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(54) **PARTICLES INCORPORATING SURFACTANTS FOR PULMONARY DRUG DELIVERY**

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(List continued on next page.)

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(74) *Attorney, Agent, or Firm*—Hamilton, Brook, Smith & Reynolds, P.C.

Related U.S. Patent Documents

(57) **ABSTRACT**

Reissue of:
(64) Patent No.: **5,855,913**
Issued: **Jan. 5, 1999**
Appl. No.: **08/784,421**
Filed: **Jan. 16, 1997**

Improved aerodynamically light particles for drug delivery to the pulmonary system, and methods for their synthesis and administration are provided. In a preferred embodiment, the aerodynamically light particles are made of a biodegradable material and have a tap density less than 0.4 g/cm³ and a mass mean diameter between 5 μm and 30 μm. The particles may be formed of biodegradable materials such as biodegradable polymers. For example, the particles may be formed of a functionalized polyester graft copolymer consisting of a linear α-hydroxy-acid polyester backbone having at least one amino acid group incorporated therein and at least one poly(amino acid) side chain extending from an amino acid group in the polyester backbone. In one embodiment, aerodynamically light particles having a large mean diameter, for example greater than 5 μm, can be used for enhanced delivery of a therapeutic agent to the alveolar region of the lung. The aerodynamically light particles incorporating a therapeutic agent may be effectively aerosolized for administration to the respiratory tract to permit systemic or local delivery of wide variety of therapeutic agents.

U.S. Applications:
(63) Continuation-in-part of application No. 08/739,308, filed on Oct. 29, 1996, now Pat. No. 5,874,064, which is a continuation-in-part of application No. 08/655,570, filed on May 26, 1996, now abandoned.

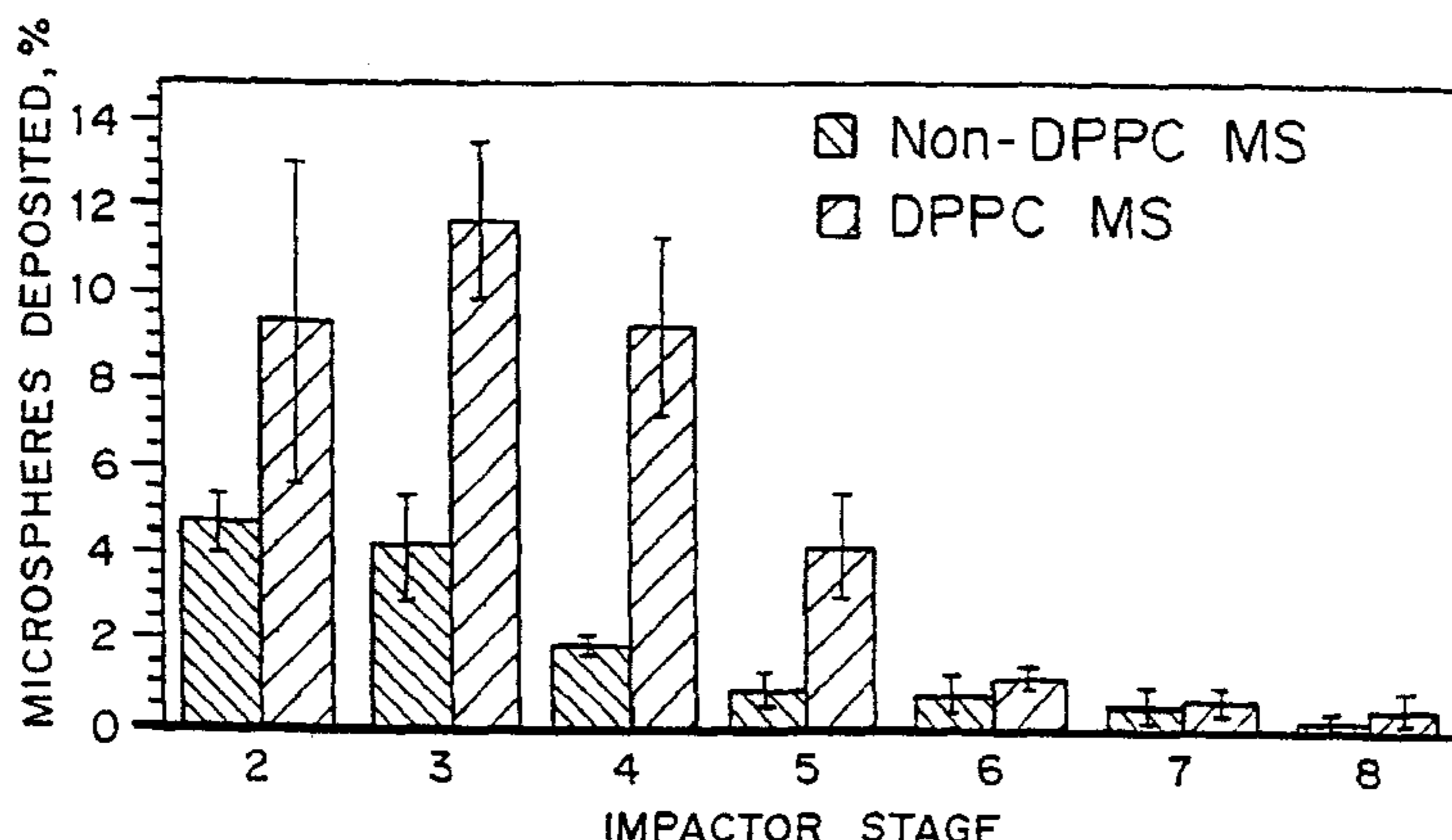
(51) **Int. Cl.**⁷ **A61K 9/14; A61K 9/16**
(52) **U.S. Cl.** **424/489; 424/499; 424/501; 424/43; 424/45; 424/46; 424/434**
(58) **Field of Search** **424/489-502, 424/434, 43-45, 46**

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33 Claims, 2 Drawing Sheets



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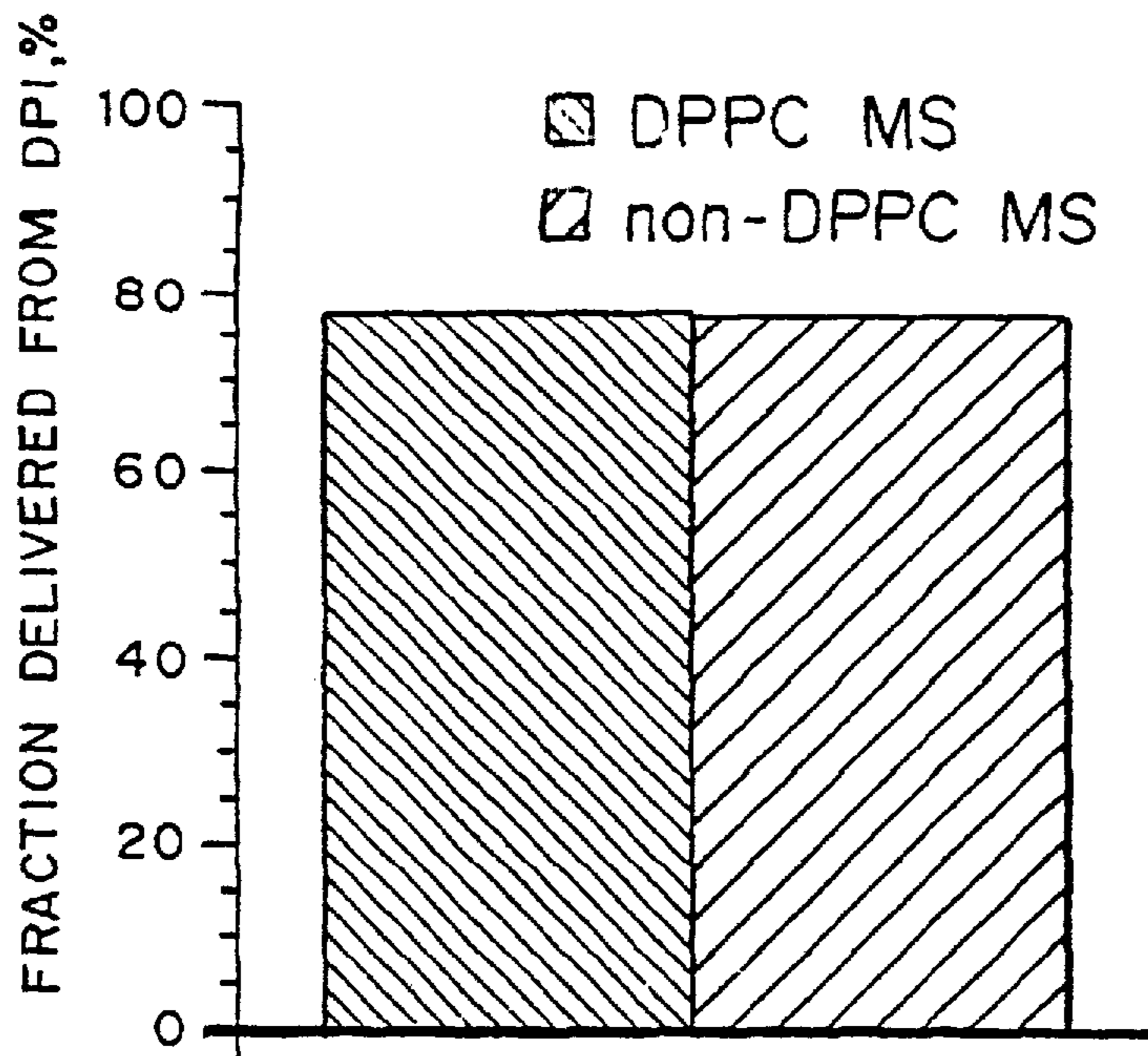


