

Chapter 1

Pulmonary drug delivery

Pulmonary drug delivery

1.1 Introduction

The respiratory tract is an attractive route for the administration of therapeutics and genomics. The past decade has been marked by intensive research efforts on pulmonary drug delivery (PDD) for local and systemic drug and gene delivery including diagnostic agents because it has several advantages over other routes of administration (1, 2). Various pharmaceuticals, biopharmaceuticals, anesthetics, smoke or steam have been successfully inhaled for medical purposes for centuries (3). Over the years inhalation therapy has established itself as a valuable tool in the local therapy of pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD) (3), cystic fibrosis (CF), and pulmonary hypertension (4). The local application of therapeutic agents to the respiratory system has several advantages over other routes of administration, i.e., a large surface area available for absorption, high to blood flow, highly vascularized tissue, rapid absorption, avoidance of first pass effect, avoidance of the effects of gastric stasis and pH, smaller doses required than by the oral route to achieve equivalent therapeutic effects useful for local treatment and systemic distribution (5, 6). Site-specific delivery facilitates a reduction of the therapeutic dose to be administered, and thus, decreases the associated adverse effects (6). In addition, inhalation represents a non-invasive alternative for systemic delivery of biopharmaceuticals, which are labile to gastric acid, and thus, improves patient compliance (7). The efficiency of inhalation therapy depends on the delivery system, the devices used and the fate of the delivered medication in the respiratory tract. Once the therapeutic agent has been deposited in the lung, elimination is instantly initiated, decreasing the initial high local concentrations of the therapeutic agent in lung tissue (7, 8). Because the concentration of the drug can decrease quickly, it often requires multiple daily inhalations which can cause difficulty for the patient's compliance (9).

Inhalation therapy involves the deposition of the drug in the lungs *via* aerosol generating devices such as pressurized metered-dose inhalers (pMDI), nebulizers, and dry powder inhalers (DPI) (7). The 'conventional' inhalation therapy does not allow targeted delivery to specific lung cells and the deposition of the therapeutic agents in different lung areas is often poorly controlled (8). To overcome these shortcomings, more sophisticated pulmonary delivery systems are desirable which include potential carrier systems, and which use micro-sized and nano-sized vehicles. These delivery systems have attracted considerable attention because of their controlled release

and targeting properties (9-15). These systems are mainly classified into immediate release (e.g., lactose-drug mixtures for DPI application) and controlled release systems (liposomes, micelles, nanoparticles and microparticles). The modulation of the various physicochemical properties of these systems has been explored to overcome the clearance mechanisms of the lungs and provide prolonged residence times of the therapeutic agent within the respiratory tract (8, 10, 12) .

1.2 Anatomy and Physiology of Human Respiratory Tract

For the development of new PDD systems, one should have detailed knowledge of lung anatomy and physiology. The respiratory tract is mainly divided into two regions i.e., the upper airway and the lower airway with the line of division being the junction of the larynx and trachea (16). The upper airway acts as an air transport system and consists of the nose, mouth, pharynx and larynx. The lower respiratory tract consists of the tracheobronchial, gas-conducting airways and the gas exchanging acini. The lower airway is divided into three zones: conducting, transitional, and respiratory zones. The conducting zone is responsible for the bulk movement of air and blood. In the central airways, air flow is rapid and turbulent and no gas exchange occurs. The transitional zone has a limited role in gas exchange (16, 17) . The respiratory zone mainly comprises of respiratory bronchioles and alveoli, where the actual gas exchange takes place (17). Figure 1.1 shows a schematic representation of the lung. The bronchial tree trunk begins with the trachea, which bifurcates to form the main bronchi: the left and right primary bronchi. Each primary bronchus divides into still smaller secondary bronchi. The secondary bronchi branch into many tertiary bronchi that further branch several times, ultimately giving rise to tiny bronchioles that sub-divide many times, finally forming terminal bronchioles and respiratory bronchioles. Each respiratory bronchiole sub-divides into several alveolar ducts that end in clusters of small thin-walled air sacs called alveoli, which open into a chamber called the alveolar sac (18) .

Two physical changes occur while moving from the trachea to the alveolar sacs in the airways that are important in influencing airway function. Firstly, the airway diameter decreases with the respiratory branches, e.g., a tracheal diameter (d) is 1.8 cm compared to an alveolar diameter of 0.04 cm. This permits adequate penetration of air to the lower airways for a given expansion of the lungs. Secondly, the surface area of the airways increases with each generation, to the extent that the total lung area at the level of the human alveolus is in the order of 140 m² (18). Alveoli are the terminal air spaces of the respiratory system and are the actual site of gas exchange

between the air and the blood. About 100 million alveoli are found in each lung (19). Each alveolus is a thin-walled polyhedral chamber of approximately 0.2 mm in diameter. Gases taken up by inhalation need to cross alveolar epithelium, the capillary endothelium and their basement membranes to reach blood; the distance makes up around 500 nm length (18). So, the alveolus, providing increased surface area, becomes principal site of gas exchange in the airways (20). The human lung consists of five lobules and ten broncho-pulmonary segments. The luminal surface of the airways is lined by ciliated cells, which are the most common and numerous cell types.

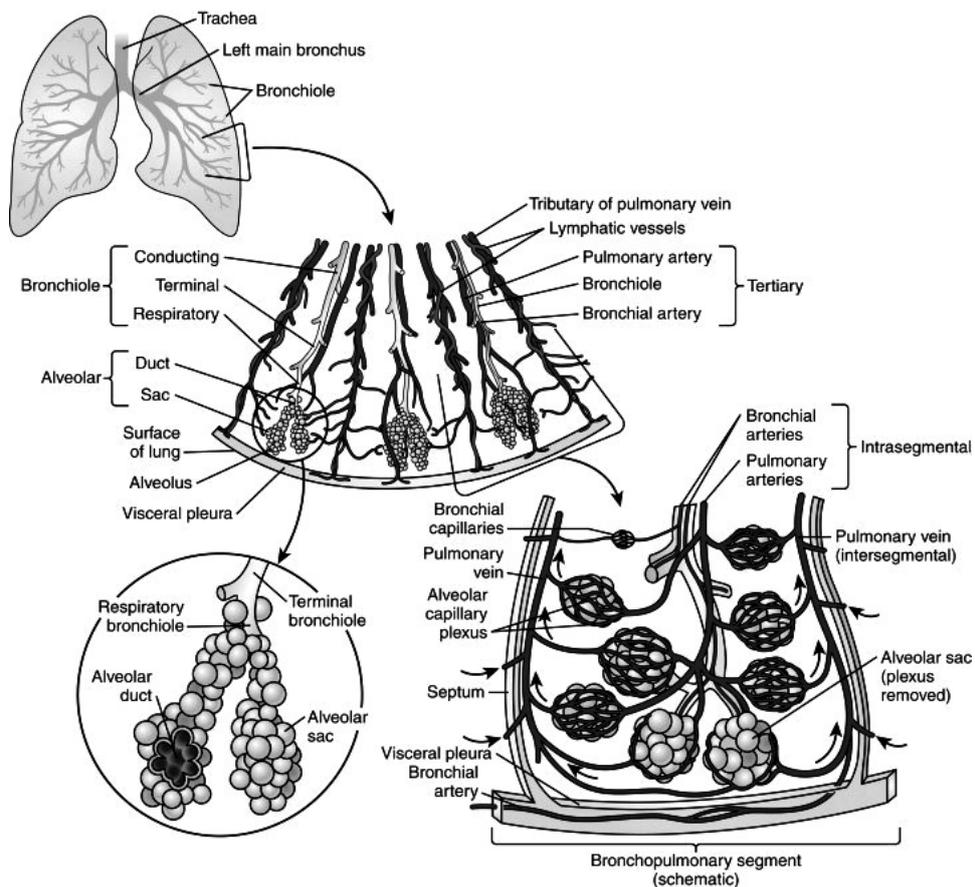


Figure 1.1 Internal structure and organization of lungs (21).

Mucus cells are intermingled among the ciliated cells. The walls of the conducting airways are coated by an adhesive, viscoelastic mucus layer (thickness: 5–55 μm) which is secreted by the mucus cells. The major components of mucus are glycoproteins and water (22). This mucus fulfils important functions, i.e., the protection of the respiratory epithelium from dehydration, the water in the mucus promotes saturation of inhaled air, mucus contains antibacterial proteins and

peptides, such as defensins and lysozyme that inhibit microbial colonization of the airways, and mucus is also involved in airway protection from inhaled xenobiotics or chemicals. Clearance of mucus from the lung is driven by the motion of the ciliated cells 'mucociliary escalator', which generates a mucus flow rate of ~5 mm/min. Thus, the mucus blanket is replaced every 20 minutes in healthy subjects (22). The mucociliary escalator serves as an important protective mechanism for removing small inhaled particles from the lungs. The composition, thickness, viscosity and clearance of the mucus is often altered in patients suffering from airway diseases such as asthma, COPD and CF (7).

The alveolar epithelium is composed of Type I and Type II alveolar cells and occasional brush cells. The Type I pneumocytes are thin cells, cover most of the surface of the alveoli (95% of the surface area) and the Type II pneumocytes are cuboidal secretory cells are interspersed among the Type I cells (23). The alveolar space is coated by a complex surfactant lining that reduces surface tension to minimize the work of breathing and prevents collapse of the alveoli during expiration (21). The majority of insoluble particles deposited in the upper airways are eliminated by mucociliary clearance (24). The most prominent defense mechanism of the respiratory region is macrophage clearance. The particles deposited in the deeper lung will be taken up by alveolar macrophages, which slowly migrate out of the lung, either following the broncho-tracheal escalator or the lymphatic system (8). The blood supply to the lung is provided by a pulmonary circulation and a systemic circulation. A drug delivered to the lower airways can enter the systemic circulation by absorption into the alveolar capillaries of the pulmonary vascular bed (21, 23).

1.3 Deposition mechanisms of inhaled particles

There are five mechanisms by which particles deposit in the respiratory tract namely, impaction, sedimentation, brownian motion, interception and electrostatic precipitation. Impaction is the inertial deposition of a particle onto an airway surface and occurs at bifurcation of airway where flow velocities are at higher and sudden change in direction of flow take place, generating considerable inertial forces. Higher velocity, high rate of breathing, and particles > 5 μ size and high density increases impaction based deposition (25). Gravitational sedimentation is an important mechanism for deposition of particles over 0.5 μ m and below 5 μ m in size in the small conducting airways where the air velocity is low. Deposition due to gravity is increased by large particle size and by longer residence times, and decreases with increasing breathing rate (25).

