

Nanomedicine in pulmonary delivery

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Abstract: The lung is an attractive target for drug delivery due to noninvasive administration via inhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action for the treatment of respiratory diseases, and the availability of a huge surface area for local drug action and systemic absorption of drug. Colloidal carriers (ie, nanocarrier systems) in pulmonary drug delivery offer many advantages such as the potential to achieve relatively uniform distribution of drug dose among the alveoli, achievement of improved solubility of the drug from its own aqueous solubility, a sustained drug release which consequently reduces dosing frequency, improves patient compliance, decreases incidence of side effects, and the potential of drug internalization by cells. This review focuses on the current status and explores the potential of colloidal carriers (ie, nanocarrier systems) in pulmonary drug delivery with special attention to their pharmaceutical aspects. Manufacturing processes, *in vitro/in vivo* evaluation methods, and regulatory/toxicity issues of nanomedicines in pulmonary delivery are also discussed.

Keywords: pulmonary delivery, colloidal carriers, nanocarrier systems, liposome, polymeric nanoparticle, solid lipid nanoparticle, submicron emulsion, dendrimer

Introduction

Burgeoning interest in colloidal carriers (nanocarrier systems) has led to increasing attention for pulmonary drug delivery. The lung is an attractive target for drug delivery due to noninvasive means to provide not only local lung effects but possibly high systemic bioavailability, avoidance of first-pass metabolism, more rapid onset of therapeutic action, and the availability of a huge surface area.^{1,2} Nanocarrier systems in pulmonary drug delivery offer many advantages. These advantages include the following: 1) the potential to achieve relatively uniform distribution of drug dose among the alveoli; 2) an achievement of enhanced solubility of the drug than its own aqueous solubility; 3) the sustained-release of drug which consequently reduces the dosing frequency; 4) suitability for delivery of macromolecules; 5) decreased incidence of side effects; 6) improved patient compliance; and 7) the potential of drug internalization by cells.^{3,4}

Nanotechnology has been described as the manipulation, precision placement, measurement, modeling or manufacture of matter in the sub-100 nm range,⁵ although, depending on the context, the term sometimes identifies particles in the 1 to 200 nm range.^{3,6} However, the 100 nm limit is constraining, as in effect it would disregard many recent achievements and a plethora of basic science (interfacial and colloidal chemistry) literature and pharmaceutical research literature reports. In addition, there are a number of literature reports in both the basic science and pharmaceutical literature

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which scientifically define dimensions of nanoparticles ranging in size from 1 to 1000 nm.^{3,7-9} In drug delivery systems, submicron size is significant because biodistribution of submicron particles are critical and some safety issues may be occurred when we use microparticles. For instance, after intravenous injection of parenteral formulation, large particles ($>5 \mu\text{m}$) can cause pulmonary embolism, which can induce fatal results,^{10,11} therefore submicron particle size is required for parenteral formulations. Although the upper limit of particle size for ophthalmic application is about $5 \mu\text{m}$, the optimum particle size is less than 1,000 nm because a scratching feeling can occur when microparticles are applied to the eyes.¹² Phagocytosis is sensitive to particle size, and it is generally thought that particles of $0.5\text{--}3 \mu\text{m}$ in diameter are taken up by macrophages,¹³ and particles of less than $0.26 \mu\text{m}$ can escape from phagocytosis by macrophages.¹ Phagocytosis is also the main mechanism responsible for the rapid clearance of particulate drug delivery systems from the body. Furthermore, cytosolic delivery of drugs can be achieved by endocytosis of submicron drug carriers. Therefore, in this review, the authors consider all particulates for which at least one dimension is 1–1000 nm.

There are numerous applications for nanotechnology, however, especially the treatment, diagnosis, monitoring and control of biological systems have recently been referred to as “nanomedicine” by the National Institutes of Health (Bethesda, MD, USA).¹⁴ In short, nanomedicine is the application of nanotechnology to medicine, and two main types of nanomedicine products are currently in clinical trials: diagnostic tests and drug delivery devices.¹⁵ Over the past decades, efforts have been focused on the development of nanomedicines such as nanoparticles, liposomes, nanoemulsions, or dendrimers for the specific delivery of drugs to the target tissues.

The modern inhalation devices can be divided into three different categories, nebulizers, pressurized metered dose inhalers (pMDI), and dry powder inhalers (DPI).¹ In most cases, nanocarriers can be delivered to the lungs by nebulization of colloidal dispersions or using pMDIs and DPis in solid form.^{1,3} Due to small size and strong particle-particle interactions of nanocarriers, particle agglomeration and settlement can occur in colloidal dispersions. Moreover, chemical instability of colloidal dispersions is another issue owing to carrier hydrolysis and drug degradation. To improve physical and chemical instability of nanocarrier dispersions, freeze-drying of nanocarriers has been explored as a means to provide a storage form. However, redispersibility and the use of stabilizers for lyophilization are still problematic in nebulization. The use of a nanocarrier itself for delivery to

lungs is severely limited because individual nanocarriers do not deposit efficiently in the lungs by diffusion, sedimentation or impaction, which results in the exhalation of a majority of the inhaled dose.³ Therefore, micron-sized powder carriers containing nanoparticles or agglomerated nanoparticles were designed to improve the inhalation aerosol delivery of nanoparticles for deep lung delivery by using MDIs and DPis.¹ A thorough discussion of incorporating nanoparticles into micron-scale structures or processing methods for agglomerated nanoparticles are beyond the scope of this review, but the topic has been recently reviewed elsewhere.^{3,4,16}

This review focuses on the current status and explores the potential of colloidal carriers (nanocarrier systems) in pulmonary drug delivery with special attention to and in depth presentation of their pharmaceutical aspects. Manufacturing processes, *in vitro/in vivo* evaluation methods, and regulatory/toxicity issues of nanomedicines in pulmonary delivery are also presented and discussed.

Drugs for inhalation

Various drugs are investigated for local or systemic pulmonary delivery.² These include small molecules, protein/peptide drug and genes (Table 1). In case of small molecule drugs, many studies were focused on local application for the treatment of chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). However, pulmonary protein/peptide delivery offers great potential for both local targeting for the treatment of respiratory diseases and systemic targeting for the treatment of diabetes mellitus or thrombosis. Gene delivery to the lungs are mainly focused on the localized delivery of drugs to the site of disease, the lungs and airways, including lung cancer, genetic disorders affecting the airways (cystic fibrosis, alpha-1-antitrypsin deficiency), obstructive lung diseases (asthma), and vaccination. Since original aerosol technology was developed for small molecule drugs, it is necessary to evolve the reengineering of nanocarrier self-assembly systems for macromolecular pulmonary delivery.¹⁷ Examples of drugs for pulmonary nanocarrier systems are shown in Figure 1.^{18-28,29-76}

Nanocarrier systems for pulmonary drug delivery

Polymeric nanoparticulate pulmonary delivery

Polymeric nanoparticles are widely studied in drug delivery system for parenteral administration;^{77,78} however

Table I Examples of drugs for pulmonary delivery using colloidal carrier self-assembly systems

