CHAPTER 2

LITERATURE REVIEW

Localized delivery of drugs to the respiratory tract has become an increasingly important and effective therapeutic method for treating a variety of pulmonary disorders, including asthma, bronchitis, and cystic fibrosis. Although the traditional form of inhalation therapy dates back to the earliest records of ancient cultures, the advantages of inhalation therapy have essentially remained the same. Several studies (Neville et al, 1977; Newman et al, 1985 and Clark, 1972) have demonstrated the clinical advantage of inhalation aerosols over systemic therapy for the treatment of lung disorders. Relatively small doses are required for effective therapy, reducing systemic exposure to drug and thus minimizing adverse effects. Lower dosage regimens may provide considerable cost savings, especially with expensive therapeutic agents. Delivering small doses of active ingredients directly to the lung effectively targets the drug, thereby maximizing therapeutic effect while minimizing adverse effects. On the other hand, the large surface area for absorption and the relatively low metabolic activity of the lungs (Clarke et al, 1984 and Lourenco et al, 1982) make this organ system a potential route for the systemic delivery of drugs that cannot be delivered by other means.

Historically, the evolution of inhalation therapy can be traced in India 4000 years ago, where the leaves of the Atropa Belladonna plant, containing atropine as Bronchodilators, were smoked as a cough suppressant (Grossman, 1994). The development of modern inhalation therapy began in the nineteenth century with the invention of the glass bulb nebulizer. The development of the first pressurized metered dose inhaler (pMDI) for asthma therapy in 1956 was a major advance in the use of aerosols for drug delivery to the lungs (Thiel, 1996). However, the required hand-lung coordination of the patient and the use of environmentally damaging CFC propellants, are major drawbacks of the traditional pMDIs. Dry powder inhalers (DPI) were introduced to overcome these drawbacks. From the first introduction of DPI in 1971 (Spinhaler), several DPI's have been added to the collection of available inhalers. DPI's represent a significant advance in pulmonary drug delivery technology for humans. They re breath-controlled and so coordination problems have been over come. DPI's are also potentially suitable for the delivery of a wider range of drugs, such as anti-asthmatics, antibiotics, peptides and proteins. DPI's can also deliver a wide range of doses from 6 μg to more than 20mg via one short inhalation (Malcolmson et al, 1998 and Hickey, 1996). This chapter gives an introduction to inhalation therapy with modern dry powder inhalers, from the anatomy of the airways, mechanism of particle depositions, recent advances in powder formulation, and technological aspects of interest for understanding DPI design.

2.1 HUMAN RESPIRATORY SYSTEM

2.1.1 Anatomy of Airways

The cardinal functions of human lung can be divided into two aspects: *ventilation and gas exchange*. The human airways consists of *upper airways*; the nasopharyngeal region including the nasal cavity down to the epiglottal level in the larynx and the *lower airways*; the tracheobronchial region which includes the ciliated airways from trachea down to the terminal bronchioles, and the alveolar region with non-ciliated airways, which is the site of the gas exchange. The branching pattern of lower airways is a complex three-dimensional system of progressively branching with gradual decreasing airway diameter distally, whereas the total cross-sectional area increases. The branching system of lower airways could be looked upon as an upside-down tree (Figure 2.1)

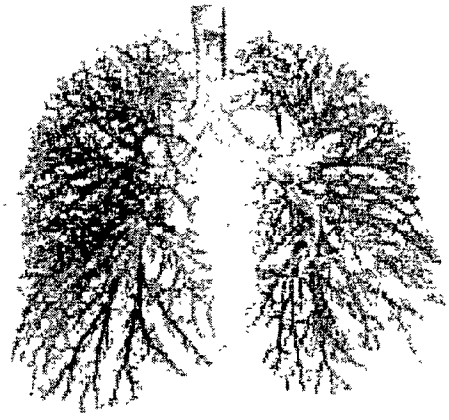


Figure 2.1 Network of airways in the lung

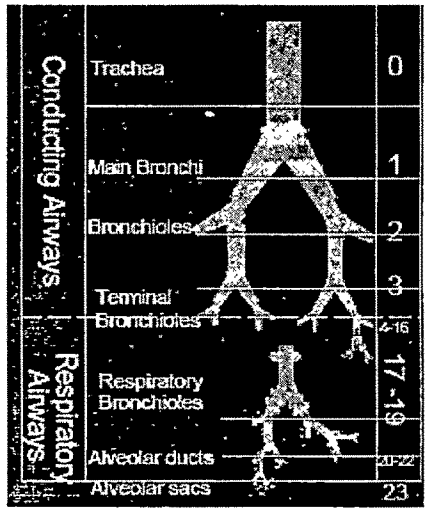


Figure 2.2 Weibel's model of Airways

This branching system provided the maximal surface area for gas exchange with in a small volume; the alveolar surface area is larger then the size of tennis court (100-150 m^2), whereas airway surface area is only about 0.5 m^2 The number of branches between the hilum and periphery varies between 8 in some segments in the upper lobe to 24 in the longest segments in the lower lobes (Horsfield, 1990); it is therefore difficult to describe the airways in a simple model. One of the most used airway model is the model proposed by Weibel (Weibel, 1963). In the Weibel model the airways multiply in a regular dichotomy, where each generation corresponds to one branch of respiratory tree. For each generation the diameter of the airway lumen decreases but the sum of the total cross sectional area increases exponentially. The larger airways consist of the generations 0-8, the smaller airways consist

of the generations 9-15 and the alveolar region consists of the remaining 16-23 generations. The conducting airways from nose to the respiratory bronchioles are lined with ciliated epithelium admixed with numerous mucus-secreting goblet cells and submucosal glands down to the small bronchi. The non-ciliated alveolar epithelium is made of type I cells, pneumocytes, which cover most of the alveolar surfaces (93%) forming the thin gas exchange barrier, and the less frequent type II cells (7%) synthesizing the surfactant.

2.1.2 Pulmonary Ventilation

The inhaled air volume depends on the extent of chest enlargement. During normal breathing, the inhaled and exhaled volumes (tidal volume) are only a part of the total lung volume. Definitions of the different parameters are given in Table 2.1. Determination of lung volumes and capacities can provide important information on the patho-physiological status of the lung. The amount of air moving in and out of the lungs can be measured through spirometry. Estimates of volume of air remaining in the lungs after expiration are made by gas dilution methods. The respiratory system of a normal adult processes 10-20 m^3 of air per day. The gas-exchange are of the lungs is about 120-160 m^2 and is perfused with over 2000 km of capillaries (Hickey, 1996). At rest, about 700 ml of tidal air is inhaled and exhaled with each breath (Hinds, 1982). During heavy work, tidal volume may be three times as much. A resting adult breathes about 12 times per minute and this rate will triple during heavy work.

2.1.3 Pulmonary Clearance

The primary function of the pulmonary defensive response to inhaled particles is to keep the respiratory surfaces of the alveoli clean and available for respiration. The elimination of particles deposited in the lower respiratory tract serves an important defense mechanism to prevent potentially adverse interactions of aerosols with lung cells. Insoluble particulates are cleared by several pathways, which are only partially understood. These pathways are known to be impaired in certain diseases and are thought to depend on the nature of the administered material (Svartengren et al, 1996). Swallowing, expectoration, and coughing constitute the first sequence of clearance mechanisms operating in the naso/oropharynx and tracheobronchial tree. However, it has been suggested that the effect of cough may extend down to the level of the respiratory bronchioli, under conditions of excess mucus production (Scherer, 1981). A major clearance mechanism for inhaled particulate matter deposited in the conducting airways is the mucociliary escalator, whereas uptake by alveolar macrophages (Schlesinger, 1985 and Oberdorster, 1988) predominates in the alveolar region. In addition to these pathways, soluble particles can also be cleared by dissolution with subsequent absorption from the lower airways.

Table 2.1 Definitions of lung volumes and capacities describing pulmonary ventilation.

Parameter	*Definition*
Tidal volume (Vt)	The volume of air inspired or expired during a normal breath
Inspiratory reserve volume (IRV)	The maximal volume of air that can be inspired after a normal tidal inspiration
Expiratory reserve volume (ERV)	The maximal volume of air that can be expired after a normal tidal expiration
Residual volume (RV)	The volume of air remaining in the lungs after a maximal expiratory effort
Inspiratory capacity (IC)	The maximal volume of air that can be inspired after a normal tidal expiration (IC = Vt + IRV)
Functional residual capacity (FRC)	The volume of air remaining in the lungs after a normal tidal expiration (FRC = ERV + RV)
Vital capacity (VC)	The maximal volume of air that can be expired from the lungs after a maximal inspiration (VC = IRV + Vt + ERV)
Total lung capacity (TLC)	The volume of air in the lungs after a maximal inspiratory effort (TLC = IRV + ERV + RV)

Lung volumes are given as the volume of air at body temperature (310° K), saturated with water vapor at that temperature (BTPS- conditions)

The rate of particle clearance from these regions differs significantly and its prolongation can have serious consequences, causing lung diseases from the toxic effects of inhaled compounds. It is now well recognized that the lungs are a site for the uptake, accumulation, and/or metabolism of numerous endogenous or exogenous compounds. The rate at which a drug is cleared and absorbed from the respiratory tract depends on the dynamic interaction of several factors, predominantly: (1) the mucociliary clearance rate, (2) site of deposition along the airways, (3) biopharmaceutical factors (particulates vs drug in solution), (4) drug release rate, and (5) the physicochemical properties of the drug, such as molecular weight, partition coefficient, and charge.

2.1.4 Mucociliary Clearance

Mucociliary clearance is a physiologic function of the respiratory tract to clear locally produced debris, excessive secretions, or unwanted inhaled particles. It consists of ciliated epithelial cells reaching from the naso/oropharynx and the upper tracheobronchial region down to the most peripheral terminal bronchioles. Beating of the cilia, together with mucus secreted by the goblet cells, contributes to an efficient clearance mechanism. The ciliary beat frequency is in the range of 1,000 –1,200 beats/min (Lippmann et al, 1984). Particles are transported at 5 mm/min if the effective stroke of cilia is 5 μm and it beats at 1,000 beats/min. The normal mucociliary transport rates in humans are 5.5 mm/min in the trachea and 2.4 mm/min in the major bronchi (Foster et al, 1980). For normal mucociliary clearance to occur it is necessary that the epithelial cells are intact, the ciliary activity and the rheology of mucus is normal, and that the depth and chemical composition of the periciliary fluid layer is optimal. Thus, the mucociliary escalator can be impaired by altering the volume of mucus secretion, the mucus viscosity and elasticity, or the ciliary beat frequency. Mucociliary clearance is known to be impaired in smokers (Foster et al, 1985) in patients with chronic bronchitis (Svartengren et al, 1996) and in acute asthmatics (Messina et al, 1991).

2.1.5 Pulmonary Endocytosis

Alveolar macrophages are considered the most important lung phagocytes. Macrophages are normal motile residents of the airways, interstitial matrix, and alveolar regions of the lungs (Brain, 1992). They are plentiful in the lung, with a ratio of 1:8 with respect to Type I cells (Stone et al, 1992), and their numbers and activity can increase substantially during inflammation or infection. Particles deposited in the alveolar region are taken up rapidly by macrophages. Phagocytic times of a few minutes (Gehr et al, 1993) up to an hour (Beck et al, 1982) have been reported. The contribution of pulmonary endocytosis to the overall lung clearance is determined by the particle size and particle shape (Lalor et al, 1996), solubility, particle burden (Oberdorster et al, 1992 and Snipes et al, 1981), and the chemical nature of the inhaled aerosol. Alveolar macrophages can clear particles from the alveolar region in 4 ways: (1) transport along the alveolar surface to the mucociliary escalator, (2) internal enzymatic degradation, (3) translocation to the tracheobronchial lymph, and/or (4) combination of the interstitial lymphatic route and mucociliary transport (Figure 2.3).

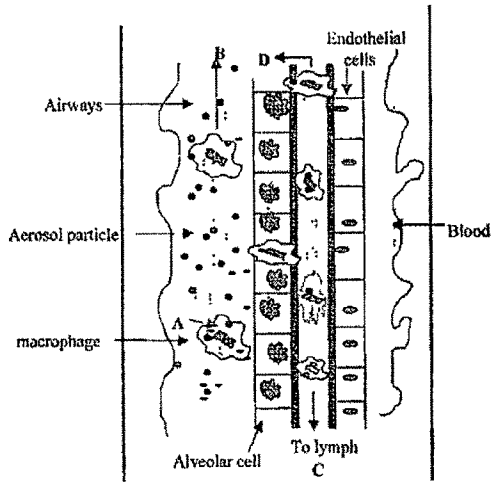


Figure 2.3 Clearance pathways of particles by alveolar macrophages

2.1.6 Pulmonary Absorption

The mechanisms of absorption through the airways and the factors affecting this process have been investigated in detail (Patton, 1996 and Olver et al, 1981). However, the translocation of molecules from the apical to the basal side of the epithelium is a subject of controversy and speculation. Absorption through membranes may be classified in general as paracellular and transcellular. Several routes of absorption have been proposed: (1) transport via membrane pores, (2) vesicular transport (through type I and/or type II lung cells), and (3) transport via the intercellular tight junctions. In general, small molecules can cross the membrane by diffusion (Schanker, 1978) or by carrier-mediated transport (Gardiner et al, 1974). A nonselective transport process like bulk flow through large pores or pinocytosis has also been postulated for the transport of small lipid-insoluble molecules (Goodman et al, 1982). In the case of macromolecules, it has been postulated that small peptides with molecular weight < 40 kilodaltons (kDa) may be absorbed by paracellular transport, while for large molecules (molecular weight >40 kDa) transcytosis may be more important. There is evidence for receptor-mediated transport of some macromolecules, such as albumin (Kim et al, 1995).

2.2 DRUG DELIVERY TO THE AIRWAYS

Drugs for inhalation therapy are administered in aerosol form. An aerosol is defined as a suspension of liquid or solid in the form of fine particles dispersed in a gas. The ability of the aerosolized drug to reach the peripheral airways is a prerequisite for efficacy. Here in lies the fundamental problem of inhalation therapy, as the anatomy and physiology of the respiratory tract have evolved to prevent the entry of particulate matter. The regional pattern of deposition efficiency determines the specific pathways and rate at which deposited particles are ultimately cleared and redistributed (Schlesinger, 1985).The pathology of disease of the lungs may considerably affect aerosol deposition. Patients with airway obstruction (eg, emphysema, asthma, chronic bronchitis) who inhaled radio labeled aerosol showed increased central (tracheobronchial) deposition and diminished penetration to the peripheral pulmonary regions (Anderson et al, 1990). The mechanisms by which particles deposit in the respiratory tract include impaction (inertial deposition), sedimentation (gravitational deposition), brownian diffusion, interception, and electrostatic precipitation (Schlesinger, 1985; Carpenter, 1999 and O'Doherty, 1993). The relative contribution of each depends on the characteristics of the inhaled particles, as well as on breathing patterns and respiratory tract anatomy. All mechanisms act simultaneously, but the first two mechanisms are most important for large-particle deposition within the airways (1 μm< MMAD<10 μm). Diffusion, however, is the main determinant of deposition of smaller particles in peripheral regions of the lung (Yu J et al, 1997).

2.2.1 Deposition Mechanisms

2.2.1.1 Impaction

Impaction occurs when a particle's momentum prevents it from changing course in an area where there is a change in the direction of bulk air flow. It is the main deposition mechanism in the upper airways, and at or near bronchial branching points. The probability of impaction and can be described by the parameter D^2F, the square of the aerodynamic diameter (D) multiplied by the inhalation flow (F). With increasing size of the particles and increasing velocity of the airflow the larger the probability of impaction.

2.2.1.2 Sedimentation

Sedimentation occurs when the velocity of airflow is low; the deposition is governed by gravity and particles sediment to the surface. Sedimentation increases with increasing diameter of the particles (D), inverse to inhalation flow (F), resulting in increasing residence time in the airways. This mechanism is most important for particles larger then 0.5μm and in the small bronchi, bronchioles and alveoli where air flow is low.

2.2.1.3 Brownian Diffusion and Electrostatic Attraction

The probability of Brownian diffusion increases with particles of smaller geometric diameter and increasing residence time. The particles random collide and by the motion deposit on the airway surface. Electrical charged particles repel or attract each other, and by the electrostatic force they deposit on the surface. The probability of deposition by electrostatic attraction increases with increasing number of electrical charges and decreasing size of the particles. These mechanisms may be important in the small airways for 0.1-1 μm particles.

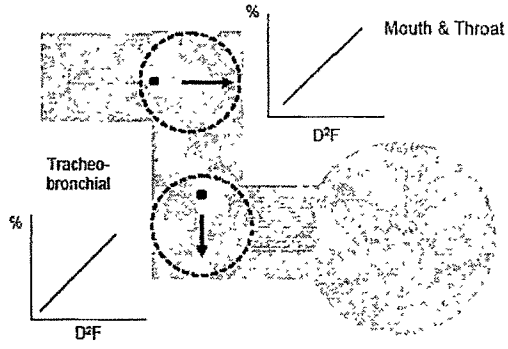


Figure 2.4 Mechanism of Impaction

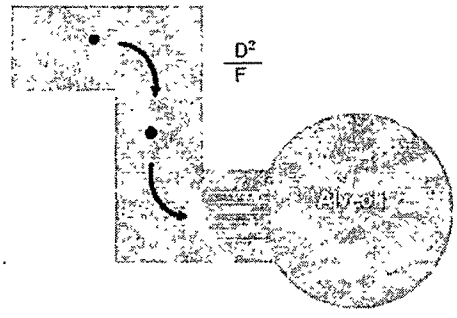


Figure 2.5 Mechanism of Sedimentation

2.2.2 Factors Controlling Deposition

The factors that control drug deposition are (1) characteristics of the inhaled particles, such as size, distribution, shape, electrical charge, density, and hygroscopicity, (2) anatomy of the airways, and (3) breathing patterns, such as frequency, tidal volume, and flow. Of these factors, aerosol particle size and size distribution are the most influential on aerosol deposition.

2.2.2.1 Particle Characteristics

The size of the particles is a critical factor affecting the site of their deposition, since it determines operating mechanisms and extent of penetration into the lungs.51 Aerosol size is often expressed in terms of aerodynamic diameter (d_{ae}). The aerodynamic diameter is defined as the equivalent diameter of a spherical particle of unit density having the same settling velocity from an air stream as the particle in question.9 Thus, particles that have higher than unit density will have actual diameters smaller than their d_{ae}. Conversely, particles with smaller than unit density will have geometric diameters larger than their d_{ae}. Aerosol size distributions may be characterized as practically monodisperse (uniform sizes, geometric

standard deviation of <1.2) or polydisperse (nonuniform sizes, geometric standard deviation ≥ 1.2). In mammals, respiratory anatomy has evolved in such a way as to actively prevent inhalation of airborne particulates. The upper airways (nose, mouth, larynx, and pharynx) and the branching anatomy of the tracheobronchial tree act as a series of filters for inhaled particles. Thus, aerosol particles >100 μm generally do not enter the respiratory tract and are trapped in the naso/oropharynx. Particles >10 μm will not penetrate the tracheobronchial tree. Particles must generally be < 5 μm in order to reach the alveolar space (Byron et al, 1994; Brain et al, 1979 and Zainudin, 1993). On the other hand, particles < 0.5 μm in diameter penetrate the lung deeply, but have a high tendency to be exhaled without deposition. However, some studies have found that breath-holding can minimize expiration of small particles (Byron, 1976 and 1987).

2.2.2.2 Airway Geometry

Airway geometry affects particle deposition in various ways. For example, the diameter sets the necessary displacement by the particle before it contacts an airway surface, cross-section determines the air velocity for a given flow, and variations in diameter and branching patterns affect mixing between tidal and reserve air (Schlesinger, 1985). Local deposition depends on the dimension of the airways. The geometry of the larynx may influence the velocity profile in the trachea and bronchi. The vocal folds act as an aperture and the sudden increase in the downstream diameter will lead to turbulent flow. Turbulent flow increases particle deposition. A pharyngeal narrowing during inhalation, not related to bronchial obstruction, has been shown to be significantly related to deposition in the upper airways (Svartengren et al, 1994). Increased airway resistance due to bronchoconstriction in diseased airways induces turbulence and increases deposition in larger airways (Svartengren et al, 1986).

2.2.2.3 Breathing Pattern

The pattern of respiration during aerosol exposure influences regional deposition, since breathing volume and frequency determine the mean flow rates in each region of the respiratory tract, which, in turn, influence the effectiveness of each deposition mechanism (Schlesinger, 1985; Byron, 1986; Hakkinen et al, 1999 and Gonda, 1990).Turbulence tends to enhance particle deposition, the degree of potentiation depending on the particle size. Rapid breathing is often associated with increased deposition of larger particles in the upper respiratory tract, while slow, steady inhalation increases the number of particles that penetrate to the peripheral parts of the lungs (Valberg et al, 1982). Byron (Byron, 1986) proposed a mathematical model that identified the effect of particle size and breathing pattern on drug deposition. Slow breathing, with or without breath-holding, showed a broad maximum

deposition in the ciliated airways (tracheobronchial region). The pulmonary maximum occurred between 1.5μm and 2.5 μm with breath-holding and between 2.5 μm and 4 μm without breath-holding. Rapid inhalation showed similar trends: the tracheobronchial region maximum falls and shifts to between 3 μm and 6 μm. Pulmonary deposition sharpens and occurs between 1.5 μm and 2 μm with breath-holding, and between 2 μm and 3 μm without breathholding. When the above considerations are taken into account, the ideal scenario for aerosol would be: (1) aerosol $d_{ae} < 5$ μm, to minimize oropharyngeal deposition, (2) slow, steady inhalation, and (3) a period of breath-holding on completion of inhalation.