Impact of surrounding air on particles $< 0.5 \mu\text{m}$ size cause a random motion in particles. Such Brownian motion can cause particle deposition by diffusion in small airways and alveoli where air flow is low in contrast to upper respiratory airways. Interception is usually significant only for fibers and aggregates. Even though, center of mass of such particles remain to be in flow stream, contact of such particles with airway wall cause deposition of such particles (25). Some freshly generated particles can be electrically charged during the mechanical generation of aerosols and may exhibit enhanced deposition due to charge induced deposition, though at a low contribution to other mechanisms (26). In fact, the deposition patterns of inhaled particles may be expressed as functions of three classes of variables: ventilatory parameters, respiratory tract morphologies and aerosol characteristics (e.g., particle size, shape, and density). The efficiencies of the different deposition mechanisms of inertial impaction, sedimentation and diffusion can, in turn, be formulated in terms of these variables.

1.4 Barriers in Pulmonary Delivery

The past 30 years have been marked by intensive research efforts on PDD for local and systemic therapy including diagnostic agents (5). The success of a PDD using aerosolized medications depends on its ability to deliver sufficient concentration of drug to the appropriate site of action in the lungs while exerting minimal side effects (27). Drugs for lung diseases and drugs that undergo extensive first-pass metabolism or gastrointestinal degradation are ideal candidates for pulmonary delivery. The lower incidence of side effects is often observed due to localized drug deposition and reduced systemic and generalized exposure to other tissues. Despite such great advantages of PDD, delivering therapeutic agents to lungs is a challenging task for the formulation scientist because of the barriers of the pulmonary region and finding a suitable delivery device.

The lungs are in direct contact with the atmosphere and thus, different lines of defense systems exist to protect the deep parts of the lungs from exposure to particles present in the inhaled air (7) (24). Several mechanisms are involved in the removal of particles from the upper respiratory tract and thus, they reduce further deposition in the lower airways. The deposition of aerosol particles in the lungs involves three mechanisms: impaction, sedimentation and diffusion (5). Deposition in the respiratory tract is affected by the particle size, the patient's inhalation parameters e.g. flow rate, ventilation volume, end-inspiratory breath holding, and the aerosol delivery system. The most crucial formulation variable for PDD is the mass median aerodynamic

diameter (MMAD) of the particles. Figure 1.2 shows the effects of particle size on the deposition efficiency of particles in respiratory system. Large particles with a MMAD of more than 5 μm experience impaction in the oropharynx and upper conducting airways because of their high momentum, while particles with an MMAD between 1 and 5 μm sediment in the deeper airways and bronchioles. Small particles with an MMAD below 0.5 μm obey the principle of Brownian diffusion, remain suspended in air and are exhaled during normal breathing (Table 1.1) (27). Apart from the size, the deposition of aerosol particles also depends on density, hygroscopicity, and the shape of the aerosolized particles. The anatomy of the airways and the breathing pattern also determines impaction, sedimentation and diffusion of particles in the airflow. It is commonly accepted that the optimal particle size (1-5 μm) is essential for the effective delivery of particles to lungs, as particles smaller than 1 μm can possibly be exhaled without being deposited (28). However, some recent investigations showed that nanoscale particles are also effectively deposited in the alveolar region because of increased diffusional mobility, especially in individuals suffering from asthma and increasingly during physical exercise (29).

Table 1.1: Deposition fate of inhaled particles in lungs

Sr. No.	Aerodynamic diameter (μm)	Fate of inhaled particle in lungs	Phagocytosis
1	<1 μm	Particle remains suspended in lungs	Less liable to phagocytosis
2	1-5 μm	Deposition of particle in the deep lungs	Highly liable to phagocytosis
3	>5 μm	Particles deposited in upper respiratory tract	Less liable to phagocytosis

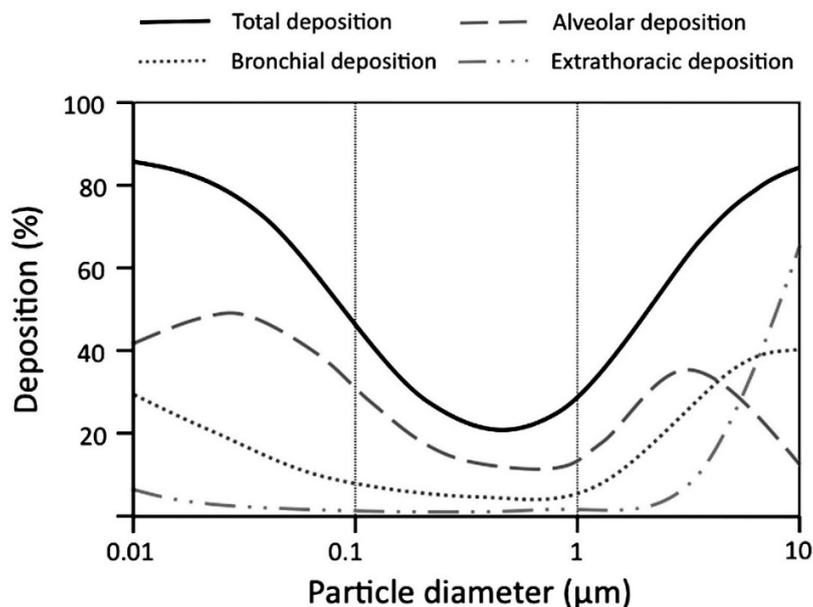


Figure 1.2 Deposition efficiency of particle in the respiratory system as function of the particle size (28) .

The respiratory mucus covers the conducting airways and captures foreign matter inhaled with each breath. The non-absorptive process involves transport of particles to the ciliated region following clearance by the mucociliary escalator. In normal airways, the respiratory cilia transport mucus at a rate of 2.5-5 mm/min towards the oropharynx where it is either swallowed or expectorated (30). The mucus acts as a physical barrier, as increased viscosity of mucus reduces drug penetration and its diffusion. Upon deposition in the lung, the particles are wetted by mucus and subsequently transported toward the oesophagus by ciliated cells. The mucociliary clearance of mucus-trapped foreign substances is an important pulmonary defense mechanism against inhaled pathogens and particles and as well it acts as a barrier to gene transfer vectors (31). The alveolar epithelium is not covered by mucus but a thin layer of alveolar fluid is secreted on the surface of the alveoli epithelium. Alveolar fluid is composed of phospholipids and lung surfactant excreted from Type II pneumocytes. The surface activity of the surfactant is mainly provided by the phospholipids, surfactant proteins B and C, which also lower the surface tension, whereas surfactant proteins A and D can opsonize foreign matter in the lungs (32). The alveolar macrophages located in the alveoli can rapidly engulf the foreign particles by phagocytosis as a defense mechanism. Every single alveolus is covered by 12-14 macrophages, which gives approximately 19,000 alveolar macrophages per microlitre of bronchoalveolar

lavage fluid (BALF) (33). Crystalline powders of pharmaceutical application which exhibit specific gravity approaching 1 and which are in the range of respirable aerodynamic diameter show rapid macrophage uptake and clearance. Such uptake has been shown to be dependent on the geometric diameter of inhaled particles, while lung deposition depends the aerodynamic diameter of particles (34).

These anatomical and physiological barriers of the lungs play an important role in pulmonary delivery of therapeutics and various approaches have been utilized to overcome these limitations. Particulate nanocarriers can be used to improve the therapeutic index, reduce metabolism, prolong half-life or reduce toxicity, increase bioavailability and site specific targeting resulting in a reduction in the biological dosage frequency and improved patient compliance.

1.5 Delivery devices

Delivery device for pulmonary delivery should produce aerosolized particles ideally in the range of 0.5-5 μ size with reproducible dosing and should maintain physical and chemical stability of drug formulation. Additionally, the system should be simple, easy to use, cheap and portable (35). Inhaled drug delivery devices can be divided into three principal categories: nebulizers, pressurized metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs), each class with its unique strengths and weaknesses.

1.5.1 Nebulizers

Nebulizers have been used in inhalation therapy since the early 19th century. Marketed respiratory solutions are generally composed of drug dissolved in aqueous, isotonic solvent systems that may contain preservatives to reduce microbial growth. There are two traditional devices: air-jet and ultrasonic nebulizers. For a typical jet nebulizer (Figure 1.3), compressed air passes through a narrow hole and entrains the drug solution from one or more capillaries mainly by momentum transfer. Large droplets impact on baffles and gets refined to the size required, while droplets with smaller size run in a streamline flow of air bypassing the impact on baffles (36). Approximately 50-60% of the particles produced are in the respirable range.

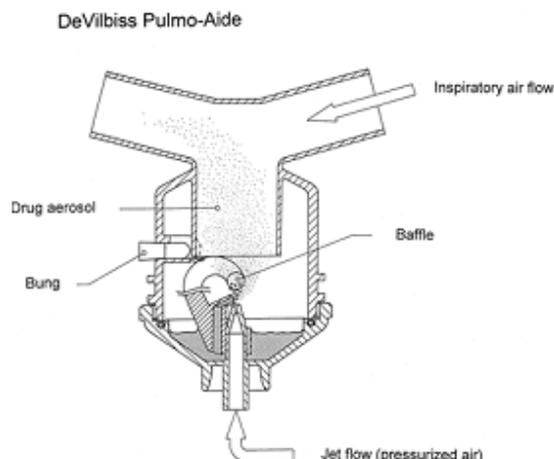


Figure 1.3: Schematic presentation of a jet nebulizer (36)

Alternatively, ultrasonic nebulizers use a high frequency vibrating plate to provide the energy required to aerosolize the liquid. The frequency of the vibrating piezoelectric crystal determines the droplet size for a given solution. Approximately 70% of the particles produced present sizes of between 1 and 5 μm . However, heat generated from frictional forces induced by movement of the transducing crystal may be harmful to thermolabile formulations. Some of the most commonly available nebulizers on the market are: Ventolin® (Salbutamol, β_2 -mimetic bronchodilator), Bricanyl® (Terbutaline, β_2 -mimetic bronchodilator), Atrovent® (Ipratropium, anticholinergic bronchodilator), Pulmozyme® (Dornase alpha, mucolytic) and Tobi® (Tobramycin, antibiotic).