Therapeutic areas and drugs	Drug types ^a	Colloidal carrier self-assembly systems and references ^b
Asthma (anti-inflammatory)		
Budesonide	S	LP ^{2,3} DN ^{4,5}
Syk antisense oligodeoxynucleotides	G	LP ⁶
Ketotifen	S	LP ⁷
Ibuprofen	S	DN ⁸
Interleukin-4 antisense oligodeoxynucleotides	G	PN ⁹
Indomethacin, ketoprofen	S	SLN ¹⁰
Vasoactive intestinal peptide	P	LP ¹¹⁻¹³ PN ¹⁴
Dexamethasone palmitate	S	LP ¹⁵
Fluticasone	S	DN ⁵
Pulmonary hypertension		
Vasoactive intestinal peptide ^c	P	LP ¹¹⁻¹³ PN ¹⁴
Vascular endothelial growth factor (VEGF) gene	G	LP ¹⁶
Nuclear factor κ B decoy oligodeoxynucleotides	G	NP ¹⁷
Nifedipine	S	DN ¹⁸
Cystic fibrosis		
Amiloride hydrochloride	S	LP ¹⁹
Secretory leukocyte protease inhibitor	P	LP ²⁰
Infections		
Tobramycin	S	LP ^{21,22} DN ²³
Rifampicin	S	LP ²⁴⁻²⁷ PN ²⁸⁻³¹ SLN ³²
Isoniazid, pyrazinamide	S	PN ^{28,29} LP ²⁷ SLN ³²
Ciprofloxacin	S	LP ^{33,34}
Amphotericin B	S	LP ^{35,36}
Itraconazole	S	DN ³⁷⁻⁴⁰
Lung cancers		
Interleukin-2	P	LP ⁴¹
p53 gene	G	PN ⁴²⁻⁴⁵
9-nitrocamptothecin	S	LP ⁴⁶
Leuprolide	P	LP ⁴⁷
Doxorubicin	S	PN ⁴⁸
Programmed cell death protein 4 (PDCD4)	P	PN ^{49,50}
Antisense oligonucleotide 2'-O-methyl-RNA	G	PN ⁵¹
Akt1 (protein kinase B) siRNA	G	PN ^{52,53}
6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride	S	PN ⁵⁴
Mutative defects in lung		
Chimeric oligonucleotide	G	PN ⁵⁵
Immune modulators		
Cyclosporine A	P	LP ⁵⁶ DN ^{57,58}
Tacrolimus	S	LP ⁵⁹
Vaccination		
HLA-A*0201-restricted T-cell epitopes from <i>Mycobacterium tuberculosis</i>	G	PN ⁶⁰
V1Jns plasmid encoding antigen 85B from <i>M. tuberculosis</i>	G	SE ⁶¹
Reactive oxygen species mediated diseases		
Superoxide dismutase	P	LP ⁶²⁻⁶⁴

(Continued)

Table 1 (Continued)

Therapeutic areas and drugs	Drug types ^a	Colloidal carrier self-assembly systems and references ^b
Parathyroid disease		
Elcatonin	P	PN ⁶⁵
Diabetes		
Insulin	P	PN, ⁶⁶ LP, ^{67,68} SLN ⁶⁹
Thrombosis		
Low molecular weight heparin	P	DD ^{70,71}
Urokinase	P	PN ⁷²

Notes: ^aS, small molecules; P, protein/peptide; G, gene; ^bDN, drug nanoparticle; PN, polymeric nanoparticle; LP, liposome; SLP, solid lipid nanoparticle; DD, dendrimer; SE, submicron emulsion; ^cCan be used for treatment of pulmonary hypertension as well as asthma.

their application to the pulmonary routes are also widely recognized.⁴ The main roles of polymeric nanoparticles in drug delivery system are to carry the drug molecules, to protect drugs from degradation, and to control drug release.⁷⁸ Therapeutically used polymeric nanoparticles are composed of biodegradable or biocompatible materials, such as poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), alginate, gelatin and chitosan. Various polymers for pulmonary drug delivery using nanocarrier systems are shown in Table 2. The chemical structures of polymers for polymeric nanoparticles used in pulmonary delivery systems are shown Figure 2.^{26,29,38,39,54–57,59,61,69,70,74,75,79–93}

Due to their biocompatibility, surface modification capability, and sustained-release properties, polymeric nanoparticles are intensively studied using various important pulmonary drugs. These pulmonary drug include anti-asthmatic drugs,^{22,26} antituberculosis drugs,^{38,39} pulmonary hypertension drugs,²⁹ and anticancer drugs.⁵⁴ However, to avoid accumulation of polymer carriers following repeated dosing, the biodegradability and toxicity of polymers over the long term should be closely examined in the formulation of polymeric nanoparticles for pulmonary delivery. Additionally, *in vitro* lung surfactant models and *in vivo* studies are required to establish the pulmonary acceptability of polymeric nanocarrier systems, as polymers and their degradation products can affect the vital surfactant properties in the alveoli which in turn will affect pulmonary immunity control and adversely affect the work of breathing.

Although cationic lipid-based gene carriers are currently being clinically evaluated further than polymer-based gene carriers,⁹⁴ cationic polymers are one of the popular carriers for gene delivery to the lungs.^{95,96} Although polyethylenimine (PEI) and polyamino acids, such as poly-L-lysine, have been shown to be effective agents for DNA delivery both *in vitro* and *in vivo*,^{97,98} cytotoxicity⁹⁹ and low transfection

efficacy problems when delivered via inhalation have to be overcome.¹⁰⁰ To solve these problems, various modifications of PEI with liposomes/PEGs or conjugations of PEI with ligands such as transferrin have been investigated extensively.^{101–107} Noninvasive pulmonary gene delivery using cationic polymers has been reviewed in detail elsewhere.⁹⁵

Liposomal pulmonary delivery

Liposomes are one of the most extensively investigated systems for controlled delivery of drug to the lung.¹⁰⁸ Liposomes seem particularly appropriate for therapeutic agent delivery to lung, since these vesicles can be prepared from compounds endogenous to the lungs, such as the components of lung surfactant, and these properties make liposomes attractive candidates as drug delivery vehicles.³⁷ The first pharmaceutical liposomal products in market include the synthetic lung surfactant Alveofact® (Dr Karl Thomae GmbH, Biberach, Germany) for pulmonary instillation for the treatment of respiratory distress syndrome (RDS).¹⁰⁹ Typically, liposomal formulations have been delivered to the lung in the liquid state, and nebulizers have been used extensively for the aerosol delivery of liposomes in the liquid state.¹¹⁰ However, concerns arise from drug stability in the liquid state and leakage when nebulizers are used to deliver a liposomal encapsulated agent.¹¹¹ Recently, liposomal dry powder formulations have been intensively examined in order to successfully circumvent these issues.^{72,112–114} Liposomal dry powder formulations have been shown to be very promising in the delivery of various types of pulmonary drugs and some of these formulations are currently in clinical trials.

Much interest has focused on cationic liposomes for pulmonary gene delivery because cationic liposomes offer the advantage of self-assembly with DNA material through favorable cationic–anionic electrostatic interactions. Additional advantages include evasion from complement

