

CHAPTER 2

LITERATURE REVIEW

The respiratory tract is one of the oldest routes for the administration of drugs. Anesthetics, aerosolized drugs, smoke or steam have been inhaled for medical purposes for centuries. Over the past decades inhalation therapy has established itself as a valuable tool in the local therapy of pulmonary disease, such as asthma or COPD (Hickey, 1992). 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 overcome. DPI's are also potentially suitable for the delivery of a wider range of drugs, such as anti-asthmatics, 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; Hickey, 1996). This chapter gives an introduction to inhalation therapy with modern dry powder inhalers, from the anatomy of the airways, mechanics of pulmonary ventilation, powder formulation, and technological aspects of interest for understanding DPI design.

2.1 Human Respiratory System

The human respiratory tract is a branching system of air channels with more than 23 bifurcations from the mouth to the alveoli. This human respiratory tract looks like an inverted tree with a single trunk. The human respiratory system can be divided into four regions, each covering one or more anatomical regions. These regions clearly differ in structure, airflow patterns, function and sensitivity to deposited particles. The most widely used morphologic model for describing the structures within the lung was initially given by Weibel et al (1963) (fig 2.1). The first region is the upper respiratory tract, which includes the nose, mouth and pharynx. The main function of this region is heating and moistening of air. Normal atmospheric air contains round 40-60% moisture and has a temperature of 20°C in the mouth, nose and throat the air is heated to 37° C and moistened till 99% relative humidity. The second region is the conduction zone. This region consists of the first 16 generations of branching. The airways of the conducting zone are described as rigid tubes that consist primarily of cartilage in the walls and that symmetrically divide or bifurcate beginning with the trachea and ending with the terminal bronchioles. The third region is the transitional zone. This region consists of generations 17 through 19 of the branching. The respiratory bronchioles each consist of a few alveoli in which limited gas exchange occurs. The fourth region is the respiratory zone. This region consist of the generations 20 through 23 of the branching, ending in the alveoli. In the highly vascularised respiratory zone gas exchange occurs by adding oxygen to and removing carbon dioxide from the blood passing the pulmonary capillary bed.

With increasing generation number, the number of branches rapidly increases, while the distance between the branches and airway diameter decrease. The summed cross sectional area from mouth to alveolar sacs rapidly increases and results in a trumpet shaped lung model, with a total absorptive surface area of up to 100 m^2 (Hickey, 1996).

2.1.1 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 (Characterized by VT, IRV, ERV, VC and IC) can be measured through spirometry. Estimates of volume of air remaining in the lungs after expiration (RV and FRC) 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 area of the lungs is about $120\text{-}160 \text{ m}^2$ 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.

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

Parameter	Definition
Tidal volume (V_t)	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 ($IC = V_t + IRV$)
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 ($VC = IRV + V_t + ERV$)
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)

2.1.2 Major components of the lung - barriers to drug absorption

As one of the primary interfaces between the organism and the environment, the respiratory system is constantly exposed to airborne particles, potential pathogens, and toxic gases in the inspired air (Plopper, 1996). As a result a sophisticated respiratory host defense system, present from the nostrils to the alveoli, has evolved to clear offending agents (Twigg, 1998). The system comprises mechanical (i.e. air filtration, cough, sneezing, and mucociliary clearance), chemical (antioxidants, antiproteases and surfactant lipids), and immunological defense mechanisms and is tightly regulated to minimize inflammatory reactions that could impair the vital gas-exchange (Nicod, 1999; Twigg, 1998).

From a drug delivery perspective, the components of the host defense system comprise barriers that must be overcome to ensure efficient drug deposition and absorption from the respiratory tract.

2.1.2.1 Epithelium

The airway epithelial cells provides a tight ciliated barrier that clears the airways from debris trapped in the airway mucus, prevents indiscriminant leakage of water and solutes into the airways, secretes components for the airway lining fluid and mucus layer, repairs injuries to the epithelium, and modulates the response of inflammatory cells, vessels, and smooth muscle (Rennard et al., 1991). The epithelium lining the tracheobronchial airways is composed of seven different cell types, i.e. basal cells, goblet cells, ciliated cells, brush cells, serous cells, Clara cells, and neuroendocrine cells (Plopper, 1996). A variety of migratory cells such as lymphocytes, leukocytes, and mast cells are also present in the epithelium (Plopper, 1996). The epithelium lining the terminal bronchioles is columnar or cuboidal and is composed of ciliated cells and Clara cells (Plopper, 1996). In the alveolar

region, four cell types are present: the epithelial type I and II cells, alveolar brush cells (type III) and alveolar macrophages (Ma et al., 1996; Plopper, 1996). The squamous type I cell covers approximately 96% of the alveolar surface area and has an average cell thickness of 0.26 μm . Characteristically the alveolar type I cell has a large cytoplasmic volume and displays only sparse cellular organelles most of which are located in the perinuclear region of the cells (Crapo et al., 1982). These morphometric features are favorable for drug transport. About 3% of the alveolar surface is covered by the much smaller cuboidal type II cells, which synthesize and secrete surface active materials (Mason R. J. et al., 1998).

The apical membranes of the epithelial cells are joined by tight junctions that divide the cell membranes into the functionally distinct apical and basolateral domains (Summers, 1991). The tight junctions are highly dynamic structures that act as barriers to fluid flow and control the transport of ions and solutes through the intercellular space (Summers, 1991). The heterogeneous composition of the lung epithelium results in a large variation of tight junctional forms with variable tightness (Godfrey, 1997; Schneeberger, 1980).

2.1.2.2 Endothelium

The lung is unique among tissues in that about 40% of its total cellular composition is capillary endothelium, which is the largest capillary endothelial surface in the body (Simionescu, 1991). The alveolar-capillary endothelium has specialized organelle-free domains to provide a particularly thin (from 200 nm down to 30-35 nm) barrier for gas exchange (Simionescu, 1991). Furthermore, the endothelial cells have a relatively large number of endocytotic vesicles (Schneeberger, 1978). The endothelial cells are joined by tight junction with few parallel arrays of contacts, which renders them leaky when the hydrostatic pressure increase (Plopper, 1996; Simionescu, 1991).

2.1.2.3 Alveolar macrophages

The alveolar macrophages are found on the alveolar surface. These phagocytic cells play important roles in the defense mechanisms against inhaled bacteria and particles that have reached the alveoli (Haley et al., 1991). Particles deposited in the lung parenchyma of rabbits and rats have been demonstrated to be phagocytized by alveolar macrophages within a few hours (Brain et al., 1984; Takenaka et al., 2001). The macrophages are cleared from the alveoli to the bronchioles by the lining fluid, and then from the airways by the mucociliary escalator (Jeffery, 1995).