But nebulization has many well-documented disadvantages, including extended administration time, high cost, low efficiency, poor reproducibility and great variability, risk of bacterial contamination and constant cleaning requirements, and sometimes the need for bulky compressors or gas cylinders (37). Moreover, in some cases, the presence of preservatives such as sodium metabisulfite, benzalkonium chloride and ethylene diamine tetraacetic acid (EDTA) has caused coughing and bronchoconstriction (38). One of the principal problems of nebulizers is that the device includes a large “dead volume” of solution. A large fraction of the amount (up to 50%) can thus remain trapped in the apparatus. Moreover, the aerosolized drug is generated continuously, leading to drug waste (38). With a continuously working compressor (continuous droplet generation), part of the aerosol cloud may be wasted into the environment through the vent when the patient stops or interrupts inhalation or does not inhale fast enough. The amount

inspired is equivalent, more or less, to half of the delivered amount. Of this inhaled amount, it is still necessary to remove a fraction of particles that are not in the “respirable range”. In conclusion, the pulmonary fractions obtained using a nebulizer may vary from 2-10% of the nominal dose. For example, 2.5 ml of Pulmozyme at 1 mg/ml is delivered by a jet nebulizer with a delivery efficiency of 10% (39).

1.5.2 Pressurized Metered-Dose Inhalers

Traditional asthma therapy has primarily used the pMDI. Since the 1950s, they have been the backbone of inhalation therapy (40). In a pMDI, a propellant pressurized to a liquid state is holding the drug either in a suspended or dissolved form. Metered release of the fluid through a valve causes expansion and evaporation of propellant leaving the drug in the form of a high velocity aerosol. Rapid release of the propellant into the valve stem along with the actuator seating forms an expansion chamber where propellant starts to boil (Figure 1.4). The liquefied propellant serves both as a source of energy for expelling the formulation from the valve in the form of rapidly evaporating droplets and as a dispersion medium for the drug and other excipients.

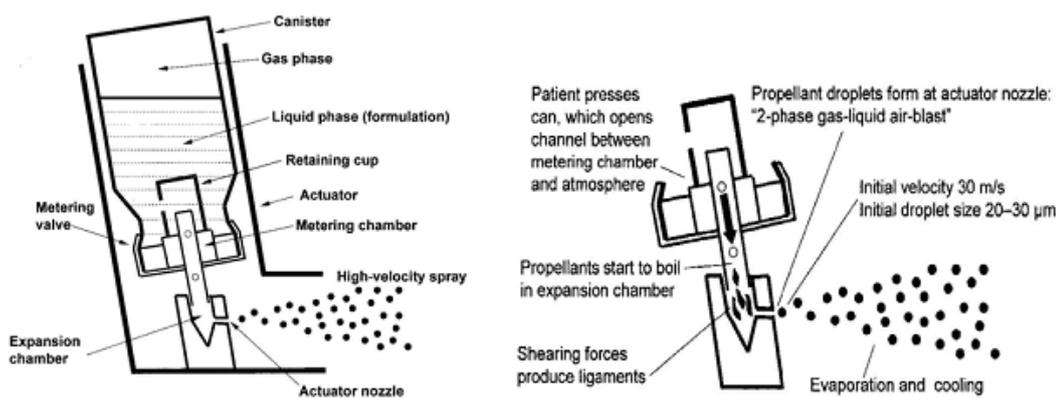


Figure 1.4: Schematic representation of a typical pressurized metered-dose inhaler (41)

Physicochemical properties of the formulation determine the particle size and size distribution of an MDI aerosol. For example, aerosol size for suspension formulations may be reduced if the formulation has a high vapour pressure, small particle size and low drug concentration. For instance, studies have shown that the aerosol size for suspension formulations may be reduced if the formulation has a high vapor pressure, a small drug particle size, or a low drug concentration (42). A surfactant such as sorbitan trioleate, oleic acid, and lecithins, at levels between 0.1% and

2.0% w/w, are typically added to aid the suspension or solubility of drug and to lubricate the metering mechanism (41). Flavors and suspended sweeteners may be present to combat the unpleasant taste. To enhance chemical stability, antioxidants (ascorbic acid) or chelating agents (EDTA) may be added to formulations (38).

The essential components of an MDI are the container, the metering valve, and the actuator. Usual valve volumes range from 25-100 μ l, which deliver a drug dose of about 50 μ g to 5 mg. The most commonly available MDIs on the market are: Flixotide® (Fluticasone, corticosteroid), Atrovent® (Ipratropium bromide, anticholinergic bronchodilator), Ventolin® (Salbutamol, β 2-mimetic bronchodilator) and Combivent® (Ipratropium bromide + Salbutamol, anticholinergic + β 2-mimetic bronchodilator). Chlorofluorocarbon (CFC)-based MDIs usually contain a mix of liquefied low boiling-point propellant (CFC 12- dichlorodifluoromethane) and a high boiling-point propellant (CFC 11- trichlorofluoromethane or CFC 114 – dichlorotetrafluoromethane). However, the success of CFC propellant-driven MDIs has been overshadowed by their contribution to ozone depletion in the upper atmosphere and the concomitant health effects (43). In fact, the international community agreed to phase out CFC propellants in 2000. So nowadays, the main emphasis in MDI developments is on the introduction of non-CFC propellants. But the non-CFC propellant hydrofluoroalkanes (HFA 134a - 1, 1, 1, 2-tetrafluoroethane and HFA 227 - heptafluoropropane) exhibit very different physicochemical properties and extremely low solvent properties. This however is advantageous in preventing the dissolution of small drug particles but may also be disadvantageous. This feature is beneficial in preventing the dissolution of small drug particles, but it is also disadvantageous in that the commonly used surface-active agents are almost totally insoluble and not able to provide any physical stabilization of drug particles in suspension. A number of approaches have been undertaken to overcome the problems of drug particles instability in HFAs, addition of cosolvents, requirement to develop new specific surface active agents, modification of surface properties of particles to reduce interfacial tension, and production of particles compatible to HFA (44). However, MDIs have several other disadvantages. There may also be chances of “cold-Freon effect” which may cause inhalation problems to patients due to cold propellant spray on the back of the throat (45). Moreover, because an MDI is pressurized, it emits the dose at high velocity, which makes premature deposition in the oropharynx more likely (46). Thus MDIs require careful coordination of actuation and inhalation. Only a small fraction of the drug (10-20%) escaping the inhaler

penetrates the patient's lungs due to a combination of high particle exit velocity and poor coordination between actuation and inhalation. The deposition of aerosolized drugs in the mouth and the oropharyngeal regions varies considerably according to the application technique, but losses using the pressurized devices are routinely greater than 70% and can exceed 90%. Particle losses that occur proximally to the lung are a long-documented problem that continues to compromise the effectiveness of current aerosol therapy protocols.

Spacer devices and reservoirs were developed to allow the deceleration of the aerosol cloud before reaching the throat and to make perfect coordination between actuation and inhalation slightly less important (Figure 1.5). Nevertheless, adding a chamber to an MDI makes it less portable, and many chambers can develop static electrical charges on the inner walls and thereby reduce the lung delivery (47). Despite these enhancements, incorrect use of MDIs is still a prevalent problem (48). About 75% of patients made at least one error when using an MDI (49).

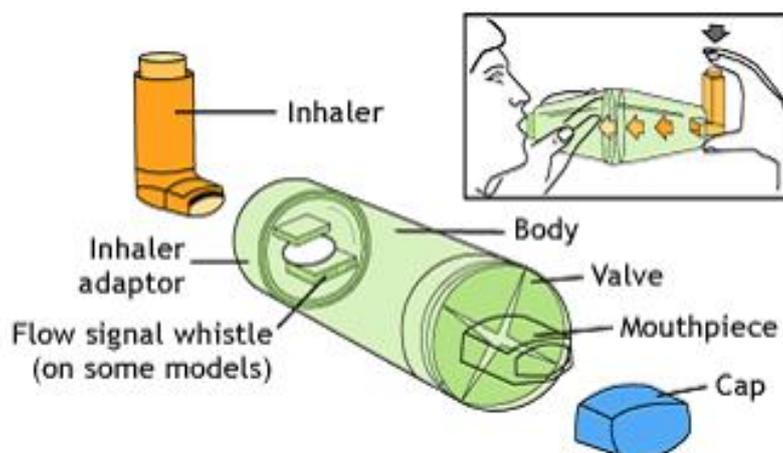


Figure 1.5: Representation of the use of a spacer device with an MDI (50)

The introduction of “breath-actuated” MDI devices has provided a convenient and portable means to achieve coordination between actuation and inhalation. Breath-actuated inhalers have been developed that sense patient's inhalation and automatically fire the inhaler. Examples are the Autohaler® (3M Pharmaceuticals, U.S.A) or Easybreathe® (Norton Healthcare, U.K.) (51).

1.5.3 Dry Powder Inhalers

As a result of the problems encountered with MDIs, the design and use of alternative inhalers that do not use propellants were developed. These devices combine powder technology with

device design in order to disperse dry particles as an aerosol in the patient's inspiratory airflow (52).

All DPIs have four basic features: a dose-metering mechanism, an aerosolization mechanism, a deaggregation mechanism, and an adaptor to direct the aerosol into the mouth. DPIs are subject to strict pharmaceutical and manufacturing standards by regulatory bodies, the most challenging of which is the demonstration of device reliability in terms of delivered dose uniformity and delivered dose deposition (53). Indeed, comparative *in-vitro* data for a generic product versus the innovator product must be provided on the complete individual stage particle size distribution profile using a multistage impactor/impinger with various impaction stages, such as the NGI (53).

For DPIs, the dose received by the patient is dependent on four interrelated factors (54)

1. The properties of the drug formulation, particularly powder flow, particle size and drug carrier interaction
2. The performance of the inhaler device, including aerosol generation and delivery
3. Correct inhalation technique for deposition in the lungs
4. The inspiratory flow rate

Therefore, a balance between the design of an inhaler device, drug formulation, and the inspiratory flow rate of patient is required (55). DPIs are a widely-accepted inhaled delivery dosage form, particularly in Europe where they are currently used by an estimated 40% of patients to treat asthma and chronic obstructive pulmonary disease. Their use will continue to grow (54). Presently, over 20 DPI devices are available on the market (Table 1.2) and more than 25 are in development (Table 1.3). Today there are essentially two types of DPIs: those in which the drug is packaged in individual doses (capsules) (Single dose or Unit-dose devices) and those that contain multiple doses in a foil-foil blister or a reservoir of drug from which the doses are metered out (Multi-unit and Multi-dose devices).

