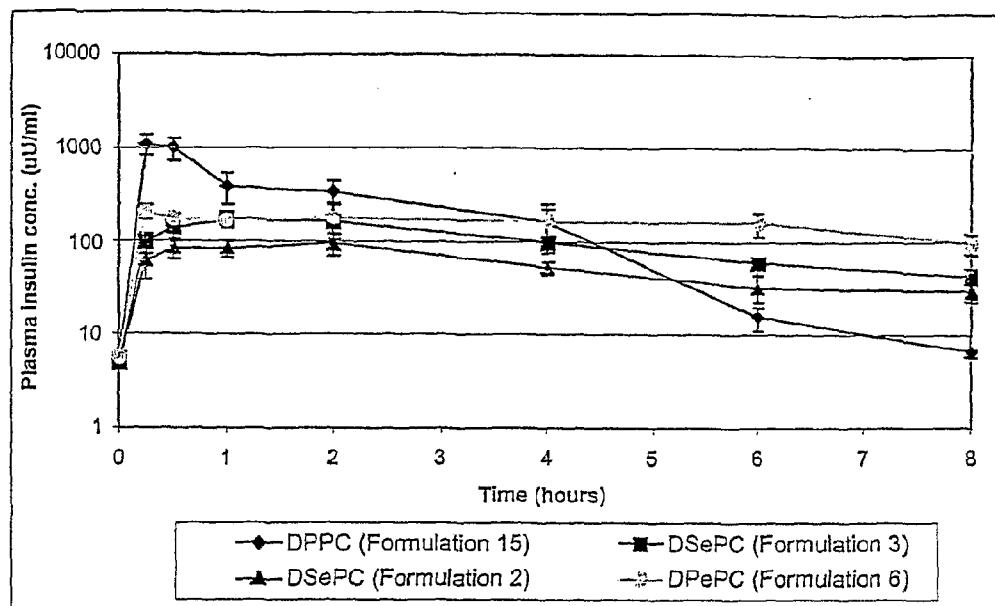


FIG. 1

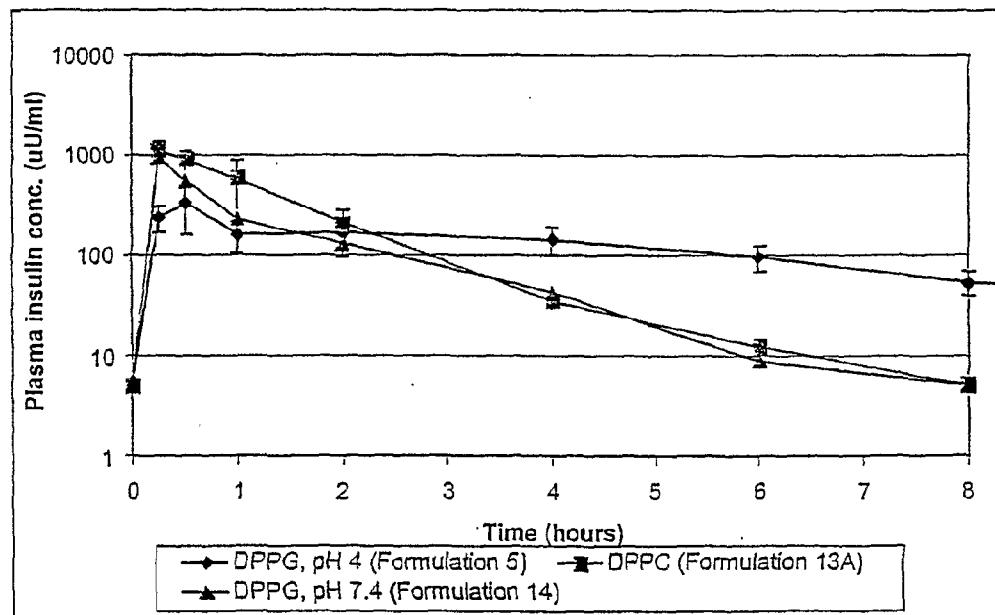


FIG. 2