FIG. 1

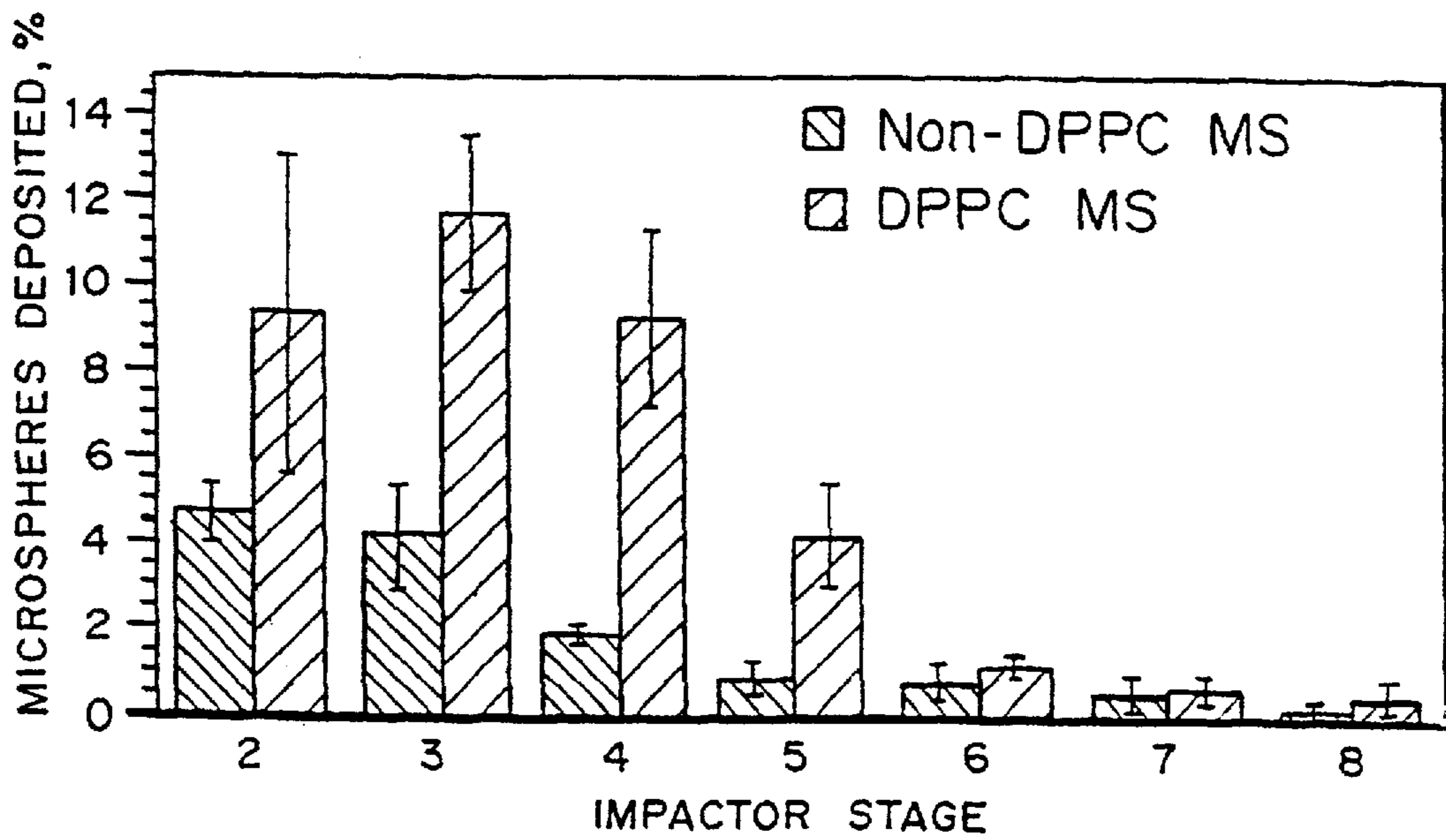


FIG. 2

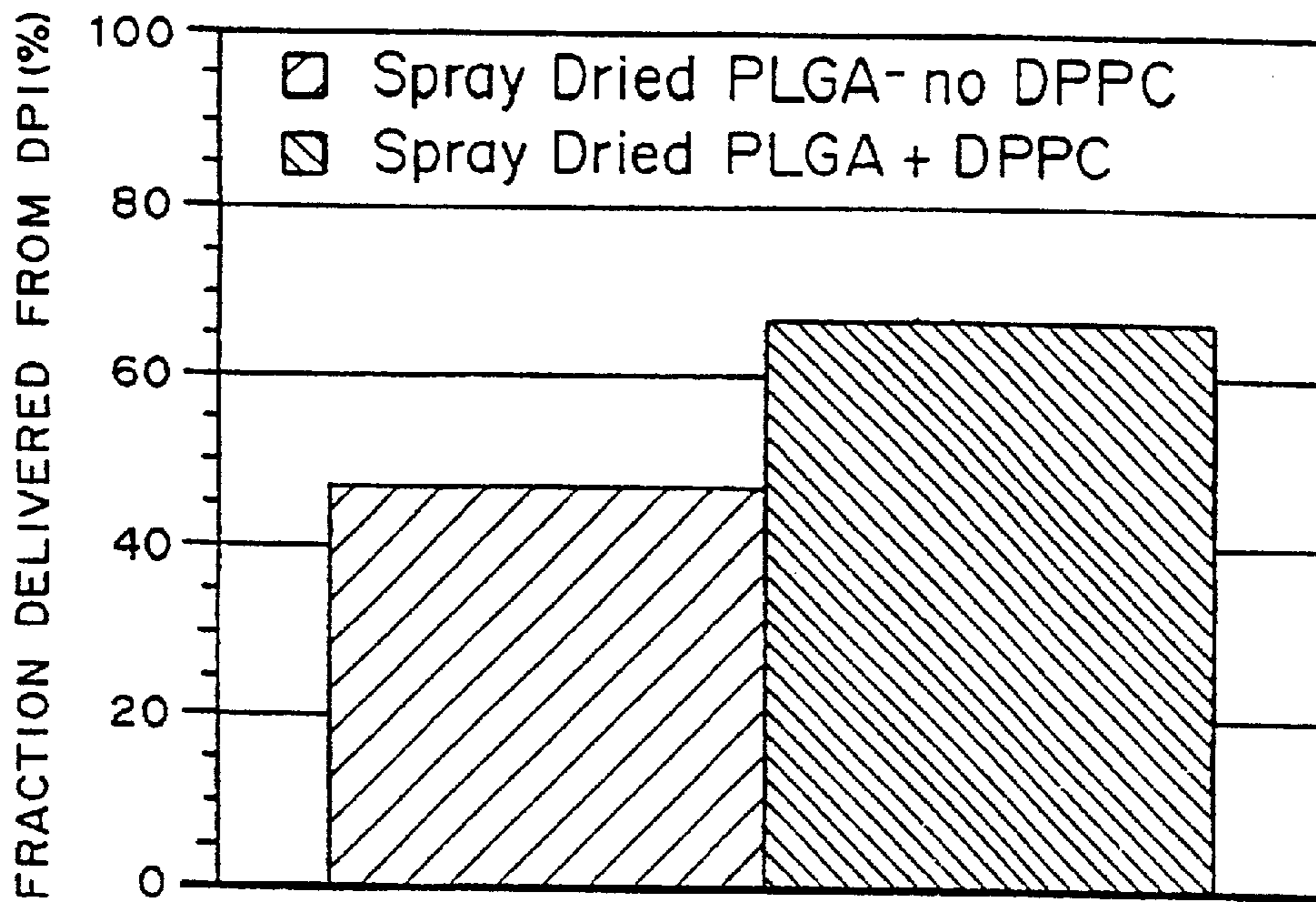


FIG. 3

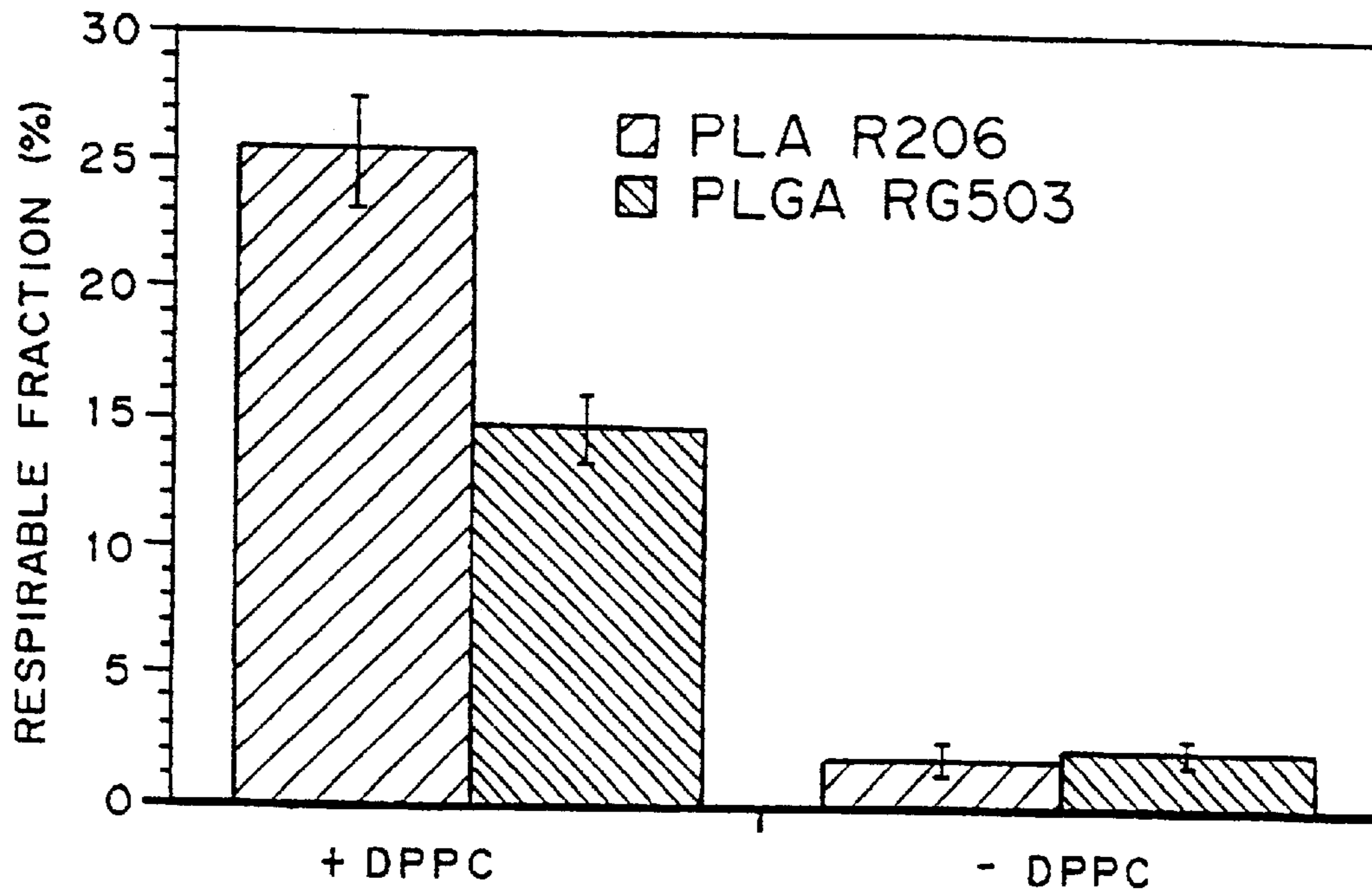


FIG. 4

**PARTICLES INCORPORATING
SURFACTANTS FOR PULMONARY DRUG
DELIVERY**

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

RELATED APPLICATIONS

This Application is a Continuation-in-Part of U.S. application having U.S. Ser. No. 08/739,308 filed on Oct. 29, 1996, now U.S. Pat. No. 5,874,064, which is a Continuation-in-Part of U.S. Ser. No. 08/655,570 filed on May 26, 1996, which is abandoned, the entire teachings of all of which are incorporated herein by reference.

The government has certain rights in this invention by virtue of Grant Number HD29129 awarded to the National Institutes of Health to Robert S. Langer.

BACKGROUND OF THE INVENTION

The present invention relates generally to particles incorporating surfactants for use in drug delivery to the pulmonary system.