2.1.2.4 Interstitium and basement membrane

The interstitium of the lung, the extracellular and extravascular space between cells in the tissue, contains a variety of cells (fibroblasts, myofibroblasts, pericytes, monocytes, lymphocytes, plasma cells), collagen, elastic fibers, and interstitial fluid (Plopper, 1996). Its main role is to separate and bind together the specific cell layers in the tissue. The main drainage pathway for the interstitial fluid is the lymphatic vessels. The outer border of the interstitium is defined by the epithelial and endothelial basement membranes (Weibel et al., 1991). The basement membrane modulates the movement of fluid, molecules, particles, and cells from the air space and blood into the interstitium (Weibel et al., 1991). However, plasma proteins and most solutes are thought to diffuse relatively unhindered through it (Patton, 1996).

2.1.2.5 Lymphatic system

The pulmonary lymphatic system contributes to the clearance of fluid and protein which has filtered from the vascular compartment into the lung tissue interstitium and helps to prevent

fluid accumulation in the lungs (Puchelle et al., 1995). The lymphatic vessels are present in the interstitium near the small airways and blood vessels, but not in the alveolar walls (Leak et al., 1983). The leaky lymphatic endothelia allow micron-sized particles (e.g. lipoproteins, plasma proteins, bacteria, and immune cells) to pass freely into the lymph fluid (Patton, 1996). The flow rate of the lymphatic fluid is normally very slow (1/500 relative the blood flow), but is increased at high pulmonary venous pressure (Patton, 1996). The lymph is filtered through regional lymph nodes and returned to the venous blood circulation at the right jugular and subclavian veins.

2.1.2.6 Epithelial lining fluid

Solid drug particles delivered to the respiratory tract need to be wetted and dissolved before they can exert their therapeutic activity. Although the humidity in the lung is near 100%, the volume of the epithelial lining fluid is small (Wiedmann et al., 2000). The thickness of the lining fluid in the airways is estimated to 5-10 μm and is gradually decreased along the airway tree until the alveoli, where the thickness is estimated to be about 0.05-0.08 μm (Patton, 1996). The volume and composition of the epithelial lining fluid is determined by active ion transport and passive water permeability of the respiratory epithelium (Puchelle et al., 1995). However, due to the inaccessibility and small volume available, the composition of the epithelial lining fluid is not fully known. Like the gastric mucosa, the airway mucosa is coated with a layer of phospholipids, which in association with mucins lubricate and protect the epithelium from offending agents (Girod et al., 1992; Puchelle et al., 1995). Phospholipids and proteins in bronchial secretions inhibit the adhesion of cilia to the mucus gel and accelerate ciliary beat frequency (Morgenroth et al., 1985). Bacteriostatic and bactericidal proteins present in the lining fluid, e.g. IgA, lactoferrin,

and lysozyme, are synthesized and secreted by submucosal gland cells and participate in the airway antibacterial defense (Puchelle et al., 1995). In the alveolar region, the surface fluid consists of a thin biphasic layer of plasma filtrates overlaid by a monolayer of pulmonary surfactant (Patton, 1996).

2.1.2.7 Surfactant

The airway and alveolar lining fluids are thus covered by at least a monolayer of lung surfactant projecting the fatty acid tails into the air space (Patton, 1996). Consequently, interactions between the phospholipids in the lung surfactant and inhaled drugs have been reported. For instance, lung surfactant was shown to enhance the solubility of glucocorticosteroids, which may affect the residence time of the steroid in the lung (Wiedmann et al., 2000). Furthermore, strong interaction of the polypeptides ditirelix and cyclosporin A with phospholipids have been demonstrated and has been suggested to limit the absorption from the lung, thus leading to a prolonged retention of the drugs in the lungs (McAllister et al., 1996). The use of exogenous surfactant as a vehicle for pulmonary drug delivery has been suggested as a means to enhance the spreading of the drug within the lungs (Van¹ t Veen et al., 1999). However, in a study with intratracheally instilled Tc-99m-tobramycin in rats it was concluded that the exogenous surfactant increased the lung clearance rate of Tc-99m-tobramycin (Van¹ Veen et al., 1999). In another study, a decrease in bactericidal activity of tobramycin and gentamicin through binding to lung surfactant was demonstrated in vitro (Van¹ Veen et al., 1995). These results reflect a complex interaction between drugs and lung surfactant, which should be considered in drug development.

2.1.2.8 Mucociliary clearance

The residence time of an inhaled drug in the lungs depends on the site of deposition. A significant proportion of the drug reaching the lungs from an inhaled aerosol is entrapped in the mucus in the conducting airways. The ability of the drug to penetrate the mucus barrier depend on particle charge, solubility, lipophilicity, and size (Bhat et al., 1995; Rubin, 1996). For instance, reduced transports across respiratory mucus layers have been demonstrated in vitro for corticosteroids (Hashmi et al., 1999) and antibiotics (Lethem, 1993).

2.1.2.9 Patho-physiological changes

Inflammatory lung diseases or repeated mucosal injury, may result in chronic structural changes to the airways (Redington, 2001). The sequestration of drugs (e.g. amines) in the lung tissue has been reported to be altered with lung injury and disease, such as inflammation, due to the changes in lung tissue composition (Audi et al., 1999; Pang et al., 1982).

Inflammatory lung diseases, such as asthma and chronic bronchitis, are associated with an impaired mucociliary clearance and hyperplasia of submucosal glands and goblet cells leading to a hypersecretion of mucus and obstruction of the airways (Lethem, 1993; Samet et al., 1994). As a consequence of the airway obstruction, a proximal shift in the airway deposition pattern of inhaled therapeutic aerosols is observed (Rubin, 1996).

There are conflicting results in the literature on the effect of inflammation and allergic reactions on the airway permeability. Some investigations state that the permeability from the air space into the systemic circulation is increased during lung inflammation (Folkesson

et al., 1991; Hogg, 1981; Ilowite et al., 1989), whereas other investigators have demonstrated an unchanged or even decreased airway absorption explained by an instantaneous epithelial restitution in response to epithelial injury (Greiff et al., 2002; O'Byrne et al., 1984; Persson et al., 1997). An increased epithelial permeability of hydrophilic compounds i.e. terbutaline (Mw 225 Da), ^{99m}Tc -labeled diethylene triamine penta-acetate (^{99m}Tc -DTPA; Mw 492 Da), and ^{113m}In -labeled biotinylated DTPA (Mw 1215 Da) has been demonstrated in smokers as compared to non-smokers (Jones et al., 1980; Mason G.R. et al., 2001; Schmekel et al., 1991).