2.2.2.4 Hygroscopic Growth

Inspired air is quickly humidified with in the airways. If a particle has hydrophilic surface, the particle absorbs water vapor from the moist air in the airways and grow in size. This is important for aerosols composed of water soluble particles.

2.2.3 Assessments of Airway Deposition

Once particles are introduced into the respiratory tract, quantifying the amount and distribution of dose is a challenging task. Not only are the technological solutions frequently complex, but also the nature of the problem like calculation of dose to the respiratory tract (either the averaged over the whole lung or the local airway or alveolar epithelial dose) is often obscure. At the outset it is essential to remember that the initial deposition pattern is continuously modified by clearance processes. An extensive menu of measurement techniques is available.

2.2.3.1 Pharmacokinetic Methods

Pharmacokinetic methods can be used to estimate the total systemic delivery via the oral and inhaled routes and thus provide valuable data which predict extra pulmonary effects. Estimation of delivery can be achieved by comparing area-under-the-curve data or urinary drug excretion (to infinity) for inhaled products. To identify the effective lung dose, methods which differentiate between drug delivered to the systemic circulation via the oral and pulmonary routes are needed. Classical pharmacokinetic studies of inhaled pharmaceutics have been difficult to perform since the delivered dose in general is very low, often below the accurate detection limits of standard assay. This approach is not necessary if oral absorption is poor (e.g. sodium cromoglycate) or when the first pass effect is substantial (e.g.fluticasone). To separate systemic delivery via the gastrointestinal and pulmonary routes, oral charcoal to block all gastrointestinal absorption or sampling during the lag time of the absorption phase has been used. For drugs whose oral bioavailability is the concentrations of drug in either plasma or urine (Hindle et al, 1993) during first 30 or 60 minutes after inhalation can be used

as an index of lung deposition, since the contribution of swallowed drug and the absorption from the GI-tract is slower than from the lung during these first time periods. The limitations of these methods are that only total lung deposition can be assessed, that expiratory maneuvers can influence airway absorption, and that the methods are drug specific. The greatest precision of lung deposition can be achieved by killing and dissecting animals. The lungs can be made rigid by freezing or drying and then sliced or dissected. It is then possible to divide the respiratory tract physically into individual pieces or specific lung compartments and analyze the particle content of each piece. Depending on one's patience and the sensitivity of the detection method, the distribution of particle retention can be described with increasing detail. These approaches permit an identification of the anatomical location of retained particles (Sweeney et al, 1991&1997 and Zeltner et al, 1991)

2.2.3.2 Modeling

Several theoretical models to predict the delivered dose to the lung have been developed with in the radiation protection field (Falk et al, 1999). These models make use of deposition predictors and clearance kinetics. If ventilation is measured well, one can estimate deposition fraction on the basis of theoretical predictions and models. A major advance of this model is that it is broadly applicable: deposition and clearance estimates can be made for most people on the basis of their age, race, and breathing level and the presence or absence of lung disease (Bailey et al, 1991 and Dahl et al, 1991). Prediction of total and regional deposited dose in healthy persons has become easier with recently developed semi-empirical deposition formulas.

2.2.3.3 Photometry and Filter Collection

Another approach to quantifying inhaled dose is to measure the difference in the particle concentration of inspired and expired air using light-scattering methods. By adding a measurement of ventilation, one can determine the deposition fraction in the respiratory tract (Kim et al, 1988). Although this technique is both sensitive and accurate, it is suitable only for well-characterized, monodisperse, nonhygroscopic aerosols that act as surrogates for challenge, environmental or therapeutic aerosols. By modifying this method, information can be obtained regarding airway size and convective transport in the respiratory tract. Alternatively, total deposition fraction can be measured by using a filter to collect the inhaled particles and then repeating the measurement using a different filter to collect the exhaled particles. The filters are then assayed for the drug or an appropriate label. An advantage of the filter method is that one can use actual aerosols that are polydisperse and hygroscopic.

However, neither the photometric nor the filter method provides information regarding the regional sites of deposition.

2.2.3.4 Bronchoalveolar Lavage

Bronchoalveolar lavage can provide information about retention of drugs that are not rapidly absorbed across the lung epithelium. It has been observed (Smaldone et al, 1991) that deposition of pentamidine was correlated with the drug concentration lavaged from the middle lobe airways 24 h after exposure.

2.2.3.5 Radioactivity

Radioactivity has been used frequently for studies of particle deposition because of its potential for noninvasive measurement and its sensitivity and resolution. Hundreds of radioisotopes are produced, and labeled drugs can be synthesized with a wide spectrum of energies, types of radiation (alpha, beta, gamma, positrons), and half-lives. Gamma emitters penetrate through tissue and are therefore suitable for making measurements externally. Alpha or beta emitters are better suited for producing autoradiographs; their short path-lengths, which make them unsuitable for external detection, produce high resolution images on film that is in contact with particle-laden tissue.

Gamma Scintigraphy

Two-dimensional gamma scintigraphy has been the most commonly used method to determine the lung deposition following inhalations. Gamma scintigraphy was first used during diagnostic testing, and its use was extended to pharmaceuticals in the 1970s. Originally, radiolabelled Teflon particles (Newman et al, 1991) were used but more recently techniques have been developed to adhere the radionuclide (usually 99 m Technetium) to either the formulation or the drug molecule (Kohler et al, 1988). The imaging of a radionuclide relies on stable activity and distribution. The technique provides a two-dimensional image of the oropharynx, lungs and the stomach together with the inhalation device. A krypton ventilation or a transmission scan is also carried out, usually some time before the study, to outline the lung fields. The method enables deposition into `central', `intermediate' and `peripheral' zones to be quantified together with a penetration index (peripheral/central zone ratio) but the clinical relevance of these is not proven.

Tomography

Tomography is used to map and follow the distribution of retained particles in three dimensions. In the two-dimensional view of the lung provided by a gamma camera, the central lung region, which is taken to represent the central airways, in actuality, also contains overlapping small airways and acini. Thus, one cannot discriminate particle deposition

among these regions, and deposition fraction in central airways may be overestimated. Alternatively, tomography can resolve particles within central airways and peripheral lung regions. There are two types of tomography appropriate for measuring regional particle retention: singlephoton emission computed tomography (SPECT) and positron emission tomography (PET).

Single-Photon Emission Computed Tomography. With SPECT, the subject inhales gamma-emitting radionuclide particles and then the gamma camera makes one scan. The camera is then rotated a few degrees and another scan is made; the process is repeated until the lung has been scanned from as many as 64 angles. Since each deposited radiolabelled particle released multiple photons, which the camera detects from different positions, there are several emission lines, with the particle at their intersection. After the scans, a computer program reconstructs the series of two-dimensional scans into a three-dimensional image, and particle retention in desired lung slices can be compared. One drawback of SPECT is that it requires levels of radioactivity about ten times higher than two-dimensional gamma camera imaging. It may also take up to 15 min to complete all the scans, which is enough time for particles within the central airways to redistribute via mucociliary clearance.

Positron Emission Tomography With PET imaging for particle retention studies, the subject would inhale particles labeled with a positron-emitting radionuclide, such as ^{11}C or ^{68}Ga. A positron travels 2 - 5 mm before it combines with an electron; the ensuing annihilation process produces two photons that travel at nearly 180° relative to each other. A series of paired scintillation detectors surrounding the subject record the site of interaction of these photons; then the line along which decay events originated can be reconstructed . Since a particle creates multiple photon pairs, which other detector pairs detect there are several emission lines, with the particle at their intersection. After the scan is complete, these multiple emission lines are analyzed by tomographic computer analysis to generate the three-dimensional particle-deposition pattern. Since PET determines the origin of photo emission on the basis of coincident detection by detector pairs, as opposed to the collimation required by SPECT, PET ignores less of the emitted radiation and is more efficient than SPECT. Other advantages are that PET is relatively fast and that many of the positron-emitting isotopes have short half-lives, so it is easier to make repeated studies in the same subject. By using drugs labeled with positron-emitting radionuclides, PET can locate the distribution of receptor sites within the respiratory tract C3583.

2.2.3.6 Magnetopneumography

Some aerosol particles are magnetizable, and sensitive magnetopneumography can measure their concentration and distribution in the lungs (Valberg et al, 1988). This technique applies a magnetic field to the whole thorax or to localized areas and detects the resultant alignment of ferromagnetic domains in retained lung particles. The greatest advantage of this technique is that the duration of measurement is not limited by radioactive decay. Measurements can be made as long as sufficient particles remain in the lungs.

2.3 DRUG DELIVERY SYSTEMS

Inhaled therapeutic aerosols are generated by different devices (Figure 2.6) that aim to deliver an aerosol to the lower airways. Inhalation devices can be classified into three categories: nebulizers, pressurized metered doe inhalers and dry powder inhalers (Dolovich , 1999 and Hicky, 1989). Aerosol generators are characterized (Gonda, 1988) using: (1) the output (mass of drug delivered per unit time), (2) the distribution of the agent in different aerodynamic size fractions, and (3) intradevice and inter-device reproducibility of operation.

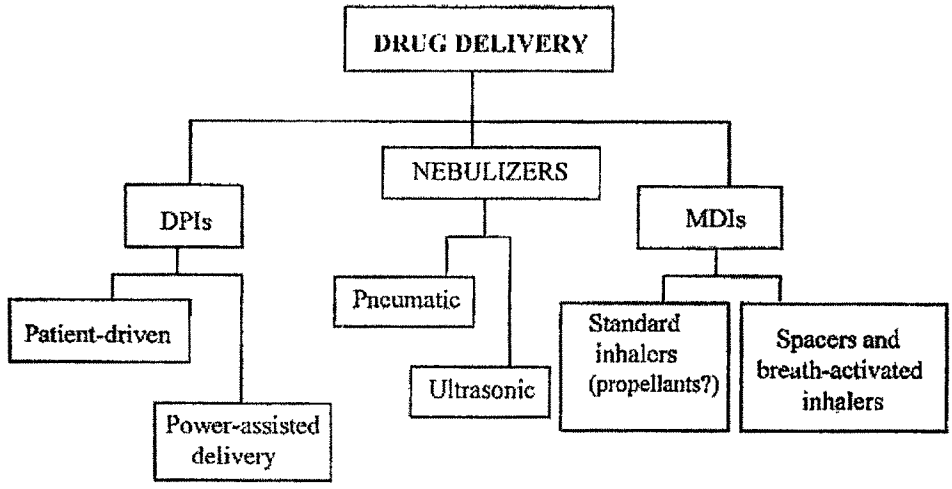


Figure 2.6 Aerosol drug delivery devices.

2.3.1 Nebulizers

Nebulizers are the oldest systems and have been used in inhalation therapy since the early 20th century (Grossman, 1994). Nebulizers are applied for drug solutions or suspensions, which are aerosolized either by air jet or ultrasonic nebulization. To generate the aerosol from an air-jet nebulizer, compressed air is forces through an orifice over, or in co-axial flow around the open end of a capillary tube. The drug solution or suspension is drawn through the capillary by means of momentum transfer. In the nozzle region, shear forces disrupt the liquid into small particles that are entrained by the air towards a baffle. Only the smallest droplets, in the desired size range, are able to follow the streamlines of the air and pass the baffle whereas larger droplets impact on the baffle and returned to the liquid reservoir. Ultrasonic nebulizers generate aerosols using high frequency ultrasonic waves by a ceramic piezoelectric crystal. The greatest disadvantages of nebulizers are their poor deposition efficiency the long inhalation time and the requirement for a power supply. Nebulizers are suitable devices for acute care of non-ambulatory patients and of infants and children (Le Brun et al, 2000).

2.3.2 Pressurized Metered Dose Inhalers

The pressurized metered dose inhaler (pMDIs) consists of four basic functional elements like container, metering valve, actuator and mouthpiece. The principle of pMDI's is based on a spray-can as used for hair spray (Thiel, 1996). A liquefied propellant serves both as an energy source to expel the formulation from the valve in the form of rapidly evaporating droplets and as a dispersion medium for the drug and other excipients (Hickey, 1996). Initial droplet size and droplet speed is too high for effective deposition in the lower respiratory tract. Evaporation and deceleration in the upper respiratory tract are essential. For pMDI's the inhalation maneuver is relevant for deposition efficacy. Especially the hand-lung co-ordination is of major importance. The use of spacer devices or breath-triggered devices overcomes this coordination problem.

2.3.3 Dry Powder Inhalers

In a United States patent from 1939, by W.B.Stuart, a description is given of what is called the first dry powder inhaler. The patent describes a device, which had been designed to aid the inhalation of aluminum dust for the chelation of inhaled silica. However, the derived was never commercialized (Clark, 1995). A patent from 1949, by M.R.Fields, described the first dry powder inhaler to be used for the administration of a pharmaceutical agent. The so-called Aero-haler was the first commercially available dry powder inhaler for the delivery of Isoprenaline sulphate (Le Brun et al, 2000). The first single dose dry powder inhaler with a

hard gelatin capsule technology was initially developed for the inhalation of relatively larger amounts of drug, being 50mg of Disodium Cromoglycate (Spinhaler, 1971) (Bell et al, 1971). Later, DPI's found their application in inhalation therapy as a CFC free alternative for the older pMDI's. However, nowadays DPI's seem to have a much larger potential (Malcolmson et al 1998; Ashurst, et al, 2000), because of the high lung deposition that can be attained and their suitability for pulmonary delivery of therapeutic peptides and proteins, which can subsequently become systemically available (Adjei et al, 1997). Table 2.2 summarizes some commercially available DPIs and new DPIs currently under development with its dispersion mechanism. DPIs are categorized mainly in two categories like breathe driven/passive DPIs and power assisted/active DPIs. Former uses patient's inspiratory inhalation flow for dispersion of dry powder while later uses some mechanical/electrical power to disperse the dry powder.

2.3.4 Factors Influencing Dry Powder Inhalers Formulation Design

2.3.4.1 Physical Properties of Powders

DPIs provide powder pharmaceuticals in aerosol form to patients. The powdered drug is either loaded by the user into the DPI before use or stored in the DPI. To generate an aerosol, the powder in its static state must be fluidized and entrained into the patient's inspiratory airflow. The powder is subject to numerous cohesive and adhesive forces that must be overcome to get dispersed. Optimization and control of flow and dispersion (deaggregation) characteristics of the formulation is of critical importance in development of DPIs. These properties are governed by adhesive forces between particles, including Van der Waals forces, electrostatic forces and the surface tension of absorbed liquid layers (Hinds, 1982). The forces are influenced by several fundamental physicochemical properties including particle density and size distribution, particle morphology (shape, habit, surface texture) and surface composition (including absorbed moisture) (Hickey et al, 1990). Inter-particle forces that influence flow and dispersion properties of inhalation powders are particularly dominant in the micronized or microcrystalline powders (particles smaller than 5 μm). Hickey *et al.* (1994) reviewed the factors influencing the dispersion of dry powders as aerosols. Several cohesive and adhesive forces are exerted on particle characteristics such as size, shape, rugosity and crystalline form, and powder characteristics such as packing density and equilibrium moisture content. Buckton *et al.* (1997) has reviewed particle surface characteristics and several studies have measured the adhesion forces in inhalation powders (Podczek, 1996). Peart and co-workers (1996) measured electrostatic charge interactions from Turbuhalers and drug powders and the results suggest that the inhaler itself and the

deaggregation mechanisms influenced the charging phenomena. Electrostatic effects in DPIs have been extensively studies by Mazumder et al (1998) and powder flow properties have also been studied (Dawson et al, 1998). Further particle characteristics have been studied such as the crystallization and amorphous content of inhalation powders (Phillips et al, 1996; Buckton, 1998) and the measurement of their surface properties by inverse gas chromatography (Thielmann et al, 2002) and computer aided image analysis to plot a Facet Signature (Kaye, 1996).

Table 2.2 Commercially available DPIs and new DPIs currently under development and its dispersion mechanism

Type of the Device & Name	*Dispersion Mechanism*
Breath Driven/ Passive Powder Inhalers: Unit- Dose	
Rotahaler (Cipla, GSK)*	Capsule separates with dispersion
Spinhaler (Fisons)*	Pierced capsule rotates on impeller vibratory dispersion
Inhalator (Boehringer Ingelheim)*	Stationary capsule pierced dispersion via capillary fluidization
Aerosolizer (Novartis)	Pierced capsule rotates in chamber dispersion aided by grid
Solo (Inhale Therapeutic Systems)	Dispersion via turbulent airflow pathway
Orbital (Brin Tech International)	Dispersion via centrifugal acceleration mechanism
Microhaler (Harris Pharm)	-
Breath Driven/ Passive Powder Inhalers: Multi-Unit Dose	
Accuhaler (GSK)*	Pierced blister dispersion via turbulent airflow pathway
Diskhaler (GSK)*	Pierced blister dispersion via turbulent airflow pathway and grid
Flowcaps (Hovione)	Capsule based device dispersion via turbulent airflow pathway
Spiros S2 (Elan Corporation)	Dispersion via free floating beads and a dosing chamber
Technohaler (Innovata Biomed)	Dispersion via turbulent airflow pathway
Breath Driven/ Passive Powder Inhalers: Multidose Reservoir	
Turbohaler (Astra Zeneca)*	Dispersion via turbulent airflow pathway
Easyhaler (Orion)*	Dispersion via turbulent airflow pathway
Clickhaler (Innovata Biomed)*	Dispersion via turbulent airflow pathway
Pulvinal (Chiesi)*	Dispersion via turbulent airflow pathway
Twisthaler (Schering Plough)	Dispersion via turbulent airflow pathway
SkyePharma DPI	Dispersion via turbulent airflow pathway
Taifun (Leiras)	Dispersion via turbulent airflow pathway
Novalizer (Sofotec GmbH)	Dispersion via turbulent airflow pathway
MAGhaler (Mundipharma)	Dispersion via turbulent airflow. Formulation present as tablet
Power Assisted/Active Powder Inhalers: Unit-Dose	
Inhance PDS (Inhale)	Gas assisted - compressed air disperses powder formulation
Omnihaler (ML Lab)	-
Pfeiffer (Pfeiffer GmbH)	-
Power Assisted/Active Powder Inhalers: Multi-Unit-Dose	
Spiros (Elan Corporation)	Electromechanical energy – battery operated impeller
Prohaler (Valois)	Gas assisted – built in pump provides compressed air

* denotes commercially available DPIs and new DPIs currently under development.

2.3.4.2 Drug Carrier

Optimization and control of particle-particle and particle-inhaler interactions is of critical importance in the development of efficient DPIs. A paradoxical situation exists in powder formulations – drug particles should be less than 5 μm aerodynamic diameters to ensure efficient lung deposition, but should also exhibit acceptable flow properties required for accurate dose metering. Thus, micronized powders are often blended with 'coarse' inert carriers e.g. lactose, glucose or alternatively palletized as loose agglomerates to improve powder flow. Lactose is often selected as a drug carrier/excipients material because of several advantageous properties like low reactivity and toxicity, low water content and its low cost. Many studies have examined the properties of lactose particles and their interaction with drug particles as part of the process to optimize DPI performance (Patel, 2000). Blending the drug with a carrier has a number of potential advantages, such as increasing the bulk of the formulation. This allows easier metering of small quantities (typically <100μg) of potent drugs, either at the manufacturing stage (if the doses are pre-metered) or within the device itself for a reservoir device. Provided the content uniformity of the blend is well controlled, this approach can improve the subsequent dosing consistency of the inhaler. The presence of the carrier material, in separating the very fine drug particles, can also improve processing (e.g. flow characteristics) of the formulation. The carrier properties (particle size distribution, particle surface characteristics) can be used to influence/control fine particle mass. An additional benefit that may be gained by the use of a carrier such as lactose is the taste/sensation on inhaling, which can assure the patient that a dose has been delivered. Clearly, the influence of the carrier material on product stability must be carefully assessed, and the range of materials available for use as carriers in inhaled products is limited for toxicological reasons. Lactose and other sugars have been studied and used and modification of these materials may allow further formulation optimization. Modifications to the lactose surface have been proposed that would improve the surface characteristics (reduce the rugosity) of the material. Ganderton (1992) claims that reducing the rugosity increases the percentage of respirable particles in conventional powder inhalers. Zeng and coworkers (1999) has found that the addition of fine lactose particles (mass median diameter 6.96 μm) increased the fine particle fraction of Salbutamol sulphate from a powder formulation delivered by a Rotahaler. They suggested that this may be because of the fine particles occupy possible drug binding sites on the larger lactose particles. Lucas *et al.* (1998) demonstrated a similar performance modifying effect with a model protein, albumin and a high-dose agglomerated preparation of Nedocromil Sodium. Other studies have looked at

similar effects of lactose size fractions and agglomerates (Boerefijn et al, 1998). The properties of lactose such as particle size and surface morphology (Clarke et al, 2000) had a profound effect on the fine particle fraction of the generated aerosol. Other excipients, like sugars, have also been studies to establish their preformulation characteristics. Braun *et al.* (1996) used two grades each of α-lactose monohydrate and dextrose monohydrate with Disodium Cromoglycate and generated aerosols using a unit-dose device, the Microhaler (Pearce, 1989).