Table 1.2: DPI devices currently available on the market (56)

Device	DPI type	Company	Delivery method	Drug(s)
Breath-actuated single unit dose				
Spinhaler	Single dose	Aventis	Capsule	Sodium cromoglycate
Rotahaler	Single dose	GlaxoSmithKline	Capsule	Salbutamol sulphate Beclomethasone dipropionate
Inhalator	Single dose	Boeringher-Ingelheim	Capsule	Fenoterol
Handihaler	Single dose	Boeringher-Ingelheim	Capsule	Tiotropium
Aerolizer	Single dose	Novartis	Capsule	Formoterol
FlowCaps	Single dose	Hovione	Capsule	Salbutamol Sulphate
TwinCaps	Single dose	Hovione	Capsule	Zanamivir
Breath-actuated multiple dose				
Turbuhaler	Multi-dose	Astra Zeneca	Reservoir	Salbutamol sulphate Terbutaline sulphate Budesonide
Diskhaler	Multi-dose	GlaxoSmithKline	Blister	Salmeterol xinafoate Beclomethasone dipropionate Fluticasone propionate Zanamivir
Diskus	Multi-dose	GlaxoSmithKline	Strip pack	Salbutamol sulphate Salmeterol xinafoate Fluticasone propionate
Aerohaler	Multi-dose	Boeringher-Ingelheim	Reservoir	Ipratopium bromide

Easyhaler	Multi-dose	Orion Pharma	Reservoir	Salbutamol sulphate Beclomethasone dipropionate
Ultrahaler	Multi-dose	Aventis	Reservoir	Ciclesonide
Pulvinal	Multi-dose	Chiesi	Reservoir	Salbutamol sulphate Beclomethasone dipropionate
Novolizer	Multi-dose	ASTA	Reservoir	Budesonide
MAGhaler	Multi-dose	Boeringher-Ingelheim	Reservoir	Salbutamol sulphate
Taifun	Multi-dose	LAB Pharma	Reservoir	Salbutamol sulphate
Eclipse	Multi-dose	Aventis	Capsule	Sodium cromoglycate
Clickhaler	Multi-dose	Innoveta Biomed	Reservoir	Salbutamol sulphate Beclomethasone dipropionate
Twisthaler	Multi-dose	Schering-Plough	Reservoir	Mometasone furoate
Active device				
Airmax	Multi-dose	Norton Healthcare	Reservoir	Formoterol Budesonide
Inhance	Single dose	Pfizer	Blister	Insulin

Table 1.3: DPIs approved or in development stage (56)

Device	DPI type	Company	Delivery method	Drug(s)
Aspirair	Multi-dose	Vectura	Powder/Active	Apomorphine hydrochloride
Actispire	Single dose	Britania	Powder/Active	Pumactant
JAGO	Multi-unit	SkyPharma	Reservoir	Salbutamol sulphate

Airmax	Multi-unit	Norton Healthcare	Reservoir	Formoterol, Budesonide
AIR	Single dose	Alkermes	Capsule	Placebo powders
MicroDose	Multi-unit	MicroDose/ 3M	Powder/Electronic	Insulin
Cyclovent	Multi-unit	Pharmachemie	Reservoir	Morphine
Conix One	Single dose	Cambridge Consultant	Foil seal	Vaccines
Microhaler	Single dose	Harris Pharmaceutical	Capsule	Sodium cromoglycate
Spiros	Multi-unit	Dura	Blister/Active	Albuterol sulphate
Miat-Haler	Multi-unit	MiatSpA	Reservoir	Formoterol, Fluticasone propionate, Budesonid
Acu-Breath	Multi-unit	Respirics	Powder	Fluticasone propionate
Swinhaler	Multi-unit	Otsuka Pharmaceutical	Powder	Budesonide
Certihaler	Multi-unit	Novartis	Powder	Formoterol

1.5.4 Unit-dose devices

The Spinhaler® (Aventis) was the first dry powder device, described in 1971 (57) which works on the mechanism to pierce the capsule. The caps fits into an impeller. The impeller rotates as the patient breaths through the device (Figure 1.6) during which powder deaggregates and relative motion imparted due to impeller rotation. This low-resistance device has presented low in vitro fine particle fractions (FPF) ($\% < 5 \mu\text{m}$) of 4-12% (58).

A similar DPI, the Rotahaler® (GlaxoSmithKline) breaks the capsules into two, the body part falling inside the device and the cap being retained in the entry port. As the patient inhales, the portion of the capsule containing the drug experiences erratic motion in the airstream, causing dislodged particles to be entrained and subsequently inhaled. Turbulence due to grid upstream of the mouthpiece deaggregates particles. A FPF of 26% has been reported for this low resistance device (58).

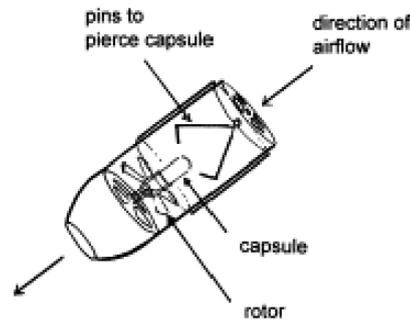


Figure 1.6: Schematic presentation of the Spinhaler (59)

The Handihaler® (Boehringer Ingelheim) delivers drug contained in a capsule through rumbling motion after piercing pins opens the capsule. The particles are dispersed through turbulence due to plastic grid during inhalation. This device is more complicated as it requires at least 7 distinct steps to deliver the dose. For some patients, two inhalations are required to completely empty the capsule and achieve the therapeutic dose (54)

Unit-dose devices are considered as not patient-friendly and not easy to use because there are several manoeuvres to accomplish before inhalation, such as taking the capsule from the package, loading it and piercing it within the device. Furthermore, there have been recent reports of patients ingesting the capsule instead of placing it in the device and inhaling the contents (60). However some studies show that adherence to correct inhaler use depends on the importance given to appropriate training prior to product use and device education by health-care providers (61).

1.5.5 Multi-dose devices

Multi-dose DPIs have been developed, either as multi-unit dose or as multi-dose reservoir devices. Inhalator M® (Boehringer Ingelheim) has a rotating drum magazine for the storage of six capsules. Capsule pierced at both ends stays stagnant while the high pressure drop across the capsule causes the emptying of the contents and shear stress and collision occurring during the process causes deaggregation (58).

The Diskhaler® (GlaxoSmithKline) makes use of individual doses packed on disc type blister packs (Figure 1.7). Piercing and subsequent flow of air through packaging depression containing the dose leads to dispersion of powder. The aerosol stream is mixed with a bypass flow entering

through two holes in the mouthpiece that, together with a grid, gives rise to turbulence that promotes deagglomeration.

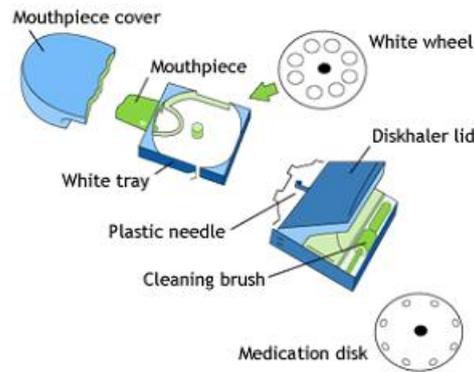


Figure 1.7: Schematic presentation of the Diskhaler (62)

The Diskus® (GlaxoSmithKline) is quite similar except that it contains a foil strip with 60 single dose blisters (Figure 1.8). FPF have been reported to be approximately 23-30% for these two low resistance devices (58).

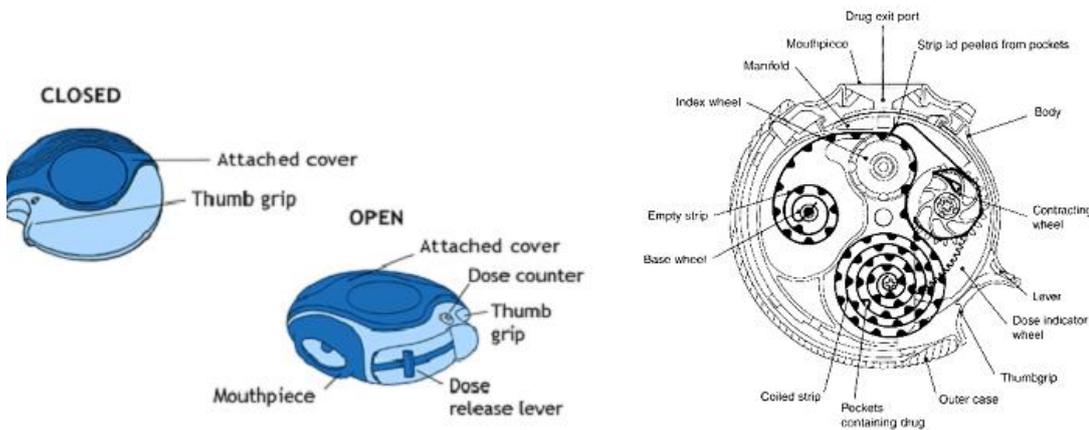


Figure 1.8: Schematic presentation of the Diskus (62)

Turbuhaler® (AstraZeneca) is another sophisticated multi-dose reservoir system that contains 200 doses of small pellets of micronized drug. The drug deaggregation takes place during metering and inhalation. One dose can be dispensed into the dosing chamber by a simple back-and-forth twisting action on the base of the reservoir (Figure 1.9).

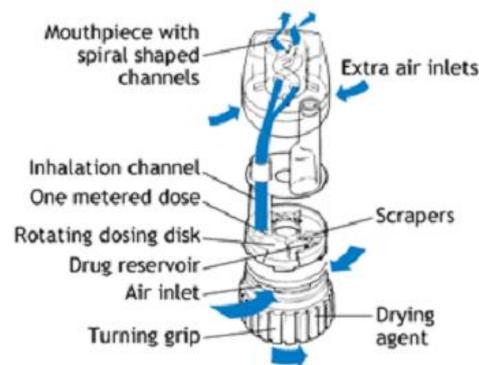


Figure 1.9: Schematic presentation of Turbuhaler (62)

The advantages of the reservoir systems are their relative ease and low cost of manufacture and the ease of including a large number of doses within the device (63). Nevertheless, reproducible dose metering remains the most difficult challenge in device design. Indeed, the variability of dose emissions from DPIs, and in particular from the reservoir system, at the recommended flow rate has been found to be relatively high, with a total relative standard deviation of more than 15% about the average emitted dose for the Pulmicort Turbuhaler (64). Furthermore, powders contained in reservoirs may be more susceptible to deterioration through entrance of moisture, and the use of a desiccant is recommended. For these reasons, pre-metered doses from multi-unit dose systems are more consistent than doses delivered from the reservoir devices, as they are individually sealed and protected from the environment until the point of use by the patient. It is also vital to ensure that no accidental or additional dose is inhaled. This has led to the incorporation of dose counters on new reservoir devices.