2.1.2.10 Particle deposition

The respiratory tract can be considered as a filter that removes particles from the inspired air (Heyder et al., 2002). The effectiveness of the filter depends on particle properties (e.g. size, shape, density, and charge), respiratory tract morphology, and the breathing pattern (e.g. airflow rate and tidal volume) (Heyder et al., 2002). These parameters determine not only the quantity of particles that are deposited but also in what region of the respiratory tract the particles are deposited. As the cross-sectional area of the airways increases, the airflow rate rapidly decreases, and consequently the residence time of the particles in the lung increases from the large conducting airways towards the lung periphery (Schulz et al., 2000). The most important mechanisms of particle deposition in the respiratory tract are inertial impaction, sedimentation, and diffusion (Figure 2.1). Inertial impaction occurs predominantly in the extrathoracic airways and in the tracheobronchial tree, where the airflow velocity is high and rapid changes in airflow direction occurs (Schulz et al., 2000). Generally, particles with a diameter larger than 10 μm are most likely deposited in the extrathoracic region,

whereas 2- to 10- μm particles are deposited in the tracheobronchial tree by inertial impaction (Schulz et al., 2000). A long residence time of the inspired air favors particle deposition by sedimentation and diffusion (Heyder et al., 2002). Sedimentation is of greatest importance in the small airways and alveoli and is most pronounced for particles with a diameter of 0.5-2 μm (Schulz et al., 2000). Ultrafine particles (<0.5 in diameter) are deposited mainly by diffusional transport in the small airways and lung parenchyma where there is a maximal residence time of the inspired air (Heyder et al., 2002). The relationship between particle size and total respiratory tract deposition has been demonstrated to be similar among species (Schlesinger, 1985).

2.1.3 Inhaler devices

For the generation of aerosol particles in the required size range for deep lung deposition, three different types of inhalation devices are available. These are the nebulizers, pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPI). pMDI's are world wide the most frequently used system and they have proven their value in therapy.

2.1.3.1 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 forced 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

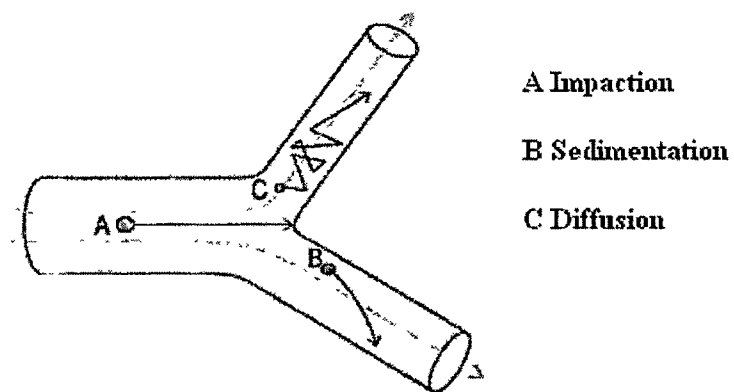


Figure 2.1. Mechanisms of particle deposition in the airways.

Table 2.2: Factors that may affect the pulmonary absorption rate and bioavailability

DEVICE AND FORMULATION	DRUG	PHYSIOLOGY
particle properties (size, density, shape, charge) deposition pattern excipients concentration osmolarity viscosity pH dose size/volume	dissolution rate solubility lipophilicity molecular weight charge hydrogen bonding potential aggregation/complex binding conformation chemical stability enzymatic stability	breathing pattern blood flow airway morphology surface area mucociliary clearance lung surfactant alveolar macrophages epithelial permeability endothelial permeability transporter proteins enzymatic/metabolic activity disease tissue composition (drug sequestration)

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).

2.1.3.2 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.

2.1.3.3 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 1 summarizes some commercially available DPI's 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.

2.1.4 Factors influencing DPI formulation design

2.1.4.1 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 studied 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 2.3 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
Bulkhaler (Asta Medica)	-
Mlat-Haler (MiatSpA)	-
Cyclovent (Pharmachemi)	-
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
MPDS-Inhale (Inhale TS)	-

Asterisk denotes commercially available DPIs and new DPIs currently under development. Name in the parenthesis indicates the manufacturer name.

2.1.4.2 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 diameter 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 $<100\mu\text{g}$) 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 (Clark *et al.*, 2000) had a profound effect on the fine particle fraction of the generated aerosol. Other excipients, like sugars, have also been studied 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).

2.1.4.3 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 parent acid and the effect of aspect ratios (longest and shortest dimensions) have been studies (Chan et al, 1989). Vidgren *et al.* (1988) have shown that spray-dried particles of Disodium Cromoglycate have better (at least in vitro) aerodynamic properties (a higher fraction of dose in a smaller size range) than micronized material.

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 single step operation.

2.1.4.4 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-doses 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).

2.1.4.5 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 ($< 5 \mu\text{m}$), 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.

2.1.5 Regulatory and pharmacopoeial requirements

The late – 1990s have seen the published agreements from the FDA (US Food and Drug Administration) (1998) and the European Inhalanda group (1999) on the tests required for the approval of new DPIs. US FDA requirements for testing dry powder inhalers are summarized in Table 2. 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.

Table 2.4: US FDA requirements for testing Dry Powder Inhalers

Drug Product
<p>This includes the device with all of its parts, any protective packaging and the formulation.</p> <ul style="list-style-type: none">ComponentsCompositionSpecifications for the formulation components like active ingredients and excipientsManufacturersMethod of manufacturing and packagingSpecifications for the drug productContainer and closure systemDrug product stability
Drug product characterization studies <ul style="list-style-type: none">Determination of appropriate storage conditionsStability of primary (unprotected) packageEffect of varying flow ratesEffect of storage on the particle size distributionDose build-up and flow resistanceEffect of orientationIn vitro dose proportionalityEffect of patient useEffect of moisturePhotostabilityProfiling of doses near device exhaustionPrimingFill weightDevice ruggednessCleaning instructions
Labelling considerations <ul style="list-style-type: none">Defines information to be included on the device label and packaging insert

The requirements from 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 $\pm 20\%$ 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.