2.3.4.3 Particle Engineering

One of the key factors involved in optimizing DPI performance is the precision particle engineering required to produce a powder formulation that delivers accurate, consistent, efficient doses of drug. Bulk drug modifications, both chemical and physical, have been attempted in order to enhance respirable dose performance. In one study (Chawla et al, 1994), spray-dried salbutamol sulfate was seen to perform as well as micronized material. In the case of disodium cromoglycate, several approaches have been successfully employed to improve flow and dispersion characteristics, including controlled adherent flocs (Bell et al, 1971; Auty et al, 1987). This approach takes advantage of the inherent cohesiveness of the particles. In a review of Staniforth (1996) who has outlined the development of improved performance dry powder inhalation systems by preformulation characterization of drug-carrier combinations. Staniforth describes the Pascal system, which is an example of carrier formulation technology using a novel single step process termed corrasion. This is a simultaneous milling, mixing and surface modification of mixtures of 98-100% α - lactose monohydrate and 0-2% of the amino acid L-leucine (Malcolmson et al, 1998). The process is designed to ensure that the drug-carrier bond is sufficiently strong to enable efficient manufacturing processes for the DPI, but also weak enough to facilitate detachment of drug from carrier surface during the inhalation process. Results claim significant increase in fine particle doses compared with conventional formulations. Lipophilic coating materials have been investigated using disodium cromoglycate as an approach to minimize hygroscopic growth (Hickey et al, 1990). In addition, crystals of the cromoglycic acid and the effect of aspect ratios (longest and shortest dimensions) have been studies (Chan et al, 1989). Other techniques such as re-crystallization from supercritical fluids for modifying drug characteristics have been discussed. More conventional ways of modifying drug particle characteristics such as spray drying have been further advanced by the use of new techniques such as supercritical fluid technologies. York and co workers (1996) have evaluated the SEDS (Solution enhanced dispersion by supercritical fluids) technique that enables a drug

solution to be processed into a micrometer sized particulate product in a singles step operation.

2.3.4.4 Metering Design

DPIs can be divided into two classes· passive and active devices. Passive devices rely solely upon the patient's inspiratory flow through the DPI to provide the energy needed for dispersion. This method has the advantage of drug release automatically coordinating with the patient's inhalation (Kjellman et al, 1981). The disadvantage is that dispersion typically is highly dependent on the patient's ability to inhale at an optimum flow rate. Depending on the inhaler design, this requirement may be difficult for some patients if the device's resistance to airflow is high (Dunbar et al, 1997). Active devices use mechanisms such as springs or batteries to store energy that can be released to facilitate powder dispersion. Whether a drug alone or a drug-carrier system is adopted, a key decision in the design of a DPI is whether to use a factory-metered dose or to include a reservoir and metering mechanism in the device itself. Early popular DPIs utilized factory-metered doses. Conventional capsule-filling technology was already well established in the early 1970s by Bell *et al* (1971) who had developed this device for the administration of powdered sodium cromoglycate. Here, the drug mixture is mixed with a bulk carrier to aid powder flow (lactose), is pre-filled into a hard gelatin capsule and loaded into the device. Following activation, capsule is pierced and the patient inhales the dose, which is dispensed from the vibrating capsule by means of inspired air. A similar kind of device (Rotahaler, Glaxo Wellcome) has been developed for the delivery of Salbutamol and Beclomethasone Dipropionate powders. Here, the drug mixture is again filled into a hard capsule and the capsule is inserted into the device, wherein it is broken open and the powder inhaled through a screened tube (Clark, 1995). Other devices dispense drug loaded into hard gelatin capsules like the Berotec (Boehringer Ingelheim) used for fenoterol (Pedersen et al, 1986).

The development of multi-does DPI has been pioneered by A.B.Draco (a division of Astra) with their Turbuhaler (Wetterlin, 1988) and by Glaxo Wellcome with the introduction of the Diskhaler (Sumby et al, 1993) and recently the Diskus (Gunawardena, 1994). The Turbuhaler device is a reservoir-based powder inhaler The drug is contained within a storage reservoir and can be dispensed into the dosing chamber by a simple back and forth twisting action on the base of the unit. The device delivers carrier-free particles of the β-agonist, Terbutaline Sulfate, as well as the steroid, Budesonide (Pedersen, 1994). The Diskhaler (Glaxo Wellcome) has been introduced for the delivery of the short-acting β-agonist, Salbutamol, as well as longer acting, Salmeterol (Brindley et al, 1995). Also, the steroids like

Beclomethasone Dipropionate and Fluticasone propionate are available as disks. These devices have a critical disk that contains a number of powder charges (four or eight), depending on a typical dosing schedule. The doses are maintained in separate aluminum blister reservoirs until just prior to inspiration, thus ensuring the integrity of the powder blend against moisture ingress. On priming the device, the aluminum blister is pierced and the powder charge is dropped into the dosing chamber. The Diskus device represents a further modification of the Diskhaler approach, with the pre-metered doses sealed in blisters on a foil strip. Instead of disk, here coiled strip is used which allows 60 doses of drug to be contained within the device. There are two main advantages in the use of a pre-metered dose. Firstly, the precision with which the dose can be metered in the factory is superior to the typical precision of metering that can be achieved within a device alone, as required by a reservoir-based powder inhaler. With an efficient delivery system the enhanced precision of metering will result in improved consistency of the delivered dose. This shows the frequency distribution of doses delivered at 60 l/min from a Terbutaline Turbuhaler and a Salmeterol Diskus (Malton et al, 1995). The pre-metered doses from the Diskus device are more consistent than the doses delivered from the reservoir device. Secondly, the pre-metered doses can be individually sealed and protected from the environment (moisture) until the point of use by the patient. Brindley *et al* have shown that the drug content per blister and the dose delivered at 60l/min from the Salmeterol Diskus device is unaffected by storage at high humidity (Brindley et al, 1995). A reservoir that contains all of the doses may be more susceptible to deterioration through ingress of moisture. Some Turbuhaler products are designed to contain a desiccant within the device, to reduce the effects of moisture uptake, although Meakin *et al* has demonstrated limitations to this approach (Meakin et al, 1995; Meakin et al, 1993). The advantages of the reservoir metering device approach are the relative ease and cost of manufacturing, since these devices can be 'dump' filled with very high manufacturing throughput. A further advantage of the reservoir approach is the relative ease of including a large number of doses within the device. Newman has also shown that the Turbuhaler inhaler performance in-vivo compares favorably with pMDIs (Newman, 1995).

2.3.4.5 Flow Path Design

In combination with the design of the formulation and the approach to metering, the third critical factor that determines product performance is the flow path design of the device, particularly the design between exposed dose to be inhaled and the exit from the mouthpiece. An ideal flow path design would allow efficient and consistent emptying from the device

across a wide range of flow rates; with sufficient turbulence to disperse/deaggregate the powder blend and thereby providing an effective pharmacological response.

Research has shown that the specific design of the DPI in terms of path length, flow angles and orifice diameters influence the resistance of the device (Britto, 1998). New DPIs may be designed with a low resistance so that all patients can be able to generate high flow rates through it. Resistance of established DPIs has been previously measured (Clark et al, 1993) and the resultant flow rates were compared. New DPIs such as the Chiesi inhaler (Pitcairn et al, 1994) (Chiesi Farmaceutici, Italy) and the Innovata Biomed Inhaler (Nantel, 1990) (Innovata Biomed Ltd. UK) are evaluated for dosing performance at a range of flow rates.

The flow path of the Diskus device is extremely short, with the powder passing through a single 'crucifix' grid to generate the necessary turbulence. As a result of the short flow path, drug losses within the device are minimized, allowing approximately 90% of the metered dose to be delivered while older devices like Turbuhaler typically delivers only 60% of the metered dose, presumably due to greater drug losses within the device (Byron, 1990). In Turbuhaler, the flow path was carefully designed to maximize turbulence, using a long flow path with spiral channels in order to generate shear forces that would disperse the drug aggregates and produce a good fine particle mass (Pedersen, 1994). At 60 l/min, the Turbuhaler can produce up to 50% of the emitted dose as respirable particles (< 5 μm), although the percentage is considerably reduced at lower flow rates (Meakin et al, 1995). A further disadvantage of a long flow path is a potential increase in the device's resistance. The higher the resistance of the device, the greater the effort a patient has to make in order to achieve a given flow rate (Clark et al, 1993). The flow rate achieved may be important in determining the performance of the device (Olsson et al, 1994). With careful flow path design, and the use of a lactose carrier, some devices such as the Diskus, are relatively insensitive to change in flow rate and deliver a consistent dose over a wide range of inhalation conditions (Prime et al, 1996). Device resistance can also affect the patient's comfort in using the inhaler. De Boer et al. (1996) established that an increase in peak inspiratory flow rate (PIFR) is obtained with decreasing inhaler resistance and that, in healthy volunteers, on average, 55% of maximum effort was regarded as comfortable as a measure of patient's convenience to inhale the dose.

2.3.5 Regulatory and Pharmacopoeial Requirements

The late – 1990s have seen the published agreements from the FDA (US Food and Drug Administration) (1998) and the European Inhalant group (1999) on the tests required for the approval of new DPIs. The US Pharmacopoeia specifications for test methods harmonize with the European Pharmacopoeial requirements are now implemented, the FDA guidelines are in consultation draft form, and provide stricter requirements than the pharmacopoeial tests. The FDA recognizes that the reproducibility of the dose and the particle size distribution are the most critical attributes of DPI. FDA requirements for testing a DPI constitute a demanding list for the approval of a new device.

A presentation of FDA Guideline for Product Development Strategy (Donawa et al, 2002) concludes the performance standards for future DPI products have to be built in. Controversy has surrounded the definition of a delivered dose from a DPI and how it should be tested. Because of the differing efficiencies of the devices and their particular formulation characteristics, each device containing the same active ingredient can deliver the same effective or respirable dose from different quantities of active ingredients. The European Pharmacopoeial Monograph defines the apparatus used for tests of uniformity of delivered dose and states that the test should be carried out at a fixed pressure drop across the inhaler of 4.0 KPa. Therefore, for devices with differing resistances, the flow rates used for testing the device will be different. This implies that the conditions used for testing the device should relate to the range of inhalation flow rates generated through the device during patient use.

It also means that the multistage apparatus for measuring the particle size distribution of the aerosol product might have to be operated at non-standard flow rates and therefore be recalibrated for each different device tested. None of the current impactors used for in vitro assessment are ideally suited to the aerodynamic particle sizing of DPIs. Several studies have demonstrated improvements in the designs of cascade impactors (Van Oort et al, 1996) and emitted-dose-measurements apparatus (Collins, 1998) used for the evaluation of the performance of DPIs. An industry consortium is developing a new impactor; the Next Generation Impactor group (Wright, 1997) phase I of the project is an evaluation of new designs.

The requirements form the Medicines Control Agency (MCA) (Summers, 1996) also include stricter controls on the uniformity of the delivered dose than the Pharmacopoeial limits and states that the applicant should be able to attain a mean of ± 20% or better from the nominal content per dose. In addition, the MCA requires each multi-dose unit to have the following two safety features: 1) A counter device or other indicator to give the patient some indication

of when it is becoming exhausted, and 2) A system to prevent inadvertent multiple dosing because of multiple actuations of the dose measuring device. The new SkyePharma powder inhaler (SkyePharma AG, Switzerland) containing a reservoir of 300 doses (Keller et al, 1997) and the Bulkhaler device (Astra Medica AG, Germany) incorporating a refillable cartridge (Berner et al, 1998) fulfill these MCA requirements. The committee for proprietary medicinal products (CPMP) has published guidelines on DPIs in 1998.

2.4 CYSTIC FIBROSIS

CF is an autosomal recessive disease caused by lack of function of a cAMP-regulated chloride channel, called CFTR (for the cystic fibrosis transmembrane conductance regulator), which normally resides at the apical surface of many epithelial cell types. Epithelial cells in the sweat glands, salivary glands, airways, nasal epithelium, vas deferens in males, bile ducts, pancreas, intestinal epithelium, as well as many other sites normally express CFTR. The function of CFTR is important in many of these organs, mutation of a single gene on chromosome 7, encoding the CF transmembrane conductance regulator, leads to multiple clinical, physiologic, and pathologic abnormalities (Collins, 1992; Quinton, 1990 and Welsh et al, 1995). Vulnerability to infection in CF occurs only in the airways, and not at other sites such as skin or urinary tract, so there is no systemic immune defect in CF. However, excess inflammation occurs at other sites: the prevalence of inflammatory bowel disease and pancreatitis is markedly increased (Lloyd-Still, 1994 and Taylor et al, 2002). Nevertheless, there is unquestionably something special about the lung, which is intended to be sterile, yet is continuously challenged by inhaled pathogens. Bacteria, when inhaled in small quantities, are ordinarily cleared without provoking significant inflammation. The lungs of patients with CF do not deal with this challenge appropriately. Obstructive pulmonary disease remains the primary cause of morbidity and mortality in CF. Initially, persistent endobronchial bacterial infections are due to *Staphylococcus aureus*, non-typeable *Haemophilus infuenzae*, and *Gram-negative bacilli*. By the end of the first decade of life, *Psa* is the predominant pathogen. Intense inflammatory response and a decrease in the host-defense mechanism lead to chronic colonization of the CF airways with *Psa*. Once colonized, it is nearly impossible to eradicate *Psa*, resulting in a vicious cycle of infection, inflammation, scarring, and lung damage, leading eventually to respiratory failure.

2.4.1 Pathophysiology

The exact mechanisms for how an abnormal gene leads to disease remain unclear. Various mechanisms have been postulated as to how defective CFTR leads to the vicious cycle of inflammation and infection that eventually leads to lung parenchymal destruction. The CFTR protein is a c-AMP-regulated chloride channel situated in the apical membrane of epithelial cells, and is thought to have a role in ion transport, mucus rheology, inflammation and bacterial adherence. There are five classes of CFTR mutation:

- Class I--No full-length CFTR protein is produced due to a premature stop codon.
- Class II--The most common class II mutation is deltaF508. These mutations cause mis-folding of CFTR protein which inhibits processing through the cell. However, if the CFTR protein can be helped to reach the apical surface of the cell, it is capable of chloride ion secretion.
- Class III--These defects disrupt the activation and regulation of CFTR at the plasma membrane.
- Class IV--There is a defect in the chloride pore leading to a reduction in chloride conductance.
- Class V--Various mutations that reduce the amount of normal CFTR protein are grouped together, e.g. mutation in the CFTR promoter. They are typically associated with a pancreatic-sufficient milder phenotype.

In Figure 2.7, mutant CFTR promotes initial bacterial infection by up regulating epithelial cell adhesion molecules for bacteria such as asialo-GM1 and by decreasing production of innate host defense molecules such as nitric oxide (NO). Defects in CFTR also lead to increased sodium absorption through the epithelial sodium channel (ENaC) and decreased chloride secretion. Water follows its concentration gradient and results in decreased depth of airway surface liquid. Bacterial persistence is promoted by alterations in airway wall architecture, impaired host defense mechanisms, an excessive inflammatory response, and adaptations made by the bacteria to the microenvironment of the cystic fibrosis airway.

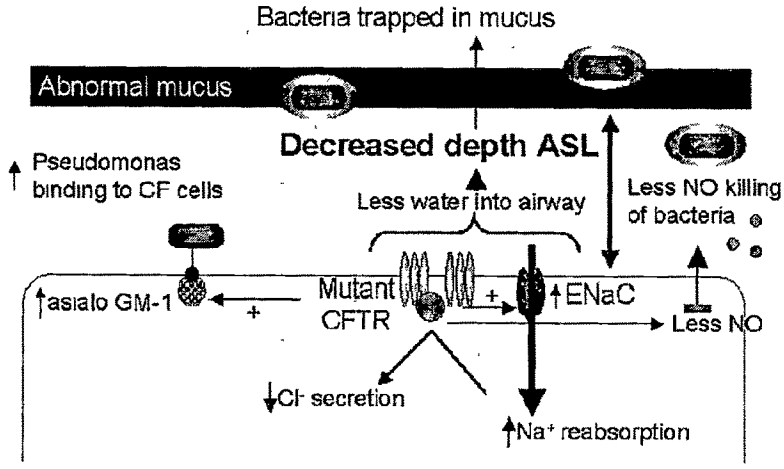


Figure 2.7 Impact of mutant cystic fibrosis transmembrane conductance regulator (CFTR) on cellular physiology.

Although the CF lung is thought to be normal at birth, evidence of inflammation occurs early with increased interleukin- 8 levels (IL-8) and activated neutrophils found in broncho-alveolar lavage fluid in infants as young as 4 weeks (Khan et al, 1995). IL-8 is a potent neutrophil chemo-attractant. Interestingly, this inflammatory response can occur in the absence of bacterial infection, providing evidence that CFTR may itself be pro-inflammatory. Other evidence to support this intrinsic association between abnormal CFTR and inflammation comes from the finding that IL-10, a protective immuno regulatory cytokine, is down regulated even after infection has been eradicated (Konstan et al, 1997). Decreased IL-10 levels may allow excessive inflammatory responses to infection in CF patients. This has led to investigation of therapies directed at immuno-modulation of the CF airway. Other studies suggest that it is infection which precedes the inflammation (Armstrong et al, 1995). Bacterial infection occurs early in the CF airway and is difficult to eradicate. The most common pathogen isolated in early life is *Staphylococcus aureus* with 50% of patients chronically infected by 10 years of age. Later on, *Psa* is common, with 90% chronically infected by adulthood. There are various hypotheses proposed to explain the predisposition of the CF airway to infection, including dysfunction of ß -defensins and alterations of the properties of the airway surface liquid (ASL) present in the lung. The high-salt theory proposes that levels of chloride and sodium are high in the ASL which leads to dysfunction of salt-sensitive anti-bacterial defense proteins such as ß-defensins. Defensins are thought to be particularly important in *Psa* killing. The dehydration hypothesis proposes that the thin layer of ASL is decreased as a result of sodium and water hyper absorption. This results in thickened sputum and poor mucociliary clearance leading to chronic infection and

inflammation. It has also been shown that defective CFTR leads to the up-regulation of binding sites for the bacteria commonly isolated in CF patients, and defective CFTR may cause cells to fail to internalize bacteria for killing.

2.4.2. Therapeutic Approaches

For the last half century, treatments aimed at reducing airway obstruction, controlling airway infection, and improving nutritional status have been the cornerstone of the successful treatment of CF. Recently, systematic anti-inflammatory therapy has come upon the scene. Beyond these basic strategies, aggressive symptomatic treatment of the complications of the lung disease is also essential. More definitive therapies directed at the basic defect, including manipulation of ion transport, activation of mutant CFTR, and gene therapy are now on the horizon.

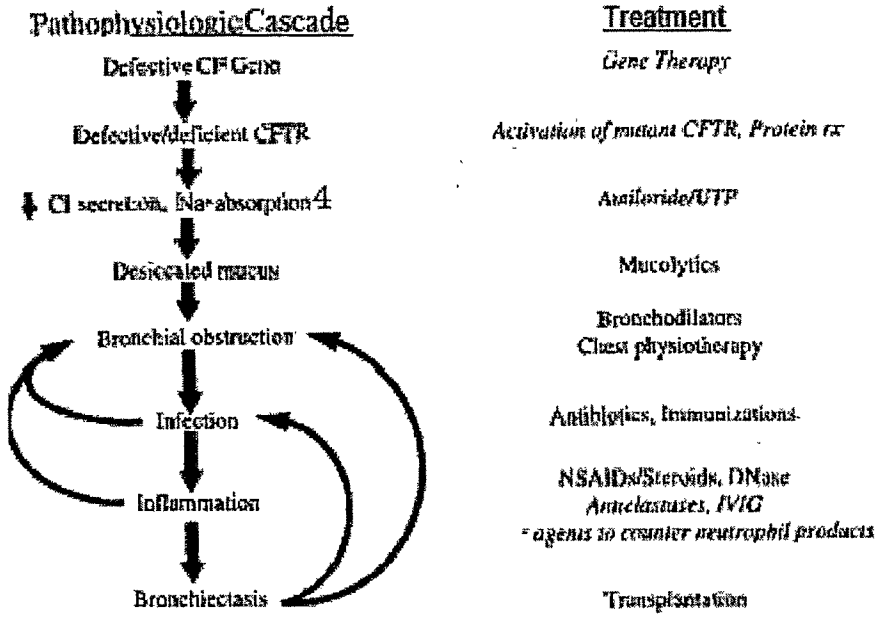


Figure 2.8 Proposed pathophysiologic cascade for the CF lung disease and the therapeutic interventions targeted at each step.

2.4.2.1 Therapies Directed at Inflammation

CF lung disease is characterized by severe neutrophil mediated airway inflammation. It would seem logical to reduce this inflammation with the use of anti-inflammatory therapies.

Steroids

Although oral corticosteroids have shown a consistent benefit on lung function (Auerbach et al, 1985), they have unacceptable side effects for long-term therapy, especially on growth. Although adverse effects preclude long-term use of steroids, short courses may attenuate the inflammatory response during and after pulmonary exacerbation. There is currently no

evidence of benefit in CF, although there is a large UK multicentred trial (CFWISE trial) ongoing which is attempting to address this question. This trial addresses the impact of withdrawing inhaled corticosteroids from patients. The study is designed in this way because of the inability to recruit patients who are steroid naive, and highlights the problem of newer therapies becoming established in routine treatment without sufficient evidence of benefit.

Non-steroidal anti-inflammatory drugs

Ibuprofen has been shown to inhibit neutrophil migration, adherence and aggregation (Hellewell et al, 1995). One trial of CF patients treated with high-dose ibuprofen demonstrated significantly less deterioration in lung function in the younger age group (Konstan et al, 1995) However, blood levels need to be closely monitored and, in theory, wrong levels could lead to worsening lung disease. Ibuprofen is currently not used widely in the UK because of concerns about efficacy and possible side-effects.