1.6 Importance of the inspiratory airflow

DPIs require little or no coordination between the actuation and inhalation due to their activation through inhalation by patient, they often result in better lung delivery than that achieved with comparable MDIs. Since DPIs are typically formulated as one-phase, solid-particle blends, they are also preferred from the standpoints of stability and processing (65). Moreover, DPI are a propellant-free and thus environmentally friendly. The effectiveness of DPIs is depend on the age, gender, disease, and the breathing cycle of the device user. One of the most important disadvantages of DPIs includes requirement of moderate inspiratory effort to draw the formulation from the device, and some patients are not capable of such effort. Low-resistance passive DPIs are less dependent on flow rate than high-resistance devices. Devices with higher

resistance need a higher inspiratory force from patients to achieve the desired air flow. This could be difficult for patients with severe asthma and for children and infants. Thus, a balance between resistance and turbulence is essential to achieve the desired therapeutic effect from DPI formulations. Additionally, it has been demonstrated that drug deposition deep in the lung from DPI formulations is determined not only by the peak flow rate but also by the flow increase rate. It was reported that a high peak flow rate did not necessarily guarantee a high aerosol deposition if the initial flow increase rate was insufficiently high (66). Most of DPIs are breath-activated, depend on inhalation for aerosol generation, several power-assisted devices i.e. pneumatic, impact force, and vibratory (67) have been developed or are currently under development. These “active” inhalers are not subject to the same limitations as passive inhalers and have a different advantage/disadvantage profile. It has been advised that if shear and turbulence could be standardized by using a dispersion mechanism that is independent of the patient’s breath, high delivery efficiency and reproducibility might be achieved.

1.7 Future research and new developments

Since the manufacture of the first DPI Spinhaler®, device technology has continued to grow and a lot of devices are now currently available on the market. However, no devices have shown notable efficiency in delivering drugs from the formulation. Additionally, the concept of powder interaction with the device on powder dispersion has generally been poorly understood. Recently, computational fluid dynamics has enhanced understanding of the impact of inhaler design on powder dispersion and deposition, and has demonstrated that small variations in device design can produce significant variations in performance (68). Nowadays, ways to improve the efficiency of drug delivery from DPIs are developed by changing formulation technology, drug and carrier particle engineering and designing new devices. Indeed, the design of a device needs to be coordinated with drug formulations (i.e., powder in capsules, disks, bulk powders or agglomerates), so that the drugs are aerosolized during inhalation and deliver a dose to the lungs that achieves maximum therapeutic benefits. It is often the case that the drug formulation and inhaler device need to be optimized together to ensure reliable and effective drug delivery. Therefore, the inhaler-drug combination is generally considered as a single medication whose in vitro performance and in vivo efficacy must be demonstrated. So, in the design of a new DPI, consideration must be given to optimizing the formulation of the powder containing the drug substance to ensure a chemically stable and consistent dose, and to optimizing the design of the

metering system within the inhaler itself to produce a convenient device that is comfortable and easy to use for the patient.

1.7.1 Formulation of dry powder inhalers

In order to obtain an effective delivery of drugs to the lungs, various ways of formulations were developed. Often, the drug is mixed with carrier particles or encapsulated in liposomes, nanoparticles and microparticles. Large porous particles were also created for DPI administration. The particle size distribution affects the deposition of drug in the respiratory tract. However, the smaller the particles, the stronger the cohesive forces, resulting in increased adhesion between the particles and subsequently poor flow properties (69). So, one way to improve the flow properties of a drug is through the addition of excipients. Excipients are used to enhance the physical or chemical stability of the active pharmaceutical ingredient, its mechanical properties, and/or its pharmaceutical properties, such as dissolution and permeation. In DPI formulations, excipients function first and foremost as carrier particles. Usually, no more than a few milligrams of drug need to be delivered (e.g. corticosteroids for asthma therapy), and excipients provide bulk, which improves handling, dispensing, and metering of the drug.

1.7.1.1 Nature of excipients

The lungs have a large surface area and thin membranes. Unlike the gastrointestinal tract, the lungs have limited buffering capacity. Many compounds that could enhance drug delivery outcomes also have the potential to irritate or injure the lungs (70). Thus, the choice of potential excipients is limited to compounds that are endogenous to the lung and can easily be metabolized or cleared. Currently, lactose is the most commonly used excipient in marketed DPIs. The reasons for this are as much historical as they are physicochemical/pharmaceutical in nature: lactose has long been used as an excipient in oral dosage forms before being deployed in DPIs. It has an established safety and stability profile, different manufacturing processes with tight controls over purity and physical properties, and is easily available at different grades and is inexpensive. Moreover, lactose is highly crystalline, less hygroscopic than other sugars and has the smooth surfaces and satisfactory flow properties desirable for a DPI carrier particle (71).

Other sugars, such as glucose, mannitol and trehalose (55, 71-73) have been shown to be feasible alternatives to lactose, and it is expected that these sugars will eventually find their way into approved products. An additional benefit that may be gained from the use of a sugar carrier

is the taste/sensation on inhaling, which can assure the patient that a dose has been taken (63). Phospholipids and cholesterol have also been used in experimental liposomal formulations (74) or as solid lipidic carriers or fillers (75). The availability of the active drug depends afterwards on the redispersion of the particles in the inspired air, as a function of the cohesive forces between drug particles and the adhesive forces between drug and carrier particles. The larger carrier particles deposit on the oropharynx, while the fine drug particles partly reach the deep lung. These adhesive forces must be carefully considered, as inadequate separation of drug and carrier is the main reason for deposition problems. So, as excipients can make up over 99% of the product by weight, their choice is a crucial determinant of overall DPI performance. Despite the apparent lack of choices, the excipient must be carefully selected, as its physicochemical properties, such as size and morphology, profoundly affect the performance of the formulation (76). In general, the morphology and roughness of carrier particles are not uniform, containing regions that exhibit different roughness parameters. Clearly, these variations in physicochemical properties in the surface of a carrier material may lead to differences in apparent adhesion properties of drug particles. Furthermore, during the dynamic process of mixing, the adherence of drug particles to the more adhesive areas of the carrier surface is likely to occur (77). Indeed, it has been proposed that the surfaces of larger particles consisted of distinct regions containing so-called “active sites”. It was further suggested that when the amount of fine carrier particles in the mixture is below the saturation limit of the large carrier particles’ adhesive potential, the fine particles will preferentially bind to these active sites. When these active sites have been completely occupied by fine particles, a binary carrier system will then exist, i.e., carrier with strongly bound fine particles, and carrier with weakly bound fine particles or free fine particles (78).

This presence of active sites has obvious implications for DPI drug delivery since retention of drug particles on these relatively high-energy sites during processing and aerosolization would result in a decrease in apparent respirable drug fraction, as suggested by Staniforth (78). Furthermore, it is suggested that the active sites present on the surface of the carrier will have a specific energy distribution with a critical, average adhesion point below which particles - drug or lactose could be removed. Current methods for overcoming such issues include “filling” the potential active sites by increasing the fine particle content present on the carrier surface or pacifying the effects of active sites by the addition of so-called “force control agents” such as

magnesium stearate (79, 80). This additional or ternary component can be added to occupy and presaturate higher-energy binding sites on the carrier before the drug is added, consequently increasing the release and the respirable fraction of the active drug.

1.7.1.2 Blending

After drug and excipient(s) have individually been brought to their desired forms, they are combined in the blending process. The flow properties of the components of the powder blend will play an important role in the efficiency of blending and, ultimately, in aerosol dispersion. Blended formulations consist of small drug particles that are mixed with large excipient carrier particles (50-200 μm). It is a critical step in the manufacture of a DPI product and is in fact subject to substantial optimization work during development. When mixing powders with different properties, particle sizes, and ratios, as is the case with DPI formulations, inadequate mixing can cause poor dose uniformity. In many cases, inadequate mixing cannot be overcome simply by increasing the mixing time. Mixer selection, rotation speed, capacity, and fill level are all subject to optimization, as they can all affect the blend homogeneity (81). Different powders may have different mixing requirements, depending on the interaction forces present between the various particles (78). For low concentration (drug-carrier ratio) blends, geometric dilutions are necessary, using multiple pre-blending steps. Various blending options are available: low-energy tumbling blending, tumbling blending with sieving to break up agglomerates of micronized drug and aid distribution with the powder mass, and high energy blending, with paddles, impeller blades and redistributing powder within the blending vessel (82). An interactive mixture of the two components is prepared by blending until the mixture can remain intact during the filling process (to produce an accurate metered dose) and then freely separate into its primary components during inhalation. There, the drug particles separate from the carrier particles and are carried deep into the lungs, while the larger carrier particles impact in the oropharynx and are cleared. Inadequate drug/carrier separation is one of the main explanations for the low deposition efficiency encountered with DPIs (83).

Though there are tremendous advantages of the pulmonary drug delivery, pulmonary administration of these therapeutics is limited by the defense mechanisms of the lung. The mucociliary escalator, coughing, and alveolar clearance are the 3 major physical ways of

removing deposited particles. In the conducting airways deposited particles are rapidly cleared by the mucociliary clearance into the pharynx. In the terminal airways (alveoli), absorptive or non-absorptive processes remove deposited particles. The absorptive process may involve either direct penetration into the epithelial cells or uptake and clearance by the alveolar macrophages. The non-absorptive process involves transport of particles to the ciliated region (conducting airways) followed by clearance by the mucociliary escalator (84). To overcome these challenges encountered during pulmonary delivery, particulate drug delivery system is another option for pulmonary administration.

1.8 Nanocarriers for pulmonary delivery

These systems can be broadly classified into immediate release [e. g. lactose-drug mixtures for dry powder inhaler (DPI) application] and controlled release systems (such as liposomes, micelles, nano- and microparticles based on polymers). Particulate nanocarriers such as liposomes, polymerosomes, micelles, microparticles and nanoparticles can be used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity, increase bioavailability, better drug targeting and delivery. Drug distribution is then controlled primarily by properties of the carrier and no longer by physicochemical characteristics of the drug substance only. Nanocarriers could provide the advantage of sustained release in the lung tissue and thus the systemic circulation, resulting in a reduction in dosage frequency and improved patient compliance (85). These nanocarriers are removed due to the binding of plasma complement, immunoglobulins, and other proteins (opsonization), leading to uptake by macrophages (absorptive process of pulmonary clearance). This clearance can be markedly delayed by the grafting of synthetic hydrophilic polymers such as poly (ethylene glycol) (PEG) onto the surface of the vehicles. PEG forms a hydrated shell hindering protein interaction with drug vehicles or drugs themselves (e.g., PEG-coated insulin), thereby greatly reducing opsonization and uptake by macrophages. Such “stealth” DDS have prolonged pharmacokinetics and lesser side effects of activation of host defense (immune response, cytokine release, complement activation) (86).