The regulatory authorities provide a comprehensive list of requirements for compliance, which must be applied to any new DPI. The complexity of the listed items generates ever-increasing demands on the development process.

2.2 Liposomes

Liposomes are microscopic vesicles composed of phospholipids bilayers surrounding aqueous compartments as described by Bangham et al (1965). They consist of one or more bilayers. The driving force for bilayer assembly is the amphiphilic nature of phospholipid molecules. Phospholipid typically consists of a hydrophilic head group attached to two hydrophobic fatty acid chains. When suspended in an excess of aqueous solution, phospholipid molecules orient themselves in ordered bilayers so that the polar heads are hydrated and hydrophobic tails are excluded from the aqueous

environment (Figure 2.2). Although suspended phospholipids may also assume other geometric(s) such as micelles and tubular aggregates in hexagonal phases, this can be controlled by several factors including lipid composition and method of preparation (Kimalberg et al, 1978; Ryman et al, 1979; Szoka et al, 1980). Entrapment of compounds is highly influenced by their physiochemical properties. Generally hydrophobic molecules are incorporated into the lipid bilayers whereas hydrophilic compounds are entrapped in the internal aqueous volume (Stamp et al, 1979).

2.2.1 Composition of liposomes

2.2.1.1 Phospholipids

Glycerol containing phospholipids are by far, the most commonly used component of liposome formulations and represent more than 50% of the weight of lipid present in biological membranes (Riaz et al, 1988). Some naturally occurring phospholipids include phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylglycerol (PG) while dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylserine (DPPS), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidic acid (DPPA), dipalmitoyl phosphatidylglycerol (DPPG), dioleoyl phosphatidylcholine (DOPC) and dioleoyl phosphatidylglycerol (DOPG) are some synthetic phospholipids.

2.2.1.2 Sterols

Cholesterol and its derivatives are often included as components of liposomal membrane. Cholesterol has been called the “mortar” of bilayer because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the phospholipid molecules, anchoring them more strongly into the structure. Its inclusion in liposomal membranes has 3 effects (i) increasing the fluidity or microviscosity of the bilayer (ii) reducing the permeability of the membrane to water-soluble molecules and (iii) solubilizing the membrane in the presence of biological fluids such as plasma.

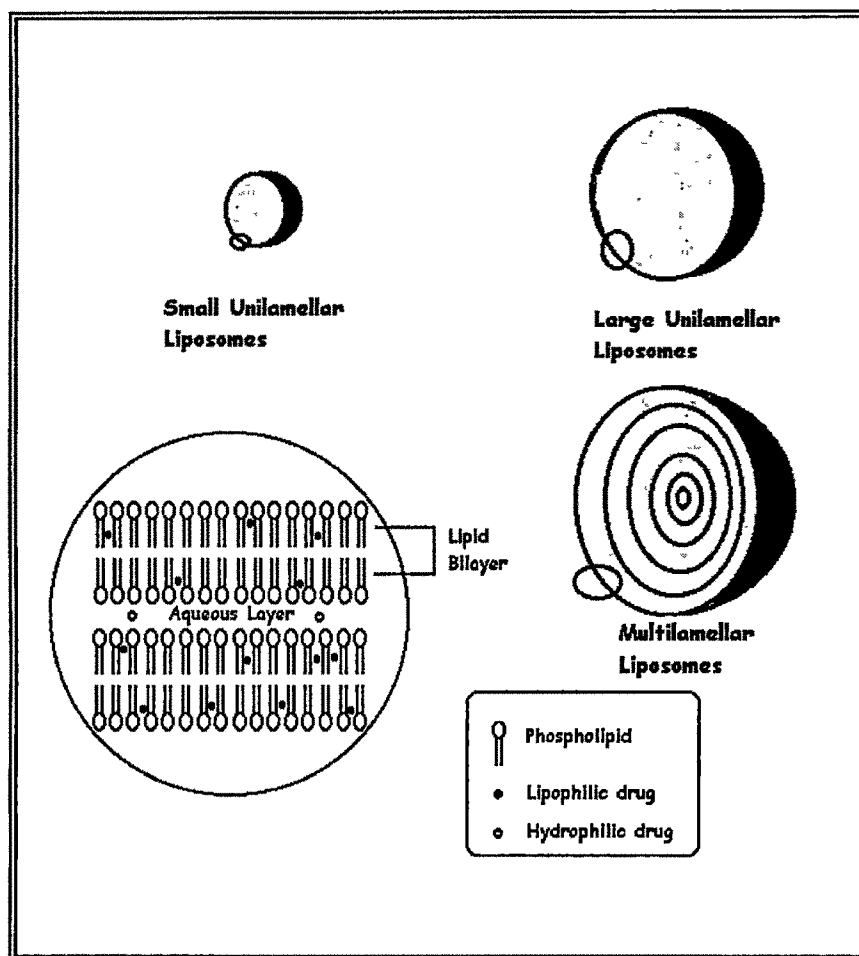


Figure 2.2: The structure of multilamellar vesicles showing the organization of phospholipid bilayers and the encapsulation of lipophilic and hydrophilic compounds.

2.2.1.3 Antioxidant

All the liposomes undergo auto-oxidation even in the presence of trace amounts of oxygen and this process is accelerated by elevated temperature, light, metal ions and some solutes. As a result, there is a dramatic, often abrupt, change in liposome permeability. Incorporation of α -tocopherol into liposomes has been reported (Hunt et al, 1981) to prolong the characteristic induction phase of auto oxidation. Addition of 0.1 mole% of α -tocopherol roughly doubles the induction time relative to liposomes containing no α -tocopherol. Addition of cholesterol enhances the effect of α -tocopherol, even though cholesterol itself is subjected to peroxidation, presumably as a result of the decreased membrane fluidity. It has been reported (Ja et al, 1990) that the release of entrapped carboxy fluorescence from liposomes was markedly retarded by the presence of α -tocopherol in the bilayer of liposomal PC membrane as compared to cholesterol-containing liposomes and pure PC liposomes. In yet another study (Etsuo, 1989) it was established that α -tocopherol suppresses the oxidation of PC liposomes by scavenging both, the aqueous radicals attacking from outside of the membrane and lipophilic radicals within the membranes. It was suggested that laterally, α -tocopherol moves fairly rapidly but it gets less efficient for it to scavenge radicals as they go deeper into the interior of the membrane.