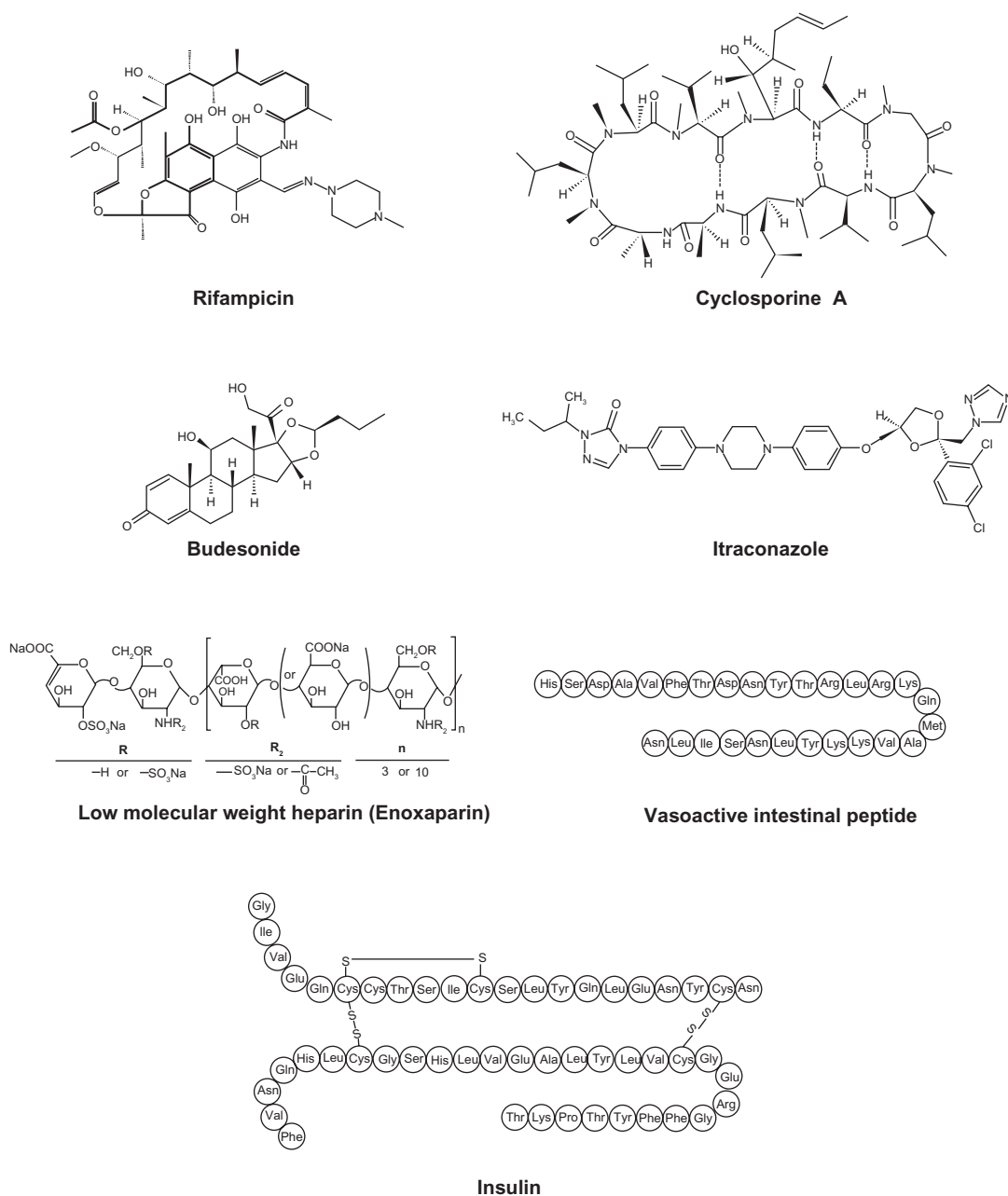


Figure 1 Examples of drugs for pulmonary colloidal carriers (nanocarrier systems).

inactivation after *in vivo* administration, the low cost and relative ease in producing nucleic acid–liposome complexes in large scale.⁹⁵ After first report of inhalation gene delivery success,¹¹⁵ many reports have been published on gene delivery using cationic liposomes by pulmonary administration.^{104,116–123} Many recent reviews^{95,124,125} present gene delivery system using cationic liposomes. Moreover, liposomes conjugated with cell-penetrating peptides are recognized as potential nanocarrier systems for intracellular delivery of macromolecules to the lung. Liposomes modified with cell-penetrating peptides, antennapedia,

the HIV-1 transcriptional activator, and octaarginine have been reported to enhance the cellular uptake of liposomes to airway cells.¹²⁶

Solid lipid nanoparticles in pulmonary delivery

Solid lipid nanoparticles (SLNs) are made from solid lipids (ie, lipids solid at room temperature), surfactant(s) and water.¹²⁷ Since the beginning of 1990s, the SLNs have been focused on an alternative to polymeric nanoparticles.¹⁰⁹ The advantages of drug release from SLNs in the

Table 2 Various polymers for colloidal pulmonary drug delivery systems

Polymers	Drugs and references	Size
Alginate		
Sodium alginate	Rifampicin, isoniazid, pyrazinamide ²⁹	235.5 nm
Chitosan		
Chitosan	Plasmid DNA ⁷⁷	91–164 nm
	Small interfering RNA ⁷⁸	40–600 nm
Chitosan/tripolyphosphate	Insulin ⁷⁹	300–388 nm
Trisaccharide-substituted chitosan	Plasmid DNA ⁸⁰	77–90 nm
Urocanic acid–modified chitosan	Programmed cell death protein 4 ⁵⁰	NA
Gelatin		
Gelatin type A	Fuoresceinamine ⁸¹	277.8 nm
Gelatin type B	Sulforhodamine 101 acid chloride ⁸²	242 ± 14 nm
PEGylated gelatin	Plasmid DNA ⁸³	100–500 nm
Polyalkylcyanoacrylate		
Polybutylcyanoacrylate	Insulin ⁶⁶	254.7 nm
	Doxorubicin ⁸⁴	173 ± 43 nm
	Ciprofloxacin ⁸⁵	156–259 nm
PLGA		
PLGA	Rifampicin, isoniazid, pyrazinamide ²⁸	570–680 nm
PEG-PLGA	Nuclear factor κ B decoy oligodeoxynucleotide ¹⁷	44 nm
Chitosan-modified PLGA	Elcatonin ⁶⁵	650 nm
Chitosan/PLGA	Antisense oligonucleotide 2'-O-methyl-RNA ⁵¹	135–175 nm
Poly[vinyl 3-(diethylamino)propylcarbamate-co-vinyl acetate-covinyl alcohol]-graft-PLGA	5(6)-carboxyfluorescein ⁸⁶	195.3 ± 7.1 nm
Proticle		
Protamine-oligonucleotide	Vasoactive intestinal peptide ¹⁴	177–318 nm
PEI		
PEI	Chimeric oligonucleotide ⁵⁵	30–100 nm
	Plasmid DNA ⁸⁷	50–100 nm
PEI- <i>alt</i> -PEG	Small interfering RNA ⁵³	NA
Glucosylated PEI	Programmed cell death protein 4 ⁴⁹	NA
Galactose-PEG-PEI	Plasmid DNA ⁸⁸	105–210 nm
Cell-penetrating peptides-PEG-PEI	Plasmid DNA ⁸⁹	113–296 nm
Poly-L-lysine		
PEGylated poly-L-lysine	Plasmid DNA ^{90,91}	211 ± 29 nm
Dendrimer		
G9 PAMAM	Plasmid DNA ⁹²	NA
G2/G3 PAMAM	Low molecular weight heparin ⁷⁰	NA
Pegylated G3 PAMAM	Low molecular weight heparin ⁷¹	17.1 ± 3.3 nm

Abbreviations: PEG, poly(ethylene glycol); PLGA, poly(lactic-co-glycolic acid); PEI, poly(ethylenimine); PAMAM, polyamidoamine; NA, not available.

lung are control of the release profile, achievement of a prolonged release and having a faster *in vivo* degradation compared to particles made from PLA or PLGA. In addition, SLNs proved to possess a higher tolerability in the lungs compared to particles made from some polymeric materials.^{109,128,129} Although SLNs for the pulmonary

delivery is not fully appreciated, toxicological profile of SLNs, when using physiological lipids, is expected to be better than that of polymer-based systems, because physiological lipids have little or no cytotoxicity.^{130,131} It is feasible that aqueous suspensions and perhaps dry powder formulations of SLN can be used for pulmonary inhalation