1.8.1 Nanoparticle for pulmonary drug formulations

Drug nanoparticle formulations are usually created in one of two ways. Particles may be precipitated out of solution (bottom-up), or they are milled from larger particles (top-down) (18).

In both mechanisms, the total surface area increases, which increases the free energy of the particles. The system compensates for this increase in free energy by dissolving crystalline nuclei and precipitating onto other particles in a process known as Ostwald Ripening (19), or by agglomerating smaller particles. Generating stable nanoparticle colloids typically necessitates the use of surfactants, which decrease the surface tension at the particle surface and thereby help to reduce the increase in free energy (18). This decreases the degree of Ostwald Ripening and agglomeration within the system. Many chemical processing technologies have been used to produce nanostructured materials suitable for pulmonary delivery. Some processes that are currently under investigation involve wet milling (18), supercritical fluid extraction (21), spray drying (22), electrospray (86), high-pressure homogenization and recrystallization via solvent displacement (87). Wet milling is a process that utilizes either ceramic or metallic milling media to grind a suspension of insoluble drug and surfactant. Wet milling has also been used to formulate budesonide for nebulized delivery to the lungs (18). Spray drying is a process that forces fluid through a nozzle, producing a mist that is dried to produce a fine powder. The technique employs a variety of different types of nozzles, some of which use ultrasound or air-jet shear to nebulize drug suspensions. Supercritical fluid extraction is a technique that is currently being developed for use in nanoparticle drug formulations. It uses supercritical fluid to extract a solvent from a drug emulsion or solution, leaving behind a suspension of drug particles (21). These processes are advantageous because they generally offer better scalability, and are therefore industrially relevant. Unfortunately, some processes (such as spray drying) often utilize co-solvents to improve drying and/or large amounts of excipient to stabilize the drug and to maintain powder properties. In addition to chemical processing technologies, multiple recent studies have examined different polymeric nanoparticle fabrication methods as applied to pulmonary drug formulations (88, 89). These techniques generally involve polyelectrolyte complex formation, double emulsion/solvent evaporation techniques, or emulsion polymerization techniques. Polyelectrolyte complexes use oppositely charged polymers to entrap drugs into a polymeric matrix nanoparticle, which then releases the drug either through polymer degradation or drug diffusion. Double emulsion/solvent evaporation techniques involve dissolving the drug and polymer in an organic solvent, which is then emulsified in an aqueous solution. The organic solvent diffuses out of the polymer phase and into the aqueous phase, and is then evaporated, leaving behind drug-loaded polymeric nanoparticles. Emulsion polymerization is similar to

emulsion/solvent evaporation except that monomer is emulsified into droplets and then polymerization is initiated. Liposomal formulations are typically produced by extruding or homogenizing a suspension of dissolved, hydrated lipids. (90). This suspension can then be delivered via nebulization, freeze-dried, or incorporated into larger particles.

1.8.2 Liposomal Nanocarrier Formulations

Liposomal nanoparticles have been investigated as potential drug carriers to the lung. Lot of lipids used for liposome preparation are already present in lungs making liposomal carriers better tolerated systems (90). Moreover, diverse groups of amphiphilic molecules available for constructing liposomes provides required variability in physicochemical parameters like particles size etc. and this in turn makes them more attractive carriers for drug delivery (91). A possible concern is that lipids themselves modify the surface tension of the lungs and have themselves been used as therapeutics (34). In general, liposomes are interesting potential drug delivery vehicles because of their stability in suspension. For instance, upon dilution or changes in ionic strength that may be encountered during administration, liposomes will theoretically remain intact. This is because liposomal formulations are not thermodynamically equilibrated systems, but are kinetically trapped systems that are unable to respond to thermodynamic perturbations (91). This property might make them suitable for formulations that require long-term storage as liquid suspensions, such as those used in nebulization. Liposomes have also been investigated as potential targeting agents for alveolar macro-phages, partially because macrophages are associated with lung surfactant metabolism (92, 93). Studies have also shown that mannosylation of liposomes and particle size influence the uptake of liposomes by macrophages (92, 93). The studies demonstrate that liposomal uptake increases as particle size increases over a range of 100 - 2,000 nm and as the degree of mannosylation increases on the surface of the particles (92, 93). This is likely due to the fact that mannose receptors are exclusively expressed on the surface of alveolar macrophages (93).

Bhavane et al. developed a novel type of drug delivery vehicle composed of liposomal nanocarriers covalently linked by enzymatically labile spacers (90). The liposomes were between 80 and 195 nm in size and loaded with ciprofloxacin with 90% efficiency. Agglomeration was induced using dimethyl 3,3-dithiobispropionimidate 2HCl (DTBP), which is a homobifunctional imidoester capable of reacting with primary amines and also contains thiol-cleavable disulfide

bonds, making it enzymatically labile (90). Agglomerated liposomes differ from unagglomerated liposomes in their release characteristics, the former being more slow releasing than latter, as well as agglomerated particles may give burst release. Upon nebulization, the agglomerated particles were reported to retain the encapsulated drug and nebulized droplets had aerodynamic diameters between 1 and 5 μm , putting them within the respirable range (90). Liposomal formulations have also been investigated in potential dry powder formulations. Chougule et al. loaded nano-sized liposomes with tacrolimus, an immunosuppressant often used to prevent rejection of allotopic lung transplants. The liposomal suspension with lactose, sucrose, or trehalose and L-leucine was spray dried to produce particles with an aerodynamic diameter of approximately 2.2 μm that demonstrated good aerosolization properties when administered from a DPI (94)

1.8.3 Biodegradable microspheres

Biodegradable microspheres produced from natural and synthetic polymers (e.g. poly (lactic-co-glycolic) acid polymer (PLGA), Poly Lactic acid (PLA)) have been extensively investigated as drug carriers for administration via a number of different routes in order to ensure targeting and sustained drug release. PLGA microspheres have been used for controlled release of a wide range of drugs including peptides and proteins, and their potential use in pulmonary delivery has been explored, principally for anti-asthmatic drugs (95). A number of microspheres have proved to be non-toxic, biodegradable and non-immunogenic following systemic injection. Duddu et al produced micron-size hollow-porous microspheres (PulmoSpheres®) using a two-step process (96). First, a fluorocarbon-in-water emulsion is prepared by high pressure homogenization, using a saturated phosphatidylcholine as the surfactant. The emulsion is then combined with a second aqueous solution containing the active agent and other wall-forming material (e.g., co-surfactants, sugars and salts). The second step involves spray drying the aqueous dispersion. Mean geometric and aerodynamic diameters of spray dried particles were about 5 and 7 μm , respectively, and the bulk density is about 0.4 g/cm^3 . Morphologically, the particle walls have a sponge-like appearance with pores (96). Particle size, morphology and density can be controlled through the selection of the blowing agent type and its concentration. High respirable fractions are obtained in vitro as a result of a significant decrease in interparticle attractive forces and improved aerodynamic properties due to the hollow porous design. One clinical evaluation on

healthy volunteers of dry powder tobramycin, using lipid-based Pulmosphere technology, has already been made, giving a mean whole lung deposition of $34 \pm 6 \%$ (37, 96) .

1.8.4 Large porous particles

In general, therapeutic dry powder aerosols are made with drug particle mass densities of approximately $1 \pm 0.5 \text{ g/cm}^3$ and mean geometric diameters of less than $5 \mu\text{m}$ to avoid deposition in the oropharyngeal cavity and in the DPI devices. Now, a new type of inhalation aerosol characterized by particles of mass density significantly lower than 1 ($0.1\text{-}0.5 \text{ g/cm}^3$) has been recently proposed, so that an aerodynamic diameter in the respirable size range can be achieved with a geometric particle size greater than $10 \mu\text{m}$ (97). These large porous particles represent an important step in pulmonary delivery for future applications (34). The increased aerosolization efficiency lowers the probability of deposition losses before particle entry in the intrapulmonary airways, thereby increasing the systemic bioavailability of an inhaled drug. This is due to a lower tendency for powder aggregation as a result of a reduced fractional surface area of particle-particle contact in the dry powder. Moreover, these large porous particles can escape the natural clearance mechanisms of the lungs, such as the mucociliary escalator and phagocytosis by alveolar macrophages, since very large particles may escape that kind of clearance, giving enough time for the drug to be effectively delivered (98). Thin-walled large porous particles have also been prepared by macroscale aggregation of nanoparticles via a spray-drying process with an additional control over physical characteristics by adding other components to the spray-dried solutions such as sugars, lipids, polymers and proteins. Nanoparticulate drug delivery systems are converted to large porous microsystems to combine the release and delivery potential of former with flow, processing and aerosolization potentials of latter (99).

1.9 Toxicity considerations of nanocarrier formulations

A major concern with nanoparticle therapeutics is the unforeseen negative health impact that nanoparticles may have. Studies in rodents have shown that intratracheally administered carbon nanoparticles accelerate vascular thrombosis (100). Additionally, it has also been demonstrated that inhaled iridium particles may migrate from the lung to the systemic circulation, which may have detrimental vascular effects (101). Inhaled carbon nanoparticles show accumulation in brain, however their toxicity needs to be determined (102). Conversely, a recent study has shown that metallic nanoparticles are no less cytotoxic than metallic microparticles, suggesting that the

small size of nanoparticles may not be the most important factor affecting their toxicity (103). This notwithstanding, the toxicity of nanoparticles is a legitimate concern and should be thoroughly investigated. In addition to the possible inherent toxic effects of nanoparticles, some materials used to formulate nanoparticles may have toxic effects and therefore may not be viable for developing therapeutic products. For example, the toxicity of polycyanoacrylates has been demonstrated by Brzoska et al (104). They prepared poly (butyl) cyanoacrylate and poly (hexyl)cyanoacrylate nanoparticles. They determined that both types of nanoparticles caused an increase in lactate dehydrogenase (LDH) activity in human pulmonary epithelial cells. The degree of toxicity was greater for the poly (butyl) cyanoacrylate and independent of the stabilizer used, which stands to reason because shorter chain polycyanoacrylates have been associated with higher cytotoxicity. The degree of toxicity also increased with increasing nanoparticle concentration, most likely due to the subsequent increase in polycyanoacrylate concentration. Polyethyleneimine (PEI) has also demonstrated cytotoxicity in lung cells (105). Bivas-Benita et al. also reported increasing cytotoxicity of PEI-DNA complexes with increasing ratio of PEI to DNA (106). Despite this, Dailey et al. have shown that PLGA nanoparticles induce less inflammation than polystyrene particles of similar size when delivered to the lungs (88). Based on this observation, nanoparticle toxicity in the lungs may be more dependent on material choice than particle size. Therefore, it is essential to investigate the toxicity of these particles and alternatives or methods can be investigated to mitigate pulmonary toxicity of these system.