2.2.1.4 Other non-structural components

Charge inducers such as Diacyl glycerol, Stearylamine and dicetyl phosphate have been incorporated into liposomes so as to impart either a negative or a positive surface charge

to these structures. Many single chain surfactants of number of single and double chain lipids having fluorocarbon chains and also compounds like quaternary ammonium salts and dialkyl phosphates (Ringdorf et al, 1988) can also be used to form liposomes.

2.2.2 Types of liposomes

Different types of liposomes can be prepared and are classified by the size and structure. A multilamellar vesicle (MLVs) consists of numerous concentric bilayers separated by aqueous spaces and range up to 15 μm in diameter. Vesicles consisting of a single bilayer encompassing a central aqueous compartment are referred to as small unilamellar vesicles (SUVs), which range upto 100 nm in diameter and large unilamellar vesicles (LUVs) ranging from 100 to 500 nm in diameter (Figure 2.2).

2.2.3 Methods of preparation of liposomes

Numerous procedures have been developed to prepare liposomes. There are at least fourteen Major published methods for making liposomes (Ostro et al, 1989; Martin et al, 1990). The seven, most commonly employed methods are, Lipid film hydration method (Bangham et al, 1965), Ethanol injection method (Batzri et al, 1973), Ether infusion method (Deamer et al. 1976), Detergent dialysis method (Kagawa et al, 1971), French press method (Barenholz et al, 1976), Rehydration-dehydration techniques (Shaw et al, 1985) Reverse phase evaporation method (Szoka et al, 1978).

2.2.4 Characterization of liposomes (New, 1990)

The behavior of liposomes in both physical and biological systems is determined to a large extent by factors such as physical size, chemical composition, quantity of entrapped solutes etc. Hence, liposome are characterized with respect to the following parameters:

2.2.4.1 Size and size distribution: There are number of methods reported in the literature to determine size and its distribution of the vesicles (Bangham et al, 1974; Meeren et al, 1992). The most commonly used ones are light microscopy preferably using electron microscope, laser light scattering or cryoelectron microscopy.

2.2.4.2 Lamellarity: The lamellarity, the average number of bilayers present in liposomes, can be determined either by ^{31}P -NMR spectroscopy or freeze fracture electron microscopy.

2.2.4.3 Entrapped/Internal volume: The internal or trapped or capture volume is expressed as aqueous entrapped volume per unit quantity of lipid ($\mu\text{l}/\mu\text{mol}$ or $\mu\text{l}/\text{mg}$). It is determined by entrapping a water-soluble marker such as 6-carboxyfluorescein, ^{14}C or ^3H -glucose or sucrose and then lysing the liposomes by the use of a detergent such as Triton X-100. Determination of the amount of marker that was trapped enables one to back calculate the volume of entrapped water.

2.2.4.4 Determination of percentage capture: The quantity of material entrapped inside liposomes can be determined more commonly by minicolumn centrifugation method, protamine aggregation method, dialysis technique or by gel chromatography.

2.2.4.5 Chemical analysis To determine the percentage of excipients: The common excipients like PC and CHOL can be estimated by the Stewart assay (1980) and Zlatkis method (Goel Bk, 1988) respectively. α -Tocopherol can be analyzed quantitatively by HPLC.

2.2.5 Stability of liposomes

A prerequisite for the successful introduction of liposomes in therapy is the long-term stability of the formulation. The stability of drug-laden liposome dispersions preferably should meet the standards of conventional pharmaceutical product. A 1-year shelf life is considered to be an absolute minimum. Both chemical and physical determines the shelf life of a product.

In the literature, on the physical stability of liposomes, attention has been focused on two processes affecting the quality and therefore acceptability of liposomes (Talsma et al, 1993). First, the encapsulated drug can leak from the vesicles into the extra-liposomal compartment (reduced retention). Second, liposomes can aggregate and/or fuse, forming larger particles. Both these processes change the disposition of the drug in vivo and thereby presumably affect the therapeutic index of the drug involved. Besides, other physical parameters may also change during storage. For instance, hydrolysis of phospholipids causes the formation of fatty acids and lysophospholipids. These compounds considerably affect the physical properties of the bilayer (Talsma et al, 1993). Apart from this, chemical degradation process may influence the safety of liposomes. Solid experimental data on the safety of partially hydrolyzed liposomes are not yet available; lysophospholipids alone have been reported to be toxic.

Several approaches have been developed to ensure the physical stability of liposomes on storage.

1. For storage of aqueous dispersions, the lipid composition of the bilayer and the aqueous solvent can be adjusted to induce optimum stability by reducing permeability/leakage. Phospholipids with long and saturated alkyl chains (distearoyl phosphatidyl choline and dipalmitoyl phosphatidyl choline or saturated hydrogenated soyabean or egg phosphatidyl choline) provide rigid bilayers with low permeabilities for small, non-bilayer-interacting compounds (Talsma et al, 1993). The incorporation of the bovine serum albumin in the liposomal membrane and treatment with glutaraldehyde has been reported to prevent leakage of the entrapped contents (Law et al, 1994). Crommelin has reported the effect of bilayer composition on permeability of carboxyfluorescein in (Crommelin et al 1984).

To formulate drugs in liposomes it is necessary to reduce the leakage of an entrapped drug. The rate of leakage of a molecule from liposomes is governed by the physio-chemical properties of a molecule. Liposomes are freely permeable to water, but cations are released at a slower rate than anions (Bangham et al, 1965), whereas aqueous hydrogen bonding may determine the leakage rate of non-electrolytes (Cohen et al, 1975).

Phospholipids in the liquid-crystalline state are more permeable to entrapped material than when they are in the gel state. Thus, loss of entrapped material is temperature dependent, generally being greatest around the phospholipid phase transition temperature (T_c) (Papahadjopoulos et al, 1973). The stability of liposomes in terms of retention of dideoxyinosine triphosphate (ddITP) was measured by Betageri (1993) at 4°, 25°, and 37°C. He observed that retention of

ddITP in liposomes was maximum when stored at 4°C followed by 25°C and 37°C.

Another way to control stability is to incorporate cholesterol into the lipid structure, since it is known to reduce leakage of various solutes through the lipid bilayer when the membrane is in a fluid-like state (Gregoriadis et al, 1979; Scherphof et al, 1984), or by polymerization of phospholipid molecules (Johnston et al, 1984; Scherphof et al, 1981). The introduction of cholesterol in liposomes of 5,6-carboxyfluorescein (CF) has been reported to reduce the rate of leakage during storage (Hernandez et al, 1990). He also observed that CF retention was greater in liposomes stored at 4°C in the presence of O₂ than those of room temperature, although liposomes stored at room temperature but in O₂-free atmosphere were more stable than those stored at room temperature in the presence of O₂.