Macrolides

The most exciting recent advance has been the finding that macrolides such as azithromycin have a role in CF. While the exact mechanisms are unknown, it is thought to be as a result of anti-inflammatory rather than antimicrobial properties. The possible mechanisms such as effects on inflammatory pathways, neutrophil function, bronchoconstriction, biofilms, mucus rheology and bacterial adherence has been reviewed recently (Jaffe et al, 2001). CF has striking similarities to diffuse panbronchiolitis, a disease found primarily in Japan. Macrolides have been used in the long term with great success in DPB with an increase in 10-year survival from 12.4%, in patients colonized with *Psa*, to more than 90% (Hoiby, 1994) Following an open study in seven children with CF treated with long-term azithromycin, which showed a significant increase in lung function (Jaffe et al, 1998), there have now been three trials completed in adults and children with CF. One recent randomized placebo-controlled crossover trial of azithromycin showed a mean increase in FEV1 of 5.4% with marked individual response (Equi et al, 2002). Patients on azithromycin required fewer courses of oral antibiotics and there were no noticeable side-effects. Due to the considerable variation in response, we would recommend a 6-month trial of azithromycin for individual patients who do not respond to conventional therapy.

2.4.2.2 Controlling Infection

Staphylococcus aureus is the most common pathogen in early life. Prophylactic anti-staphylococcal therapy remains controversial but it has been shown by a Cochrane review to be beneficial in young children less than 3 years of age. There are concerns that the use of broad-spectrum anti- staphylococcal agents, e.g. cephalexin, may lead to an increase in the acquisition of *Psa*. The first isolation of *Psa* is treated aggressively with nebulized colistin and oral ciprofloxacin for at least 3 weeks or intravenous antibiotics. Ultimately, most patients become chronically infected with *Psa* which is associated with a decline in lung function. In patients chronically infected with *Psa*, continuous nebulized antibiotics such as amikacin (Schaad et al, 1987), colistin, gentamicin and tobramycin have been shown to decrease the frequency of respiratory exacerbations and to decrease the decline in lung function (Mukhopadhyay et al, 1996). There have been two large multi-centred trials of intermittent inhaled preservative-free tobramycin (TOBI) in patients with CF and *Psa* infection which showed an average improvement in FEV1 of 10% and a decreased risk of hospitalization (Ramsey et al, 1999). Currently, only TOBI and colistin (Colomycin) are approved for inhalation in CF.

2.4.2.3 Reducing Airway Obstruction

Physiotherapy

It is well accepted that physiotherapy is important to clinical well-being in CF, although the evidence of benefit is largely anecdotal. Physiotherapy techniques such as positive expiratory pressure (PEP) mask, oscillatory PEP (flutter, cornet), oscillating jackets and intrapulmonary percussive ventilator have been developed in an attempt to aid sputum clearance and treatment independence for the patient. Incorporating forced expiratory manoeuvres into techniques has been the greatest recent advance in physiotherapy.

Exercise

Exercise is beneficial in mobilizing sputum and is complimentary to airway clearance. Further research is needed to assess other long-term benefits of physical training.

Bronchodilators

Bronchodilators, usually beta-adrenergic agonists or cholinergic blockers given by wet nebulization or metered-dose inhalers, are often used prior to chest physiotherapy to dilate small airways and facilitate mucus clearance. For a population of patients with CF, bronchodilators can be shown to be of benefit, but in a given individual, bronchodilators may be beneficial, of no important, or actually harmful. Regular use of bronchodilators is indicated for patients who have significant increases in pulmonary function measures after

bronchodilator inhalation. For patients with paradoxical deterioration of pulmonary function after bronchodilators, these agents are contraindicated. Such a response probably occurs in patients with floppy airways who depend on smooth muscle tone to prevent expiratory collapse.

Mucolytics

Sputum contains mucus, inflammatory cells, cellular debris, bacteria, actin and large amounts of DNA from neutrophil degradation. Mucolytics went into eclipse two decades ago because the available drugs, N-acetylcysteine and bovine pancreatic DNase, were bronchial irritants and had unpleasant side effects. However, the idea of hydrolyzing extracellular DNA remained appealing because much of the viscoelasticity of CF secretions is due to DNA released from dead neutrophils and bacteria. Aerosolized recombinant human deoxyribonuclease1 (rhDNase) cleaves DNA in CF sputum and decreases sputum viscosity, which results in decreased sputum tenacity and easier expectoration. Other drugs designed to improve the properties of sputum now in preclinical investigation include gelsolin and thymosin $ß_4$, proteins that act on actin, another inflammatory cell product that contributes to the viscosity of sputum. Both agents decrease the viscosity of CF sputum *in vitro* (Pamela et al, 1996).

2.4.2.4 Nutrition

The importance of optimizing nutrition and growth cannot be emphasized enough as there is a close relationship between nutrition, lung function and CF survival. A recent study showed that during first year of observation, adolescents who had a greater than 5% predicted decrease in weight for height had a concomitant mean loss of FEV1 of 16.5%.Those patients who gained relative weight had a parallel increase in FEV1 of 2.1% predicted (Steinkamp et al, 2002).

2.4.2.5 Therapies Directed at the Basic Defect

Gene therapy

Gene therapy involves insertion of DNA encoding for normal CFTR into respiratory cells, which potentially corrects the abnormalities caused by the defective protein. The incorporation of a vector as a delivery vehicle is normally used to increase gene transfer, and may be either recombinant viruses or synthetic vectors such as liposomes or polymers. There have now been several phase 1 clinical trials of gene therapy which have demonstrated evidence of gene transfer and partial correction of the chloride defect, but efficacy is currently insufficient to warrant phase II/III trials.

Stem-cell therapy

There has been great interest in the recent findings that stemcells may have the potential to differentiate into different lineages under certain conditions. A study by Krause et al. presented evidence that a purified population of bone marrow cells, transplanted into an irradiated mouse, differentiated into type II pneumocytes which expressed surfactant B and cytokeratin (Krause et al, 2001). This has therapeutic implications for CF as there is the possibility of targeting gene therapy at the stem cell to treat lung disease.

CFTR mutations and protein repair

New treatments are likely to be directed at specific classes of CFTR mutation and correction of the basic defect. Certain aminoglycoside antibiotics (gentamicin not tobramycin) possess the ability to bind to rRNA and skip over the stop mutation, leading to production of full length protein, and may be of benefit in those patients possessing class I premature stop mutations. Clinical trials have shown that nasally administered gentamicin drops can increase nasal epithelial chloride transport (Wilschanki et al, 2000). Several chemical chaperones have been investigated to help trafficking of CFTR produced by class II mutations. Isoflavonoids such as Genistein bind to CFTR and stimulate chloride activity, and these compounds may be useful for class III mutations. Adenosine nucleotides are being investigated for repairing class IV defects. Therapies to increase the activity of CFTR produced by class V mutations are being investigated.

Correction of ion transport

Therapies to correct the impaired chloride secretion, sodium hyper absorption and rehydrate the ASL are being investigated. Amiloride, a sodium channel inhibitor which reduces sodium and water absorption in respiratory cells, has produced variable results in trials, and other longer-acting agents such as benzamil are under investigation. Functional chloride secretion channels, other than CFTR, provide an alternative pathway to increase chloride secretion. P2Y2 agonists such as adenosine triphosphate (ATP) and uridine triphosphate (UTP) can activate these alternative channels and these agents are being explored.

2.4.2.6 Lung Transplant

Lung transplant is a final therapeutic option for some patients with end-stage lung disease. CF patients with FEV1 of less than 30% have greater than 50% mortality at 2 years (Aurora et al, 2000). Current survival after transplant is 70% at 1year and 40% at 5 years. The survival of CF lung recipients does not differ significantly from non-CF transplant recipients, and most survivors enjoy a markedly improved quality of life. The primary obstacle to transplant is the availability of organs, and the majority of patients accepted for transplant

will die before a donor becomes available. In order to overcome this, there has been a growing interest in bilateral living lobar lung donation, which has had some success in the USA, with1-year survival quoted as 72% in one study (Cohen et al, 2001) but brings ethical concerns.

2.5 AEROSOLIZED ANTIBIOTICS IN CYSTIC FIBROSIS

As an interim attempt at delivering drugs by direct contact with the airways, inhaled antibiotic preparations were prepared from intra venous medications that were not originally intended for delivery to the airways. Several of these preparations contained preservatives, such as phenol and bisulfites that may contribute to airway irritation, coughing, and bronchoconstriction. Initial attempts at aerosolization with neomycin and other polymyxins were made about 50 years ago. Then, in the 1950s, penicillin G was aerosolized to treat patients with pneumococcal pneumonia. Unfortunately, this acidic solution was associated with unpleasant side effects, such as stinging and bronchospasm, probably due to its pH and to ingredients in the solution that were not intended for inhalation. Soon after its introduction, the antibiotic gentamicin was used in nebulizers to treat patients with CF. Patients received treatment by sleeping inside humidified tents into which the antibiotic was directed. Over the next 20 years, other antimicrobial agents that were not formulated or intended for aerosolization, including ticarcillin disodium, ceftazidime, and carbenicillin, also were used with variable results. These agents were administered in doses ranging from 500 to 2,000 mg as in hypertonic solutions, which proved to be quite irritating to bronchial smooth muscle and produced cough and bronchospasm in patients. Aerosolized amphotericin B, a colloidal suspension, also has been used by a number of clinicians in managing CF. However, colloidal suspensions, by definition, do not nebulize well. When diluted with normal saline solution, they will often precipitate. Sterile water for injection should be used when the aerosolized delivery of amphotericin B is attempted. Gentamicin for parenteral administration contains phenol as a preservative. In addition to an unpleasant taste, phenol is a neurotoxin and is listed by the National Institute for Occupational Safety and Health as being hazardous for occupational exposure. In addition, phenol may increase the time required for nebulization of a solution (Nikolaizik et al, 1996). Further, some IV formulations of gentamicin contain methylparaben and propylparaben, which are detergents that can alter particle size and dispersal characteristics, as well as sodium bisulfite and ethylenediaminetetraacetic acid, which are both known to cause bronchoconstriction (Nikolaizik et al, 1996). Colistin sulphomethate is used extensively in Europe for inhalation therapy in the treatment of patients with CF. However, in the United States, only the prodrug sterile colistimethate

sodium (USP) is available, and it must be converted to its active form. An additional drawback to the aerosolization of this agent is the significant foaming that occurs with nebulization. This makes ascertaining the exact dose of the drug to be delivered to the patient difficult and cumbersome. Bronchospasm also has been associated with this agent.

2.5.1 Challenges in Aerosolized Antibiotics Administration in Cystic Fibrosis

Several challenges must be taken into account when treating infectious respiratory exacerbations in patients with CF via the direct delivery of antibiotics to the lumen of the airways. One of the most important considerations is drug distribution. Particle size and mode of administration have been shown to play an important role in pulmonary drug distribution, and the use of different delivery devices can result in variations in particle sizes for the same drug. Moreover, the adequate treatment of respiratory infections *in situ* depends on a sufficient concentration of drug particles reaching the site of infection in the airways. This is a particular challenge in CF patients. Factors such as the physical and chemical composition of mucus and the degree of parenchymal destruction and bronchiectasis in CF patients can significantly alter drug distribution and bioavailability. Two classes of antagonistic sputum components have been identified. They include small molecules, which physically decrease antibiotic penetration into bacteria, and large glycoprotein molecules, which bind and sequester aminoglycosides. Soluble sputum components such as monovalent and divalent cations antagonize aminoglyco-side bioactivity. For effective bacteriocidal activity, sufficient quantities of an antibiotic must penetrate the thick, purulent endobronchial secretions to reach the lumen of the airways in CF patients (Robert, 2001).

Maintenance treatment with inhaled, nebulized antibiotics is common practice in patients with CF. Two different types of nebulizers are frequently in use: the jet and the ultrasonic nebulizer (Le Brun et al, 2000a). The need for a compressor unit or pressurized air for the jet type and electricity for the ultrasonic nebulizer immobilizes the patient to great extent. Regular cleaning and adequate disinfection of the equipment is required in order to prevent contamination with microorgamisms and subsequent colonization of the patient's oropharynx (Le Brun et al, 2000b and Grassi et al, 1995). An even greater drawback of nebulizers is their low efficiency. The droplet formation is influenced by many factors and a favourable size distribution for deposition in the target area is not always obtained (O'Callaghan et al, 1997 and Le Brun et al, 1999). Output rates are often low and the patient's breathing pattern is a major determinant for the fraction of the dose wasted to the environment (Denyer et al, 1998). As a result, only 2–12% of the dose for jet nebulizers, and 1–32% of the dose for ultrasonic

devices is deposited in the lungs (O'Callaghan et al, 1997 and Newman, 1987). Dry powder inhalation (DPI) may provide an alternative for drug nebulization.

Dry powder aerosols of micronized antibiotics has been attempted, but were limited by inefficient delivery devices and/or poorly dispersible lactose formulations (Goldman et al, 1990 and Labiris et al, 1999). Poor dispersibility of micronised formulations is because of their tendency to form aggregate due to hydrophobic or electrostatic interactions between the fine particles. These changes in the particle size and increases in cohesive forces over time tend to provide powders that give undesirable pulmonary distribution profiles upon activation of the device. More particularly, fine particle aggregation disrupts the aerodynamic properties of the powder, thereby preventing large amounts of the aerosolized medicament from reaching the deeper airways of the lung where it is most effective. A major focus has been on the particle engineering to deliver large amount of therapeutic agents to the lungs. These particles provide a low area of contact and reduced cohesive forces between them. This lower surface energy imparts increase in flowability and fine particle fraction and have been shown to improve peripheral lung deposition by reducing deposits in the extrathoracic and tracheobronchial airways, making them ideally suited for inhaled therapies used in the treatment of diseases involving infection of the airway (cystic fibrosis, bronchiectasis) without substantial systemic component and systemic delivery of therapeutic agents (insulin). Furthermore some of them had shown reduced clearance by alveolar macrophage action, thereby improving the bioavailability of inhaled pharmaceuticals (Vanbever et al, 1999).

2.6 STRATEGIES FOR ENGINEERED DRY POWDER AEROSOL FORMULATIONS

2.6.1 Use of Crystalline Instead of Amorphous Materials

A common problem encountered in making stable dry powder inhalation products is the amorphous nature of micronized drugs (and even the excipients) which is a result of the grinding, milling or spray drying process used to produce the required finely divided particles. Amorphous materials are physically unstable and in high humidity will recrystallize uncontrollably, forming solid bridges between particles. Consequently the powder dispersibility will decline during storage. However, if these amorphous materials can be rendered crystalline in a post-production treatment, the powder stability will be increased. This has been achieved by conditioning the amorphous particles through exposure to a controlled environment [e.g., 35–85% relative humidity (RH) or organic solvent vapor] to induce crystallization. The process can be carried out in a closed container (e.g., a column or a flask) or in a fluidized bed dryer at controlled

temperature and humidity, and has been employed to transform the spray dried amorphous spherical lactose carrier to the crystalline alpha monohydrate form used for sodium cromoglycate and budesonide (Kussendrager et al, 2002).

2.6 .2 Use of Suitable Excipients in Blend Formulations

Blends have been prepared by mixing excipient(s) with the active drug to form a binary or ternary blend. There are number of strategies using excipients to improve the powder properties for the purpose of: (I) Promoting release of the active drug from carrier particles. (II) Improving powder flowability. (III) Improving moisture resistance and storage stability. (IV) Combination of I, II and III.

2.6.2.1 Fine Particle Excipient

Fine lactose particles (~ 5 mm) have been used to enhance the dispersibility of salbutamol sulfate and nedocromil sodium (Lucas et al, 1996). The fine lactose powder was mixed with the coarse lactose carrier before adding in the salbutamol sulfate or mixed with the pre-blended coarse lactose carrier and salbutamol sulfate to form multiplets. For nedocromil sodium, the drug was directly blended with the fine lactose particles without the coarse carrier. It was suggested that the fine lactose particles disrupt the strong cohesion of nedocromil sodium. In both cases, the drug particles will be easier to detach from the powder formulation due to reduced cohesion force. However, the deposition of fine carriers in the lung may raise clinical and regulatory concerns.

2.6.2.2 Ternary Additive with Lubricant or Anti-adherent Properties

In this process, the additive is first mixed with the carrier to form 'composite' excipient particles (Staniforth et al, 2002), followed by adding in the active drug. This procedure of mixing the ingredient is considered important as it allows the additive material to first occupy the high energy binding site on the carrier, thus making the weakly bound active particles easier to release from the carrier during dispersion. Magnesium stearate in particular, a hydrophobic excipient, has been widely employed for this purpose. Only a small amount (< 0.5 %w/w) of the ternary additive is usually required to impart an improvement to the powder formulation (Musa et al, 2000a &2000b). This is expected as the surface-to-volume ratio of the coarse carrier particles is relatively low and only the surface of the carrier needs to be covered to have the surface properties modified. Besides Mg stearate, other hydrophobic compounds such as leucine, lecithin, stearic acid and their derivatives were also reported to be suitable additives (Staniforth et al, 2002; Lucas et al, 1999; Fults et al, 1997 and Staniforth, 1997 & 1996). In a special application, the fine excipient material was used to coat the active drug so as to modify its surface electrical properties (Nilsson et al, 2002).

2.6.3. Use of Particles with Designed Features

Since the aerodynamic diameter depends on the particle density, whereas the inter-particulate cohesion force and hygroscopicity depends on the surface morphology and chemical nature, these parameters can be modified to enhance the aerosol performance and moisture stability of the powder.

2.6.3.1 Co-spray Drying with a Suitable Excipient

The choice of excipients is demonstrably critical for powder dispersion. Sodium chloride increased the dispersibility of spray dried protein rhDNase with increase of powder crystallinity. NaCl crystals were observed on the surface of the protein particles (Chan et al, 1997). The dispersibility enhancement was attributed to decreased cohesion as a result of changes in surface energy and morphology of the crystalline particles. It has been reported that the effect of moisture on powder dispersion can be instantaneous (Chew et al, 2000). A possible way to reduce the hygroscopic effect is to use hydrophobic excipients. For example, spray dried particles of L-isoleucine, a hydrophobic amino acid, were shown to have superior physical stability at 40 8C/75% RH for 6 months (Yamashita et al, 1996). Di-leucine and tri-leucine have also been used to co-spray dry with therapeutic peptides to improve the aerosol performance by enriching the particle surface with these hydrophobic amino acids (Lechuga-Ballesteros et al, 2001). More recently, sodium cromoglcate has been co-spray dried with hydrophobic amino acids, in particular leucine, to increase the powder dispersibility (Chew et al, 2002). Surface analysis by X-ray photoelectron spectroscopy showed an enrichment of leucine on the surface of the cromoglycate particles, indicating that the improvement on dispersion is due to formation of a more hydrophobic particle surface.

2.6.3.2. Wrinkled Particles

Surface morphology has been explored to improve dispersibility. *Non-porous solid* albumin particles with wrinkled surface were shown to give a significantly higher fine particle fraction (i.e. mass fraction of particles less than 5 microns in the aerosol as referenced against the total recovered dose) (FPF) than the non-wrinkled spherical particles (Figure 2.9) (Chew et al, 2001). This was attributed to a reduced cohesion as a result of less close contact between particles. A distinct advantage of these wrinkled particles is that their dispersion performance is less dependent on the inhaler device and air flow.

2.6.3.3. Large Porous Particles

Large, porous particles (AIR^{TM}, Figure 2.10, mean diameter 5–20 μm, specific surface area ~50–100 m^2 /g, Alkermes, MA, USA) have been prepared to improve fine particle fraction due to the low density (<0.4 g/ml) giving rise to a small aerodynamic diameter, as observed

in particles containing insulin (20 %w/w) and poly (lactic acid-co-glycolic acid) (PLGA) (80 %w/w) (Edwards et al, 1997). The superior aerosol performance of porous particles was observed and was attributed to decreased cohesiveness of these particles.

2.6.3.4. Pulmospheres and Pulmosols

Pulmospheres™ and Pulmosols™ (Figure 2.11 and 2.12, Nektar Therapeutics, San Carlos, CA, USA) are also porous particles with excellent dispersibility, but smaller in size (3–5 μm). These particles have been used to deliver immunoglobulin to the respiratory tract (Bot et al, 2000). In addition, gentamycin, budesonide and albuterol particles have also been prepared by this technology (Weers et al, 1998).

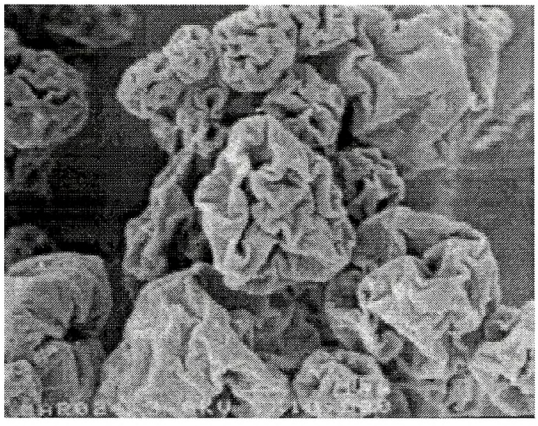


Figure 2.9 Wrinkled particles

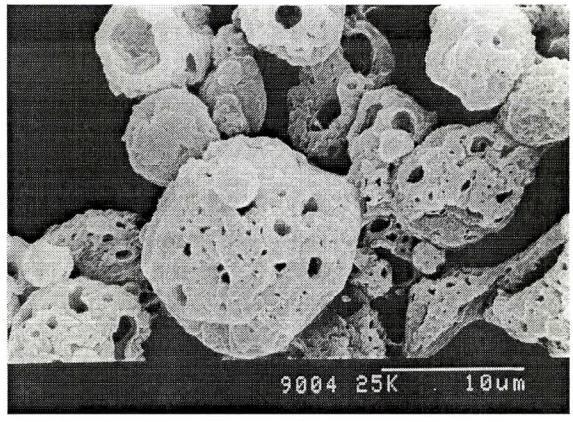


Figure 2.10 Large porous particles

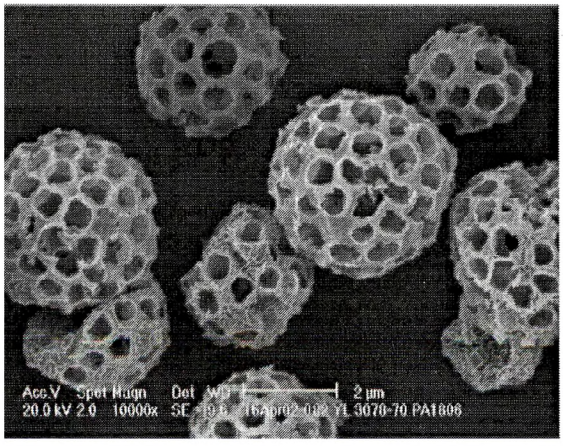


Figure 2.11 Pulmospheres

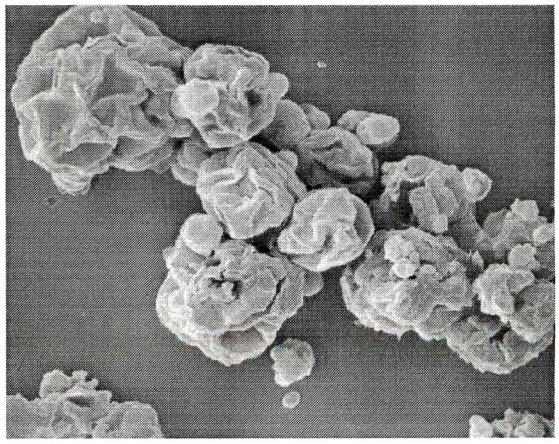


Figure 2.12 Pulmosols

2.7. EMERGING POWDER PRODUCTION METHODS

While the conventional methods of powder production for inhalation products may have been sufficient in the past, they are not suitable to produce powders with the required flow and dispersion characteristics to meet the need of enhanced powder performance. Various methods that have been explored or are in their advanced stage of development will be discussed here.