Biodegradable particles have been developed for the controlled-release and delivery of protein and peptide drugs. Langer, R., *Science* 249: 1527-1533 (1990). Examples include the use of biodegradable particles for gene therapy (Mulligan, R. C., *Science*, 260: 926-932 (1993)) and for 'single-shot' immunization by vaccine delivery (Eldridge et al., *Mol. Immunol.*, 28: 287-294 (1991)).

Aerosols for the delivery of therapeutic agents to the respiratory tract have been developed. Adjei, A. and Garren, J. *Pharm. Res.*, 7: 565-569 (1990); and Zanen, P. and Lamm, J.-W. J. *Int. J. Pharm.*, 114: 111-115 (1995). The respiratory tract 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. The upper and lower airways are called the conducting airways. The terminal bronchioli then divide into respiratory bronchioli which then lead to the ultimate respiratory zone, the alveoli, or deep lung. 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). The deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic drug delivery.

Inhaled aerosols have been used for the treatment of local lung disorders including asthma and cystic fibrosis (Anderson et al., *Am. Rev. Respir. Dis.*, 140: 1317-1324 (1989)) and have potential for the systemic delivery of peptides and proteins as well (Patton and Platz, *Advanced Drug Delivery Reviews*, 8:179-196 (1992)). However, pulmonary drug delivery of macromolecules; these include protein denaturation during aerosolization, excessive loss of inhaled drug in the oropharyngeal cavity (often exceeding 80%), poor control over the site of deposition, irreproducibility of therapeutic results owing to variations in breathing patterns, the often too-rapid absorption of drug potentially resulting in local toxic effects, and phagocytosis by lung macrophages.

Considerable attention has been devoted to the design of therapeutic aerosol inhalers to improve the efficiency of inhalation therapies. Timsina et. al., *Int. J. Pharm.*, 101: 1-13 (1995); and Tansey, I. P., *Spray Technol. Market*, 4:

26-29 (1994). Attention has also been given to the design of dry powder aerosol surface texture, regarding particularly the need to avoid particle aggregation, a phenomenon which considerably diminishes the efficiency of inhalation therapies. French, D. L., Edwards, D. A. and Niven, R. W., *J. Aerosol Sci.*, 27: 769-783 (1996). Dry powder formulations ("DPFs") with large particle size have improved flowability characteristics, such as less aggregation (Visser, J., *Powder Technology* 58: 1-10 (1989)), easier aerosolization, and potentially less phagocytosis. Rudt, S. and R. H. Muller, *J. Controlled Release*, 22: 263-272 (1992); Tabata, Y., and Y. Ikada, *J. Biomed. Mater. Res.*, 22: 837-858 (1988). Dry powder aerosols for inhalation therapy are generally produced with mean diameters primarily in the range of <5 μm . Ganderton, D., *J. Biopharmaceutical Sciences*, 3:101-105 (1992); and Gonda, I. "Physico-Chemical Principles in Aerosol Delivery," in *Topics in Pharmaceutical Sciences* 1991, Crommelin, D. J. and K. K. Midha, Eds., Medpharm Scientific Publishers, Stuttgart, pp. 95-115, 1992. Large "carrier" particles (containing no drug) have been co-delivered with therapeutic aerosols to aid in achieving efficient aerosolization among other possible benefits. French, D. L., Edwards, D. A. and Niven, R. W., *J. Aerosol Sci.*, 27: 769-783 (1996).

The human lungs can remove or rapidly degrade hydrolytically cleavable deposited aerosols over periods ranging from minutes to hours. In the upper airways, ciliated epithelia contribute to the "mucociliary escalator" by which particles are swept from the airways toward the mouth. Pavia, D. "Lung Mucociliary Clearance," in *Aerosols and the Lung: Clinical and Experimental Aspects*, Clarke, S. W. and Pavia, D., Eds., Butterworths, London, 1984. Anderson et al., *Am. Rev. Respir. Dis.*, 140: 1317-1324 (1989). In the deep lungs, alveolar macrophages are capable of phagocytosing particles soon after their deposition. Warheit, M. B. and Hartschy, M. A., *Microscopy Res. Tech.*, 26: 412-422 (1993); Brain, J. D., "Physiology and Pathophysiology of Pulmonary Macrophages," in *The Reticuloendothelial System*, S. M. Reichard and J. Filkins, Eds., Plenum, New York, pp. 315-327, 1985; Dorries, A. M. and Valberg, P. A., *Am. Rev. Resp. Disease* 146: 831-837 (1991); and Gehr, P. et al. *Microscopy Res. and Tech.*, 26: 423-436 (1993). As the diameter of particles exceeds 3 μm , there is increasingly less phagocytosis by macrophages. Kawaguchi, H. et al., *Biomaterials* 7: 61-66 (1986); Krenis, L. J. and Strauss, B., *Proc. Soc. Exp. Med.*, 107:748-750 (1961); and Rudt, S. and Muller, R. H., *J. Contr. Rel.*, 22: 263-272 (1992). However, increasing the particle size also has been found to minimize the probability of particles (possessing standard mass density) entering the airways and acini due to excessive deposition in the oropharyngeal or nasal regions. Heyder, J. et al., *J. Aerosol Sci.*, 17: 811-825 (1986).

Local and systemic inhalation therapies can often benefit from a relatively slow controlled release of the therapeutic agent. Gonda, I., "Physico-chemical principles in aerosol delivery," in: *Topics in Pharmaceutical Sciences* 1991, D. J. A. Crommelin and K. K. Midha, Eds., Stuttgart: Medpharm Scientific Publishers, pp. 95-117 (1992). Slow release from a therapeutic aerosol can prolong the residence of an administered drug in the airways or acini, and diminish the rate of drug appearance in the bloodstream. Also, patient compliance is increased by reducing the frequency of dosing. Langer, R., *Science*, 249:1527-1533 (1990); and 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).

Controlled release drug delivery to the lung may simplify the way in which many drugs are taken. Gonda, I., *Adv. Drug*

Del. Rev., 5: 1–9 (1990); and Zeng, X. et al., *Int. J. Pharm.*, 124: 149–164 (1995). Pulmonary drug delivery is an attractive alternative to oral, transdermal, and parenteral administration because self-administration is simple, the lungs provide a large mucosal surface for drug absorption, there is no first-pass liver effect of absorbed drugs, and there is reduced enzymatic activity and pH mediated drug degradation compared with the oral route. Relatively high bioavailability of many molecules, including macromolecules, can be achieved via inhalation. Wall, D. A., *Drug Delivery*, 2: 1–20 (1995); Patton, J. and Platz, R., *Adv. Drug Del. Rev.*, 8: 179–196 (1992); and Bryon, P., *Adv. Drug. Del. Rev.*, 5: 107–132 (1990). As a result, several aerosol formulations of therapeutic drugs are in use or are being tested for delivery to the lung. Patton, J. S., et al., *J. Controlled Release*, 28: 79–85 (1994); Damms, B. and Bains, W., *Nature Biotechnology* (1996); Niven, R. W., et al., *Pharm. Res.*, 12(9): 1343–1349 (1995); and Kobayashi, S., et al., *Pharm. Res.*, 13(1): 80–83 (1996).

Drugs currently administered by inhalation come primarily as liquid aerosol formulations. However, many drugs and excipients, especially proteins, peptides (Liu, R., et al., *Biotechnol. Bioeng.*, 37: 177–184 (1991)), and biodegradable carriers such as poly(lactide-co-glycolides) (PLGA), are unstable in aqueous environments for extended periods of time. This can make storage as a liquid formulation problematic. In addition, protein denaturation can occur during aerosolization with liquid formulations. Mumenthaler, M., et al., *Pharm. Res.*, 11: 12–20 (1994). Considering these and other limitations, dry powder formulations (DPF's) are gaining increased interest as aerosol formulations for pulmonary delivery. Damms, B. and W. Bains, *Nature Biotechnology* (1996); Kobayashi, S., et al., *Pharm. Res.*, 13(1): 80–83 (1996); and Timsina, M., et al., *Int. J. Pharm.*, 101: 1–13 (1994). However, among the disadvantages of DPF's is that powders of ultrafine particulates usually have poor flowability and aerosolization properties, leading to relatively low respirable fractions of aerosol, which are the fractions of inhaled aerosol that escape deposition in the mouth and throat. Gonda, I., in *Topics in Pharmaceutical Sciences* 1991, D. Crommelin and K. Midha, Editors, Stuttgart: Medpharm Scientific Publishers, 95–117 (1992). A primary concern with many aerosols is particulate aggregation caused by particle-particle interactions, such as hydrophobic, electrostatic, and capillary interactions. An effective dry-powder inhalation therapy for both short and long term release or therapeutics, either for local or systemic delivery, requires a powder that displays minimum aggregation, as well as a means of avoiding or suspending the lung's natural clearance mechanisms until drugs have been effectively delivered.

There is a need for improved inhaled aerosols for pulmonary delivery of therapeutic agents. There is a need for the development of drug carriers which are capable of delivering the drug in an effective amount into the airways or the alveolar zone of the lung. There further is a need for the development of drug carriers for use as inhaled aerosols which are biodegradable and are capable of controlled release of drug within the airways or in the alveolar zone of the lung. There also is a need for particles for pulmonary drug delivery with improved aerosolization properties.

It is therefore an object of the present invention to provide improved carriers for the pulmonary delivery of therapeutic agents. It is a further object of the invention to provide inhaled aerosols which are effective carriers for delivery of therapeutic agents to the deep lung. It is another object of the invention to provide carriers for pulmonary delivery which

avoid phagocytosis in the deep lung. It is a further object of the invention to provide carriers for pulmonary drug delivery which are capable of biodegrading and releasing the drug at a controlled rate. It is yet another object of the invention to provide particles for pulmonary drug delivery with improved aerosolization properties and optimized particle—particle interactions.

SUMMARY OF THE INVENTION

Particles incorporating surfactants for drug delivery to the pulmonary system, and methods for their synthesis and administration are provided. Exemplary surfactants include naturally occurring phosphatidylcholines, such as dipalmitoylphosphatidylcholine (“DPPC”). In a preferred embodiment, the particles are aerodynamically light particles, which are made of a biodegradable material, and have a tap density less than 0.4 g/cm^3 , as described in U.S. Ser. No. [08/655,570,] 08/739,308, filed Oct. 29, 1996, the disclosure of which is incorporated herein. The aerodynamically light particles generally have a mean diameter between $5 \mu\text{m}$ and $30 \mu\text{m}$. The particles may be formed of biodegradable materials such as biodegradable polymers, proteins, or other water soluble or non-water soluble materials. Other examples include particles formed of water-soluble excipients, such as trehalose or lactose, or proteins, such as lysozyme or insulin. The particles incorporating a surfactant can be used for enhanced delivery of a therapeutic agent to the airways or the alveolar region of the lung. The particles may be effectively aerosolized for administration to the respiratory tract to permit systemic or local delivery of a wide variety of therapeutic agents. They also optionally may be co-delivered with larger carrier particles, not carrying a therapeutic agent, having, for example, a mean diameter ranging between about $50 \mu\text{m}$ and $100 \mu\text{m}$.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph comparing the mass fraction of the initial dose that is released from a dry powder inhaler device, after in vitro aerosolization of poly (D,L-lactic-co-glycolic acid) (“PLGA”) microspheres made by a double emulsion procedure with and without the incorporation of L- α -phosphatidylcholine dipalmitoyl (“DPPC”).