Akbarieh et al (1992) developed the liposomal delivery system for the targeting and controlled release of praziquantel. They observed that although the trapping efficiency of cholesterol-poor liposomes was higher than that of cholesterol rich liposomes, drug latency was much lower after 3 weeks under different storage conditions. The findings clearly showed that the proper selection of bilayer components e.g. inclusion of a high cholesterol ratio and longer acyl chain phosphatidyl choline into the bilayer, which yield a more solid membrane, is essential for the development of stable liposomal preparations for praziquantel delivery. Also, studies on doxorubicin containing liposomes revealed that bilayer

stability is strongly dependent on the lipid composition of liposomes (Storm et al, 1989).

2. Freezing the liposome dispersion is also an approach to achieve prolonged liposome shelf-life (Crommelin et al, 1987). Lyophilization and rehydration, which include a freezing and thawing cycle, represent another method, used by many laboratories for better stability of liposomal formulations (Venkataram et al, 1990). Several groups have published reports on freezing, drying (Hauser et al, 1987) or freeze-drying of liposomes. Cryoprotectants play an important role in the physical stabilization of liposomes during freezing, drying or freeze-drying. The 100% CF retention could be found (Talsma et al, 1993) using cryoprotectant after a full freezing-thawing cycle. Studies made on the stability of liposomes with time, when they were either freeze-dried or in solution have been reported (Cromellin et al, 1984).
3. In addition, two other techniques can solve the problem of drug leakage during storage, proliposomes and remote loading (Talsma et al, 1993) that permit liposome dispersion preparation in situ. Several reports have been published in this context. Chemical analysis mainly concerns hydrolysis of the ester bonds in phospholipids and oxidation of their unsaturated acyl chains if present. Hydrolysis of phospholipid to free fatty acid and lysophospholipids can disturb the phospholipid bilayer structure and may disrupt it, leading to leakage of encapsulated products. Oxidation of unsaturated phospholipids and cholesterol may be initiated by the action of light and heavy metals (New et al, 1990). According to Hernandez-caselles (1990), the presence of A-tocopherol decreased

the breakdown of phosphatidyl choline to lysophosphatidyl choline and also reduced the level of peroxidation. Although the mechanism of the action of α -tocopherol is not clear, it is suggested that this may happen through specific binding to the phospholipid molecule (Villalain et al, 1986). α -tocopherol acetate was found to be much less effective than α -tocopherol in preventing lipid peroxidation (Fukuzawa et al, 1981). Further information about chemical stability can be found in reviews of hydrolytic and oxidation reactions in phospholipids (Talsma et al, 1993).

2.2.6 Liposomes as drug delivery systems

In the recent past, controlled release concept and technology have received increasing attention in the face of growing awareness to toxicity and ineffectiveness of drugs when administered or applied by conventional methods. Liposomes as drug delivery systems are among research topics that are being vigorously investigated in both academic and industrial laboratories, with different outlooks and common goals and end products. The scientific literature is rich with comprehensive review of liposomes as drug delivery systems (Gulati et al, 1998; Pergini et al, 1998).

Over the last twenty years, the liposome has changed its status from being a novel plaything for the laboratory worker to a powerful tool for an industrialist with the gap between the ideal desired characteristics of liposomes and what is technically feasible becoming narrower all the time. Vastly improved technology in terms of drug capture, vesicle stability on storage, scale-up production and the design of formulations for special tasks has facilitated the application of a wide range of drugs in the treatment and prevention of diseases in experimental animals and clinically.

Liposomes may prove to be efficient carrier for targeting the drug to the site of action because of the following properties (Taylor et al, 1994): Amphiphilic nature, flexibility in structural characteristics, localized drug effect, controllability of drug release rate, stability in vivo, direct cell liposome interaction, sterilizability, ability to protect drug and body from each other, non-toxicity, non immunogenicity, biocompatibility and biodegradability and accommodation of molecules with wide range of solubility and molecular weight.

At the same time, there are certain problems associated with liposome as drug delivery systems (Deasy et al, 1984) such as difficulty in procuring pure phospholipids, difficulty in scale-up, poor stability over a long shelf-life, expensive, batch to batch variation in performance, low drug loading, difficulty in avoiding the reticulo-endothelial system and possibility of unwanted vascular obstruction caused by large liposomes. However, research into the use of liposomes in drug delivery has led to vastly improved technology in terms of drug capture, vesicle stability, storage, scaled up production and the design of formulations for specialized tasks. Table 2.1 shows the liposome application according to their mode of action.

Until the early 1980s, researchers paid little attention to the pharmaceutical aspect of liposomes. But, in the past decade, clinical and animal studies have demonstrated the ability of liposomes to encapsulate and effectively deliver a diverse assortment of drugs, including antibiotics, antineoplastics, steroids, bronchodilators, nucleotides and peptides (Ranade, 1989). The development of the liposome-based pharmaceuticals progressed rapidly from the laboratory to the point of commercialization and several products now undergoing clinical trials are expected to be approved during the next decade.

Due to their high degree of biocompatibility, liposomes were initially considered as delivery systems for intravenous administration. The first parenterally applied formulation Ambisome (Vestar Inc., San Dimas, CA), a liposomal amphotericin formulation for the treatment of disseminated fungal infections that frequently occur in immunosuppressed patients, was launched in Ireland in 1990 that showed both high therapeutic activity and reduced toxicity (Talsma et al, 1992) as compared to the original product. More recently in 1995, a sterically stabilized liposomal formulation containing the anticancer drug, doxorubicin (Lasic et al, 1993) has been launched in United States.

It has since become apparent that liposomes can also serve as an effective tool for other delivery systems that include oral (Sveinsson et al, 1993) ophthalmic (Velpandian et al, 1999), aerosol (Conley et al, 1997), dermal/transdermal (Fresta et al, 1997; Trafny et al, 1999) applications, as immunological adjuvants, as carriers of antigens, leishmaniasis, lysosomal storage diseases, cell biological application etc. The recent research is concentrated on the use of liposomes to deliver hemoglobin and act as red blood cell substitutes. The scientists are also engaged in designing of liposomal prodrug using principle of specific enzyme cleavage and facilitated spontaneous hydrolysis. Another field of liposomal research in producing sterically stabilized liposomes for prolonged circulation in blood stream. Liposomes are currently being studied as drug carriers for a variety of drugs that include recombinant proteins (Sugarman et al, 1992), gene transfer and immuno diagnostic applications (Tolstoshev et al, 1993). Of these, non-invasive route of administration continuously demands significant efforts in designing the liposomes that will no doubt continue to contribute significantly to more efficient use of "old drugs" with better and established therapeutic index vis-à-vis minimum side effects.