2.7.1 Spray Drying and Related Droplet Evaporation Methods

2 .7.1.1 Spray Drying

Spray drying was explored in the 1980s as an alternative means of making fine particles with desirable flow and dispersion characteristics without the need of using coarse carriers or forming soft pellets. Anti-asthmatic drugs including salbutamol sulfate, terbutaline sulfate, isoprenaline sulfate and sodium cromoglycate were investigated. However, it was not until the early 1990s when the potential of the pulmonary route for therapeutic proteins delivery has been recognized, then an enormous effort was focused on spray drying of pharmaceuticals. In spray drying, a drug solution is atomized to fine droplets which are evaporated in a warm air current to form dry particles (Masters, 1979). Although the drying air temperature can be relatively high (e.g.,>100°C), the actual temperature of the evaporating droplets is significantly lower due to cooling by the latent heat of vaporization. Thus, thermal degradation of the active ingredient is not so much a concern as it first appears. In addition to drug production, spray drying has been used to produce carrier particles. Spray drying is not limited to aqueous solutions. Non-aqueous systems have also been used to prepare porous particles suitable for aerosol delivery (Dellamary et al, 2000; Edwards et al 2001 and Weers, 2000). The properties of the spray dried powders are controlled by both the process and formulation parameters. Earlier studies have looked into the effects of the active ingredient, atomizing nozzle type, powder collection technique and droplet drying time (Masters, 1979; Forrester et al, 1986 and Maa et al, 2000). The liquid feed can be atomized by rotary nozzle, two-fluid nozzles, or ultrasonic nozzles, depending on the droplet size required. Powder collection is usually achieved by using a cyclone but a filter bag can also be used. The latter is not preferred since particulate of the filter material may contaminate the powder. The driving force for drying is controlled by the water content and the difference in the inlet and outlet temperatures of the drying air. Drying time of the droplets depends on the residence time of the droplets in the spray drier which, in turn, is determined by the spray drier dimension and the drying air flow rate. It is important to note that these parameters are closely interrelated. Changing a process parameter will therefore lead to a change in the

others. For example, while reducing the air flow will lengthen the time for the droplets to evaporate, the drying efficiency will be reduced simultaneously because less air is available to evaporate the droplets. A lower drying air flow will also decrease the collection efficiency of the cyclone. However, higher air flow will evaporate the droplets more rapidly, resulting in a less crystalline product due to insufficient time allowed for crystallization. This is a significant drawback as amorphous materials are hygroscopic, more cohesive and difficult to flow and disperse. To facilitate crystallization, the drying time can be prolonged by inserting a secondary drying apparatus between the primary drying chamber and the cyclone as described (Chickering et al, 2001). Another limitation of spray drying is its unsuitability for substances sensitive to mechanical shear of atomization (Maa et al, 2000). Drugs that are unstable to liquid–air interface and decomposed by oxidation should be avoided, but the problem could be minimized by using an inert gas instead of air in the process, or if feasible, using an anti-oxidant. It should be noted that, depending on the particle size range, powder nature, and the cyclone collection efficiency, the process yield for inhalable particles can be unacceptably low.

2.7.1.2 Freeze Drying

In the freeze-drying process water is sublimed from the composition after it is frozen. The particular advantage associated with this process is that biologicals and pharmaceuticals that are relatively unstable in an aqueous solution can be dried without elevated temperatures (thereby eliminating the adverse thermal effects), and then stored in a dry state where there are few stability problems. With respect to the present invention such techniques are particularly compatible with the incorporation of biologicals and pharmaceuticals in particulates or microstructures without compromising physiological activity. The freeze dried cake containing particles can be micronized using techniques known in the art to provide respirable sized particles. Accordingly, to the extent that freeze-drying processes may be used to provide engineered particles having the desired features and size.

2.7.1.3 Spray Freeze Drying

Spray freeze drying was explored for pharmaceutical application in early 1990s. It involves spraying the drug solution into a freezing medium (usually liquid nitrogen) followed by lyophilization. Compared with spray drying, this process produces light and porous particles with enhanced aerosol performance, and the production yield is almost 100%. The method has been applied to prepare rhDNase and anti-IgE antibody (Maa et al, 1999 & 2001) particles for inhalation, but can be used for anti-asthmatic compounds. However, this is an

expensive process and would only be justifiable for expensive drugs as it requires the additional use of liquid nitrogen and the freeze drying step is more time consuming.

2.7.1.4 Controlled Evaporation of Droplets

Like spray drying, this is a single-step continuous process involving atomizing the drug solution into a carrier gas for drying (Watanabe et al, 2001& 2002). Unlike spray drying, this method provides better control over the temperature history and residence time of droplets. In the actual setup, the solution is atomized using an ultrasonic nebulizer. The droplets suspended in a carrier gas are then fed into a turbular flow reactor housed in a constant temperature oven for evaporation. Since the feed rate and temperature are adjustable, the temperature history and residence time of the droplets can be controlled. The method has the potential to control the particle morphology and polymorphic form and has been used to produce beclomethasone dipropionate particles.

2.7.1.5 Evaporation of Low-Boiling-Point Solutions

This involves simply dissolving the active ingredient in a low-boiling-point organic solvent followed by atomizing the solution and evaporating the resulting droplets to produce the dry particles (Morton, 2001). The concept of this approach is similar to both spray drying and rapid expansion of supercritical fluid.

2.7.2 Solvent precipitation

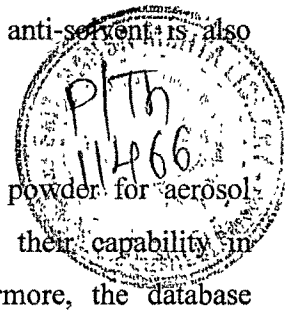
2.7.2.1 Sono-crystallization

Inhalable particles can potentially be obtained by rapid precipitation from aqueous solutions using anti-solvents. However, due to dispersion in the nucleation rate and crystal growth, it is difficult to reproducibly generate particle size in the micron range for aerosol delivery. Recently, ultrasonic radiation has been applied to control the precipitation process (Lancaster et al, 2000). The setup can simply comprise an ultrasound probe in a mechanically stirred reaction tank where the anti-solvent is mixed with the drug solution to precipitate the fine drug particles. The ultrasound frequency is crucial and 20–25 kHz (or higher) was reported to be suitable.

2.7.2.2 Microprecipitation by opposing liquid jets

In this method, precipitation occurs in a region of extreme turbulence and intense mixing created by a jet of drug solution opposing a jet of anti-solvent coming through two opposing nozzles mounted in a small chamber (Begon et al, 2001). As two liquid jets mix, the anti-solvent will cause the drug to precipitate as fine particles. The crucial process parameters include the speed of the liquid jets and concentration of the drug solution. A high jet stream speed or a high drug concentration was found to give finer particles but higher residual

solvent level and vice versa. The volume ratio of drug solution to anti-solvent is also expected to affect the precipitation process.



2.7.2.3 Supercritical fluid (SCF) technology

SCF technology has been proven to be effective for producing fine powder for aerosol applications (York et al, 1996 and Sievers et al, 2000). However, their capability in controlling the particle size requires further demonstration. Furthermore, the database concerning the process effects on protein stability needs to be built. From the formulation perspective, its feasibility in systems involving multiple components remains unclear.

2.7.3 Specialized Milling

Dry milling tends to produce partially amorphous materials with surface charge causing particle agglomeration. These problems can be dealt with by specialized milling methods.

2.7.3.1. Fluid Energy Milling at Elevated Humidity

In order to reduce the amorphous content in the material produced by milling, the latter can be carried out at elevated humidity to facilitate in situ crystallization. The milled products have been reported to be predominantly crystalline with particle size distribution similar to those produced by the conventional milling process. The setup involves a control of the relative humidity (e.g., 30–70%) of the milling chamber by humidifying the feed gas (e.g., by superheated steam to minimize condensation) used for milling the powder (Vemuri et al, 2000).

2.7.3.2. Wet Milling Nanotechnology

Nanocrystal® (Elan Pharmaceutical Technologies, King of Prussia, PA, USA) technology is an aqueous-based milling process to reduce particle size to below 400 nm. A conventional ball mill can be used for the process, and the materials selected for the grinding media (e.g., glass, zirconium oxide) were reported to be not crucial. However, the size of the grinding media are preferably 1 mm or less in order to be effective in attribution and imparting less wear to the mill (Liversidge et al, 1992). Since the particles are produced in water, any amorphous regions in the particles would undergo recrystallization. Thus the wet-milled powder is anticipated to be crystalline and more stable to moisture than powders produced by dry milling. A surface modifier (e.g., polyvinyl pyrrolidone (PVP), lecithin, cellulose derivatives) is added during or after milling to prevent agglomeration of the nanoparticles. Although these stabilizers can be of GRAS materials, long-term inhalation can be a concern unless proven safe. Another major drawback of wet milling is that, depending on the type of mill and the drug, a lengthy processing time may be required (up to 5 days or longer).

2.8 AERODYNAMICALLY LIGHT AND LARGE PARTICLES

ALLP have a tap density less than about 0.4 g/cm^3 and geometric diameter of at least 5μm. These biodegradable particles can be used for improved and /or controlled systemic or local delivery of therapeutic agents to the respiratory tract via aerosolization. Features of the particle which can contribute to low tap density include irregular surface texture and porous structure. Administration of the low density particles to the lung by aerosolization permits deep lung delivery of relatively large diameter therapeutic aerosols. A rough surface texture also can reduce particle agglomeration and provide a highly flowable powder, which is ideal for aerosolization via dry powder inhaler devices, leading to lower deposition in the mouth, throat and inhaler device.

2.8.1 Density and Size of Aerodynamically Light and Large Particles

2.8.1.1 Particle Size

The mass mean diameter of the particles can be measured using a laser diffractometry, Coulter Counter and time of flight measurements. The diameter of particles in a sample will range depending upon depending on factors such as particle composition and methods of synthesis. The distribution of size of particles in a sample can be selected to permit optimal deposition within targeted sites within the respiratory tract. The ALLP incorporating a therapeutic drug, are more capable of escaping inertial and gravitational deposition in the oropharyngeal region, and are targeted to the airways or the deep lung. The use of larger particles (mean diameter at least about 5 μm) is advantageous since they are able to aerosolize more efficiently than smaller, non-light aerosol particles such as those currently used for inhalation therapies. In comparison to smaller non-light particles, the larger (at least about 5 μm) aerodynamically light particles also can potentially more successfully avoid phagocytic engulfment by alveolar macrophages and clearance from the lungs, due to size exclusion of the particles from the phagocytes' cytosolic space. Phagocytosis of particles by alveolar macrophages diminishes precipitously as particle diameter increases beyond 3 μm (Kawaguchi et al, 1986; Krenis et al, 1961 and Rudt et al, 1992). For particles of statistically isotropic shape (on average, particles of the powder possess no distinguishable orientation), such as spheres with rough surfaces, the particle envelope volume is approximately equivalent to the volume of cytosolic space required within a macrophage for complete particle phagocytosis. ALLP thus are capable of a longer term release of a therapeutic agent. Following inhalation, aerodynamically light biodegradable particles can deposit in the lungs (due to their relatively low tap density), and subsequently undergo slow degradation and drug release, without the majority of the particles being phagocytosed by alveolar macrophages.

The drug can be delivered relatively slowly into the alveolar fluid, and at a controlled rate into the blood stream, minimizing possible toxic responses of exposed cells to an excessively high concentration of the drug. The ALLP thus are highly suitable for inhalation therapies, particularly in controlled release applications.

The particles may be fabricated with the appropriate material, surface roughness, diameter and tap density for localized delivery to selected regions of the respiratory tract such as the deep lung or upper airways. For example, higher density or larger particles may be used for upper airway delivery, or a mixture of different sized particles in a sample, provided with the same or different therapeutic agent may be administered to target different regions of the lung in one administration.

2.8.1.2 Particle Density and Deposition

"Aerodynamically light particles" refers to particles having a tap density less than about 0.4 g/cm^3. Tap density is a standard measure of the envelope mass density. The envelope mass density of an isotropic particle is defined as the mass of the particle divided by the minimum sphere envelope volume within which it can be enclosed. Inertial impaction and gravitational settling of aerosols are predominant deposition mechanisms in the airways and acini of the lungs during normal breathing conditions (Edwards, 1995). The importance of both deposition mechanisms increases in proportion to the mass of aerosols and not to particle (or envelope) volume. Since the site of aerosol deposition in the lungs is determined by the mass of the aerosol (at least for particles of mean aerodynamic diameter greater than approximately 1 μm), diminishing the tap density by increasing particle surface irregularities and particle porosity permits the delivery of larger particle envelope volumes into the lungs, all other physical parameters being equal. The low tap density particles have a small aerodynamic diameter in comparison to the actual envelope sphere diameter.

2.8.2 Aerodynamically Light and Large Particles Composition

ALLP are designed with aid of novel excipients, which are toxicologically innocuous when inhaled as a dispersed powder and do not significantly; interact with the active agent in a manner that adversely affects the desired physiological action of the agent. Excipients that are particularly useful in this regard are surfactants and/or co-surfactants at various concentrations. Moreover, a dispersibility enhancing agent (s) also have been incorporated and evaluated to investigate the synergistic effect with respect to deep lung targeting efficiency in terms of fine particle fraction (FPF)/respirable fraction. The amount of excipient that is useful in the composition of this invention is an amount that serves to uniformly distribute the active agent throughout the composition so that it can be uniformly dispersed

when it is to be delivered to a subject in need thereof. It must also serve to dilute the active agent to a concentration at which the active agent can provide the desired beneficial or curative effects. At the same time, it minimizes the adverse effects due to reduced dose requirement. The amount of excipient (s) in the formulation varies between 30%w/w to 80% w/w of the total composition.

2.8.2.1 Surfactants/Co-surfactants

The excipient (s) may include one or more combinations of surfactants/ co-surfactants and inert bio-acceptable materials for inhalation. Surfactants which can be incorporated into dry powder formulations to improve their aerosolization properties include phospholipids, from both natural and synthetic origin are particularly compatible and may be used in varying proportions (Vanbever et al, 1999). The surfactants improve surface properties by reducing particle-particle interactions, and render the surface of the particles less adhesive. The use of surfactants endogenous to the lung can potentially reduce opsonization (and thereby reducing phagocytosis by alveolar macrophages), thus providing a long -lived particle in the lung releasing drug in a controlled and predetermined manner and also avoid the use of non-physiologic surfactants (Carmen et al, 1998) . Due to their excellent biocompatibility characteristics, phospholipids /combinations of phospholipids and surfactants/co- surfactants are suitable for use in the pharmaceutical embodiments. Compatible phospholipids having gel to liquid crystalline phase transition greater than 35°C and preferably above 55°C, either water soluble or dispersible such as soya/egg /synthetic or semi synthetic lecithins are used. Other phospholipids includes, dipalmitoylphosphatidylcholine,disteroylphosphatidylcholine, diarachidoylphosphatidylcholine dibehenoylphosphatidylcholine, short-chain phosphatidylcholines, long-chain saturated phosphatidylethanolamines, long-chain saturated phosphatidylserines, long-chain saturated phosphatidylglycerols, long-chain saturated phosphatidylinositols, glycolipids etc.

Co-surfactants those are useful in the design of ALLP include, group comprising of sorbitan esters such as sorbitan trioleate (Span 85), sorbitan sesquioleate, sorbitan monooleate, sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, oleyl polyoxyethylene ether, stearyl polyoxyethylene ether, lauryl polyoxyethylene ether, glycerol esters, and sucrose esters. Preferred block copolymers include diblock and triblock copolymers of polyoxyethylene and polyoxypropylene, including poloxamer 188, poloxamer 407 and poloxamer 338. Ionic surfactants such as sodium sulfosuccinate and fatty acid soaps may also be utilized (Tarara et al, 2003).

2.8.2.2 Anti-adherents

The additive materials also include an anti-adherent, which reduces tendency of the particles to bond strongly either to each other or to the device itself, reduces powder cohesion & adhesion thereby promotes better flow characteristics which leads to improvements in the dose reproducibility. Materials those thought of as anti-adherent includes one or more compounds selected from amino acids and derivatives thereof. Amino acids, peptides and polypeptides and their derivatives are physiologically acceptable and act as anti-adherent materials when added to the active material. It is particularly advantageous for the additive material to comprise an amino acid (Lucas et al, 1999). Amino acids have been found to give high respirable fraction of the active material and also good flow properties of the powder. Amino acids having relatively low solubilities in water, e.g., from about 10 mg/ml to about 75 mg/ml are useful as effective anti adherents. Not to be bound by any theory, reduced aqueous solubility lends to decreasing moisture sorption and delayed crystallization in the resulting spray dried powder, both of which are desirable characteristics for a respirable powder (e.g., in this regard, leucine is preferred over histidine which is preferred over alanine which is preferred over glycine). Also preferred are amino acids having somewhat large Van der Waals volumes, e.g., greater than about 100 A^3, e.g., isoleucine, leucine, lysine, methionine and phenylalanine. Increasing Van der Waals volume tends to correlate with increased glass transition temperature (Tg) of the resulting pharmaceutical powder, thus indicating greater storage stability. Also preferred are amino acids having a glass transition temperature greater than 40°C and preferably 70°C or greater. The inclusion of such amino acids in spray dried/lyophilized powders typically improve the aerosol performance, and in particular, mass median aerodynamic diameter (MMAD) and emitted dose (ED), by about 10-25%, whilst the L-form of the amino acids is preferred over D- and DL-forms. Improvement in aerosol performance is due to its surface activity, which tends to concentrate on the surface of particles, i.e. the concentration of leucine on the surface of spray dried/lyophilized particles is typically greater than in the bulk powder. Other surface active amino acids which tend to concentrate on the surface of spray dried protein particles include asparagine, isoleucine, phenylalanine, tryptophan, tyrosine, norleucine and valine, derivatives of amino acids and/or peptides. For example, may be a salt or an ester such as aspartame, N acetyl-L cysteine, acesulfame K or other sweeteners like saccharin sodium or cyclamate. The powder preferably comprises between about 5% and 20%, more preferably between about 10% and 15% by weight of additive material based on the weight of the powder. Other anti adherents may include or consist of one or more water insoluble surface active materials, for

example solid state fatty acids such as lauric acid, palmitic acid, stearic acid, erucic acid, behenic acid, or derivatives (such as esters and salts) thereof. Specific examples of such materials are: magnesium stearate; sodium stearyl fumarate; sodium stearyl lactylate etc (Staniforth, 2003). Other possible additive materials include talc, titanium dioxide, aluminium dioxide, silicon dioxide and starch.

2.8.2.3 Polymers and Rigidifying agents

ALLP optionally comprises of synthetic or natural polymers or combinations thereof. The particles may be formed from any biocompatible and preferably biodegradable polymer, copolymer, or blend, which is capable of forming particles having a tap density less than about 0.4 g/cm^3. Surface eroding polymers such as polyanhydrides may be used to form the aerodynamically light particles. Bulk eroding polymers such as those based on polyesters including poly(hydroxy acids) like polyglycolic acid (PGA) or polylactic acid (PLA) or copolymers thereof may be used to form the aerodynamically light particles, wherein the polyester has incorporated therein a charged or functionalizable group such as an amino acid. Other polymers include polyamides, polycarbonates, polyalkylenes, poly vinyl compounds, polymers of acrylic and methacrylic acids, celluloses and other polysaccharides, and peptides or proteins, or copolymers or blends thereof which are capable of forming aerodynamically light particles with a tap density less than about 0.4 g/cm^3. Polymers may be selected with or modified to have the appropriate stability and degradation rates in vivo for different controlled drug delivery applications.