Nanocarrier drug formulations offer many advantages over traditional aerosol powders and liquid pulmonary dose formulations. The bioavailability of poorly water-soluble drugs can be greatly enhanced by the large surface area of drug nanocarrier formulations. Additionally, nanoparticles can be formulated in such a way to offer enhanced control over the morphology of dry powder drug formulations and the ability to produce structures with both a low-density microstructure for delivery to the deep lung and nanostructure for enhanced dissolution and bioavailability. The literature suggests many different formulation approaches for drugs that use a variety of excipients to fabricate nanocarrier or nanocarrier complexes suitable for pulmonary delivery. Many chemical processing techniques such as lyophilization, supercritical fluid extraction and spray drying have been successfully used for therapeutic nanoparticle processing. Additionally, a range of formulation techniques are available, for example, emulsion polymerization, double emulsion/solvent evaporation, polyelectrolyte complexation,

nanoprecipitation and liposomal loading that suits different requirements. These techniques offer the ability to produce nanocarriers with a high degree of control over particle size, however, residual solvents, presence of cytotoxic components, drug loading problems as well as scale up issues need to be addressed to have commercial applicability of such formulations. With the perfection of pulmonary nanocarrier drug formulations, the lungs may become a preferred route of drug delivery for many more local and systemic therapeutic interventions.

1.10 References

1. Groneberg DA, Eynott PR, Doring F, Dinh QT, Oates T, Barnes PJ, et al. Distribution and function of the peptide transporter PEPT2 in normal and cystic fibrosis human lung. *Thorax*. 2002;57(1):55-60.
2. Groneberg DA, Nickolaus M, Springer J, Doring F, Daniel H, Fischer A. Localization of the peptide transporter PEPT2 in the lung: implications for pulmonary oligopeptide uptake. *The American journal of pathology*. 2001;158(2):707-14.
3. Hiller FC. In: Hickey AJ, editor. *Pharmaceutical Inhalation Aerosol Technology*. New York: Marcel Dekker Inc.; 1992. p. 385.
4. Olschewski H, Simonneau G, Galie N, Higenbottam T, Naeije R, Rubin LJ, et al. Inhaled iloprost for severe pulmonary hypertension. *The New England journal of medicine*. 2002;347(5):322-9.
5. Courrier HM, Butz N, Vandamme TF. Pulmonary drug delivery systems: recent developments and prospects. *Critical reviews in therapeutic drug carrier systems*. 2002;19(4-5):425-98.
6. Groneberg DA, Witt C, Wagner U, Chung KF, Fischer A. Fundamentals of pulmonary drug delivery. *Respiratory medicine*. 2003;97(4):382-7.
7. Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. *Nature reviews Drug discovery*. 2007;6(1):67-74.
8. Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. *Particle and fibre toxicology*. 2010;7:2.
9. Gessler T, Seeger W, Schmehl T. Inhaled prostanoids in the therapy of pulmonary hypertension. *Journal of aerosol medicine and pulmonary drug delivery*. 2008;21(1):1-12.
10. Kurmi BD, Kayat J, Gajbhiye V, Tekade RK, Jain NK. Micro- and nanocarrier-mediated lung targeting. *Expert opinion on drug delivery*. 2010;7(7):781-94.

11. Langer R, Vacanti JP. Tissue engineering. *Science* (New York, NY). 1993;260(5110):920-6.
12. Chow AH, Tong HH, Chattopadhyay P, Shekunov BY. Particle engineering for pulmonary drug delivery. *Pharmaceutical research*. 2007;24(3):411-37.
13. Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. *Trends in biotechnology*. 2007;25(12):563-70.
14. Azarmi S, Roa WH, Lobenberg R. Targeted delivery of nanoparticles for the treatment of lung diseases. *Advanced drug delivery reviews*. 2008;60(8):863-75.
15. Rytting E, Nguyen J, Wang X, Kissel T. Biodegradable polymeric nanocarriers for pulmonary drug delivery. *Expert opinion on drug delivery*. 2008;5(6):629-39.
16. JB. West PW. Ventilation-perfusion relationships.in *The Lung: Scientific Foundations*. In: R.G. Crystal JBW, P.J. Barnes and E.R. Weibel, Raven Press, editor. 1991. p. 1289.
17. Yu J, Chien YW. Pulmonary drug delivery: physiologic and mechanistic aspects. *Critical reviews in therapeutic drug carrier systems*. 1997;14(4):395-453.
18. Thompson AJHaDC. In: A.J. Hickey MD, editor. *Pharmaceutical Inhalation Aerosol Technology*. 2nd Edition ed. New York, NY, USA2004. p. 1.
19. Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. *American journal of respiratory cell and molecular biology*. 1992;6(2):235-43.
20. Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number and cell characteristics of the normal human lung. *The American review of respiratory disease*. 1982;126(2):332-7.
21. C.A. D'Angelis JCaRMR. In: Zimmerman BPFaJJ, editor. *Pediatric Critical Care*. 4th Edition ed. Philadelphia, PA, USA2011.
22. Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. *Advanced drug delivery reviews*. 2009;61(2):86-100.
23. Thompson RJAaDC. *Inhalation Aerosols: Physical and Biological Basis for Therapy*. New York, NY, USA1996. p. 83.
24. van der Schans CP. Conventional chest physical therapy for obstructive lung disease. *Respiratory care*. 2007;52(9):1198-206; discussion 206-9.

25. Y. MTBaY. Deposition mechanics of pharmaceutical particles in human airways:. In: AJ H, editor. Inhalation aerosols, Physical and biological basis for therapy. 94. New york, NY, pp 3-271996.
26. Lippmann M, Schlesinger RB. Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. *Journal of toxicology and environmental health.* 1984;13(2-3):441-69.
27. Martonen TB, Katz IM. Deposition patterns of aerosolized drugs within human lungs: effects of ventilatory parameters. *Pharmaceutical research.* 1993;10(6):871-8.
28. Lam JK, Liang W, Chan HK. Pulmonary delivery of therapeutic siRNA. *Advanced drug delivery reviews.* 2012;64(1):1-15.
29. Daigle CC, Chalupa DC, Gibb FR, Morrow PE, Oberdorster G, Utell MJ, et al. Ultrafine particle deposition in humans during rest and exercise. *Inhalation toxicology.* 2003;15(6):539-52.
30. Yeates DB, Aspin N, Levison H, Jones MT, Bryan AC. Mucociliary tracheal transport rates in man. *Journal of applied physiology.* 1975;39(3):487-95.
31. P.B. McCray J, G. Wang, B. Davidson, M. Bodner, S.M. Herrmann, D.J. Jolly, inventor; The University of Iowa Research Foundation Chiron Corp, assignee. Methods and compositions for increasing the infectivity of gene transfer vectors. U patent US 6855549. 2005.
32. Goerke J. Pulmonary surfactant: functions and molecular composition. *Biochimica et biophysica acta.* 1998;1408(2-3):79-89.
33. Sutinen S, Riska H, Backman R, Sutinen SH, Froseth B. Alveolar lavage fluid (ALF) of normal volunteer subjects: cytologic, immunocytochemical, and biochemical reference values. *Respiratory medicine.* 1995;89(2):85-92.
34. Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, et al. Large porous particles for pulmonary drug delivery. *Science (New York, NY).* 1997;276(5320):1868-71.
35. Dolovich MB, Ahrens RC, Hess DR, Anderson P, Dhand R, Rau JL, et al. Device selection and outcomes of aerosol therapy: Evidence-based guidelines: American College of Chest Physicians/American College of Asthma, Allergy, and Immunology. *Chest.* 2005;127(1):335-71.

36. Le Brun PP, de Boer AH, Heijerman HG, Frijlink HW. A review of the technical aspects of drug nebulization. *Pharmacy world & science : PWS*. 2000;22(3):75-81.
37. Newhouse MT, Nantel NP, Chambers CB, Pratt B, Parry-Billings M. Clickhaler (a novel dry powder inhaler) provides similar bronchodilation to pressurized metered-dose inhaler, even at low flow rates. *Chest*. 1999;115(4):952-6.
38. Dalby R.N. TSL, Hickey A.J. Medical devices for the delivery of therapeutic aerosols to the lungs. In: AJ H, editor. *Inhalation aerosols, Physical and biological basis for therapy*. vol. 94. New york, NY1996. p. 441-73.
39. Cipolla D, Gonda I, Shire SJ. Characterization of aerosols of human recombinant deoxyribonuclease I (rhDNase) generated by jet nebulizers. *Pharmaceutical research*. 1994;11(4):491-8.
40. Crompton G. A brief history of inhaled asthma therapy over the last fifty years. *Primary care respiratory journal*. 2006;15(6):326-31.
41. Newman SP. Principles of metered-dose inhaler design. *Respiratory care*. 2005;50(9):1177-90.
42. Polli GP, Grim WM, Bacher FA, Yunker MH. Influence of formulation on aerosol particle size. *Journal of pharmaceutical sciences*. 1969;58(4):484-6.
43. Molina MJ, Rowland FS. Stratospheric sink for chlorofluoromethanes: chlorine atom-catalysed destruction of ozone. *Nature*. 1974;249(5460):810-12.
44. Pethick C, Smith H. *Bose-Einstein condensation in dilute gases*: Cambridge university press; 2002.
45. Crompton GK. Problems patients have using pressurized aerosol inhalers. *European journal of respiratory diseases Supplement*. 1982;119:101-4.
46. Ganderton D. General factors influencing drug delivery to the lung. *Respiratory medicine*. 1997;91 Suppl A:13-6.
47. Geller DE, Pitlick WH, Nardella PA, Tracewell WG, Ramsey BW. Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest*. 2002;122(1):219-26.
48. Giraud V, Roche N. Misuse of corticosteroid metered-dose inhaler is associated with decreased asthma stability. *The European respiratory journal*. 2002;19(2):246-51.