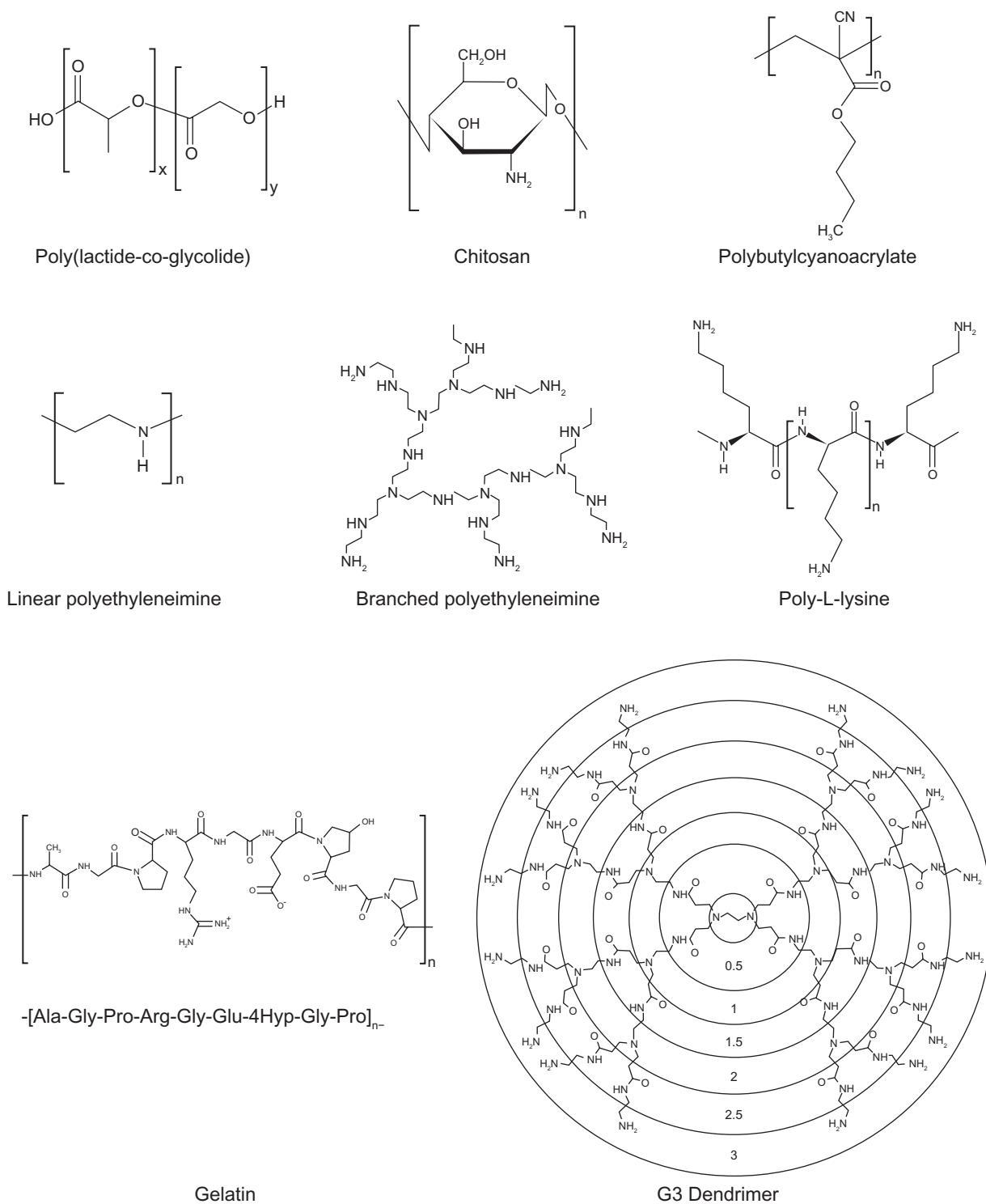


Figure 2 Chemical structures of polymers for polymeric nanoparticles in pulmonary delivery systems.

aerosol administration of drugs using nebulizers and dry powder inhalers.¹⁰⁹

Several studies have been published on the pulmonary applications of SLNs as local delivery carriers for small molecules⁴² or as systemic delivery carriers for

macromolecules.^{73,132} Pandey and Khuller⁴² studied the chemotherapeutic potential of SLNs incorporating rifampicin, isoniazid and pyrazinamide against experimental tuberculosis, and observed the slow and sustained-release of drugs from the SLNs *in vitro* and *in vivo*. Gene delivery

of SLN-based gene vectors was introduced by Rudolph and colleagues.¹³² They reported that SLN gene vectors mediate gene expression in the mouse lungs upon aerosol application, which was increased by the TAT peptide, and aerosol application of fragile gene delivery systems can be achieved by a mild nebulization technology based on a novel perforated membrane technology. Liu and colleagues⁷³ studied novel nebulizer-compatible SLNs containing insulin for pulmonary delivery. They concluded that SLNs could be successfully applied as a pulmonary carrier system for insulin, which might provide novel solutions to the currently unmet medical needs for systemic delivery of proteins.

Deposition and clearance of SLNs were assessed by Videira and colleagues^{133,134} after inhalation of aerosolized insoluble particles using gamma-scintigraphy imaging analysis. It was observed that a few minutes after deposition, inhaled material began to translocate to regional lymph nodes indicating that inhalation can be an effective route to deliver drug-containing lipid particles to the lymphatic systems and lipid particles can be used as potential drug carriers for lung cancer therapy, as well as for vaccine delivery.^{133,134}

Submicron emulsions in pulmonary delivery

Until now, the submicron emulsion system has not yet been fully exploited for pulmonary drug delivery and very little has been published in this area.⁶⁵ Cationic submicron emulsion loaded with *Mycobacterium tuberculosis* Ag85B DNA vaccine was explored vaccine for the purpose of pulmonary mucosal vaccination.⁶⁵ Emulsion systems have been introduced as alternative gene transfer vectors to liposomes.¹³⁵ Other emulsion studies for gene delivery (nonpulmonary route) have shown that binding of the emulsion/DNA complex was stronger than liposomal carriers.¹³⁶ This stable emulsion system delivered genes to endothelial cells in the mouse nasal cavity more efficiently than commercially available liposomes.¹³⁷ Bivas-Benita and colleagues⁶⁵ have reported that cationic submicron emulsions are promising carriers for DNA vaccines to the lung since they are able to transfect pulmonary epithelial cells, which possibly induce cross priming of antigen-presenting cells and directly activate dendritic cells, resulting in stimulation of antigen-specific T-cells. Therefore the nebulization of submicron emulsions will be a new and upcoming research area. However, extensive studies are required for the successful formulation of inhalable submicron emulsions due to possible adverse

effects of surfactants and oils on lung alveoli function (adverse interactions with lung surfactant).

Dendrimer-based nanoparticles for lung delivery

Dendrimers are polymers, which have hyperbranched structures, with layered architectures.¹³⁸ The research in dendrimer-mediated drug delivery has mainly been focused on the delivery of DNA drugs into the cell nucleus for gene or antisense therapy, and many studies have been reported on the possible use of dendrimers as nonviral gene transfer agents.¹³⁸ Several studies have been published regarding pulmonary applications of dendrimers as systemic delivery carriers for macromolecules.^{74,75,93,139}

Kukowska-Latallo and colleagues⁹³ investigated the ability of polyamido amine (PAMAM) dendritic polymers (dendrimers) to augment plasmid DNA gene transfer *in vivo* and evaluates the targeting of the lung by alternative routes of administration. They suggested that vascular administration seemed to achieve expression in the lung parenchyma, mainly within the alveoli, while endobronchial administration primarily targeted bronchial epithelium, indicating that each delivery route requires different vectors to achieve optimal transgene expression, that each approach appears to target different cells within the lung.

Rudolph and colleagues¹³⁹ compared the properties of branched polyethylenimine (PEI) 25 kDa and fractured PAMAM dendrimers for topical gene transfer to the airways *in vivo*. Their results demonstrated that gene transfer mediated by PEI under optimal conditions was two orders of magnitude higher compared to fractured dendrimers. Therefore, branched PEI 25 kDa was superior to fractured dendrimers for gene delivery to the airways.

Bai and colleagues⁷⁴ produced low molecular weight heparin (LMWH)–dendrimer complex through electrostatic interactions using various PAMAM dendrimers, then evaluated both the safety and the efficacy of the drug–dendrimer formulations in preventing deep vein thrombosis *in vivo* and *in situ*. They concluded that cationic dendrimers can be used as pulmonary delivery carriers for a relatively large molecular weight anionic drug. These carriers bind anionic drug molecules most likely via electrostatic interactions and increase drug absorption through charge neutralization. In addition, preliminary safety studies using the frog palate model and bronchoalveolar lavage analysis suggest that dendrimers could be viable carriers for pulmonary delivery of LMWH. In another study the same group⁷⁵ also investigated pegylated dendrimers (mPEG–dendrimer) in order

to increase pulmonary absorption and circulation time of the drug. Briefly, half-life and absorption of LMWH administered via the pulmonary route can be increased by encapsulating the drug in dendrimeric micelles, and their study suggests that LMWH loaded in the mPEG–dendrimer could potentially be used as noninvasive delivery system for the treatment of thromboembolic disorder. However, the potential of dendrimers in the pulmonary drug administration route still remains as a challenge that needs further research to achieve lower cytotoxicity and higher biocompatibility.