Besides the aforementioned polymer materials and surfactants, it may be desirable to add other excipients to ALLP formulation to improve particle rigidity, production yield, delivery efficiency and deposition, shelf-life and patient acceptance. Such optional excipients include, but are not limited to: coloring agents, taste masking agents, buffers, hygroscopic agents, antioxidants, and chemical stabilizers. Further, various excipients may be incorporated in, or added to, the particulate matrix to provide structure and form to the perforated microstructures (Tarara et al, 2003). Other rigidifying excipients may include, carbohydrates including monosaccharides, disaccharides and polysaccharides. For example, monosaccharides such as dextrose (anhydrous and monohydrate), galactose, mannitol, D-mannose, sorbitol, sorbose and the like; disaccharides such as lactose, maltose, sucrose, trehalose, and the like; trisaccharides such as raffinose and the like; and other carbohydrates such as starches (hydroxyethylstarch), cyclodextrins and maltodextrins. Amino acids are also suitable excipients with glycine preferred. The inclusion of both inorganic (e.g. sodium chloride, calcium chloride, etc.), organic salts (e.g. sodium citrate, sodium ascorbate,

magnesium gluconate, sodium gluconate, tromethamine hydrochloride, etc.) and buffers is also contemplated. The inclusion of salts and organic solids such as ammonium carbonate, ammonium acetate, ammonium chloride or camphor are also contemplated.

2.8.3 Formation of Aerodynamically Light and Large Particles

With regard to the formation ALLP, the particles are formed by techniques including spray drying, vacuum drying, solvent extraction, emulsification or lyophilization, and combinations thereof and any similar process using suitable equipment. The first step in particulate production typically comprises feed stock preparation. The selected drug is either dissolved or dispersed in water to produce a concentrated solution. Alternatively, the drug may be incorporated in the form of a solid particulate dispersion. The concentration of the active or bioactive agent used is dependent on the amount of agent required in the final powder and the performance of the delivery device employed.

Spray drying is a one-step process that converts a liquid feed to a dried particulate form. Spray drying has been used to provide powdered material for various administrative routes including inhalation (Hicky, 1996). In general, spray drying consists of bringing together a highly dispersed liquid, and a sufficient volume of hot air to produce evaporation and drying of the liquid droplets. The preparation to be spray dried or feed (or feed stock) can be any solution, course suspension, slurry, colloidal dispersion, or paste that may be atomized using the selected spray drying apparatus. The feed stock will comprise a colloidal system such as an emulsion, reverse emulsion, microemulsion, multiple emulsion, particulate dispersion, or slurry. Typically the feed is sprayed into a current of warm filtered air that evaporates the solvent and conveys the dried product to a collector. The spent air is then exhausted with the solvent.

While the resulting spray-dried powdered particles typically are approximately spherical in shape, nearly uniform in size and frequently are hollow, there may be some degree of irregularity in shape depending upon the incorporated medicament and the spray drying conditions. In many instances dispersion stability and dispersibility of the particles appears to be improved if an inflating agent (or blowing agent) is used in their production. The system may comprise an emulsion with the inflating agent as the disperse or continuous phase. The inflating agent is preferably dispersed with a surfactant solution, using, for instance, a commercially available microfluidizer at a pressure of about 5000 to 15,000 psi. This process forms an emulsion, stabilized by an incorporated surfactant, typically comprising submicron droplets of water immiscible blowing agent dispersed in an aqueous continuous phase. The blowing agent is preferably a fluorinated compound (e.g. perfluorohexane, perfluorooctyl

bromide, perfluorodecalin, perfluorobutyl ethane) which vaporizes during the spray-drying process, leaving behind generally hollow, porous aerodynamically light particles. Other suitable liquid blowing agents include nonfluorinated oils, chloroform, Freons, ethyl acetate, alcohols and hydrocarbons. Nitrogen and carbon dioxide gases are also contemplated as a suitable blowing agent.

Besides incorporation of blowing agents, inorganic and organic substances which can be removed under reduced pressure by sublimation in a post-production step are also compatible in the formation of ALLP. These sublimating compounds can be dissolved or dispersed as micronized crystals in the spray drying feed solution and include ammonium carbonate and camphor.

Along with spray drying, ALLP may be formed by lyophilization. Lyophilization is a freeze-drying process in which water is sublimed from the composition after it is frozen. The particular advantage associated with the lyophilization process is that biologicals and pharmaceuticals that are relatively unstable in an aqueous solution can be dried without elevated temperatures (thereby eliminating the adverse thermal effects), and then stored in a dry state where there are few stability problems. The lyophilized cake containing a fine foam-like structure can be micronized using techniques to provide 3 to 10 μm sized particles.

ALLP may also be formed using a method where a feed solution (either emulsion or aqueous) containing wall forming agents is rapidly added to a reservoir of heated oil (e.g. perflubron or other high boiling FCs) under reduced pressure. The water and volatile solvents of the feed solution rapidly boils and are evaporated. This process provides a perforated structure from the wall forming agents similar to puffed rice or popcorn. Preferably the wall forming agents are insoluble in the heated oil. The resulting particles can then separated from the heated oil using a filtering technique and subsequently dried under vacuum.

Double emulsion method may be employed for the preparation of ALLP. In the double emulsion method the medicament is first dispersed in a polymer dissolved in an organic solvent (e.g. methylene chloride) by sonication or homogenization. This primary emulsion is then stabilized by forming a multiple emulsion in a continuous aqueous phase containing an emulsifier such as polyvinylalcohol. Evaporation or extraction using conventional techniques and apparatus then removes the organic solvent.

2.9 DEVELOPMENT OF DRY POWDER INHALATION FORMULATION

Of critical importance in the development of DPI products is the evaluation, optimization, and control of flow and dispersion (deaggregation) characteristics of the formulation. These properties are a function of the principal adhesive forces that exist between particles

including Van der Waals forces, electrostatic forces, and the surface tension of the adsorbed liquid layer (Hinds et al, 1982). These forces are influenced by several fundamental physicochemical properties including particle density and size distribution, particle morphology (shape, habit, surface, texture), and surface composition (including adsorbed moisture). In combination with dry powder formulations, particles pose the additional problem of offering electrostatically charged surfaces for collection of drug particles. Interparticle forces, which influence flow and dispersion properties, are particularly dominant in micronized or microcrystalline powders required for inhalation therapy (<5 μm). It has been demonstrated that powder adhesion, mediated in part by Vander Walls forces, is directly related to the presence of particles <10 μm. In the case of sodium chromoglycate, several approaches have been successfully used to improve flow and dispersion characteristics, including the use of drug blends with coarse-particle lactose and controlled aggregation of the undiluted drug to form loosely adherent flocs (Moren et al, 1985).

It is imperative, during early development, to characterize the moisture sorption and desorption attributes of the drug in relation to available salt forms. Assuming solubility is sufficient to ensure adequate absorption, and then a non-hygroscopic form should be explored. This would confer a number of advantages, including improved flow properties and dispersion as well as enhanced physical stability in the drug and final dosage form due to minimal moisture transfer between the drug, immediate container (e.g. gelatin capsule cell), and the environment. Further more, improved chemical stability may result in the case of hydrolytically labile drug (Yoshioka et al, 1990). Hygroscopic growth during administration would also be minimized. Although inherently attractive, the approach of using non hygroscopic drug forms must be applied with caution because, in the case of insulating particles, the level of adsorbed moisture may not be sufficient to dissipate attractive electrostatic forces, resulting in particle adhesion.

Particle morphology, including attributes such as crystal habit, surface texture and porosity also influence particle adhesion. An anisometric particle, that is those with extreme "elongation" or "flatness" ratios, tend to build up packing of high porosity, but they are also more readily deformed by compression than packaging of isometric particles. Anisometric particles tend to align along their long axis during flow and thus, exhibit less internal friction than isometric particles. Powder flow tends to be adversely affected by surface roughness and porosity. Generation of microcrystals that fall within the able range (<5 μm) by recrystallisation or precipitation is rarely possible. Instead, the drug must be micronized in a ball mill or a jet mill, significantly altering the morphology. It is important to evaluate the

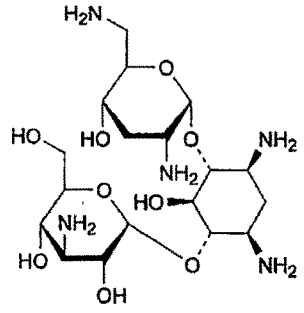
drug after milling to ensure consistency with the parent in terms of polymorphic forms. However, prediction of powder rheology based on the potential interplay a number of physicochemical properties is extremely complicated. Instead, flow and dispersion properties are generally characterized using appropriate derived properties including, but not limited to, angle of repose, bulk density, compressibility, and dustability. It is important to identify and control critical parameters, both fundamental and derived, to ensure optimum and consistent product performance. Environmental factors including temperature, humidity, and light essential considerations during formulation development. Therefore, it is imperative to evaluate the influence of these factors on the physical and chemical stability of the formulation during early preformulation studies. Light exposure may usually be controlled by judicious choice of product packaging; however, temperature and humidity are not so easily controlled, and they often act in concert to promote product degradation. The effects of elevated temperature and humidity on product stability can be assessed after stress storage. Yoshioka and Cartensen (1990) recently proposed several useful kinetic models for the accelerated testing of solid pharmaceuticals based on isothermal storage at controlled elevated temperature and controlled elevated humidity. Temperature or humidity cycling experiments is also useful, particularly for assessing potential physical charges.

Chemical degradation after stress storage is assessed using an appropriate stability-indicating assay. In addition, physical changes are evaluated using an array of techniques available to the preformulation scientist, including polarized light microscopy (aggregation, crystal growth), differential scanning calorimetery, infrared spectroscopy, X-ray diffractometery, solution calorimetery, thermogravimetric analysis, and hot-stage microscopy (moisture uptake, polymorph interconversion, pseudopolymorph formulation). Stressed stored samples should also be evaluated for evidence of caking and discoloration.

2.10 DRUG PROFILES

2.10.1 Tobramycin sulfate

Tobramycin sulfate, a water-soluble antibiotic of the aminoglycoside group, is derived from the actinomycete *Streptomyces tenebrarius*. Tobramycin sulfate is O-3-amino-3-deoxy-a-D-glucopyranosyl(1—>4)-O-[2,6-diamino-2,3,6-trideoxy-a-D-ribo-hexopyranosyl-(1—>6)]-2-deoxy-L-streptamine, sulfate (2:5)(salt) and has the molecular formula $(C_{18}\ H_{37}N_5O_9)_2 \cdot 5\ H_2SO_4$ with a molecular weight of 1425.45.



$. 5\ H_2SO_4$

CAS Registry number: [79645-27-5]

2.10.1.1 Physico-chemical Properties

A white to almost white , freely soluble in water, very slightly soluble in alcohol .The melting point occurs at approximately 217°C. The pH of a 4% aqueous solution is 6-8.0. pK_a are 6.7, 8.3 and 9.9.It has octanol/water (Log P) partition coefficient of 6.899.

2.10.1.2 Pharmacology and Pharmacokinetics

Tobramycin is actively transported across the bacterial cell membrane, irreversibly binds to one or more specific receptor proteins on the 30 S subunit of bacterial ribosomes, and interferes with an initiation complex between messenger RNA (mRNA) and the 30 S subunit. DNA may be misread, thus producing nonfunctional proteins; polyribosomes are split apart and are unable to synthesize protein. This results in accelerated aminoglycoside transport, increasing the disruption of bacterial cytoplasmic membranes, and eventual cell death

Rapidly and completely absorbed after intramuscular administration. Poorly absorbed from intact gastrointestinal tract after oral administration, but may accumulate in patients with renal failure. On local or topical application, it may also be absorbed in significant amounts from body surfaces (except urinary bladder) following local irrigation or topical application. Intraperitoneal and intrapleural administration results in rapid absorption (USP DI, 2001).

Tobramycin is rapidly absorbed following intramuscular administration. Peak serum concentrations of tobramycin occur between 30 and 90 minutes after intramuscular administration. Following an intramuscular dose of 1 mg/kg of body weight, maximum serum

concentrations reach about 4 mcg/mL, and measurable levels persist for as long as 8 hours. Therapeutic serum levels are generally considered to range from 4 to 6 mcg/mL. In patients with normal renal function, except neonates, tobramycin administered every 8 hours does not accumulate in the serum. However, in those patients with reduced renal function and in neonates, the serum concentration of the antibiotic is usually higher and can be measured for longer periods of time than in normal adults. Following parenteral administration, little, if any, metabolic transformation occurs, and tobramycin is eliminated almost exclusively by glomerular filtration. Renal clearance is similar to that of endogenous creatinine. Ultrafiltration studies demonstrate that practically no serum protein binding occurs. In patients with normal renal function, up to 84% of the dose is recoverable from the urine in 8 hours and up to 93% in 24 hours. Peak urine concentrations ranging from 75 to 100 mcg/mL have been observed following the intramuscular injection of a single dose of 1 mg/kg. After several days of treatment, the amount of tobramycin excreted in the urine approaches the daily dose administered. When renal function is impaired, excretion of Nebcin is slowed, and accumulation of the drug may cause toxic blood levels. The serum half-life in normal individuals is 2 hours. An inverse relationship exists between serum half-life and creatinine clearance, and the dosage schedule should be adjusted according to the degree of renal impairment .Tobramycin can be detected in tissues and body fluids after parenteral administration. Concentrations in bile and stools ordinarily have been low, which suggests minimum biliary excretion. Tobramycin has appeared in low concentration in the cerebrospinal fluid following parenteral administration, and concentrations are dependent on dose, rate of penetration, and degree of meningeal inflammation. It has also been found in sputum, peritoneal fluid, synovial fluid, and abscess fluids, and it crosses the placental membranes. Concentrations in the renal cortex are several times higher than the usual serum levels.

Microbiology

Gram-positive: Staphylococcus aureus

Gram-negative: Citrobacter species,Enterobacter species ,Escherichia coli ,Klebsiella species,Morganella morganii ,Pseudomonas aeruginosa ,Proteus mirabilis ,Proteus vulgaris ,Providencia species ,Serratia species.

Aminoglycosides have a low order of activity against most gram-positive organisms, including *Streptococcus pyogenes, Streptococcus pneumoniae,* and *enterococci.* Although most strains of enterococci demonstrate *in vitro* resistance, some strains in this group are susceptible.*In vitro* studies have shown that an aminoglycoside combined with an antibiotic

that interferes with cell-wall synthesis affects some enterococcal strains synergistically. The combination of penicillin G and tobramycin results in a synergistic bactericidal effect *in vitro* against certain strains of *Enterococcus faecalis.* However, this combination is not synergistic against other closely related organisms, eg, *Enterococcus faecium.* Speciation of enterococci alone can not be used to predict susceptibility. Susceptibility testing and tests for antibiotic synergism are emphasized. Cross resistance between aminoglycosides may occur.

2.10.1.3 Therapeutic use

Tobramycin is indicated for the treatment of serious bacterial infections caused by susceptible strains of the designated microorganisms in the following diseases :

Septicemia in the pediatric patient and adult caused by. *P. aeruginosa, E. coli,* and *Klebsiella* spp

Lower respiratory tract infections caused by *P.aeruginosa, Klebsiella* spp, *Enterobacter* spp, *Serratia* spp, *E.coli,* and *S.aureus* (penicillinase and non-penicillinase producing strains)

Serious central-nervous-system infections (meningitis) caused by susceptible organisms

Intra-abdominal infections, including peritonitis, caused by *E. coli, Klebsiella* spp, and *Enterobacter* spp.

Skin, bone, and skin structure infections caused by *P.aeruginosa, Proteus* spp, *E. coli, Klebsiella* spp, *Enterobacter* spp, and *S.aureus*

Complicated and recurrent urinary tract infections caused by *P.aeruginosa, Proteus* spp (indole-positive and indole-negative),*E. coli, Klebsiella* spp, *Enterobacter* spp, *Serratia* spp, *S.aureus, Providencia* spp, and *Citrobacter* spp

2.10.1.4 Adverse effects

Neurotoxicity

Adverse effects on both the vestibular and auditory branches of the eighth nerve have been noted, especially in patients receiving high doses or prolonged therapy, in those given previous courses of therapy with an ototoxin, and in cases of dehydration. Symptoms include dizziness, vertigo, tinnitus, roaring in the ears, and hearing loss. Hearing loss is usually irreversible and is manifested initially by diminution of high-tone acuity. Tobramycin and gentamicin sulfates closely parallel each other in regard to ototoxic potential.

Nephrotoxicity

Renal function changes, as shown by rising BUN, NPN, and serum creatinine and by oliguria, cylindruria, and increased proteinuria, have been reported, especially in patients with a history of renal impairment who are treated for longer periods or with higher doses than those recommended. Adverse renal effects can occur in patients with initially normal renal funotion.

Other reported adverse reactions possibly related to tobramycin include anemia, granulocytopenia, and thrombocytopenia; and fever, rash, exfoliative dermatitis, itching, urticaria, nausea, vomiting, diarrhea, headache, lethargy, pain at the injection site, mental confusion, and disorientation.

2.10.1.5 Methods for Estimation

Spectrophotometric method: Colorimetric methods based on reaction of tobramycin with copper sulfate and another based on reaction with 2, 4-dinitrofluorobenzene has been reported for the quantification of tobramycin.

High Pressure Liquid Chromatography (HPLC): Since tobramycin has poor absorbance in UV and visible region, it is derivatized with absorbance enhancing or fluorescence-producing agents like *o*-pthaladehyde, 2, 4, 6 trinitrobenzenesulfonic acid etc. HPLC method of analysis is official method of analysis in U.S.P, B.P and European pharmacopoeia.

Gas liquid Chromatography (GLC): A silanized pyrex column utilized nitrogen as a carrier gas.

Microbiological assay: Potency is determined by turbid metric method using Bacillus subtilis (ATCC 6633).

Fluorescenceimmunoassay (FIA): FIA uses the principle of competitive protein binding and has been used to quantify tobramycin in body fluids. Competitive binding reactions are set up with fluorogenic tobramycin reagent, a limiting amount of antibody against the drug and biological sample to be analyzed.

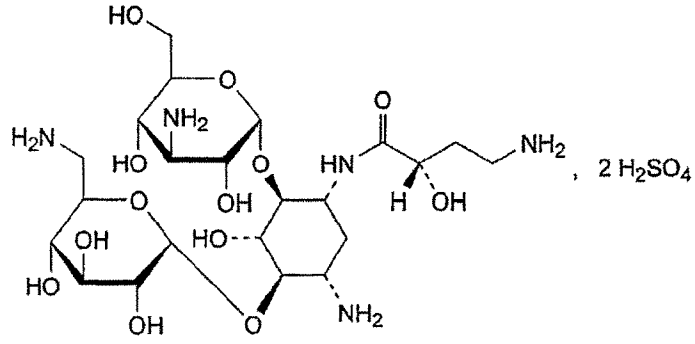
Fluorescence Polarization immunoassay (FPI): FPI is a method that combines the principle of competitive protein binding with the principle of fluorescence polarization.

Radioimmunoassay (RIA): A very sensitive method of estimation in body fluids. ^{125} I-used as labeling agent.

Radioenzymatic assay (REA): The method involves specific enzymatic transfer of radioactive modifying group to the drug.

2.10.2 Amikacin sulfate

Amikacin sulfate is a semi-synthetic aminoglycoside antibiotic derived from kanamycin. D-Streptamine,O-3-amino-3-deoxy-a-b-glucopyranosyl)1>6)-O-[6-amino-6-deoxy-a-D-glucopyranosyl(1>4)]-N1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-(S)-,sulfate (1:2)(salt), and has the molecular formula $C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$ with a molecular weight of 781.75.



CAS Registry number: [39831-55-5]

2.10.2.1 Physico-chemical Properties

A white or almost white crystalline powder, freely soluble in water, practically insoluble in acetone and in alcohol. The melting point occurs between 203 and 204°C. There are four reported pK_a values; 8.4, 6.7, 9.7 and 8.4 (Kane et al, 2001). If the four amino groups of amikacin considered being equivalent, tha apparent pK_a value is 8.1. The pH of a 1% aqueous solution is 2.0-4.0. It has octanol/water partition coefficient (Log P) of 9.048.

2.10.2.2 Pharmacology and Pharmacokinetics

Amikacin is actively transported across the bacterial cell membrane, irreversibly binds to one or more specific receptor proteins on the 30 S subunit of bacterial ribosomes, and interferes with an initiation complex between messenger RNA (mRNA) and the 30 S subunit. DNA may be misread, thus producing nonfunctional proteins; polyribosomes are split apart and are unable to synthesize protein. This results in accelerated aminoglycoside transport, increasing the disruption of bacterial cytoplasmic membranes, and eventual cell death

Amikacin rapidly and completely absorbed after intramuscular administration. Poorly absorbed from intact gastrointestinal tract after oral administration, but may accumulate in patients with renal failure. On local or topical application, it may also be absorbed in significant amounts from body surfaces (except urinary bladder) following local irrigation or topical application. Intraperitoneal and intrapleural administration results in rapid absorption (USP DI, 2001).

Pharmacokinetic studies in normal adult subjects reveal the mean serum half life to be slightly over 2 hours with a mean total apparent volume of distribution of 24 liters (28% of the body weight). By the ultra filtration technique, reports of serum protein binding range from 0 to 11%. The mean serum clearance rate is about 100 mL/min and the renal clearance rate is 94 mL/min in subjects with normal renal function. Amikacin is excreted primarily by glomerular filtration. Patients with impaired renal function or diminished glomerular filtration pressure excrete the drug much more slowly (effectively prolonging the serum half-life). Therefore, renal function should be monitored carefully and dosage adjusted accordingly. Following administration at the recommended dose, therapeutic levels are found in bone, heart, gallbladder, and lung tissue in addition to significant concentrations in urine, bile, sputum, bronchial secretions, interstitial, pleural, and synovial fluids. Spinal fluid levels in normal infants are approximately 10% to 20% of the serum concentrations and may reach 50% when the meninges are inflamed. Amikacin has been demonstrated to cross the placental barrier and yield significant concentrations in amniotic fluid. The peak fetal serum concentration is about 16% of the peak maternal serum concentration and maternal and fetal serum half-life values are about 2 and 3.7 hours, respectively.