49. Khassawneh BY, Al-Ali MK, Alzoubi KH, Batarseh MZ, Al-Safi SA, Sharara AM, et al. Handling of inhaler devices in actual pulmonary practice: metered-dose inhaler versus dry powder inhalers. *Respiratory care*. 2008;53(3):324-8.
50. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *The European respiratory journal*. 2008;31(1):143-78.
51. Rubin BK, Fink JB. Optimizing aerosol delivery by pressurized metered-dose inhalers. *Respiratory care*. 2005;50(9):1191-200.
52. Delaby I, Ernst B, Froelich D, Muller R. Droplet deformation in immiscible polymer blends during transient uniaxial elongational flow. *Polymer Engineering & Science*. 1996;36(12):1627-35.
53. Newman SP, Busse WW. Evolution of dry powder inhaler design, formulation, and performance. *Respiratory medicine*. 2002;96(5):293-304.
54. Atkins PJ. Dry powder inhalers: an overview. *Respiratory care*. 2005;50(10):1304-12; discussion 12.
55. Steckel H, Thies J, Müller BW. Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide. *International journal of pharmaceuticals*. 1997;152(1):99-110.
56. Islam N, Gladki E. Dry powder inhalers (DPIs)--a review of device reliability and innovation. *International journal of pharmaceuticals*. 2008;360(1-2):1-11.
57. Bell JH, Hartley PS, Cox JS. Dry powder aerosols. I. A new powder inhalation device. *Journal of pharmaceutical sciences*. 1971;60(10):1559-64.
58. Dunbar CA, Hickey AJ, Holzner P. Dispersion and Characterization of Pharmaceutical DryPowder Aerosols. *Kona Powder and Particle Journal*. 1998;16:7-45.
59. Concessio NM, Hickey AJ. Descriptors of irregular particle morphology and powder properties. *Advanced drug delivery reviews*. 1997;26(1):29-40.
60. Tezky T, Holquist C. Misadministration of capsules for inhalation. *Drug Topics*. 2005;4:48-9.
61. Melani AS, Zanchetta D, Barbato N, Sestini P, Cinti C, Canessa PA, et al. Inhalation technique and variables associated with misuse of conventional metered-dose inhalers and newer dry powder inhalers in experienced adults. *Annals of allergy, asthma & immunology*. 2004;93(5):439-46.

62. How to Use Your Inhaler 2008. Available from: <http://www.asthma.ca/adults/treatment/howToUse.php>.
63. Sumby B, Slater A, Atkins PJ, Prime D. Review of dry powder inhalers. *Advanced drug delivery reviews*. 1997;26(1):51-8.
64. Hindle M, Byron PR. Dose emissions from marketed dry powder inhalers. *International journal of pharmaceuticals*. 1995;116(2):169-77.
65. Ashurst II, Malton A, Prime D, Sumby B. Latest advances in the development of dry powder inhalers. *Pharmaceutical science & technology today*. 2000;3(7):246-56.
66. De Boer A.H. GD, Hagedoorn P. . Inhalation characteristics and their in vitro drug delivery. Part 2: Effect of peak flow rate (PIFR) and inspiration time of the in vitro drug release from three different types of commercial dry powder inhaler. *Int J Pharm*. 1996;138:45-56.
67. Tobyn M, Staniforth JN, Morton D, Harmer Q, Newton ME. Active and intelligent inhaler device development. *International journal of pharmaceuticals*. 2004;277(1-2):31-7.
68. Coates MS, Fletcher DF, Chan HK, Raper JA. Effect of design on the performance of a dry powder inhaler using computational fluid dynamics. Part 1: Grid structure and mouthpiece length. *Journal of pharmaceutical sciences*. 2004;93(11):2863-76.
69. Feeley JC, York P, Sumby BS, Dicks H. Determination of surface properties and flow characteristics of salbutamol sulphate, before and after micronisation. *International journal of pharmaceuticals*. 1998;172(1-2):89-96.
70. Telko MJ, Hickey AJ. Dry powder inhaler formulation. *Respiratory care*. 2005;50(9):1209-27.
71. Smyth HC, Hickey A. Carriers in drug powder delivery. *Am J Drug Deliv*. 2005;3(2):117-32.
72. Tee SK, Marriott C, Zeng XM, Martin GP. The use of different sugars as fine and coarse carriers for aerosolised salbutamol sulphate. *International journal of pharmaceuticals*. 2000;208(1-2):111-23.
73. Steckel H, Bolzen N. Alternative sugars as potential carriers for dry powder inhalations. *International journal of pharmaceuticals*. 2004;270(1-2):297-306.
74. Shah SP, Misra A. Development of liposomal amphotericin B dry powder inhaler formulation. *Drug delivery*. 2004;11(4):247-53.

75. Sebti T, Amighi K. Preparation and in vitro evaluation of lipidic carriers and fillers for inhalation. *European journal of pharmaceutics and biopharmaceutics*. 2006;63(1):51-8.
76. Islam N, Stewart P, Larson I, Hartley P. Effect of carrier size on the dispersion of salmeterol xinafoate from interactive mixtures. *Journal of pharmaceutical sciences*. 2004;93(4):1030-8.
77. Young PM, Edge S, Traini D, Jones MD, Price R, El-Sabawi D, et al. The influence of dose on the performance of dry powder inhalation systems. *International journal of pharmaceutics*. 2005;296(1-2):26-33.
78. Staniforth JN, Rees JE, Lai FK, Hersey JA. Interparticle forces in binary and ternary ordered powder mixes. *The Journal of pharmacy and pharmacology*. 1982;34(3):141-5.
79. Zeng XM, Martin GP, Marriott C, Pritchard J. The influence of carrier morphology on drug delivery by dry powder inhalers. *International journal of pharmaceutics*. 2000;200(1):93-106.
80. Ferrari F, Cocconi D, Bettini R, Giordano F, Santi P, Tobyn M, et al. The surface roughness of lactose particles can be modulated by wet-smoothing using a high-shear mixer. *AAPS PharmSciTech*. 2004;5(4):e60.
81. Alexander A, Shinbrot T, Johnson B, Muzzio FJ. V-blender segregation patterns for free-flowing materials: effects of blender capacity and fill level. *International journal of pharmaceutics*. 2004;269(1):19-28.
82. Malcolmson RJ, Embleton JK. Dry powder formulations for pulmonary delivery. *Pharmaceutical science & technology today*. 1998;1(9):394-8.
83. Zeng XM, Martin GP, Marriott C, Pritchard J. The influence of carrier morphology on drug delivery by dry powder inhalers. *International journal of pharmaceutics*. 2000;200(1):93-106.
84. Roy I, Vij N. Nanodelivery in airway diseases: Challenges and therapeutic applications. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2010;6(2):237-44.
85. Fernandes CA, Vanbever R. Preclinical models for pulmonary drug delivery. *Expert opinion on drug delivery*. 2009;6(11):1231-45.
86. Agu RU, Ugwoke MI. In vitro and in vivo testing methods for respiratory drug delivery. *Expert opinion on drug delivery*. 2011;8(1):57-69.

87. Jacobs C, Muller RH. Production and characterization of a budesonide nanosuspension for pulmonary administration. *Pharmaceutical research*. 2002;19(2):189-94.
88. Dailey LA, Jekel N, Fink L, Gessler T, Schmehl T, Wittmar M, et al. Investigation of the proinflammatory potential of biodegradable nanoparticle drug delivery systems in the lung. *Toxicology and applied pharmacology*. 2006;215(1):100-8.
89. Dailey LA, Kleemann E, Wittmar M, Gessler T, Schmehl T, Roberts C, et al. Surfactant-free, biodegradable nanoparticles for aerosol therapy based on the branched polyesters, DEAPA-PVAL-g-PLGA. *Pharmaceutical research*. 2003;20(12):2011-20.
90. Vincent JH, Johnston AM, Jones AD, Bolton RE, Addison J. Kinetics of deposition and clearance of inhaled mineral dusts during chronic exposure. *British journal of industrial medicine*. 1985;42(10):707-15.
91. Chono S, Tanino T, Seki T, Morimoto K. Influence of particle size on drug delivery to rat alveolar macrophages following pulmonary administration of ciprofloxacin incorporated into liposomes. *Journal of drug targeting*. 2006;14(8):557-66.
92. Edwards DA, Dunbar C. Bioengineering of therapeutic aerosols. *Annual review of biomedical engineering*. 2002;4:93-107.
93. A.V. Annapragada PSaFM. In: A.L. Adjel and P.K. Gupta MD, editor. *Inhalation Delivery of Therapeutic Peptides and Proteins*. New York, NY, USA1997. p. 27.
94. Chougule M, Padhi B, Misra A. Nano-liposomal dry powder inhaler of tacrolimus: preparation, characterization, and pulmonary pharmacokinetics. *International journal of nanomedicine*. 2007;2(4):675-88.
95. Tee SK, Marriott C, Zeng XM, Martin GP. The use of different sugars as fine and coarse carriers for aerosolised salbutamol sulphate. *International journal of pharmaceutics*. 2000;208(1-2):111-23.
96. Duddu SP, Sisk SA, Walter YH, Tarara TE, Trimble KR, Clark AR, et al. Improved lung delivery from a passive dry powder inhaler using an Engineered PulmoSphere powder. *Pharmaceutical research*. 2002;19(5):689-95.
97. Musante CJ, Schroeter JD, Rosati JA, Crowder TM, Hickey AJ, Martonen TB. Factors affecting the deposition of inhaled porous drug particles. *Journal of pharmaceutical sciences*. 2002;91(7):1590-600.

98. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. *Journal of applied physiology* (Bethesda, Md : 1985). 1998;85(2):379-85.
99. Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards DA. Trojan particles: large porous carriers of nanoparticles for drug delivery. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(19):12001-5.
100. Tetley TD. Health effects of nanomaterials. *Biochemical Society transactions*. 2007;35(Pt 3):527-31.
101. Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdorster G, et al. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhalation toxicology*. 2004;16(6-7):453-9.
102. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, et al. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *Journal of toxicology and environmental health Part A*. 2002;65(20):1531-43.
103. Cha KE, Myung H. Cytotoxic effects of nanoparticles assessed in vitro and in vivo. *Journal of microbiology and biotechnology*. 2007;17(9):1573-8.
104. Brzoska M, Langer K, Coester C, Loitsch S, Wagner TO, Mallinckrodt C. Incorporation of biodegradable nanoparticles into human airway epithelium cells-in vitro study of the suitability as a vehicle for drug or gene delivery in pulmonary diseases. *Biochemical and biophysical research communications*. 2004;318(2):562-70.
105. Kleemann E, Neu M, Jekel N, Fink L, Schmehl T, Gessler T, et al. Nano-carriers for DNA delivery to the lung based upon a TAT-derived peptide covalently coupled to PEG-PEI. *Journal of controlled release*. 2005;109(1-3):299-316.
106. Bivas-Benita M, Romeijn S, Junginger HE, Borchard G. PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium. *European journal of pharmaceutics and biopharmaceutics*. 2004;58(1):1-6.