FIG. 2 is a graph comparing the mass fraction of the aerosolized dose that is deposited in different stages of a cascade impactor after in vitro aerosolization of PLGA microspheres made by a double emulsion procedure with and without the incorporation of DPPC.

FIG. 3 is a graph showing the aerosolization behavior of PLGA microspheres made by spray drying with and without the incorporation of DPPC showing the mass-fraction of the initial dose that is released from the dry powder inhaler device after in vitro aerosolization.

FIG. 4 is a graph comparing the in vitro aerosolization behaviors of PLA and PLGA microspheres made by spray drying with and without the incorporation of DPPC showing the mass-fraction of the aerosolized dose that is deposited in stages of a cascade impactor corresponding to the “respirable-fraction”.

DETAILED DESCRIPTION OF THE INVENTION

Particles incorporating a surfactant for pulmonary drug delivery are provided. In one embodiment, the particles are aerodynamically light, biodegradable particles, having a tap density less than about 0.4 g/cm^3 . The particles can be used

for controlled systemic or local drug delivery to the respiratory tract via aerosolization. Administration of the low density particles to the lung by aerosolization permits deep lung delivery of relatively large diameter therapeutic aerosols, for example, greater than 5 μm in mean diameter. The particles can be fabricated with a rough surface texture to reduce particle agglomeration and improve flowability of the powder. The particles incorporating a surfactant have improved aerosolization properties. The 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.

Surfactants

Surfactants which can be incorporated into particles to improve their aerosolization properties include phosphoglycerides. Exemplary phosphoglycerides include phosphatidylcholines, such as the naturally occurring lung surfactant, L- α -phosphatidylcholine dipalmitoyl ("DPPC"). The surfactants advantageously improve surface properties by, for example, reducing particle-particle interactions, and can render the surface of the particles less adhesive. The use of surfactants endogenous to the lung may avoid the need for the use of non-physiologic surfactants.

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 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 drug and increase bioavailability of the drug.

As used herein, a particle "incorporating a surfactant" refers to a particle with a surfactant on at least the surface of the particle. The surfactant may be incorporated throughout the particle and on the surface during particle formation, or may be coated on the particle after particle formation. The surfactant can be coated on the particle surface by adsorption, ionic or covalent attachment, or physically "entrapped" by the surrounding matrix. The surfactant can be, for example, incorporated into controlled release particles, such as polymeric microspheres.

Providing a surfactant on the surfaces of the particles can reduce the tendency of the particles to agglomerate due to interactions such as electrostatic interactions, Van der Waals forces, and capillary action. The presence of the surfactant on the particle surface can provide increased surface rugosity (roughness), thereby improving aerosolization by reducing the surface area available for intimate particle-particle interaction. The use of a surfactant which is a natural material of the lung can potentially reduce opsonization (and thereby reducing phagocytosis by alveolar macrophages), thus providing a longer-lived controlled release particle in the lung.

Surfactants known in the art can be used including any naturally occurring lung surfactant. Other exemplary surfactants include diphosphatidyl glycerol (DPPG); hexadecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; sorbitan trioleate (Span 85); glycocholate; surfactin; a poloxomer; a sorbitan fatty acid ester such as sorbitan trioleate; tyloxapol and a phospholipid.

Particle Materials

The particles preferably are biodegradable and biocompatible, and optionally are capable of biodegrading at a controlled rate for delivery of a drug. The particles can be made of a variety of materials. Preferably, the particles are "aerodynamically light particles", which as used herein, refers to particles having a tap density less than about 0.4 g/cm^3 . Both inorganic and organic materials can be used. For example, ceramics may be used. Polymeric and non-polymeric materials, such as fatty acids, may be used to form aerodynamically light particles. Optionally the particles may be formed of the surfactant plus a therapeutic or diagnostic agent. Different properties of the particle which can contribute to the aerodynamic lightness include the composition forming the particle, and the presence of irregular surface structure, or pores or cavities within the particle.

Polymeric Particles

Polymeric particles may be formed from any biocompatible and preferably biodegradable polymer, copolymer, or blend. Preferred particles are those which are capable of forming aerodynamically light particles having a tap density less than about 0.4 g/cm^3 .

Surface eroding polymers such as polyanhydrides may be used to form the particles. For example, polyanhydrides such as poly[(p-carboxyphenoxy)-hexane anhydride] (PCPH) may be used. Biodegradable polyanhydrides are described in U.S. Pat. No. 4,857,311, the disclosure of which is incorporated herein by reference.

In another embodiment, bulk eroding polymers such as those based on polyesters including poly(hydroxy acids) can be used. For example, polyglycolic acid (PGA), polylactic acid (PLA), or copolymers thereof may be used to form the particles. The polyester may also have a charged or functionalizable group, such as an amino acid. In a preferred embodiment, particles with controlled release properties can be formed of poly(D,L-lactic acid) and/or poly(D,L-lactico-glycolic acid) ("PLGA") which incorporate a surfactant such as DPPC.

Other polymers include polyamides, polycarbonates, polyalkylenes such as polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly vinyl compounds such as polyvinyl alcohols, polyvinyl ethers, and polyvinyl esters, polymers of acrylic and methacrylic acids, celluloses and other polysaccharides, and peptides or proteins, or copolymers or blends thereof. Polymers may be selected with or modified to have the appropriate stability and degradation rates in vivo for different controlled drug delivery applications.

In one embodiment, aerodynamically light particles are formed from functionalized polyester graft copolymers, as described in Hrkach et al., *Macromolecules*, 28:4736-4739 (1995); and Hrkach et al., "Poly(L-Lactic acid-co-amino acid) Graft Copolymers: A Class of Functional Biodegradable Biomaterials" in *Hydrogels and Biodegradable Polymers for Bioapplications*, ACS Symposium Series No. 627, Raphael M. Ottenbrite et al., Eds., American Chemical Society, Chapter 8, pp. 93-101, 1996.

Other Particle Materials

Materials other than biodegradable polymers may be used to form the particles including other polymers and various excipients. The particles also may be formed of the drug or diagnostic agent and surfactant alone. In one embodiment, the particles may be formed of the surfactant and include a therapeutic agent, to improve aerosolization efficiency due to reduced particle surface interactions, and to potentially reduce drug loss due to phagocytosis by alveolar macrophages.

Other materials include, but are not limited to, gelatin, polyethylene glycol, trehalose, and dextran. Particles with degradation and release times ranging from seconds to months can be designed and fabricated, based on factors such as the particle material.

The polymers may be tailored to optimize different characteristics of the particle including: i) interactions between the agent to be delivered and the polymer to provide stabilization of the agent and retention of activity upon delivery; ii) rate of polymer degradation and, thereby, rate of drug release profiles; iii) surface characteristics and targeting capabilities via chemical modification; and iv) particle porosity.

Formation of Polymeric Particles

Polymeric particles may be prepared using single and double emulsion solvent evaporation, spray drying, solvent extraction, solvent evaporation, phase separation, simple and complex coacervation, interfacial polymerization, and other methods well known to those of ordinary skill in the art. Particles may be made using methods for making microspheres or microcapsules known in the art.

Methods developed for making microspheres for drug delivery are described in the literature, for example, as described in Doubrow, M., Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992. Methods also are described in Mathiowitz and Langer, *J. Controlled Release* 5,13-22 (1987); Mathiowitz et al., *Reactive Polymers* 6, 275-283 (1987); Mathiowitz et al., *J. Appl. Polymer Sci.* 35, 755-774 (1988), the teachings of which are incorporated herein. The selection of the method depends on the polymer selection, the size, external morphology, and crystallinity that is desired, as described, for example, by Mathiowitz et al., *Scanning Microscopy* 4: 329-340 (1990); Mathiowitz et al., *J. Appl. Polymer Sci.* 45, 125-134 (1992); and Benita et al., *J. Pharm. Sci.* 73, 1721-1724 (1984), the teachings of which are incorporated herein.

In solvent evaporation, described for example, in Mathiowitz et al., (1990), Benita; and U.S. Pat. No. 4,272,398 to Jaffe, the polymer is dissolved in a volatile organic solvent, such as methylene chloride. Several different polymer concentrations can be used, for example, between 0.05 and 1.0 g/ml. The drug, either in soluble form or dispersed as fine particles, is added to the polymer solution, and the mixture is suspended in an aqueous phase that contains a surface active agent such as poly(vinyl alcohol). The aqueous phase may be, for example, a concentration of 1% poly(vinyl alcohol) w/v in distilled water. The resulting emulsion is stirred until most of the organic solvent evaporates, leaving solid microspheres, which may be washed with water and dried overnight in a lyophilizer. Microspheres with different sizes (1-1000 microns) and morphologies can be obtained by this method.

Solvent removal was primarily designed for use with less stable polymers, such as the polyanhydrides. In this method, the drug is dispersed or dissolved in a solution of a selected polymer in a volatile organic solvent like methylene chloride. The mixture is then suspended in oil, such as silicon oil, by stirring, to form an emulsion. Within 24 hours, the solvent diffuses into the oil phase and the emulsion droplets harden into solid polymer microspheres. Unlike the hot-melt microencapsulation method described for example in Mathiowitz et al., *Reactive Polymers*, 6:275 (1987), this method can be used to make microspheres from polymers with high melting points and a wide range of molecular weights. Microspheres having a diameter for example between one and 300 microns can be obtained with this procedure.

Aerodynamically Light Particles

Aerodynamically light particles, having a tap density less than about 0.4 g/cm^3 , may be fabricated, as described in U.S. Ser. No. [08/655,570,] 08/739,308, filed Oct. 29, 1996, the disclosure of which is incorporated herein.

Aerodynamically Light Particle Size

The mass mean diameter of the particles can be measured using a Coulter Multisizer II (Coulter Electronics, Luton, Beds, England). The aerodynamically light particles in one preferred embodiment are at least about 5 microns in diameter. 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 optimal deposition within targeted sites within the respiratory tract.

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

In one embodiment, in the particle sample, the interquartile range may be $2 \mu\text{m}$, with a mean diameter for example, between about 7.5 and $13.5 \mu\text{m}$. Thus, for example, between at least 30% and 40% of the particles may have diameters within the selected range. Preferably, the percentages of particles have diameters within a $1 \mu\text{m}$ range, for example, $6.0-7.0 \mu\text{m}$, $10.0-11.0 \mu\text{m}$ or $13.0-14.0 \mu\text{m}$.

The aerodynamically light particles incorporating a therapeutic drug, and having a tap density less than about 0.4 g/cm^3 , with mean diameters of at least about $5 \mu\text{m}$, 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 particles (mean diameter at least about $5 \mu\text{m}$) is advantageous since they are able to aerosolize more efficiently than smaller, non-light aerosol particles such as those currently used for inhalation therapies.