Manufacture of nanoparticulate inhalation aerosols

In formulation preparation, several processing technologies have been used to obtain nanoparticles for use as pulmonary inhalation aerosols with desirable attributes, such as narrow particle size distribution, enhanced stability, controlled and targeted release, and improved bioavailability. Jet-milling of the drug under nitrogen gas is the traditional way of creating respirable aerosol particles in the solid-state.^{140,141} The basic procedure of jet-milling is to grind a bulk crystallized particles into small particles by one of the following mechanical forces: pressure, friction, attrition, impact, or shear. However, up until very recently, this technology had a limitation to generate particles in the nanosized range, although a few cases have been reported to produce nanoparticles, such as insulin nanoparticles¹⁴² and budesonide nanoparticles,¹⁴³ by wet milling process. The recent availability of new nanojet milling instruments which produce nanoparticles by jet milling may increase the routine use of creating nanoparticles by this method.

More sophisticated and advanced manufacturing technologies are utilized to produce respirable aerosol nanoparticles. These respirable particles may be encapsulated in microparticles in the respirable aerodynamic size range of 1–5 microns or the nanoparticles may be designed to aggregate to a favorable aerodynamic size range. These manufacturing nanotechnologies include spray drying (sometimes referred to as advanced spray drying or nanospray drying), spray-freeze drying, supercritical fluid technology, double emulsion/solvent evaporation technology, antisolvent precipitation, particle replication in nonwetting templates (PRINT), and thermal condensation using capillary aerosol generator.

Spray-drying

Spray-drying is an advanced pharmaceutical manufacturing process used to efficiently produce respirable colloidal

particles in the solid-state.^{144,145} In the process, the feed solution is supplied at room temperature and pumped to the nozzle where it is atomized by the nozzle gas. The atomized solution is then dried by preheated drying gas in a special chamber to remove water moisture from the system, thus forming dry particles. These prepared particles are collected with a cyclone separation device. A schematic representation of a spray drying process is shown in Figure 3.

The spray-drying process is suitable for thermolabile materials such as proteins and peptides, because mechanical high energy input is avoided in this process.^{146–148} Moreover, the spray-drying system can be modified to meet specific needs. For example, Maa and colleagues¹⁴⁹ replaced the bag-filter unit of a spray-drying system with a vacuum to reduce the drying airflow resistance. It allows the protein (recombinant humanized anti-IgE antibody) to be dried at a much lower temperature than usual and the production scale to be increased.¹⁴⁶ In another study, poly(lactic-co-glycolic acid) particles containing proteins were successfully dried by ultrasonic atomization of feed solution into an atmosphere under reduced pressure.¹⁴⁷ Solution spray-drying ensures compositional homogeneity of the drug powder, since the drug and the excipients are dissolved prior to the process. More importantly, spray-drying can result in uniform particle morphology.^{114,149,150} In industry, spray-drying is a continuous production method, scalable for commercial production volumes.

Lactose monohydrate is the only US Food and Drug Administration (FDA)-approved sugar carrier for dry powder aerosol formulations and one of a few FDA approved excipients.¹⁵¹ For example, lactose with either gelatin or poly(butylcyanoacrylate) nanoparticles were spray dried to produce particles for pulmonary delivery.⁸⁴ Lactose in solid form can be either crystalline as lactose monohydrate or amorphous as lactose. Crystalline lactose can exist in one of two distinct forms: α -lactose monohydrate (Figure 4a) and anhydrous β -lactose (Figure 4b). Amorphous lactose may contain both α - and β -lactose molecules that are arranged in a more or less random state. α -lactose monohydrate and anhydrous β -lactose are superior in offering better physical stability than amorphous lactose as the later has a tendency to recrystallize spontaneously.^{152,153} A disadvantage of lactose monohydrate, a reducing sugar, as a sugar carrier and/or excipient in the solid-state is that it participates in Maillard decomposition reactions with certain small molecular weight pulmonary drugs (eg, budesonide, formoterol), proteins and peptides.¹⁵⁴ Recently, D-mannitol, a nonreducing sugar alcohol, whose molecular structure is shown in Figure 4c, has

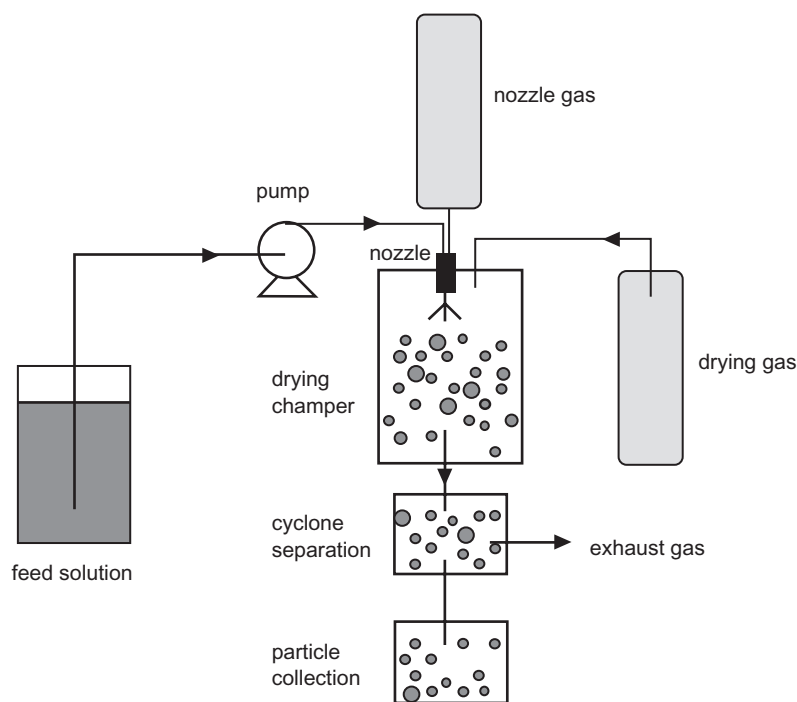


Figure 3 A schematic representation of the spray-drying process.

been used as an alternative sugar carrier of pulmonary drugs in dry powder inhalation aerosols, since it does not participate in the solid-state decomposition Maillard reaction.¹⁵⁵

Spray-freeze-drying (SFD)

Spray-freeze-drying (SFD) is an advanced particle engineering method which combines spray-drying and freeze-drying processing steps. This technique involves the atomization of an aqueous drug solution into a spray chamber filled with a cryogenic liquid (liquid nitrogen) or halocarbon refrigerant such as chlorofluorocarbon or fluorocarbon.¹⁵⁶ The water is removed by sublimation after the liquid droplets solidify.¹⁵⁷ SFD is capable of producing porous particles with high fine particle fraction (FPF) at subambient temperatures.¹⁵⁷ They are usually low-density composite amorphous particles with high specific surface area.¹⁵⁸ Thermolabile protein and peptide substances, such as insulin¹⁵⁹ and plasmid DNA,¹⁶⁰ can also be formulated into dry powder inhalation products by SFD.