Microbiology

Gram-negative: Amikacin is active *in vitro* against Pseudomonas species, Escherichia coli, Proteus species (indole-positive and indole-negative), Providencia species, Klebsiella-Enterobacter-Serratia species, Acinetobacter (formerly Mima-Herellea) species, and Citrobacter freundii. When strains of the above organisms are found to be resistant to other aminoglycosides, including gentamicin, tobramycin and kanamycin, many are susceptible to amikacin *in vitro.*

Gram-positive: Amikacin sulfate is active *in vitro* against penicillinase and nonpenicillinase-producing Staphylococcus species including methicillin-resistant strains. However, aminoglycosides in general have a low order of activity against other *Gram-positive organisms:* viz, Streptococcus pyogenes, enterococci, and Streptococcus pneumoniae (formerly Diplococcus pneumoniae). Amikacin resists degradation by most aminoglycoside inactivating enzymes known to affect gentamicin, tobramycin, and kanamycin. *In vitro* studies have shown that amikacin sulfate combined with a beta-lactam antibiotic acts synergistically against many clinically significant Gram-negative organisms.

2.10.2.3 Therapeutic Use

Amikacin sulfate injection is indicated in the short-term treatment of serious infections due to susceptible strains of Gram-negative bacteria, including Pseudomonas species, Escherichia

coli, species of indole-positive and indole-negative Proteus, Providencia species, Klebsiella-Enterobacter-Serratia species, and Acinetobacter (Mima-Herellea) species. Clinical studies have shown amikacin sulfate injection to be effective in bacterial septicemia (including neonatal sepsis); in serious infections of the respiratory tract, bones and joints, central nervous system (including meningitis) and skin and soft tissue; intra-abdominal infections (including peritonitis); and in burns and post operative infections (including post vascular surgery). Clinical studies have shown amikacin also to be effective in serious complicated and recurrent urinary tract infections due to these organisms. Aminoglycosides, including amikacin sulfate injection, are not indicated in uncomplicated initial episodes of urinary tract infections unless the causative organisms are not susceptible to antibiotics having less potential toxicity.

Amikacin has also been shown to be effective in staphylococcal infections and may be considered as initial therapy under certain conditions in the treatment of known or suspected staphylococcal disease such as, severe infections where the causative organism may be either a Gram-negative bacteriumora staphylococcus, infections due to susceptible strains of staphylococci in patients allergic to other antibiotics, and in mixed staphylococcal/Gram-negative infections. In certain severe infections such as neonatal sepsis, concomitant therapy with a penicillin-type drug may be indicated because of the possibility of infections due to Gram-positive organisms such as streptococci or pneumococci.

2.10.2.4 Adverse Effects

All aminoglycosides have the potential to induce auditory, vestibular, and renal toxicity and neuromuscular blockade. They occur more frequently in patients with present or past history of renal impairment, of treatment with other ototoxic or nephrotoxic drugs, and in patients treated for longer periods and/or with higher doses than recommended.

Neurotoxicity-Ototoxicity: Toxic effects on the eighth cranial nerve can result in hearing loss, loss of balance, or both. Amikacin primarily affects auditory function. Cochlear damage includes high frequency deafness and usually occurs before clinical hearing loss can be detected.

Neurotoxicity-Neuromuscular Blockage: Acute muscular paralysis and apnea can occur following treatment with aminoglycoside drugs.

Nephrotoxicity: Elevation of serum creatinine, albuminuria, presence of red and white cells, casts, azotemia, and oliguria have been reported. Renal function changes are usually reversible when the drug is discontinued.

2.10.2.5 Methods for Estimation

Microbiological assay: Potency is determined by turbid metric method using staphylococcus aureus (ATCC 6538).

High Pressure Liquid Chromatography (HPLC): Since amikacin has poor UV absorbance, it is derivatized as a part of HPLC procedure. HPLC method of analysis is official method of analysis in U.S.P, B.P and European pharmacopoeia.

Gas liquid Chromatography (GLC): GLC estimation involves derivatization of amikacin with N-trimethylsilyl imidazole followed by N-heptafluorobutyryl imidazole.

Fluoroscenceimmunoassay (FIA): Amikacin in body fluids is estimated by FIA, where amikacin is labeled by the fluorogenic enzyme substrate ß-galactosylumbelliferone.

Radioimmunoassay (RIA): A very sensitive method of estimation in body fluids. ^{125} I-used as labeling agent.

Radioenzymatic assay (REA): Acetyltransferase method is used. It is a rapid and accurate method of estimation.

Spectrophotometric-Enzymatic assay: The method uses a purified kanamycin acetyltransferase followed by derivatization of acetylation byproduct.

2.11 REFERENCES

Adjei A. L., and Gupta P. K. Inhalation Delivery of Therapeutic Peptides and Proteins Marcel Dekker Inc., New York. 1997.

Anderson P.J., Wilson J.D., and Hiller F.C. Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. Chest. 1990; 97(5):1115–1120.

Armstrong D.S., Grimwood K., and Carzino R. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. Brit. Med. J. 1995; 310: 1571 -1572.

Ashurst I., Malton A., Prime D., and Sumby B. Latest advances in the development of dry powder inhalers. Pharm. Sci. Technol. Today. 2000;3: 246-256.

Aurora P., Wade A., and Whitmore P. A model for predicting life expectancy of children with cystic fibrosis. Eur. Respir. J. 2000; 6: 1056-1060.

Auerbach H. S., Williams M., and Kirkpatrick J. A. Alternate-day prednisolone reduces morbidity and improves pulmonary function in cystic fibrosis. Lancet. 1985; 2: 686 - 688.

Auty R.M., Brown K., Neale M.G., and Snashall P.D. Respiratory tract deposition of sodium cromoglycate is highly dependent upon technique of inhalation using the Spinhaler. Brit. J. Dis. Chest.1987; 81: 371-380.

Begon D., Guillaume P., Kohl M. Process for producing fine medicinal substances, WO 0114036. 2001.

Bot A .I., Tarara T.E., Smith D.J., Bot S.R., Woods C.M., and Weers J.G. Novel lipid-based hollow-porous microparticles as a platform for immunoglobulin delivery to the respiratory tract. Pharm. Res. 2000; 17:275–283.

Berner B., Fyrnys B., De Boer A., Gottenauer W., Wolf-Heuss E. Asta Medica multidose dry powder inhaler. In: Respiratory Drug Delivery VI(Eds Byron P. R., Dalby R. N., and Farr S. J.). Interpharm Press, IL.1998; 475-478.

Byron P. R. Aerosol formulation, generation and delivery using metered systems. In: Respiratory Drug Delivery (Ed Byron P. R.). CRC Press, Boca Raton, FL.1990;167-205.

Byron P.R. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J. Pharm. Sci.1986; 75(5):433–438.

Byron P. Some future perspectives for unit dose inhalation aerosols. Drug Dev. Ind. Pharm. 1976;12:993–1015.

Byron P. Pulmonary targeting with aerosols. Pharm. Tech. 1987;11: 42–56.

Byron P.R., and Patton J.S. Drug delivery via the respiratory tract. J. Aerosol Med. 1994;7(1):49–75.

Britto L. Optimal design of dry powder inhalers using fundamental fluid dynamics principles. In: Respiratory Drug Delivery VI (Eds Byron P. R., Dalby R. N., Farr S. J.). Interpharm Press, IL.1998; 312-313.

Bell J.H., Hartley P.S., and Cox J.S.G. Dry powder aerosols 1: A new powder inhalation device J. Pharm.Sci. 1971; 60: 1559-1564.

Brindley A., Sumby B.S., Smith I.J., Prime D., Haywood P.A., and Grant A.C. Design, manufacture and dose consistency of the Serevent Diskus inhaler. Pharm. Tech. Eur. 1995;7:14-22.

Bailey M. R., Birchall A., Cuddihy R.G., James A.C., and Roy M. Respiratory tract clearance model for dosimetry and bioassay of inhaled radionuclides. Radiat. Prot. Dosim. 1991;38:153-158.

Booth S.J. Dose reliability of the Servent Diskhaler systems. Pharm. Tech. Int. 1993; June: 20-27.

Brindley A., Sumby B.S., Smith I.J., Prime D., Haywood P.A., and Grant A.C. Design, manufacture and dose consistency of the Serevent Diskus inhaler. Pharm. Tech. Eur. 1995; 7: 4-22.

Braun M.A., Oschmann R., and Schmidt P.C. Influence of excipients and storage humidity on the deposition of Disodium cromolgycate (DSCG) in the twin impinger. Int. J. Pharm.1996;135: 53-62.

Boerefijn R., Ning Z., and Ghadiri M. Disintegration of weak lactose agglomerates for inhalation applications Int. J. Pharm. 1998; 172: 199-209.

Buckton G. Conversions between different amorphous states and crystal polymorphs of significance for inhalation systems. In: Respiratory Drug Delivery VI (Eds Byron P.R., Dalby R. N., and Farr S. J). Interpharm Press, IL.1998;145-151.

Buckton G. Characterization of small changes in the physical properties of powders of significance for dry powder inhaler formulations. Adv. Drug Del. Rev. 1997; 26:17- 27.

Brain J.D., and Valberg P.A. Deposition of aerosols in the respiratory tract. Am. Rev. Respir. Dis. 1979;120(6):1325–1373.

Beck B.D., Brain J.D., and Bohannon D.E. An in vivo hamster bioassay to assess the toxicity of particulates for the lungs. Toxicol. Appl. Pharmacol. 1982; 66(1):9–29.

Brain J.D. Mechanisms, measurement, and significance of lung macrophage function. Environ. Health Perspect. 1992; 97:5–10.

Carpenter R.L. Aerosol deposition modeling using ACSL. Drug Chem. Tox. 1999;22(1):73–90.

Clark T.J. Effect of beclomethasone dipropionate delivered by aerosol in patients with asthma. Lancet. 1972;1(7765):1361–1364.

Clarke S.W., and Newman S.P. Therapeutic aerosols 2. Drugs available by the inhaled route. Thorax 1984;39(1):1–7.

Clarke M.J., Tobyn M.J., Staniforth J.N.In: Respiratory Drug Delivery VII(Eds Byron P. R., Dalby R. N., and Farr S. J.). Interpharm Press, IL, 2000; 645.

Clark A. R. Medical Aerosol Inhalers: Past, Present and Future. Aerosol Sci. Technol. 1995; 22:374-391.

Clark A. R., and Hollingworth A. M. The relationship between powder inhaler resistance and peak inspiratory condition in healthy volunteers: Implications for in-vitro testing J. Aerosol Med.1993; 6: 99-110.

Chan H. K., and Gonda I . Respirable form of crystals of cromoglycic acid. J. Pharm. Sci. 1989; 78: 176-180.

Chan H .K., Clark A., Gonda I., Mumenthaler M. and Hsu, C. Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. Pharm. Res. 1997; 14: 431–437.

Chawla A., Taylor K.M.G., Newton J.M., and Johnson M.C.R. Production of spray-dried salbutamol sulphate for use in dry powder aerosol formulation. Int. J. Pharm.1994; 108: 233-240.

Collins S. Determination of the emitted dose from dry powder inhalers II: comparison of the pharmacopoeial forum apparatus and the funnel apparatus. In: Drug Delivery to the Lungs IX, The Aerosol Society, UK.1998; 60-63.

Collins F.S. Cystic fibrosis: molecular biology and therapeutic implications. Science. 1992; 256:774-779.

Cohen R. G., and Starnes V. A. Living donor lung transplantation. World J. Surg. 2001; 25: 244-250.

Chew N .Y.K., and Chan H.-K. Effect of humidity on the dispersion of dry powders. In: Respiratory Drug Delivery VII, Vol. II (Eds Dalby R.N., Byron P.R., Peart J., and Farr S.J.). Serentec Press, North Carolina. 2000; 615–618.

Chew N .Y.K., and Chan H.-K. Effect of amino acids on the dispersion of spray dried cromoglycate powders as aerosol. In: Proceedings of Respiratory Drug Delivery VIII, Vol. II (Eds Dalby R.N., Byron P.R., Peart J., and Farr S.J.). Davis Horwood International, North Carolina. 2002;619–622.

Chew N .Y.K., and Chan H.-K. Use of solid corrugated particles to enhance powder aerosol performance. Pharm. Res. 2001; 18 : 1570–1577.

Chickering D.E. Keegan M.J., Randall G., Bernstein H. and Straub J. Spray drying method. US Pat. 6223455. 2001.

Carmen E., Isabel S., Rick A. R., Kevin M. S., Justin H., and Robert L. Relating the phagocytosis of microparticles by alveolar macrophages to surface chemistry: the effect of 1,2-dipalmitoylphosphatidylcholine. J. Contr. Rel. 1998; 51:143–152.

Dawson M.L., and Clarke J.G. Dynamic powder flow as a predictor of powder processability. In: Drug Delivery to the Lungs IX, The Aerosol Society, UK. 1998; 68-71.

Dahl A.R.,Schlesinger R. B., Heck H.D., Medinsky M. A., and Lucier G.W. Comparative dosimetry of inhaled materials: differences among animal species and extrapolation to man. Symposium overview, Fundam. Appl. Toxicol.1991;16:1-13.

Dunbar C., and Hickey A. J. Effect of particle morphology on emitted dose of fatty acid-treated disodium cromoglycate powder aerosols. Pharm. Technol. 1997;21: 116.

Dolovich J. New delivery systems and propellants. Can. Respir. J. 1999;6(3):290–295.

De Boer A.H., Winter H.M.I., and Lerk C.F. Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers Part I Inhalation characteristics, work of breathing and volunteers preference in dependence of the inhaler resistance Int. J. Pharm.1996;130: 231-244.

Donawa M. E., and Horhota S.T. In: Respiratory Drug Delivery VIII (Eds Byron P. R., Dalby R, N, and Farr S. J.). Interpharm Press, IL. 2002; 371.

Denyer J., Nikander K. Halolitew a novel liquid drug aerosol delivery system. Respir. Drug Deliv. VI.1998; 311–314.

Dellamary L .A., Tarara T.E., Smith D.J., Woelk C.H., Adractas A., Costello M.L., Gill H., and Weers J. Hollow porous particles in metered dose inhalers. Pharm. Res. 2000; 17 :168–174.

Elborn J.S., Shale D.J., and Britton J.R. Cystic fibrosis: current survival and population estimates to the year 2000. Thorax. 1991; 46:881–885.

Equi A., Balfour-Lynn I. M., and Bush A. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet. 2002; 360: 978-984.

Edwards D .A., Hanes J., Caponetti G., Hrkach J., Ben-Jebria A., Eskew M.L., Mintzes J., Deaver D., Lotan N., and Langer R. Large porous particles for pulmonary drug delivery. Science. 1997; 276:1868–1871.

Edwards D.A., Batycky R.P., and Johnston L. Highly efficient delivery of a large therapeutic mass aerosol. WO 0195874 A2. 2001.

Edwards, D. A. The macrotransport of aerosol particles in the lung: Aerosol deposition phenomena. J. Aerosol Sci. 1995; 26:293-317.

Falk R., Philison K., Svartengren M., Bergmann R., Hofman W., Jervis N., Baily M., and Camner P. Assessment of long term bronchiolar clearance of particles from measurements of lung-retention and theoretical estimates of regional deposition. Exp. Lung Res. 1999; 25(6): 495-516.

Forrester B.R., and Boardman T.D. Inhalation pharmaceuticals. US Pat. 4590206. 1986.

Foster W.M,· Langenback E., and Bergofsky E. Measurement of tracheal and bronchial mucus velocities in man: relation to lung clearance. J Appl. Physiol. 1980;48(6):965–971.

Foster W.M., Langenback E.G., and Bergofsky E.H. Disassociation in the mucociliary function of central and peripheral airways of asymptomatic smokers. Am. Rev. Respir. Dis. 1985; 132(3):633–639.

Fults K .A., Miller I.F., and Hickey A.J. Effect of particle morphology on emitted dose of fatty acid-treated disodium cromoglycate powder aerosols. Pharm. Dev. Technol.1997; 2 :67–79.

Ganderton D. The generation of respirable clouds from coarse powder aggregates J. Biopharm. Sci.1992; 3: 101-105.

Grossman J. The evolution of Inhaler technology, J. Asthma.1994; 31: 55-64.

Gehr P., Geiser M., Im Hof V., Schurch S., Waber U., and Baumann M. Surfactant and inhaled particles in the conducting airways: structural, stereological, and biophysical aspects. Microsc. Res. Technol. 1993; 26(6):423–436.

Gonda I.Therapeutic aerosols. In: Pharmaceutics: the science of dosage form design (Ed Aulton M.). Churchill Livingstone, Edingburgh.1988;341.

Gonda I. Aerosols for delivery of therapeutic and diagnostic agents to· the respiratory tract. Crit. Rev. Ther. Drug Carrier Syst. 1990; 6(4): 273–313.

Goldman J.M., Bayston S.M., O'Connor S., and Meigh R.E. Inhaled micronized gentamicin powder: a new delivery system. Thorax. 1990; 45 : 939–940.

Grassi G., Majani U., Pinna G., Rampulla C., Ricci S., and Corsico R. Prevention of respiratory infections: evaluation of contamination of two types of aerosol equipments, Eur. Resp. J. 1995;18 (19): 235S.

Gardiner T., and Schanker L. Absorption of disodium cromoglycate form the rat lung: evidence of carrier transport. Xenobiotica 1974; 4:725–731.

Goodman B.E., and Wangensteen D. Alveolar epithelium permeability to small solutes: developmental changes. J. Appl. Physiol. 1982; 52(1):3–8.

Hickey A. Inhalation therapy. Drug Topics 1989;133:60–69.

Hickey A.J.Inhalation Aerosols: Physical and Biological Basis of Therapy, Marcel Dekker Inc., New York. 1996.

Hickey A.J., Gonda I., Irwin W.J., and Fildes F.J.T. Factors influencing the dispersion of dry powders as aerosols. J. Pharm. Sci. 1990;79:1009-1014.

Hickey A.J., Concessio N. M., Van M. M., and Platz A. M. Factors influencing the dispersion of dry powders as aerosols. Pharm.Tech. 1994;18: 58-64.

Horsfield K. Diameters, generations, and orders of branches in the bronchial tree.J. Appl. Physiology. 1990; 2 (68): 457-461.

Hinds W.C. In: Aerosols Technology: Properties, Behavior and measurement of Air born Particles, Wiley, New York. 1982 ;127, 424.

Hakkinen A.M., Uusi-Heikkila H., Jarvinen M., Saali K., and Karhumaki L. Using an inhalation-synchronized dosimeter in healthy adults. Clin. Physiol. 1999;19(3):269–274.

Hindle M., Newton D.A., and Chrystyn H. Investigations of an optimal inhaler technique with the use of urinary salbutamol excretion as a measure of relative bioavailability to the lung. Thorax. 1993; 48 (6):607-610.

Hellewell P. G., Young S. K., and Henson P. M. Paradoxical effect of ibuprofen on neutrophil accumulation in pulmonary and cutaneous inflammation. Am. J. Respir. Crit. Care Med. 1995; 151: 1218-1227.

Hoiby N. Diffuse panbronchiolitis, cystic fibrosis: East meets West. Thorax. 1994; 49: 531-532.

Hinds W.C. In.:Aerosols Technology: Properties, Behavior and measurement of Air born Particles, Wiley, New York. 1982;127, 424.

Jaffe A., Francis J., Rosenthal M. Long-term azithromycin may improve lung function in children with cystic fibrosis. Lancet. 1998; 351: 420.

Jaffe A., and Bush A. Anti-inflammatory effects of macrolides in lung disease. Pediatr. Pulmonol. 2001; 31: 464-473.

Kane R.S.,Glink P.T.,Chapman R.G.,McDonald J.S.,Jensen P.K.,Gao H., Pasa T.L.,Smith R.D., and whitesides G.M. Basicity of amino groups of aminoglycoside Amikacin using capillary electrophoresis and coupled CE-MS-MS techniques. Anal. Chem. 2001; 73(16) :4028-36.

Kawaguchi H.,Koiwai N., Ohtsuka Y.,.Miyamoto M., Sasakawa S. Phagocytosis of latex particles by leucocytes. I. Dependence of phagocytosis on the size and surface potential of particles. Biomaterials.1986; 7 (1): 61-66.

Krause D. S., Thies N. D., and Collector M. I. Multi-organ, multilineage engraftment by a single bone-marrow derived stem cell. Cell. 2001; 105: 369-377.

Khan T. Z., Wagener J. S., and Bost T. Early pulmonary inflammation in infants with cystic fibrosis. Am. J. Respir. Crit. Care Med. 1995; 151: 1075--1082.

Konstan M.W., and Berger M. Current understanding of the inflammatory process in cystic fibrosis: onset and etiology. Pediatr. Pulmonol. 1997; 24: 137 -142

Konstan M.W., Byard P. J., and Hoppel C. L. Effect of high-dose ibuprofen in patients with cystic fibrosis. N. Eng. J. Med. 1995; 332: 848-854.

Keller M., Muller-Walz R., Gilchrist P., Lefrancoise G., and Haeberlin B. The jago dry powder inhaler prototype 10, in vitro assessment compared to Pulmocort Turbohlaer. In: Respiratory Drug Delivery VII (Eds Byron P. R., Dalby R. N., and Farr S.J.). Interpharm Press, IL.1997; 511.

Kaye B.H. Measuring parameters relevant to the flow and packing of powders. In: Respiratory Drug Delivery V (Eds Byron P.R, Dalby R.N., Farr S.J). Interpharm Press, IL.1996 95-101.

Kjellman NIM., Wirenstrand B. Salbutamol powder inhaltion for bronchial asthma Allergy.1981; 36: 437-438.