In comparison to smaller non-light particles, the larger (at least $5 \mu\text{m}$) aerodynamically light particles 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 $3 \mu\text{m}$. Kawaguchi, H. et al., *Biomaterials* 7: 61-66 (1986); Krenis, L. J. and Strauss, B., *Proc. Soc. Exp. Med.*, 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.

Aerodynamically light particles thus are capable of a longer term release of a therapeutic agent in the lungs. Following inhalation, aerodynamically light biodegradable

particles can deposit in the lungs (due to their relatively low tap density), and subsequently undergo slow degradation and drug release, without the majority of the particles being phagocytosed by alveolar macrophages. The drug can be delivered relatively slowly into the alveolar fluid, and at a controlled rate into the blood stream, minimizing possible toxic responses of exposed cells to an excessively high concentration of the drug. The aerodynamically light particles thus are highly suitable for inhalation therapies, particularly in controlled release applications.

The preferred mean diameter for aerodynamically light particles for inhalation is at least about 5 μm , for example between about 5 and 30 μm . The particles may be fabricated with the appropriate material, surface roughness, diameter and tap density for localized delivery to selected regions of the respiratory tract such as the deep lung or upper airways. For example, higher density or larger particles may be used for upper airway delivery, or a mixture of different 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 administration.

Density and Deposition of Aerodynamically Light Particles

As used herein, the phrase "aerodynamically light particles" refers to particles having a tap density less than about 0.4 g/cm^3 . The tap density of particles of a dry powder may be obtained using a GeoPycTM (Micrometrics Instrument Corp., Norcross, Ga. 30093). Tap density is a standard measure of the envelope mass density. The envelope mass density of an isotropic particle is defined as the mass of the particle divided by the minimum sphere envelope volume within which it can be enclosed. Features which can contribute to low tap density include irregular surface texture and porous structure.

Inertial impaction and gravitational settling of aerosols are predominant 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 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 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:

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

where the envelope mass ρ is in units of g/cm^3 . Maximal deposition of monodisperse aerosol particles in the alveolar region of the human lung (~60%) occurs for an aerodynamic diameter of approximately $d_{aer} = 3 \mu\text{m}$. 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 inhaled powder that will exhibit maximum deep-lung deposition is:

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

where d is always greater than 3 μm . For example, aerodynamically light particles that display an envelope mass density, $\rho = 0.1 \text{ g}/\text{cm}^3$, will exhibit a maximum 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.

Targeting of Particles

Targeting molecules can be attached to the particles via reactive functional groups on the particles. For example, targeting molecules can be attached to the amino acid groups of functionalized polyester graft copolymer particles, such as PLAL-Lys particles. Targeting molecules permit binding interaction of the particle with specific receptor sites, such as those within the lungs. The particles can be targeted by attachment of ligands which specifically or non-specifically bind to particular targets. Exemplary targeting molecules include antibodies and fragments thereof including the variable regions, lectins, and hormones or other organic molecules capable of specific binding, for example, to receptors on the surfaces of the target cells.

Therapeutic Agents

Any of a variety of therapeutic, prophylactic or diagnostic agents can be incorporated within the particles, or used to prepare particles consisting solely of the agent and surfactant. The particles can be used to locally or systemically deliver a variety of therapeutic agents to an animal. Examples include 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. Nucleic acid sequences include genes, antisense molecules which bind to complementary DNA to inhibit transcription, and ribozymes. The agents to be incorporated 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, and antibodies. In some instances, the proteins may be antibodies or antigens which otherwise would have to be administered by injection to elicit an appropriate response. Compounds with a wide range of molecular weight can be encapsulated, for example, between 100 and 500,000 grams or more per mole.

Proteins are defined as consisting of 100 amino acid residues or more; peptides are less than 100 amino acid residues. Unless otherwise stated, the term protein refers to both proteins and peptides. Examples include insulin and other hormones. Polysaccharides, such as heparin, can also be administered.

The polymeric aerosols are useful as carriers for a variety of inhalation therapies. They can be used to encapsulate small and large drugs, release encapsulated drugs over time periods ranging from hours to months, and withstand extreme conditions during aerosolization or following deposition in the lungs that might otherwise harm the encapsulated therapeutic.

The particles may include a therapeutic agent for local delivery within the lung, such as agents for the treatment of asthma, emphysema, or cystic fibrosis, or for systemic treatment. For example, genes for the treatment of diseases

such as cystic fibrosis can be administered, as can beta agonists for asthma. Other specific therapeutic agents include, but are not limited to, insulin, calcitonin, leuprolide (or gonadotropin-releasing hormone ("LHRH")), granulocyte colony-stimulating factor ("G-CSF"), parathyroid hormone-related peptide, somatostatin, testosterone, progesterone, estradiol, nicotine, fentanyl, norethisterone, clonidine, scopolomine, salicylate, cromolyn sodium, salmeterol, formeterol, albuterol, and vallyum.

Administration

The particles incorporating a surfactant and a therapeutic agent may be administered alone or in any appropriate pharmaceutical carrier, such as a liquid, for example saline, or a powder, for administration to the respiratory system. They can be co-delivered with larger carrier particles, not including a therapeutic agent, the latter possessing mass mean diameters for example in the range 50 μm –100 μm .

Aerosol dosage, formulations and delivery systems 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, "Aerosol dosage forms and formulations," in: *Aerosols in Medicine, Principles, Diagnosis and Therapy*, Moren, et al., Eds. Esvier, Amsterdam, 1985, the disclosures of which are incorporated herein by reference.

The greater efficiency of aerosolization by particles incorporating a surfactant permits more drug to be delivered. The use of biodegradable polymers permits controlled release in the lungs and long-time local action or systemic bioavailability. Denaturation of macromolecular drugs can be minimized during aerosolization since macromolecules are contained and protected within a polymeric shell. Coencapsulation of peptides with peptidase-inhibitors can minimize peptide enzymatic degradation. Pulmonary delivery advantageously can eliminate the need for injection. For example, the requirement for daily insulin injections can be avoided.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1

Synthesis of Aerodynamically Light Poly[(p-carboxyphenoxy)-hexane anhydride] ("PCPH") Particles

Aerodynamically light poly[(p-carboxyphenoxy)-hexane anhydride] ("PCPH") particles were synthesized as follows. 100 mg PCPH (MW~25,000) was dissolved in 3.0 mL methylene chloride. To this clear solution was added 5.0 mL 1% w/v aqueous polyvinyl alcohol (PVA, MW ~25,000, 88 mole % hydrolyzed) saturated with methylene chloride, and the mixture was vortexed (Vortex Genie 2, Fisher Scientific) at maximum speed for one minute. The resulting milky-white emulsion was poured into a beaker containing 95 mL 1% PVA and homogenized (Silverson Homogenizers) at 6000 RPM for one minute using a 0.75 inch tip. After homogenization, the mixture was stirred with a magnetic stirring bar and the methylene chloride quickly extracted from the polymer particles by adding 2 mL isopropyl alcohol. The mixture was continued to stir for 35 minutes to allow complete hardening of the microparticles. The hardened particles were collected by centrifugation and washed several times with double distilled water. The particles were freeze dried to obtain a free-flowing powder void of clumps. Yield, 85–90%.

The mean diameter of a typical batch prepared by this protocol is 6.0 μm , however, particles with mean diameters ranging from a few hundred nanometers to several millimeters may be made with only slight modifications. Scanning electron micrograph photos of a typical batch of PCPH particles showed the particles to be highly porous with irregular surface shape. The particles have a tap density less than 0.4 g/cm³.

A surfactant such as DPPC may be incorporated into the polymer solution prior to particle formation, or optionally the particles can be ionically or covalently coated by surfactant on the particle surface after particle formation, or the surfactant may be absorbed onto the particle surface.

Example 2

Synthesis of Spray-Dried Particles Aerodynamically Light Particles Containing Polymer and Drug Soluble in Common Solvent

Aerodynamically light 50:50 PLGA particles were prepared by spray drying with testosterone encapsulated within the particles according to the following procedures. 2.0 g poly (D,L-lactic-co-glycolic acid) with a molar ratio of 50:50 (PLGA 50:50, Resomer RG503, B.I. Chemicals, Montvale, N.J.) and 0.50 g testosterone (Sigma Chemical Co., St. Louis, Mo.) are completely dissolved in 100 mL dichloromethane at room temperature. The mixture is subsequently spray-dried through a 0.5 mm nozzle at a flow rate of 5 mL/min using a Buchi laboratory spray-drier (model 190, Buchi, Germany). The flow rate of compressed air is 700 nl. The inlet temperature is set to 30° C. and the outlet temperature to 25° C. The aspirator is set to achieve a vacuum of –20 to –25 bar. The yield is 51% and the mean particle size is approximately 5 μm . Larger particle size can be achieved by lowering the inlet compressed air flow rate, as well as by changing other variables. The particles are aerodynamically light, as determined by a tap density less than or equal to 0.4 g/cm³. Porosity and surface roughness can be increased by varying the inlet and outlet temperatures, among other factors.

Aerodynamically Light Particles Containing Polymer and Drug in Different Solvents

Aerodynamically light PLA particles with a model hydrophilic drug (dextran) were prepared by spray drying using the following procedure. 2.0 mL of an aqueous 10% w/v FITC-dextran (MW 70,000, Sigma Chemical Co.) solution was emulsified into 100 mL of a 2% w/v solution of poly (D,L-lactic acid) (PLA, Resomer R206, B.I. Chemicals) in dichloromethane by probe sonication (Sonics & Materials, Model VC-250 sonicator, Danbury, Conn.). The emulsion is subsequently spray-dried at a flow rate of 5 mL/min with an air flow rate of 700 nl/h (inlet temperature=30° C., outlet temperature=21° C., –20 mbar vacuum). The yield is 56%. The particles are aerodynamically light, as determined by a tap density less 0.4 g/cm³.

Aerodynamically Light Protein Particles

Aerodynamically light lysozyme particles were prepared by spray drying using the following procedure. 4.75 g lysozyme (Sigma) was dissolved in 95 mL double distilled water (5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air was 725 nl/h. The flow rate of the lysozyme solution was set such that, at a set inlet temperature of 97°–100° C., the outlet temperature is 55°–57° C. The aspirator was set to achieve a vacuum of –30 mbar. The enzymatic activity of lysozyme was found to be unaffected by this process and the yield of the aerodynamically light particles (tap density less than 0.4 g/cm³) was 66%.

Aerodynamically Light High-Molecular Weight Water-Soluble Particles

Aerodynamically light dextran particles were prepared by spray drying using the following procedure. 6.04 g DEAE dextran (Sigma) was dissolved in 242 mL double distilled water (2.5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air was 750 nl/h. The flow rate of the DEAE-dextran solution was set such that, at a set inlet temperature of 155° C., the outlet temperature was 80° C. The aspirator was set to achieve a vacuum of -20 mbar. The yield of the aerodynamically light particles (tap density less than 0.4 g/cm³) was 66%.

Aerodynamically Light Low-Molecular Weight Water-Soluble Particles

Aerodynamically light trehalose particles were prepared by spray drying using the following procedure. 4.9 g trehalose (Sigma) was dissolved in 192 mL double distilled water (2.5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air 650 nl/h. The flow rate of the trehalose solution was set such that, at a set inlet temperature of 100° C., the outlet temperature was 60° C. The aspirator was set to achieve a vacuum of -30 mbar. The yield of the aerodynamically light particles (tap density less than 0.4 g/cm³) was 36%.