Supercritical fluid technology (SCF)

The basic feature of the supercritical fluid process is the controlled crystallization of drugs from dispersion in supercritical fluids, carbon dioxide. This method has demonstrated a wide range of application in producing pulmonary inhalable formulations.^{23,161,162} Supercritical fluid technology

can be divided into several classes. The most important two are supercritical antisolvent precipitation (SAS)^{163,164} and supercritical fluid extraction of emulsions (SFEE).^{165,166}

The fundamental mechanism of SAS is based on rapid precipitation when a drug solution is brought into contact with a supercritical CO₂. SFEE is based on extraction of the organic phase in oil-in-water or multiple emulsions using supercritical CO₂.^{165,166} The schematic representation of SAS and SFEE processes is shown in Figure 5. Because most of the drugs (eg, asthma drugs) are not soluble in CO₂, SAS processes provide an easy and excellent way to produce dry powder inhalation formulations.^{163,164} SFEE can provide uniform crystalline drug nanoparticles, composite nanoparticles containing polymeric materials and the drugs, and nanosuspensions.^{165,166} For instance, Chattopadhyay and colleagues²³ used a continuous SFEE method to produce nanoparticle suspensions containing one of three lipids (tripalmitin, tristearin, or gelucire 50/13), and one of two model drugs (indomethacin or ketoprofen). The first step of this process was to produce nanoemulsions by mixing organic phase containing lipids, a selected drug and chloroform with aqueous phase containing sodium glycocholate, under high pressure homogenization. Then the nanoemulsions were introduced to an extraction chamber countercurrently to a stream of supercritical CO₂. The CO₂ extracted the organic solvent from the dispersed droplets,

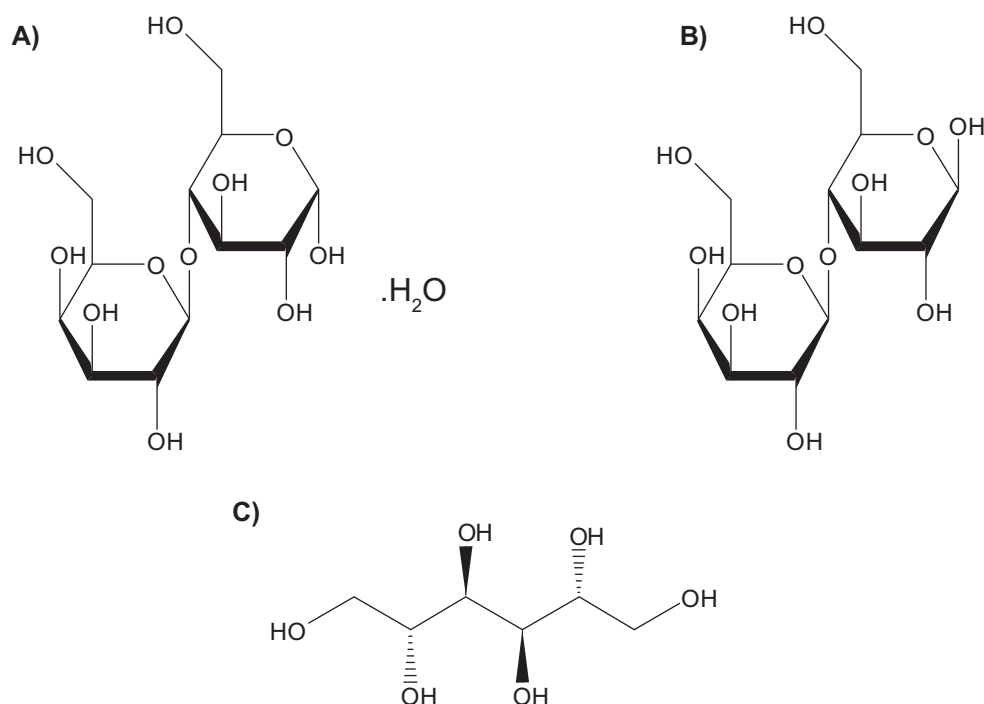


Figure 4 Molecular structures of sugar carriers: **A)** α -lactose monohydrate, **B)** anhydrous β -lactose and **C)** D-mannitol.

forming nanoparticles with a volume mean diameter between 10–30 nm with a high drug loading efficiency for the gelucire particles (~80%–90%). In another example, nanoparticles containing cholesterol acetate (CA), griseofulvin (GF), and megestrol acetate (MA) were produced by extraction of the internal phase of oil-in-water emulsions using supercritical carbon dioxide.¹⁶⁵ This method offered advantages such as the control of particle size, crystallinity, and surface properties. Meanwhile, it shortened the processing time, improved the product purity, and reduced the large waste streams.

Double emulsion/solvent evaporation technique

Respiratory nanoparticle formation from double emulsion/solvent evaporation system involves preparation of oil/water (o/w) emulsions with subsequent removal of the oil phase (ie, typically a volatile organic solvent) through evaporation. The emulsions are usually prepared by emulsifying the organic phase containing the drug, polymer and organic solvent in an aqueous solution containing emulsifier. The organic solvent diffuses out of the polymer phase and into the aqueous phase, and is then evaporated, forming drug-loaded polymeric nanoparticles. By this method, biodegradable polymers, including poly(L-lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactide-co-glycolide) acid (PLGA),

have been intensively investigated as carriers for solid drug nanoparticles.¹⁶⁷

Antisolvent precipitation

Crystalline drug particles with narrow size distribution could be prepared by direct controlled crystallization.¹⁶⁸ This process involves antisolvent precipitation of drug solution in a water-miscible organic solvent, followed by addition of a bridging solvent, which is immiscible or partially miscible with water. Growth-retarding stabilizing additives, such as hydroxypropylmethylcellulose (HPMC), is usually added in the medium to yield particles with small size. The precipitated drug crystals exhibit a high FPF and low amorphous content.¹⁶⁹

Particle replication in nonwetting templates (PRINT)

PRINT is a top-down particle fabrication technique developed by Dr. Joseph DeSimone and his group. This technique is able to generate uniform populations of organic micro- and nanoparticles with complete control of size, shape and surface functionality, and permits the loading of delicate cargos such as small organic therapeutics, peptides, proteins, oligonucleotides, siRNA, contrast agents, radiotracers, and fluorophores.^{170–172} All these features are essential for controlling *in vivo* transport, biodistribution,

and drug-release mechanisms of nanoparticles. The principle of PRINT is to utilize a low surface energy fluoropolymeric mold that enables high-resolution imprint lithography, an emerging technique from the microelectronics industry, to fabricate a variety of organic particles. PRINT is therefore an adaptation of lithographic techniques found in the microelectronics industry to fabricate carriers of precise size for use in nanomedicine. Through the use of an appropriately designed master template, PRINT can precisely manipulate particle size ranging from 20 nm to more than 100 μm . The shape of particles can be sphere, cylinder, discs, and toroid with defined aspect ratios. PRINT is a promising and novel technology in nanoparticulate design and manufacture for use in pulmonary delivery.

Thermal condensation aerosols

Thermal condensation is a cutting edge technology which uses capillary aerosol generator (CAG) to produce high concentration condensation submicron to micron sized aerosols from drug solutions. The drug solution is pumped through a heated capillary tube. Precisely controlled heating of the capillary causes the solution to evaporate. Formulation vapor exiting the tip of the capillary tube mixes with the cooler surrounding air, and then becomes supersaturated and initiates nucleation. The condensation of surrounding vapor onto the generated nuclei results in an aerosol. Various drugs, such as perphenazine,¹⁷³ prochlorperazine,¹⁷⁴ rizatriptan¹⁷⁵ and benzyl,¹⁷⁶ have been aerosolized using this technique. Propylene glycol (PG) is a popular solvent chosen to dissolve the drugs.¹⁷⁷ However, in some cases, rapid heating of thin films of pharmaceutical compounds can also vaporize the molecules, leading to formation of aerosol particles of optimal size for pulmonary drug delivery.¹⁷⁸ By controlling the film thickness, the purity of aerosols can be enhanced by reducing the amount of aerosol decomposition.¹⁷⁹

***In vitro* evaluation methods for pulmonary drug delivery systems**

In vitro characterization of nanoparticulate aerosol systems

Nanoparticles for pulmonary drug delivery can be evaluated by comprehensive characterization methods. Table 3 lists several important techniques as well as their functions in the *in vitro* characterization study of the behavior of nanoparticulate pulmonary inhalation aerosols.¹⁸⁰