Kim C. S., Lewars G. A., and Sackner M.A. Measurement of total lung aerosol deposition as an index of lung abnormality. J. Appl. Physiol.1988; 64:1527-1537.

Kohler D., Fleischer W., and Mathys H. New method for easy labelling of beta-2-agonists in the metered dose inhaler with technetium ^{99m}. Respiration. 1988; 53: 65-73.

Kussendrager K.D., and Ellison M.J.H. Carrier material for dry powder inhalation.WO 0207705.2002.

Kim K., Matsukama Y., Lee V., and Crandall E. Characteristics of albumin absorption across rat alveolar epithelial cell monolayers .FASEB J. 1995;9:A569.

Lourenco R., and Cotromanes E. Clinical aerosols II. Therapeutic aerosols. Arch. Int. Med. 1982;142(13):2299–2308.

Lippmann M., and Schlesinger R.B. Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. J. Toxicol. Environ. Health 1984; 13(2–3):441–469.

Lalor C., Atkins K., Concessio N., Pettis R., and Hickey A. Lung deposition and alveolar macrophage uptake of microparticles from suspension aerosols in guinea pigs. Society of Toxicology, 35th Annual Meeting, Anaheim, CA; 1996.

Le Brun P.P. H., De Boer A.H., Heijerman H.G.M., and Frijlink H.W. A review of the technical aspects of drug nebulization Pharm. World Sci. 2000; 22: 75-81.

Le Brun P.P.H., De Boer A.H., Heijerman H.G.M., and Frijlink H.W. A review of the technical aspects of drug nebulization. Pharm. World Sci. 2000a; 22: 75–81.

Le Brun, P.P.H., Brimicombe R.W., Van Doorne H., and Heijerman H.G.M. The cleaning and disinfection of nebulizers used at home and in cystic fibrosis. Eur. Hosp. Pharm. 2000b;6: 58–63.

Le Brun P.P.H., De Boer A.H., Gjaltema D., Hagedoorn P., Heijerman H.G.M., and Frijlink H.W. Inhalation of tobramycin in cystic fibrosis. Part 1: the choice of a nebulizer. Int. J. Pharm. 1999; 189 :205–214.

Lloyd-Still J.D. Crohn's disease and cystic fibrosis. Dig. Dis. Sci. 1994; 39:880-885.

Lucas P.,Clarke M.J., Anderson K., Tobyn M.J., and Staniforth J.N. The role of fine particle excipients in pharmaceutical dry powder aerosols. In: Proceedings of Respiratory Drug Delivery VI (Dalby R.N., Byron P.R., and Farr S.J.). Interpharm Press, Buffalo Grove, IL. 1996; 243–250.

Lucas P., Anderson K., Potter U.J., and Staniforth J.N. Enhancement of small particle size drug powder aerosol formulations using an ultra low density additive. Pharm. Res. 1999;16:1643–1647.

Lucas P., Clarke K.A, Tobyn M.J., and Staniforth J.N. The role of fine particle excipients in pharmaceutical dry powder aerosols In: Respiratory Drug Delivery VI(Eds Byron P. R., Dalby R. N., and Farr S. J.). Interpharm Press, IL. 1998;243-250.

Lechuga-Ballesteros D., and Kuo M.-C. Dry powder compositions having improved dispersivity. WO 0132144. 2001.

Lucas P., Anderson K., Potter U.J., and Staniforth J.N. Enhancement of small particle size dry powder aerosol formulations using an ultra low density additive. Pharm. Res. 1999; 16:1643–1647.

Labiris R.N., Crowther A.M., Holbrook H., Crystyn S.M., and MacLeod M.T.N. Dry powder versus intravenous and nebulized gentamicin in cystic fibrosis and bronchiectasis. Am. J. Respir. Crit. Care Med. 1999; 160 :1711–1716.

Lancaster R .W., Sigh H., Theophilus A.L. Apparatus and process for preparing for preparing crystalline particles. WO 0038811.2000.

Liversidge G.G., Cundy K.C., Bishop J.F., and Czekai D.A. Surface modified drug Nanoparticles. US Pat. 5145684.1992.

Messina M.S., O'Riordan T.G., and Smaldone G.C. Changes in mucociliary clearance during acute exacerbations of asthma. Am. Rev. Respir. Dis. 1991; 143(5 Pt 1):993–997.

Mazumder M.K., Bhattacharyya D., Guo W., and Hickey A.J. Electrostatic effects on the flowability of pharmaceutical powders and their evaluation technique. In: Respiratory Drug Delivery VI (Eds Byron P.R., Dalby R.N.,and Farr S.J.). Interpharm Press, IL. 1998; 369-372.

Malton A., Sumby B.S., and Smith I.J. A comparison of in vitro drug delivery from two multidose powder inhalation devices. Eur. J. Clin. Res. 1995; 7: 177-193.

Meakin B.J., Cainey J.M., and Woodcock P.M. Drug delivery characteristics of Bricanyl Turbuhlaer dry powder inhaler. Int. J. Pharm. 1995;119: 91-102.

Meakin B.J., Cainey J.M., and Woodcock P.M. Effect of exposure to humidity on terbutaline delivery from Bricanyl Turbohaler dry powder inhaler devices. Eur. Respir. J. 1993;6: 760-763.

Mukhopadhyay S., Singh M., and Cater J.I. Nebulised anti pseudomonal antibiotic therapy in cystic fibrosis: a meta analysis of benefits and risks. Thorax. 1996; 51: 364-368.

Masters K. Spray Drying Handbook, 3rd Edition, Halsted Press, New York. 1979.

Weers J. Dispersible powders for inhalation applications. Innovations in Pharm. Tech. 2000; 01: 111–116.

Musa P., and Ventura, P.C. Improved powdery pharmaceutical compositions for inhalation comprising low percentage lubricant. WO 0053157. 2000a.

Musa R., Bilzi P., and Ventura P. C. Modified carrier particles for use in dry powder inhalers. WO 0053158. 2000b.

Maa Y.-F., Prestrelski S.J. Biopharmaceutical powders: particle formation and formulation considerations. Curr. Pharm. Biotechnol. 2000; 1:283–302.

Maa Y.-F., Nguyen P.-A., Sweeney T., Shire S.J., and Hsu C.C. Protein inhalation powders: spray drying vs. spray freeze drying. Pharm. Res. 1999; 16 :249–254.

Maa Y.-F., and Nguyen P.-A. Method of spray freeze drying proteins for pharmaceutical administration. US Pat. 6284282. 2001.

Morton D .A.V. Method of manufacturing particles. WO 0187277. 2001.

Malcolmson R.J., Embleton J.K. Dry powder formulations for pulmonary delivery. Pharm. Sci. Technol. Today.1998;1: 394-398.

Moren F. In:Aerosols in Medicine: Principles, Diagnosis and Therapy (Eds Moren F., Newhouse M.T., and Dolovich M.B.). Elsevier, New York.1985; 265.

Neville A., Palmer J.B., Gaddie J., May C.S., Palmer K.N., and Murchison L.E. Metabolic effects of salbutamol: comparison of aerosol and intravenous administration. Br. Med. J. 1977;1(6058):413–414.

Newman S., and Clarke S. Aerosols in medicine: principles, diagnosis and therapy. In: Aerosol in therapy (Eds Moren F., Newhouse M., Dolovich M.). Amsterdam: Elsevier, 1985.

Newman S.P., Pavia D., MoreÂn F., Sheahun N.F., and Clarke S.W. Deposition of aerosol in the human respiratory tract. Thorax. 1981; 36: 52-55.

Newman S.P. A comparision of lung deposition patterns between different asthma inhalers. J. Aerosol. Med. 1995;8: S21-S27.

Nantel N.P. Paediatric inspiratory flows through a novel multidose DPI. In: Respiratory Drug Delivery V (Eds Byron P.R., Dalby R.N., and Farr S.J.). Interpharm Press, IL.1996;386-388.

Nikolaizik W.H., Jenni-Galovic B., and Schoni M.H. Bronchial constriction after nebulized tobramycin preparations and saline in patients with cystic fibrosis. Pneumology 1996;155:608–611.

Newman S.P. Deposition and Effects of Inhalation Aerosols. ABDRACO, Lund. 1983.

Nilsson T. L. and Nilsson. Medical electropowders for inhalers. WO 0211801. 2002.

Oberdorster G. Lung clearance of inhaled insoluble and soluble particles. J. Aerosol Med. 1988; 1:289–330.

Oberdorster G., Ferin J., Gelein R., Soderholm S.C., and Finkelstein J. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. Environ. Health Perspect. 1992;97:193–199.

Olver R.E., Schneeberger E.E, and Walters D.V. Epithelial solute permeability, ion transport and tight junction morphology in the developing lung of the fetal lamb. J.Physiol. (Lond) 1981; 315:395–412.

O'Doherty M.J., and Miller R.F. Aerosols for therapy and diagnosis. Eur. J. Nucl. Med. 1993;20(12):1201–1213.

Olsson B., and Asking L. Critical aspects of the function of inspiratory flow driven inhalers J. Aerosol Med.1994;7: S43-S47.

O'Callaghan C., and Barry P.W. The science of nebulized drug delivery. Thorax.1997; 152 (2): S31–S44.

Patton J. Mechanisms of macromolecule absorption by the lungs. Adv. Drug. Del. Rev. 1996; 19:3–36.

Podczek F. Relationship between centrifugal adhesion measurement and aerosol performance of dry powder inhalations. In: Drug Delivery to the Lungs VII, The Aerosol Society, UK.1996;70-73.

Peart J., Staniforth J.N., Byron P.R., and Meakin B.J. Electrosatic charge interaction in pharmaceutical dry powder aerosols. In: Respiratory Drug Delivery V (Eds.Byron P. R., Dalby R. N.,and Farr S. J.). Interpharm Press, IL. 1996; 85-93.

Phillips E.M., Byron P.R., and Naini V. Characterizing variable amorphous content in powders for inhalation. In:Respiratory Drug Delivery V (Eds Byron P. R., Dalby R. N., Farr S. J.), Interpharm Press, IL. 1996;253-261.

Pearce J.O. Dispensers for powdered medication. EP patent no. 0333334A2. 1989

Peart J., Staniforth J.N., Byron P.R., Meakin B.J. Electrosatic charge interaction in pharmaceutical dry powder aerosols In: Respiratory Drug Delivery V (Eds Byron P. R., Dalby R. N., and Farr S. J.). Interpharm Press, IL. 1996; 85-93.

Pedersen S. Clinical efficacy and safety of budesonide Turbuhaler as compared to MDIs in children. J. Aerosol Med. 1994; 7: S-67.

Pitcairn G., Lunghetti G., Venture P., and Newman S. A comparision of the lung deposition of salbutamol inhaled from a new dry powder inhaler at two inhaled flow rates. Int. J. Pharm.1994;102: 11-18.

Pedersen S. Clinical efficacy and safety of budesonide Turbuhaler as compared to MDIs in children J. Aerosol Med.1994;7: S-67.

Prime D., Slater A. L., Haywood P. A., and Smith I. J. Assessing dose delivery from the Flixotide Diskus inhaler – A multi-dose powder inhaler. Pharm. Tech. Eur.1996; March: 23-26.

Pai V.B., and Nahata M.C. Efficacy and Safety of Aerosolized Tobramycin in Cystic Fibrosis. Pediatric Pulmonology. 2001;32:314-327.

Pamela B., Davis M.D., and Michael W. K. Cystic Fibrosis. Am. J. Rerpir. Crit. Care Med. 1996; 154:1229-1256.

Patel A.N. In: Respiratory Drug Delivery VII (Eds Byron P.R., Dalby R. N., Farr S. J.). Interpharm Press, IL. 2000; 381.

Quinton P.M. Cystic fibrosis: a disease of electrolyte transport. FASEB J. 1990;4:2709-2717.

Ramsey B.W., Pepe M. S., and Quan J. M. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. N. Engl. J. Med. 1999; 340: 23-30.

Robert J. K. Formulation of Aerosolized Therapeutics. Chest. 2001; 120: S94–S98.

Rudt, S. and Muller, R. H. In vitro phagocytosis assay of nano- and microparticles by chemiluminescence. I. Effect of analytical parameters, particle size and particle concentration. J. Contr. Rel.1992; 22: 263-271.

Scherer P.W. Mucus transport by cough. Chest. 1981;80(6): 830–833.

Schlesinger R.B. Clearance from the respiratory tract. Fundam. Appl. Toxicol. 1985; 5(3):435–450.

Stone K.C., Mercer R.R., Gehr P., Stockstill B., and Crapo J.D. Allometric relationships of cell numbers and size in the mammalian lung. Am. J. Respir. Cell Mol. Biol. 1992; 6(2):235–243.

Snipes M.B., and Clem M.F. Retention of microspheres in the rat lung after intratracheal instillation. Environ. Res. 1981; 24(1):33–41.

Schanker L.S. Drug absorption from the lung. Biochem. Pharmacol. 1978; 27(4):381–385.

Schlesinger R.B. Clearance from the respiratory tract. Fundam. Appl. Toxicol. 1985; 5(3):435–450.

Schlesinger R.B. Comparative deposition of inhaled aerosols in experimental animals and humans: A review. J. Toxicol. Environ. Health. 1985;15(2):197–214.

Svartergren K., Lindestad P.A., Svartengren M.,Bylin G., Philiposon K., and Camner P. Deposition of in haled particles in mouth and throat of asthmatic subjects. Eur. Respir. J. 1994; 7(8):1467-1473.

Svartengren K., Ericsson C.H., Svartengren M., Mossberg B., Philipson K., and Camner P. Deposition and clearance in large and small airways in chronic bronchitis. Exp. Lung Res. 1996; 22(5):555–576.

Svartergren M., Philiposon K., Linnman L., and Camner P., Regional deposition of particles in human lung after induced bronchoconstriction. Exp. Lung Res. 1986; 10(3): 223-233.

Sweeney T. D., Brain J. D., Leavitt S.A., and Godleski J.J. Emphysema alters the deposition pattern of inhaled particles in hamsters. Am. J. Phythol.1987;128:19-28.

Sweeney T. D., and Brain, J. D. Pulmonary deposition: Determinants and measurement techniques. Toxicol. Pathol.1991; 19:384-392.

Snell J.L., and Ganderton D. Assessing lung deposition of inhaled medications: Consensus statement from a workshop of the British Association of Lung Research. Respir. Med. 1999; 93: 123-133.

Smaldone G. C., Fuhrer J., Steigbigel R. T., and McPeck M., Factors determining pulmonary deposition of aerosolized pentamidine in patients with human immunodeficiency virus infection. Am. Rev. Respir. Dis.1991; 143 :727-737.

Staniforth J.N. Pre-formulation aspects of dry powder aerosols In:Respiratory Drug Delivery V (Eds Byron P.R., Dalby R.N., and Farr S.J.). Interpharm Press, IL. 1996;65-73.

Sumby B.S., Churcher K.M., Smith I.J., Grant A.C., Truman K.G., Marriott R.J., and Gunawardena K.A., Pleace K.J., and Clay M.M. Salmeterol delivered from a new multi-dose powder inhaler (Diskus Accuhaler inhaler) or Diskhaler inhaler in adult asthmatics Amer. J. Respir. Crit. Care Med.1994;149: A21.

Summers M.P. The dry powder inhaler: regulatory issue. In: Recent Advances in Dry Powder Inhalers, Management Forum Ltd. UK. 1996;142.

Steinkamp G., and Wiedemann B. Relationship between nutritional status and lung function in cystic fibrosis: cross sectional and longitudinal analyses from the German CF quality assurance (CFQA) project. Thorax. 2002; 57: 596-601.

Staniforth J .N., Morton D.A.V., and Musa R. Method of making particles for use in a pharmaceutical composition. WO 0200197 A1. 2002.

Staniforth J .N. Powders for use in dry powder pharmaceutical inhalers. WO 9703649 A1. 1997.

Staniforth J.N. Carrier particles for use in dry powder inhalers. WO 9623485 A1. 1996.

Staniforth J.N. Carrier particles for use in dry powder inhalers. US pat 652126. 2003.

Tarara, T.E., Weers J.G., Kabalnov A., Schutt E.G., Dellamary L.A. US pat. appl. 2003006402.2003.

Sievers R .E., Sellers S.P., Clark G.S., Villa J.A., Mioskowski B.,Carpenter J. Supercritical fluid carbon dioxide technologies for fine particle formation for pulmonary drug delivery. In: 219th ACS National Meeting, San Francisco, CA. 2000; March 26–30.

Thiel C.G. From Susie's question to CFC free: an inventor's perspective on forty years of MDI development and regulation. Resp. Drug Deliv. V, Arizona.1996; 115-123.

Thielmann F., Levoguer C., and Domingue J. In: Respiratory Drug Delivery VIII (Eds Byron P.R., Dalby R. N., Farr S. J.). Interpharm Press, IL. 2002; 611.

Taylor C.J. and Aswani N. The pancreas in cystic fibrosis. Paediatr. Respir. Rev. 2002; 3:77-81.

USP Dispensing Information (USP DI). Micromedex Thomson Healthcare. 2001.

Valberg P.A., Brain J.D., Sneddon S.L., and Le Mott S.R. Breathing patterns influence aerosol deposition sites in excised dog lungs. J. Appl. Physiol. 1982;53(4):824–837.

Valberg P. A., and Brain J. D. Lung particle retention and lung macrophage function evaluated using magnetic aerosols. J. Aerosol Med.1988; 1:331-349.

Van Oort M., and Downey B. Cascade impaction of MDIs and DPIs: Induction port, inlet cone and preseparator lid designs recommended for inclusion in the general test chapter, aerosols, US Pharm., Forum, NJ.1996;22, 2204-2210.

Vanbever R., Mintzes J., Wan J., Nice J., Chen D., Batycky R., Langer R., and Edwards D. A. Formulation and physical characterization of large porous particles for inhalation. Pharm. Res. 1999;16:1735–1742.

Vemuri N., Authelin J.-R., and Hosek P. The milling process for the production of finely milled medicinal substances. WO 0032165.2000.

Vanbever R., Mintzes J.D., Wang J., Nice J., Chen D., Batycky R., Langer R., and Edwards D.A. Formulation and physical characterization of large porous particles for inhalation. Pharm. Res. 1999; 16 :1735–1742.

Weibel E.R. Morphometry of the Human Lung. Newyork: SpringerVerlag, Academic press, Berlin. 1963;136-153.

Wetterlin K. Turbuhaler : A new powder inhaler for adminstration of drugs to the airways Pharm. Res. 1988; 5: 506-508.

Wright P. Next generation impactor – Autumn 1997 update. In: Drug Delivery to the Lungs VIII, The Aerosol Society,UK. 1997; 107-110.

Welsh M.J., Tsui L.C., Boat T.F., and Beaudet A.L. Cystic fibrosis. In: The metabolic and molecular bases of inherited disease (Eds Scriver C.R., Beaudet A.L., Sly W.S., and Valle D.). McGraw Hill, New York. 1995; 3799-3876.

Wilschanki M., Famini C., and Blau H. A pilot study of the effects of gentamicin on nasal potential difference measurements in cystic fibrosis patients carrying stop mutations. Am. J. Respir. Crit. Care Med. 2000; 161: 860-865.

Weers J.G., Schutt E.G., Dellamary L.A., Tarara T.E., and Kabalnov A. Stabilized preparations for use in metered dose inhalers. US Pat. 6309623. 1998.

Watanabe W., Ahonen P., Kauppinen E., Jarvinen R., Brown D., Jokiniemi J., Muttonen W. Inhalation particles. WO 0149263. 2001.

Watanabe W., Ahonen P., Kauppinen E., Jarvinen R., Brown D., Jokiniemi J., and Muttonen W. Novel method for the synthesis of inhalable multicomponent drug powders with controlled morphology and size. In: Proceedings of Respiratory Drug Delivery VIII, Vol. II (Eds Dalby R.N., Byron P.R.,Peart J., and Farr S.J.). Davis Horwood International, North Carolina. 2002;795–797.

Yu J., and Chien Y.W. Pulmonary drug delivery: physiologic and mechanistic aspects. Crit. Rev. Ther. Drug Carrier Syst. 1997;14(4):395–453.

York P., Hanna M. Particle engineering by supercritical fluid technologies for powder inhaltion drug delivery. In: Respiratory Drug Delivery V(Eds Byron P.R., Dalby R. N., Farr S. J.). Interpharm Press, IL. 1996; 231.

Yamashita C., Nishibayashi T., Akashi S., Toguchi H., and Odomi M. A novel formulation of dry powder for inhalation of peptides and proteins. In: Proceedings of Respiratory Drug Delivery VI (Eds Dalby R.N., Byron P.R., and Farr S.J.). Interpharm Press, Buffalo Grove, IL. 1996; 483–485.

York P., and Hanna M. Particle engineering by supercritical fluid technologies for powder inhalation drug delivery. In: Proceedings of Respiratory Drug Delivery VI (Eds Dalby R.N., Byron P.R., and Farr S.J.). Interpharm Press, Buffalo Grove, IL. 1996; 231–239.

Yoshioka S., and Carstensen J.T. Rational storage conditions for accelerated testing of stability of solid pharmaceuticals. J. Pharm. Sci. 1990; 79 (10): 943-944.

Zainudin B.M. Therapeutic aerosol: principles and practices. Med. J. Malaysia 1993;48(3):259–268.

Zeng X.M., Martin G.P., Tee S., Ghoush A.A., and Marriott C. Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation. Int. J. Pharm.1999;182:133-144.

Zeltner T. B., Sweeney T. D., Skornik W.A., Feldman H.A., and Brain J. D. Retention and clearance of 0.9-mm particles inhaled by hamsters during rest and exercise. J. Appl. Physiol.1991; 70: 1137- 1145.