Table 2.5. Major modes of liposomal action and related applications

Mode of action	Application
Intracellular uptake (lysosomes, endosomes/cytoplasm)	Microbial disease, Metal storage disease, Gene manipulation, uptake by some tumour, cells, macrophage activation to a tumoricidal/microcidal state, efficient antigen presentation by antigen presenting cells (vaccines).
Slow release of drugs near the target area	Tumors near fixed macrophages.
Avoidance of tissue, sensitive to drugs	Cardiotoxicity of doxorubicin
Circulating reservoirs	Blood surrogates
Facilitation of drug uptake by certain routes	Drug delivery to skin, lungs, eyes, mucosal tissues.

2.3 Liposomes as pulmonary drug delivery system

Traditional forms of inhalation therapy date back to the earliest records of ancient cultures. Although the delivery of the therapeutic compounds directly to the respiratory tract is more sophisticated today, the advantages of inhalation therapy have essentially remained the same (Newmen et al, 1985). First, the onset of action is very rapid. An oral dose of bronchodilator may take 2-3 hrs to be fully effective while an inhaled dose usually takes a minimum of 15-30 min. Second, relatively small doses are required for effective therapy. A few hundred micrograms of inhaled bronchodilator is as effective as an oral dose of 5-10 mg. This also reduces exposure of drug to the systemic circulation and potentially minimizes adverse effects. Lower dosage regimens may provide considerable cost saving especially with expensive therapeutic agents.

In the treatment of pulmonary disorders and, in some cases, for systemic action, inhalation is the preferred route of administration. Delivering small doses of active ingredient directly to the lung effectively localizes the drug there by maximizing therapeutic effect while minimizing unwanted side effects. Despite these advantages and the widespread use of therapeutic aerosols, there are several shortcomings associated with drug delivery to the respiratory tract. Although the onset of action is very rapid, the duration is often short-lived as the drug can be quickly removed from the lung through various clearance mechanisms (Taylor, 1990). The relationship between the functional anatomy of the respiratory tract and aerosol kinetics makes drug delivery to the lung highly inefficient. Typically only 10% of inhaled dose reaches the lower airways (Newmen et al, 1985). Furthermore, pulmonary tissue has a highly developed cytochrome P-450 system capable of inactivating inhaled compounds (Brown, 1974). Regardless of

the type of aerosol device employed, i.e., metered dose inhalers (MDI), nebulizer, or insufflation device, most patients will require dosing every 6-8 hrs and in some instances more frequently.

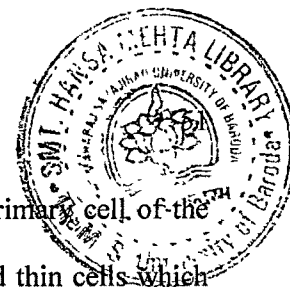
Improving drug delivery to the pulmonary system has been an area of increasing interest among several disciplines. There has been extensive effort to define the factors that influence the deposition of aerosols within the respiratory tract, clearance mechanisms from the lung, circulation in the airways, and absorption and metabolism of compounds by the lung (Chediak et al, 1990; Byron et al, 1990). With this information, strategies to improve drug delivery to the respiratory system have developed. Particulate carriers such as liposomes have many attractive features as pulmonary drug delivery systems particularly with respect to controlled delivery. The objective of this chapter is to review the problems and constraints of controlled drug delivery to the respiratory system and take a comprehensive look at the application of particulate systems as a means of improving inhalation therapy. It will deal briefly with the relevant physiology and pharmacology of the airways as well as the physical behavior of particle deposition in order to provide a basis to evaluate and assess the potential role of particulate carriers, particularly liposomes, in pulmonary drug delivery.

2.3.1 Constraints of pulmonary drug delivery

The respiratory tract can be divided into upper and lower airways with the line of division being the junction of the larynx and trachea (Forrest et al, 1985). As such the upper airways or nasopharyngeal region consists of the nose, mouth larynx, and pharynx. Below the contour of the nasopharyngeal region, the lower airways resemble a series of tubes undergoing regular dichotomous branching (Wiebel et al, 1991). Successive

branching from the trachea to the alveoli reduces the diameter of the tubes but markedly increases the surface area of the airways, a design intrinsic to its role in gas exchange. The lower airways can be divided into three physiological zones: conducting, transitional, and respiratory zone (Wiebel, 1991). The conducting zone consists of the larger tubes responsible for the bulk movement of air and blood. In the central airways, airflow is rapid and turbulent and having no alveoli, no gas exchange occurs. The transitional zone plays only a limited role in the gas exchange as compared as the respiratory zone in which the airflow is smooth and laminar in nature (Clarke et al, 1984).

The characteristic D shape of the trachea is maintained by longitudinally stacked cartilage supported by smooth muscles fibers. Interspersed throughout the pseudostratified epithelial layer of the trachea and the main bronchi are a number of cell types including ciliated, basal, goblet, and Kulchitsky cells that are supported by a fully developed basement membrane. A large number of mucus and serum producing glands are located in the submucosa. The human lung consists of 5 lobes and 10 bronchopulmonary segments. Distal to each segment are lung lobules, composed of three to five terminal bronchioles. Each bronchiole supplies the smallest structural unit of the lung, the acinus, which consists of alveolar ducts, alveolar sacs, and alveoli. The structural morphology of the main bronchi is similar to that of the trachea except smooth muscle is interspersed between cartilage segments. At the level of small bronchi and bronchioles the amount and the organization of the cartilage diminishes as the number of bronchial bifurcation increases. In the smaller bronchioles ciliated squamous epithelium replaces ciliated columnar epithelium and another secretory cells, the Clara cell, replaces goblet cells.