Inertial impaction is the standard method to measure the particle or droplet aerodynamic size from pharmaceutical aerosol delivery systems.^{181,182} It describes the phenomenon of the deposition of aerosol particles on the walls of an airway conduct. The impaction (obstruction) tends to occur where the airway direction changes. The big particles have high momentum (inertia) and are more likely to travel in the initial direction of airflow, while those with low momentum adjust to the new direction of flow and pass around the obstruction. Inertial impaction employs Stokes' law to determine the aerodynamic diameter of particles being evaluated. This has the advantage of incorporating shape and density effects into a single term.¹⁴¹

In addition to the conventional commercially available cascade impactors, MSP Corporation (Minneapolis, MN, USA) has recently developed a new commercially available nanomicroorifice uniform deposition impactor (Nano-MOUDI™) device with high accuracy in sampling and collecting size-fractionated airborne particle samples and pharmaceutical aerosol particle samples for gravimetric and/or chemical analysis. The Nano-MOUDI™ differs from conventional cascade impactors by using microorifice nozzles to reduce the jet velocity, pressure drop, particle bounce, and evaporative loss. These impactors also have a uniform deposit feature by rotating the impaction plates with respect to the

Table 3 Methods to characterize the physicochemical and aerodynamic properties of particles (microparticles and nanoparticles) for pulmonary inhalation delivery

Technology	Function
Inertial impaction	Measurement of the aerodynamic size of particles
XRPD	Measurement of molecular long-range vs short-range order
DSC	Measurement the phase transitions and phase behavior
SEM	Visualization of the surface morphology of particles
AFM	Surface nanotopographical imaging, surface free energy measurements, and measure of interparticulate forces
IGC	Determination of the surface free energy of bulk powders
Karl Fisher titration	Analytical quantification of water content in pharmaceutical powders, liquids, and semi-solids

Abbreviations: AFM, atomic force microscopy; DSC, differential scanning calorimetry; IGC, indocyanine green; SEM, scanning electron microscopy; XRPD, X-ray particle diffraction.

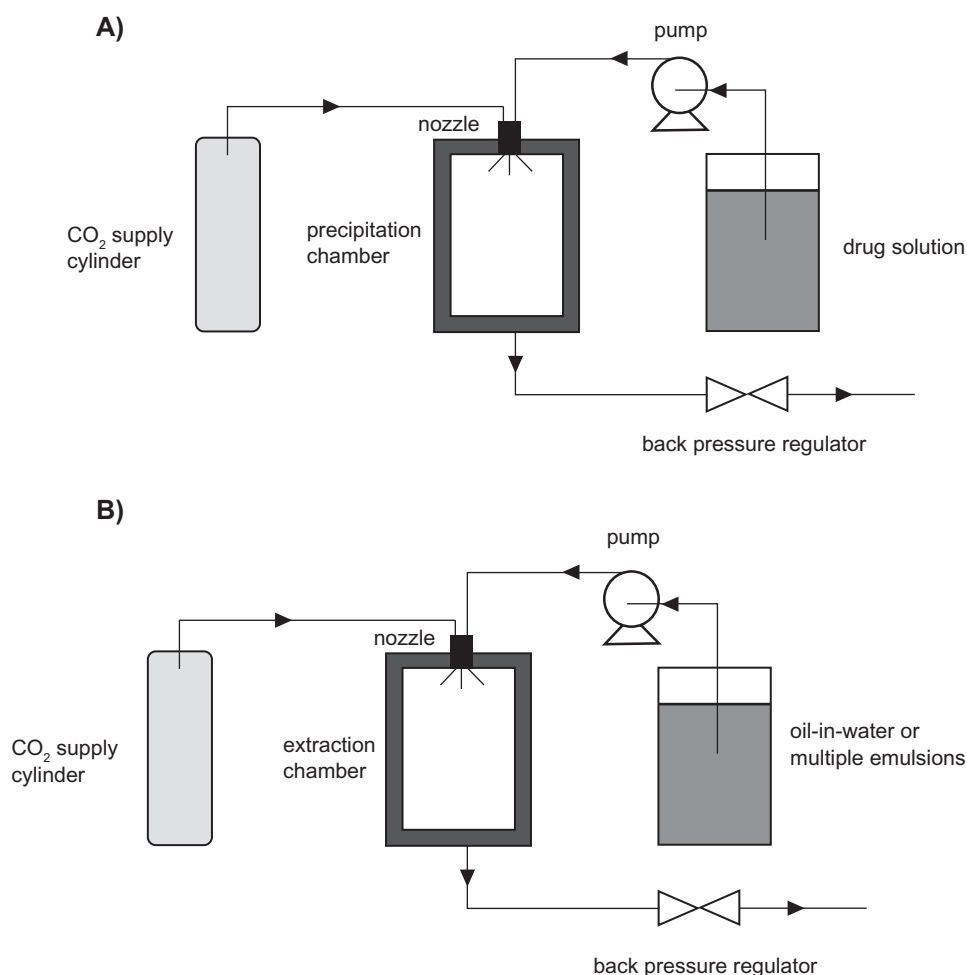


Figure 5 Schematic representations of **A)** SAS and **B)** SFEE processes.

nozzles to spread out the particle deposit uniformly on the collection substrates. The uniform deposit prevents heavy particles build-up under each nozzle to reduce particle bounce and blow-off that may otherwise occur. Nano-MOUDI™ is also superior to conventional cascade impactors in its aerodynamic design features. It is designed to prevent cross-flow interference between adjacent nozzles, which results in sharp cut-size characteristics not available with other cascade impactors. Because of its superior aerodynamic design and outstanding performance characteristics, Nano-MOUDI™ has an increasing application in environmental air quality and air pollution studies.^{183–190} We believe its application in pharmaceutical nanoaerosol drug delivery characterization and modeling is very promising, as well.

X-ray powder diffraction (XRPD) is one of the most important characterization tools used in solid state chemistry and materials science, since it directly measures the degree of molecular long-range vs short-range order. Molecular long-range order is indicative of crystallinity,

while short-range order is indicative of noncrystallinity such as liquid crystallinity and amorphicity. It gives information about the extent and nature of crystallinity and molecular order for solid-state materials, ie, how the atoms pack together in the crystalline state and what the interatomic distance and angle are.^{191–194}

Differential scanning calorimetry (DSC) is a powerful and routinely used pharmaceutical thermal analytical method for phase behavior study on polymorphs, hydrates, binding interactions, amorphicity, thermotropic and lyotropic phase transitions of pharmaceutical materials, including nanoparticles. DSC directly measures the gain and loss of enthalpy, that is, order-to-disorder (eg, melting) and disorder-to-order (eg, crystallization) phase transitions.¹⁹⁵

Scanning electron microscopy (SEM) is used to visualize the surface morphology of particles with a high magnification. The resolution allows identification of specific surface features and asperities that lead to mechanical interlocking (ie, structural cohesion) and that contain high-energy “active”

sites on the surface which influence surface energetic properties and interparticulate interactions and ultimately influence aerosol dispersion performance.¹⁸⁰ Surface and interfacial/interparticulate forces are of great importance in the properties of nanoparticles and in the properties of aerosols. Atomic force microscopy (AFM) is used in surface nanotopographical imaging, measurement of surface energy, and measurement of interparticulate forces. This microscopy works in mesoscopic scale resolution (10^{-6} – 10^{-9} m).¹⁹⁶ Inverse gas chromatography (IGC) measures the surface free energy of bulk powders such as polymers, fibers, and composite materials.¹⁹⁷

Karl Fischer titration is used to analytically quantify small amounts of water present in the inhalation powder which has important consequences on capillary condensation (ie, capillary force is an important interparticulate force in inhalation aerosol particles), solid-state phase behavior, solid-state properties, and solid-state stability of pharmaceutical particles in the solid-state.¹⁹⁸

***In vitro* pulmonary cell culture models**

The lung can be anatomically divided into several parts: the trachea, the main bronchi, the conducting bronchioles, the terminal bronchioles and the alveoli.^{199,200} Drug delivery via inhalation may be absorbed throughout the conducting airway from the trachea down to the terminal bronchioles and ultimately the distal alveoli.²⁰¹ The airway and alveolar epithelium of the lung, which have different cell types, provide barrier capability to drug absorption.