Aerodynamically Light Low-Molecular Weight Water-Soluble Particles

Polyethylene glycol (PEG) is a water-soluble macromolecule, however, it cannot be spray dried from an aqueous solution since it melts at room temperatures below that needed to evaporate water. As a result, PEG was spray-dried at low temperatures from a solution in dichloromethane, a low-boiling organic solvent. Aerodynamically light PEG particles were prepared spray drying using the following procedure. 5.0 g PEG (MW 15,000-20,000, Sigma) was dissolved in 100 mL double distilled water (5.0% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air 750 nl/h. The flow rate of the PEG solution was set such that, at a set inlet temperature of 45° C., the outlet temperature was 34°-35° C. The aspirator was set to achieve a vacuum of -22 mbar. The yield of the aerodynamically light particles (tap density less than 0.4 g/cm³) was 67%.

A surfactant such as DPPC may be incorporated into the polymer solution prior to particle formation, or optionally the particles can be ionically or covalently coated by surfactant on the particle surface after particle formation, or the surfactant may be absorbed onto the particle surface.

Materials and Methods

In Examples 3 and 4 below, the following materials and methods were used.

Materials

The polymers: poly(D,L-lactic-co-glycolic acid) with a molar ratio of 50:50 and reported molecular weights of 100,000 Daltons (PLGA RG506) and 34,000 Daltons (PLGA RG503), and poly(D,L-lactic acid) with a reported molecular weight of 100,000 Daltons (PLA R206) were obtained from Boehringer Ingelheim (distributed by B.I. Chemicals, Montvale, N.J.). Fluorescently labelled FITC-Dextran with an average molecular weight of 19,000, and L, α -phosphatidylcholine dipalmitoyl (DPPC) were purchased from Sigma Chemical Company, St. Louis, Mo.

Microsphere Preparation: Double Emulsion

A double-emulsion solvent-evaporation procedure (Cohen, S., et al., *Pharm. Res.*, 8(6): 713-720 (1991); and Tabata, Y., et al., *Pharm. Res.*, 10(4): 487-496 (1993)), was

modified to prepare microspheres for aerosolization. Briefly, 300 μ l of an aqueous FITC-Dextran solution (50 mg/ml) was emulsified on ice into a 4.0 ml polymer solution is methylene chloride (200 mg polymer) by sonication at output 3 (Model VC-250, Sonics & Materials Inc., Danbury, Conn.) using a microtip for 5-10 s to form the inner-emulsion. The first emulsion was poured into 100 ml 1.0% aqueous PVA solution and homogenized (Model LD4 Homogenizer, Silverson Machines Ltd, England) at 6000 RPM using a 5/8" tip for 1 min to form the double emulsion. The microspheres were continuously stirred for 3 hours to allow hardening, collected by centrifugation, washed several times with double-distilled water, and freeze-dried into a freely flowing powder. Microspheres containing DPPC were prepared by dissolving DPPC in the polymer solution at 3 mg/ml prior to the initial emulsification.

Microsphere Preparation: Spray Drying

The model hydrophilic drug, dextran labeled with fluorescein isothiocyanate (FITC-Dextran), was encapsulated into PLA or PLGA by a novel emulsion/spray method. For example, 2.0 ml of an aqueous 10% w/v FITC-Dextran (MW=70,000, Sigma Chemical Co.) solution was emulsified into 100 ml of a 2% w/v solution of PLA in dichloromethane by probe sonication. The emulsion was subsequently spray-dried using a Büchi Mini Spray Drier (Model 190, Büchi Instruments, Germany) at a flow rate of 5 ml/min with an inlet air flow rate of 700 nl/h, inlet temperature of 30° C., outlet temperature of 21° C., and vacuum of -20 mbar. When DPPC was incorporated it was dissolved in the polymer solution at a concentration of 2 mg/ml prior to emulsification and spray drying.

Microsphere Size Distribution Analysis

Microsphere size distributions were determined using a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England). Approximately 10 drops Coulter type IA non-ionic dispersant were added, followed by 2 mL isoton II solution (Coulter), to 5-10 mg microspheres, and the spheres were dispersed by brief vortex mixing. This suspension was added to 50 mL isoton 11 solution until the coincidence of particles was between 5 and 8%. Greater than 500,000 particles were counted for each batch of spheres.

Drug Distribution by Confocal Microscopy

For confocal microscopy, a few milligrams of microspheres containing FITC-Dextran as the drug were suspended in glycerin by brief probe sonication (Vibra-cell Model VC-250 Sonicator, 1/8" microtip probe, Sonics & Materials Inc., Danbury, Conn.) at output 4 (50 W). A drop of the suspension was placed onto a glass slide and a glass cover slip was applied and held in place with finger nail polish. The suspension was allowed to settle for one hour before being viewed by confocal microscopy (Bio-Rad MRC-600 Confocal, Axioplan microscope).

Microsphere Morphology by Scanning Electron Microscopy (SEM)

Microsphere morphology was observed by scanning electron microscopy (SEM) using a Stereoscan 250 MK3 microscope from Cambridge Instruments (Cambridge, Mass.) at 15 kV. Microspheres were freeze-dried, mounted on metal stubs with double-sided tape, and coated with gold prior to observation.

Microsphere Density Analysis

Microsphere bulk density was estimated by tap density measurements and confirmed by mercury intrusion analysis at Porous Materials, Inc. (Ithaca, N.Y.).

Determination of Amount FITC-Dextran and DPPC Encapsulated

The amount of model drug, FITC-Dextran, encapsulated into microspheres was determined by dissolving 10.0 mg microspheres in 3.0 ml 0.8N NaOH overnight at 37° C., filtering with a 0.45 μ m filter (Millipore), and measuring the

fluorescence relative to a standard curve (494 nm excitation and 525 nm emission) using a fluorimeter. The drug loading was determined by dividing the amount of FITC-Dextran encapsulated by the theoretical amount if it all were encapsulated. The amount of lung surfactant, DPPC, encapsulated into microspheres was determined by dissolving 10.0 mg of microspheres in chloroform and using the Stewart Assay. New, R. R. C., "Characterization of Liposomes," in *Liposomes: A Practical Approach*, R. New, Editor, IRL Press, New York, 105-161 (1990).

In Vitro Aerosolization and Inertial Deposition Behavior

The in vitro microparticle aerodynamic characteristics were studied using an Andersen Mark I Cascade Impactor (Andersen Samplers, Atlanta, Ga.) at an air flow rate of 28.3 l/min. The metal impaction plates were coated with a thin film of Tween 80 minimize particle bouncing Turner, J. and S. Hering, *J. Aerosol Sci.*, 18: 215-224 (1987). Gelatin capsules (Eli Lilly) were charged with 20 mg of microparticles and loaded into a Spinhaler® inhalation device (Fisons, Bedford, Mass.). The aerosolization experiments were done in triplicate. In each experiment, 10 inhalers were discharged for 30 seconds into the impactor. A 60-second interval was observed between every two consecutive aerosolizations. Fractions of microspheres deposited on each of nine stages, corresponding to stages 0-7, and the filter (F) of the impactor, were collected in volumetric flasks by carefully washing the plates with NaOH solution (0.8N) in order to provide degradation of the polymer and complete dissolution of the fluorescent material. After 12 hours of incubation at 37° C., the solutions were filtered with a 0.45 μm filter and the amount of fluorescent material in each stage was measured at 494 nm (excitation) and 525 nm (emission) using a fluorimeter. Respirable fraction of the delivered dose was calculated according to the fluorescence measurements as percentages of the total fluorescence (i.e., that amount collected in stages 0 - Filter) compared with that collected in stages 2 - Filter of the Impactor.

In Vivo Particle Distribution Following Aerosolization in Rats

Male Sprague Dawley rats (150-200 g) were anesthetized using a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The anesthetized rat was placed ventral side up on a surgical table provided with a temperature controlled pad to maintain physiological temperature. The animal was cannulated above the carina with an endotracheal tube connected to a Harvard ventilator (Rodent Ventilator Model 685, South Natick, Mass.). The animal was force ventilated for 20 minutes at 300 ml/min. 50 mg of microspheres made with or without DPPC were introduced into the endotracheal tube. Following the period of forced ventilation, the animal was sacrificed and the lungs and trachea were separately washed using bronchialalveolar lavage as follows: a tracheal cannula was inserted, tied into place, and the airways were washed with 10 ml aliquots of phenol red-free Hanks balanced salt solution (Gibco, Grand Island, N.Y.) without Ca²⁺ and Mg²⁺ (HBSS). The lavage procedure was repeated until a total volume of 30 ml was collected. The lavage fluid was centrifuged (400 g) and the pellets collected and resuspended in 2 ml HBSS. 100 μl was removed for particle counting using a hemacytometer. The remaining solution was mixed with 10 ml of 0.4N NaOH. After incubation at 37° C. for 12 hours, the fluorescence of each solution was measured (wavelengths of 494 nm excitation, 525 nm emission) using a fluorimeter.

Example 3

Fabrication of PLGA microspheres by a Double Emulsion Process Which Encapsulate a Model High-Molecular-Weight Drug, FITC-Dextran.

Scanning electron microscopy "SEM" photographs showing surface morphology of microspheres (MS) made by the

double emulsion process with and without the lung surfactant, DPPC were obtained. By SEM, the microspheres made with and without DPPC by the double emulsion process had very similar surface characteristics and size distribution, as confirmed by size distribution measurements, shown below in Table 1.

The efficient entrapment of DPPC within microspheres (83% of theoretical ±11% standard deviation, n=6) was confirmed by dissolving an aliquot of MS in chloroform and detecting the DPPC concentration in solution by the Stewart Assay, as shown in Table 1. Particles made by double emulsion with DPPC are easily resuspended in aqueous solution after lyophilization and are lump-free when dry as determined by light microscopy. Particles made by the double emulsion process without DPPC resuspend easily, however, they appear somewhat agglomerated when dry by light microscopy.

TABLE 1

Characteristics of Microparticles used for In Vitro and In Vivo Aerosolization ^a				
Sample	Mass-Mean (True) Diameter, (μm)	DPPC Load (μg/mg spheres)	DPPC Loading Efficiency, (%)	FITC-Dextran (Model Drug) Loading Efficiency, (%)
MS without DPPC	8.5 ± 0.76	0	N/A	95.8
MS with DPPC	8.2 ± 0.18	45 ± 6	83 ± 11	82.4

^aValues are given ± standard deviation.

Confocal microscopy was used to evaluate the distribution of the model drug, FITC-Dextran (M_w 19,000), throughout microspheres made without DPPC and with DPPC. In each case, the drug is evenly dispersed throughout the polymer matrix, which can lead to prolonged delivery of macromolecules after placement in an aqueous environment.

The density of the microspheres as determined by mercury intrusion analysis is shown in Table 2 (and confirmed by tap density measurements).