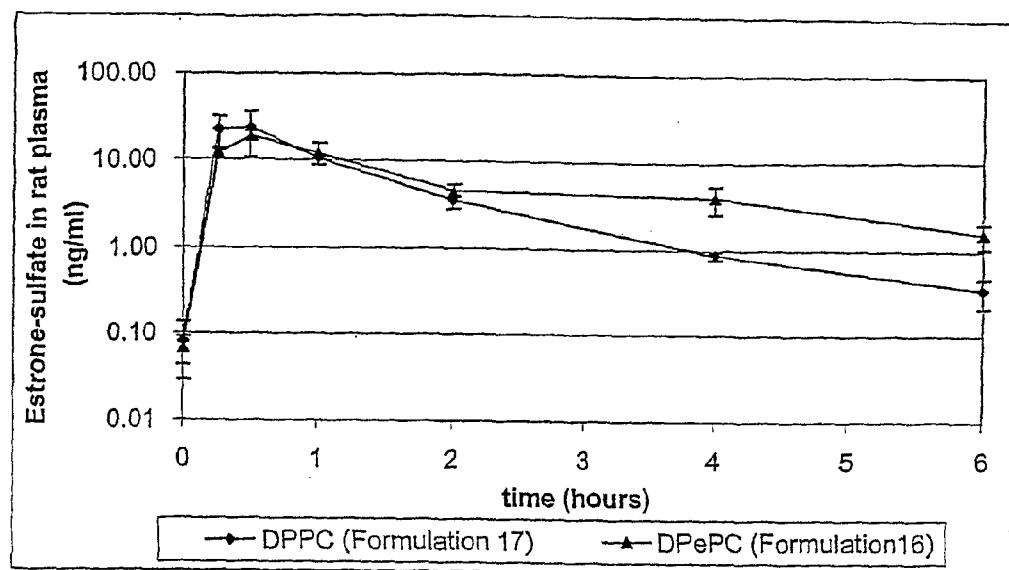


FIG. 3

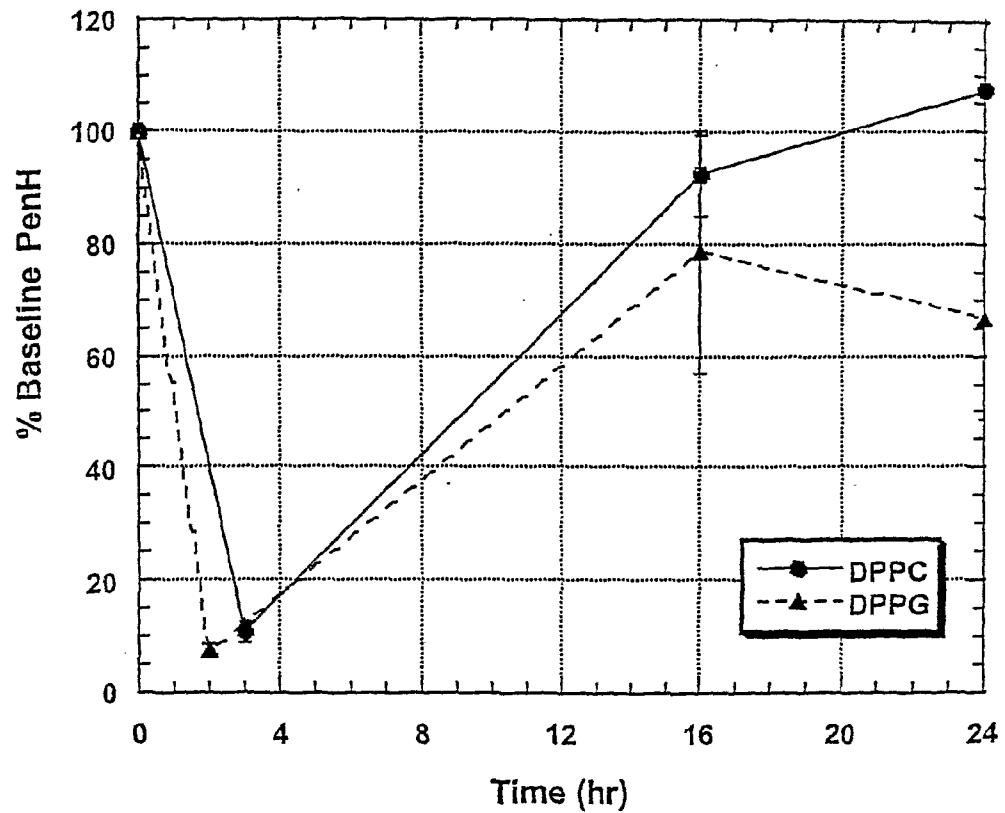


FIG. 4