In order to better understand the drug absorption process in different regions (eg, tracheal, bronchial and alveolar) of the respirable barriers, *in vitro* pulmonary cell culture models have been developed.^{201–205} The cell lines used include A427, A549, HBE14o, and the Calu line (-1, -2, and -3).^{205–210} These are immortal (continuous) cell lines and hence have different membrane structural features compared with mortal cell lines which influence drug absorption and efflux. For example, A549 cell line represents the alveolar type II pulmonary epithelial cell, and has been reported to be an ideal model to study the metabolic and macromolecule mechanisms of drug delivery at the alveolar pulmonary epithelium because the endocytic ability of the pulmonary epithelium and localization of cytochrome P450 systems is largely a function of type II pneumocytes.²⁰⁹ Calu-3 and HBE14o model the upper airways (bronchi) and many studies have been conducted on them. For example, the permeability data of small lipophilic molecule (eg, testosterone) and high molecular weight substance (eg, fluorescein isothiocyanate-transferrin)

across Calu-3 cell line has been examined by Foster and colleagues.²⁰⁸ The authors demonstrated this cell line is useful for studying the contributions of bronchial epithelial cells to the mechanisms of drug delivery at the respiratory epithelium. Similar conclusions were drawn when the apparent permeability of the glucocorticosteroid budesonide was investigated.²⁰⁶

Primary cultured cells have also been used in pulmonary absorption and transport studies. These cell cultures have advantages over continuous cells by exhibiting tight junctions. It is important for cell cultures to form a sufficient and appropriately tight polarized monolayer, so that the transport kinetics of test molecules can be properly assessed.²¹¹

***In vivo* evaluation methods for pulmonary drug delivery systems**

Pharmacokinetic study after nebulization of colloidal dispersion of drugs

Colloidal drug dispersions have been delivered to male ICR mice via nebulization using a restraint-free small animal inhalation dosing chamber. Then, the mice were sacrificed at pre-determined time points post-dosing. Blood samples were taken by cardiac puncture, and the lungs were harvested for analysis by analytical instrument. From these experiments, lung and serum (blood) pharmacokinetics of colloidal drug dispersions can be determined as reported previously.^{212–214}

Biodistribution study using radiolabeled SLNs

To assess the inhaled radiolabelled SLNs biodistribution, nanoparticles (200 nm) were radiolabeled with ^{99m}Tc and biodistribution studies were carried out following aerosolisation and administration of a ^{99m}Tc-HMPAO-SLNs suspension to a group of adult male Wistar rats. After administration of radiolabeled SLNs, dynamic image acquisition was performed in a gamma-camera, followed by static image collection. Then radiation counting was performed in organ samples, collected after the animals were sacrificed. From these experiments, biodistribution of SLNs can be traced and evaluated.^{133,134}

Fluorescence/bioluminescence imaging systems for pulmonary gene delivery

Visualization and evaluation of both the pulmonary delivery and the gene expression properties after dry powder inhalation in mice were recently reported by Mizuno and colleagues.²¹⁵ It was shown that the pulmonary delivery and

the gene expression can be evaluated using fluorescence of indocyanine green (ICG) as a fluorescent label and the detection of luciferase activity, respectively, by using a non-destructive real-time *in vivo* imaging system. They concluded that the dry powder containing both ICG and pCMV-Luc was useful as a dual imaging system to visualize pulmonary delivery and gene expression in mice.

Regulatory and toxicity issues

Inhaled microparticles and nanoparticles of different sizes can target into different regions of respiratory tract, including nasopharyngeal, tracheal, bronchial, and alveolar regions, with several mechanisms. Meanwhile, the surface chemistry, charge, shape and aggregation status of nanoparticles also have influences on their disposition efficacy.²¹⁶ For example, inhaled large particles and nanoparticles deposit in the respiratory tract by distinctive mechanisms. Large particles deposit via inertial impaction, gravitation settling and interception mechanisms, while nanoparticles deposits via diffusion due to displacement when they collide with air molecules. The presence of albumin²¹⁷ and phospholipids (eg, lecithin)²¹⁸ in alveolar epithelial lining fluid is important to facilitate epithelial cell uptake of nanoparticles after deposition in the alveolar space. Nanoparticles coated with them may translocate across the alveolo-capillary barrier, whereas uncoated particles do not. Therefore, when evaluating the efficacy and fate of nanoparticles by inhalation delivery, all the variables should be taken into consideration.¹

After delivery into the lung, some nanoparticles may be translocated to extrapulmonary sites and reach other target organs by cellular endocytosis, transcytosis, neuronal,²¹⁹ epithelial²²⁰ and circulatory²²¹ translocation and distribution, which makes them desirable for medical therapeutic or diagnostic application. However, these features can also pose potential toxicity. Transcytosis absorbs nanoparticles and translocate them into the interstitial sites, where they gain access to the blood circulation via lymphatics, resulting in distribution throughout the body. Neuronal translocation involves uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and central nerve systems (CNS) structures. For example, nanoparticles facilitating drug delivery to the CNS in the brain raises the question of fate of nanoparticles after their translocation to the specific cell types or to subcellular structures. This kind of questions includes whether mitochondrial localization induces oxidative stress and how persistent the coating or the core of nanoparticles

is, which is essential in nanoparticle toxicology and safety evaluation.²²²

The clearance of nanoparticles in the alveolar region is predominantly mediated by alveolar macrophages, through phagocytosis of deposited nanoparticles. After macrophages recognize the deposited nanoparticles and phagocytize them, macrophages with internalized nanoparticles gradually move toward the mucociliary escalator, and then the clearance process is started. The retention half-time of solid particles in the alveolar region based on this mechanism is very slow, up to 700 days in humans. Moreover, unlike larger particles, results from several studies show the apparent inefficiency of alveolar macrophage phagocytosis of nanosized particles.^{223–225} The ultrafine nanoparticles are not easily phagocytized by macrophages and, consequently, are not readily cleared in the alveolar region.²²⁶ These nanoparticles are either in epithelium cells or are further translocated to the interstitium, which may cause a long-term accumulation in the lung and subsequent toxicity issues.

According to the FDA guidance for dry powder inhaler (DPI) drug products, α -lactose monohydrate is the only approved sugar that can be used as a large carrier particle in dry powder inhalation aerosol products to fluidize and disperse the respiratory drug while itself not being delivered to the lung. Other novel materials, including phospholipids, specifically lecithin and amino acids (lysine, polylysine) have also been developed for use in pulmonary formulations as excipients that are delivered to the lung. A thorough assessment of these alternatives associated with any inhalable substance is required, from a variety of sources: human, animal, and/or *in vitro* test models.²²⁷

Conclusions

Inhalable colloidal carriers (nanocarrier systems) offer numerous advantages. The decrease in particle size leads to an increase in surface area leading to enhanced dissolution rate, as well as relatively uniform distribution of drug dose among the alveoli. In addition, by suspending the drugs in nanoparticles, one can achieve a dose that is higher than that offered by a pure aqueous solution, which is thermodynamically limited by the aqueous solubility of the drug. Nanocarrier systems can provide the advantage of sustained-release in the lung tissue, resulting in reduced dosing frequency and improved patient compliance. Local delivery of inhalable nanocarriers may be a promising alternative to oral or intravenous administration, thus decreasing the incidence of side effects associated with a high drug serum concentration. As with all formulations designed for

pulmonary drug delivery, the potential long-term risk of excipient toxicity and nanoscale carrier itself are issues that need to be considered in the successful product development of pulmonary drug delivery systems. Nevertheless, their inherently small size and surface modification properties enable further opportunities for innovative controlled drug release and pulmonary cell targeting therapeutic platforms. The integration of nanotechnology and pulmonary delivery has the potential to improve the targeting, release, and therapeutic effects of drugs and needle-free inhalation vaccines with significant potential capability of overcoming the physicochemical and biological hurdles.

Disclosure

The authors report no conflicts of interest in this work.

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