TABLE 2

Comparison of Porous Microparticles with Bulk (PLGA 50:50) Polymer		
Sample	Density, ρ _{MS} (g/cc)	Respirable Size Range, d _{resp} (μm)
Bulk PLGA	1.35	0.69-4.05
MS without DPPC	0.37 ± 0.03	1.3-7.7
MS with DPPC	0.30 ± 0.06	1.46-8.58

Using the concept of aerodynamic diameter (Gonda, I., in *Topics in Pharmaceutical Sciences* 1991, D. Crommelin and K. Midha, Editors, Stuttgart: Medpharm Scientific Publishers, pp. 95-117 (1992)), it is possible to determine the size range of the microspheres which are theoretically respirable given their mass density, ρ_{MS}. Specifically, it can be shown below in Equation 2 that:

$$\frac{0.8}{\sqrt{\rho_{MS}}} \leq d_{resp} \leq \frac{4.7}{\sqrt{\rho_{MS}}} \quad (2)$$

where d_{resp} corresponds to the diameter of particles (in μm) theoretically able to enter and remain in the airways without inertial or gravitational deposition (particles smaller than

this range are exhaled), and where ρ_{MS} is in units of g/cc. The theoretical respirable size range of the microspheres also is shown in Table 2. The optimal size range (i.e., d_{resp}) for a non-porous PLGA 50:50 microsphere is 0.69–4.05 μm (Table 2). The optimal respirable size range for microspheres without DPPC is 1.3–7.7 μm and, for microspheres with DPPC, 1.46–8.58 μm (Table 2). The upper limit on size of respirable particles is increased from 4.05 to greater than 8.5 μm when DPPC is used in the PLGA microsphere preparation. Therefore, the use of low density DPPC microspheres allows the use of larger particles for aerosolization, which may have advantages for drug delivery, such as less particle-particle interaction due to decreased surface area to volume ratio, and lower susceptibility to phagocytosis by alveolar macrophages. In addition, a primary effect of DPPC is to render the particles less adhesive and therefore allow improved aerosolization, as demonstrated below.

FIGS. 1 and 2 show the results of an in vitro aerosolization of the PLGA microspheres made by a double emulsion process with and without DPPC. The microspheres were aerosolized as a dry powder released from a Spinhaler® dry powder inhaler (DPI). FIG. 1 illustrates the mass-fraction of the initial dose that is released from the dry powder inhaler device (DPI Efficiency) using an Andersen Mark I Cascade Impactor. DPI efficiencies approaching 80% were obtained with microspheres made with and without DPPC. Although the DPI efficiencies for the two batches were nearly the same, a great difference can be seen between microspheres made with and without DPPC when their deposition within the cascade impactor is observed (FIG. 2).

FIG. 2 shows the mass fraction of aerosolized particles that is deposited in stages 2 through Filter (2-Filter) of the Andersen cascade impactor, considered the stages corresponding to the respirable fraction of the microspheres. Stages 0 and 1 correspond roughly to the mouth and throat, and to the upper airways of the lung, respectively. Stages 2-F correspond to successively deeper fractions of the lung. It can be seen that a much greater percentage of microspheres make it to the latter stages of the impactor (considered deeper portions of the lungs) when DPPC is used in their preparation. Overall, greater than 35% (37.0±2.1) of aerosolized particles made with DPPC are considered respirable compared with 13.2±2.9% without DPPC, as shown in Table 3. The large difference in respirable fraction between the DPPC and non-DPPC particles is at least in part attributed to reduced particle-particle interaction due to the use of DPPC.

In order to estimate the theoretical respirable fraction (RF) of the microspheres, and compare it with experimentally measured in vitro and in vivo RF's, size distribution measurements were analyzed to determine the percentage of particles (by mass) of each type (DPPC and non-DPPC) that were within the theoretical respirable size range (i.e., d_{resp} Table 2). As shown in Table 3, a higher percentage of particles made with DPPC are expected to be respirable compared with non-DPPC particles (63 to 51%, respectively). This theoretical respirable fraction is based on the mass fraction of microspheres with diameters in the respirable size range, d_{resp} as defined by Eq. (2), and therefore takes into account the different sizes and densities of the two batches of microspheres.

TABLE 3

Sample	Comparison of Microparticle Aerosolization Properties In Vitro	
	Theoretical Respirable Fraction (i.e., Mass % of microspheres in Respirable Size Range) ^a	Measured Respirable Fraction (% In Vitro) ^b
microspheres without DPPC	51 ± 6	13.2 ± 2.9
microspheres with DPPC	63 ± 2	37.0 ± 2.1

^aBased on theoretical respirable size range (d_{resp} Table 2) and size distribution analyses.

^bMeasured using an Andersen Mark I Cascade Impactor.

To determine whether agglomeration forces during particle aerosolization from the Spinhaler device might be playing a role even after the particles enter the impactor system (i.e., primarily non-DPC particles remain agglomerated in the inspired stream, resulting in deposition in the first two impactor stages: stages 0 and 1), in vivo aerosolization experiments were performed in which particles were permitted to fall by gravity into the inspiration stream of a Harvard ventilator system joined with the trachea of an anesthetized rat. In this model, approximately 63% of the inhaled DPPC-PLGA particles deposit in the airways and distal lung regions, whereas 57% of the non-DPPC particles are able to penetrate beyond the trachea in the lungs. These respirable fractions are much nearer to the predicted respirable fractions based upon particle diameter and mass density (Table 3).

Particle aggregation thus is less with DPPC-containing PLGA particles than without DPPC, even though the particles are of similar size and surface morphological features. The use of DPPC thus appears to reduce interparticle attractions, such as van der Waals and electrostatic attractions. It is also possible that the presence of DPPC reduces moisture absorption which may cause particle-particle interaction by capillary forces.

In addition to the biocompatibility features of DPPC and improvement of surface properties of microspheres for aerosolization, it is possible that the release of DPPC from the slow-eroding PLGA microspheres in the alveolar region of the lungs can more effectively insure the maintenance of normal surfactant fluid composition thereby minimizing the possibility of local toxic side effects. The alveolar surfactant fluid layer is, on average, 10 nm thick (Weibel, E. R., *Morphometry of the Human Lung*, New York: Academic Press (1963).

Example 4

Fabrication of PLGA Microspheres by Spray Drying which Encapsulate a Model High Molecular Weight Drug, FITC-Dextran.

Microspheres were made by spray drying using a variety of polymeric carriers with and without the incorporation of DPPC. The results are summarized in Table 4.

TABLE 4

Characterization of Spray Dried Microparticulates				
Sample	Mass-Mean (True) Diameter, (μm)	DPPC Load ($\mu\text{g}/\text{mg}$ spheres) and Efficiency (%)	FITC-Dextran Loading Efficiency, (%)	% of Surface Coated with DPPC by ESCA
R206 + DPPC	5.4	a	54.9	a
R206 - DPPC	4.4	—	64.8	—
RG503 + DPPC	2.0	62.8	65.2	46.5%
RG503 - DPPC	3.0	—	78.2	—
RG506 + DPPC	4.3	89.1	62.7	42-62%
RG506 - DPPC	b	—	100	—

a Not Determined

b No reliable determination because the powder was highly aggregated.

Aerosolization properties of the microspheres also were examined, as shown in Table 5. Microspheres made by spray drying with and without DPPC have similar size distributions (Table 5) and mass densities (0.49 ± 0.04 g/cc). However, the aerosolization performance of spray-dried aerosols made with and without DPPC is markedly different. FIG. 3 shows that the fraction of low-molecular-weight PLGA RG503 microparticles that are aerosolized from the dry powder inhaler (i.e., the % of particles that leave the DPI upon simulated inhalation, defined as the DPI Efficiency) is 70.4% when the particles are made with DPPC compared with only 46.8% for particles made without DPPC. Furthermore, the deposition of all types of polymer microparticles following aerosolization into an Andersen impactor is greatly improved using DPPC-coated particles (Table 5). Without the use of DPPC, $\leq 2\%$ of the particles aerosolized reach the latter stages of the impactor (those corresponding to the respirable fraction, stages 2-Filter). On the other hand, a maximum of 25.6% of DPPC-coated microspheres reach stages 2-Filter, as shown in FIG. 4. Higher respirable fractions may be obtained with particles that contain low molecular weight drugs that are soluble in methylene chloride and therefore do not require the use of water during their preparation.

TABLE 5

Summary of Aerosolization Data of microspheres Prepared by Spray Drying with or without DPPC				
Sample	% Aerosolized Particles that reach stages 1 - Filter	% Aerosolized Particles that reach stages 2 - Filter	% Aerosolized Particles that reach stages 3 - Filter	DPI Efficiency
R206 + DPPC	40.4 \pm 8.4	25.6 \pm 2.3	18.0 \pm 2.7	38.6 \pm 3.7
R206 - DPPC	7.4 \pm 2.1	1.8 \pm 0.5	1.1 \pm 0.3	41.0 \pm 4.8
RG503 + DPPC	36.0 \pm 9.2	14.7 \pm 1.53	10.4 \pm 0.46	70.4 \pm 2.4
RG503 - DPPC	3.3 \pm 0.6	2.1 \pm 0.3	2.0 \pm 0.3	46.8 \pm 8.0
RG506 + DPPC	13.7 \pm 9.1	7.1 \pm 4.1	4.1 \pm 2.5	76.6 \pm 8.4
RG506 - DPPC	1.8 \pm 0.6	1.6 \pm 0.6	1.4 \pm 0.7	74.0 \pm 7.2

R206 = PLA, molecular weight approximately 100,000.

RG503 = PLGA 50:50, molecular weight approximately 34,000.

RG506 = PLGA, molecular weight approximately 100,000.

Modifications and variations of the present invention will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the following claims.

What is claimed is:

1. A particulate composition for drug delivery to the pulmonary system comprising:

biodegradable particles incorporating a therapeutic, prophylactic or diagnostic agent and a surfactant, wherein

the particles have a tap density less than 0.4 g/cm³ and a mean diameter between 5 μm and 30 μm effective to yield an aerodynamic diameter of the particles of between approximately one and three microns.

2. The system of claim 1 wherein at least 50% of the particles have a mass mean diameter between 5 μm and 30 μm .

3. The composition of claim 1 wherein at least 50% of the particles have a mean diameter between 5 μm and 15 μm and a tap density less than 0.1 g/cm³.

4. The composition of claim 1 further comprising a pharmaceutically acceptable carrier for administration to the lungs.

5. The composition of claim 1 wherein the particles comprise a biodegradable polymer.

6. The composition of claim 1 wherein the particles comprise a polyester.

7. The composition of claim 1 wherein the particles comprise an excipient or a fatty acid.

8. The composition of claim 1 wherein the particles have an irregular surface structure.

9. The composition of claim 1 wherein the surfactant is coated on the surface of the particle.

10. The composition of claim 1 wherein the surfactant is incorporated within and on the surface of the particle.

11. The composition of claim 1 wherein the therapeutic agent is selected from the group consisting of proteins, polysaccharides, lipids, nucleic acids and combinations thereof.

12. The composition of claim 1 wherein the therapeutic agent is selected from the group consisting of a ribonucleic acid and a deoxyribonucleic acid.

13. The composition of claim 1 wherein the therapeutic agent is selected from the group consisting of insulin, calcitonin, leuprolide and albuterol.

14. The composition of claim 1 wherein the surfactant is selected from the group consisting of a fatty acid, a phospholipid, and a poloxamer.

15. The composition of claim 1 wherein the surfactant is a phosphoglyceride.

16. The composition of claim 1 wherein the surfactant is dipalmitoyl L- α -phosphatidylcholine.

17. A method for drug delivery to the pulmonary system comprising:

administering to the respiratory tract of patient in need of treatment an effective amount of biodegradable particles incorporating a therapeutic, prophylactic or diagnostic agent and a surfactant,

wherein the particles have a tap density less than about 0.4 g/cm³ and a mean diameter of between 5 μm and 30 μm effective to yield an aerodynamic diameter of the particles of between approximately one and three microns.

18. The method of claim 17 wherein at least 50% of the administered particles have a mean diameter between 5 μm and 15 μm .

19. The method of claim 18 wherein at least 50% of the administered particles have a mean diameter between 5 μm and 15 μm and a tap density of less than about 0.1 g/cm³.

20. The method of claim 17 wherein the particles comprise a biodegradable polymer.

21. The method of claim 17 wherein the particles comprise a polyester.

22. The method of claim 17 wherein the particles comprise an excipient.

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23. The method of claim **21** wherein the particles have an irregular surface structure.

24. The method of claim **17** for delivery to the alveolar zone of the lung wherein at least 90% of the particles have a mean diameter between about 9 μm and 11 μm and a tap density less than 0.1 g/cm^3 .

25. The method of claim **17** wherein the therapeutic agent is selected from the group consisting of proteins, polysaccharides, lipids, nucleic acids and combinations thereof.

26. The method of claim **17** wherein the therapeutic agent selected from the group consisting of a ribonucleic acid and a deoxyribonucleic acid.

27. The method of claim **17** wherein the therapeutic agent is selected from the group consisting of insulin, calcitonin, leuprolide and albuterol.

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28. The method of claim **17** wherein the particles are administered in combination with a pharmaceutically acceptable carrier for administration to the respiratory tract.

29. The method of claim **17** wherein the surfactant is selected from the group consisting of a fatty acid, a phospholipid, and a poloxamer.

30. The method of claim **17** wherein the surfactant is a phosphoglyceride.

31. The method of claim **17** wherein the surfactant is dipalmitoyl L- α -phosphatidylcholine.

32. The method of claim **17** wherein the surfactant is coated on the surface of the particle.

33. The method of claim **17** wherein the surfactant is incorporated within and on the surface of the particle.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : Re. 37,053
DATED : February 13, 2001
INVENTOR(S) : Justin Hanes, David A. Edwards, Carmen Evora and Robert Langer


It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Assignee section it reads: "Massachusetts Institute of Technology, Cambridge, MA (US)".....

It should read: ---Massachusetts Institute of Technology, Cambridge, MA (US) and Penn State Research Foundation, University Park, PA (US)---.....

Signed and Sealed this
Twenty-second Day of May, 2001

Attest:



NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : RE 37,053 C1
DATED : December 3, 2002
INVENTOR(S) : Justin Hanes et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,

Item [73], Assignees, delete "**The National Institutes of Health**" and insert
-- **Massachusetts Institute of Technology** --.

Signed and Sealed this

Eighth Day of April, 2003

A handwritten signature in black ink, appearing to read "James E. Rogan", written over a horizontal line.

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : RE37,053 E
APPLICATION NO. : 09/351341
DATED : February 13, 2001
INVENTOR(S) : Justin Hanes et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Column 1, lines 18-20, delete "The government has certain rights in this invention by virtue of Grant Number HD29129 awarded to the National Institutes of Health to Robert S. Langer." and insert -- This invention was made with government support under Grant No. HD029129, awarded by the National Institutes of Health. The government has certain rights in this invention. --

Signed and Sealed this
Tenth Day of May, 2011

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive style with a large initial "D" and "K".

David J. Kappos
Director of the United States Patent and Trademark Office



US00RE37053C1

(12) **REEXAMINATION CERTIFICATE** (4674th)

United States Patent

Hanes et al.

(10) **Number:** **US RE37,053 C1**

(45) **Certificate Issued:** ***Dec. 3, 2002**

(54) **PARTICLES INCORPORATING SURFACTANTS FOR PULMONARY DRUG DELIVERY**

(75) Inventors: **Justin Hanes**, Baltimore, MD (US); **David A. Edwards**, State College, PA (US); **Carmen Evora**, La Laguna (ES); **Robert Langer**, Newton, MA (US)

(73) Assignees: **The National Institutes of Health**, Bethesda, MD (US); **The Penn State Research Foundation**, University Park, PA (US)

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Appl. No.: **09/351,341**
Filed: **Jul. 12, 1999**

(*) Notice: This patent is subject to a terminal disclaimer.

Certificate of Correction issued May 22, 2001.

Related U.S. Patent Documents

Reissue of:

(64) Patent No.: **5,855,913**
Issued: **Jan. 5, 1999**
Appl. No.: **08/784,421**
Filed: **Jan. 16, 1997**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/739,308, filed on Oct. 29, 1996, now Pat. No. 5,874,064, which is a continuation-in-part of application No. 08/655,570, filed on May 26, 1996, now abandoned.

(51) **Int. Cl.**⁷ **A61K 9/14; A61K 9/12**

(52) **U.S. Cl.** **424/489; 424/499; 424/45; 424/46; 424/434; 424/426; 424/491**

(58) **Field of Search** **424/489, 499, 424/45, 46, 434, 426, 491**

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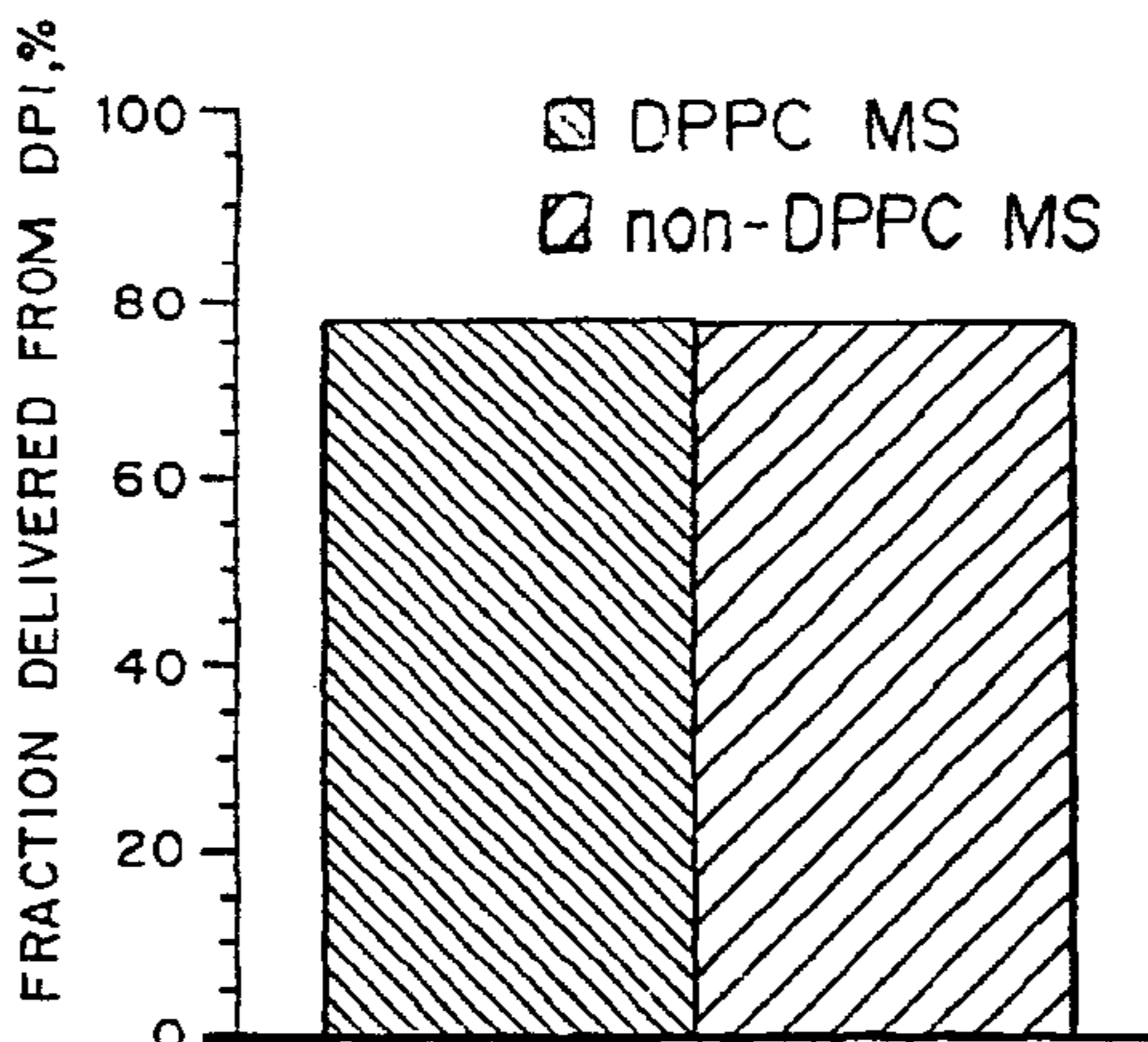
File History for U.S. patent application No. 08/423,515, filed Apr. 14, 1995, to Platz et al.

Gonda, I. "Physico-chemical principles in aerosol delivery," in *Topics of Pharmaceutical Sciences* 1991 (eds. D.J.A. Cronmelin and K.K. Midha).

Primary Examiner—Jose' G. Dees

(57) **ABSTRACT**

Improved aerodynamically light particles for drug delivery to the pulmonary system, and methods for their synthesis and administration are provided. In a preferred embodiment, the aerodynamically light particles are made of a biodegradable material and have a tap density less than 0.4 g/cm³ and a mass mean diameter between 5 μm and 30 μm. The particles may be formed of biodegradable materials such as biodegradable polymers. For example, the particles may be formed of a functionalized polyester graft copolymer consisting of a linear α-hydroxy-acid polyester backbone having at least one amino acid group incorporated therein and at least one poly(amino acid) side chain extending from an amino acid group in the polyester backbone. In one embodiment, aerodynamically light particles having a large mean diameter, for example greater than 5 μm, can be used for enhanced delivery of a therapeutic agent to the alveolar region of the lung. The aerodynamically light particles incorporating a therapeutic agent may be effectively aerosolized for administration to the respiratory tract to permit systemic or local delivery of wide variety of therapeutic agents.



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**REEXAMINATION CERTIFICATE
ISSUED UNDER 35 U.S.C. 307**

NO AMENDMENTS HAVE BEEN MADE TO
THE PATENT

2

AS A RESULT OF REEXAMINATION, IT HAS BEEN
DETERMINED THAT:

The patentability of claims 1-**33** is confirmed.

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