The acinus represents a marked change in morphology. The primary cell of the epithelium is the type I pneumocyte. Type I pneumocytes are flattened thin cells which extend to cover about 90% of the entire alveolar surface. Type II pneumocytes are more numerous but have a smaller total volume. These cells are responsible for the storage and secretion of lung surfactant that continuously line the alveolar ducts and sacs to maintain surface tension at the air water interface. Less prevalent cell types include type III pneumocyte (alveolar brush cell) and alveolar macrophages. No glandular structures, goblet or ciliated cells are found at this level although sphincters like muscles line the alveolar ducts. The cells of the alveolar epithelium are closely joined to form continuous lining for alveoli and is rather impermeable to all but small molecules. The endothelial cells of the alveolar capillaries are in many respects similar to the type I pneumocyte. Both are extremely flat and extended, have nuclei close to septal corners, and are tightly apposed to form tight junctions. The alveolar blood- barrier in its simplest form consists of a single epithelial cell, a basement membrane, and a single endothelial cell. The distance between air and blood is less than 1 μm . While this morphological arrangement readily facilitates gas exchange, it can still represent a major barrier to large molecule.

2.3.2 Controlled drug delivery to the lung

Controlled drug delivery to the respiratory tract, whether for local or systemic activity, provides an interesting challenge. The anatomical design and the physiological processes of the respiratory tract represent major barriers to efficient drug delivery and effective prolonged pharmacotherapy. Regardless of which type of aerosol delivery device is employed, generally only about 10% of the dose reach the lung (Newmen et al, 1985). A large percentage of the dose deposits in the oropharyngeal region and is

eventually swallowed. The fraction of the dose that actually reaches the lung is subjected to a variety of efficient clearance mechanisms. An obvious strategy to improve aerosol therapy is to increase the fraction of the dose reaching the lungs. To do so one must optimize the formulation and then ensure its proper utilization. To provide a prolonged effect, it is necessary to prevent rapid clearance and metabolism of the compound by the lung. Particulate systems such as liposomes represent a delivery system that can potentially fulfill these objectives. The evaluation of the particulate systems for inhalation therapy must critically examine the pharmaceutical aspects of the dosage form, define and establish their clinical application, and verify and improvement over existing treatments.

2.3.3 Pharmacokinetic and pharmacological studies

One of the perceived benefits of liposomes as a drug carrier is based on their ability to alter favorably the pharmacokinetic profile of the encapsulated species and thus provide selective and prolonged pharmacological effects at the site of administration. Administration of liposomes to the respiratory tract is particularly attractive because of the accessibility of the lung as a target organ, the compatibility of liposomes and lung surfactant components, and the need for sustained local therapy following inhalation. Consequently, numerous studies have explored the effect of liposomal encapsulation on the distribution and fate of compounds administered directly to the lung by either intratracheal instillation or inhalation. In some cases, the correlation between altered pharmacokinetic and improved pharmacological responses has been established.

The first study was reported by McCullough and Juliano and compared the effectiveness and distribution of “free” and encapsulated β -cytosine arabinoside (ARA-

C) following intratracheal administration to rats (McCullough et al, 1979). MLVs were composed of egg PC/CHOL/ stearylamine (25:7:1 weight ratio). "Free" ARA-C was rapidly cleared from the lung ($t_{1/2}$ =40 min) and entered the systemic circulation while liposomal ARA-C displayed little redistribution to other tissues and persisted throughout the lung ($t_{1/2}$ =8hr). Further more liposome-encapsulated ARA-C selectively suppressed the incorporation of thymidine and DNA synthesis in the lung but not in the gut and bone marrow as observed with the "free" compound (Juliano et al, 1980). The liposomal form favorably altered the distribution of ARA-C and successfully produced a prolonged local pharmacological effect without causing any untoward effects in other tissues.

Negatively charged MLVs containing superoxide dismutase or catalase administered intratracheally prior to hyperoxia exposure showed elevated enzyme levels, improved survival rate, and less lung injury as compared to the control groups (Padmanabham et al, 1984). The protective effect was attributed to the elevated levels of enzymes maintained in the lungs by liposome encapsulation. In vitro study by Sone and coworkers concluded that macrophages treated with liposome-encapsulated muramyl dipeptide had significantly higher tumoricidal activity and maintained its longer action than macrophages treated with muramyl dipeptide (Sone et al, 1984). Similar success was reported using a lipophilic derivative of the dipeptide (Phillips et al, 1985). Acyl tripeptide and its analogs were 800 times more effective in liposomal form than the unencapsulated form in potentiating the tumoricidal activity of macrophages (Sone et al, 1984).

In contrast to studies with CF, neither the dose nor the ratio of the drug to lipid had a significant effect on the *in vitro* release of Bronchodilators (Fielding, 1989). However,

changes in the lipid composition of vesicles greatly influenced the release rate of the encapsulated bronchodilators as well as the lipophilic steroid. The retention of the drug in the lung was prolonged by an increase in the chain length and saturation of the fatty acyl side chains and by the inclusion of the cholesterol. Consequently, the authors suggested liposomal formulation with a wide range of release rate could be designed by careful manipulation of lipid composition. In pharmacological studies with bronchodilators, it was reported that the formulations with a half-life of clearance greater than 17 hr provided no protection against histamine-induced bronchoconstriction while those with half-time less than 2 hr provided essentially the same effect as the free drug (Fielding et al, 1988).

Taylor et al. performed studies in humans that compared the systemic absorption of sodium chromoglycate in solution and liposomal form after inhalation therapy (Taylor et al, 1988).

The pharmacokinetic profile and pharmacological activities of aerosolized pentamidine in solution and liposomal form have been compared by Debs et al (1987). MLVs composed of PC/PS/CHOL (8:2:5 molar ratio) were labeled with fluorescent PE and extruded through a 0.2 μm filter prior to nebulization. It was found that greater than 95% of the labeled lipid was removed from the lung by 48 hr as compared to both free and encapsulated pentamidine, which were not cleared at all during this time period. The actual clearance of the phospholipid may be misleading because the labeled lipid could possibly have been metabolized and quenched within the lung rather than cleared. Approximately 75% of the encapsulated pentamine remained associated with liposomes following administration yet there was limited absorption of both the free and

encapsulated drug to the systemic circulation. As a result, there was also low distribution to the liver and kidney. Based on these data, the use of liposomes as a carrier for pentamine may be of little additional benefit because the sequestering of free drug in the lungs provide similar clearance to that of encapsulated drug. However, it was interesting that the authors noted a subtle difference in lung deposition between the two preparations of pentamine. The fraction of the dose recovered in the cell pellet 1 hr after the administration was significantly increased for liposomes-encapsulated pentamine and was probably due to uptake by pulmonary macrophages. Assessment of the therapeutic efficacy of aerosolized pentamidine preparations in immunosuppressed rats indicated that both free and encapsulated drug eradicated *Pneumocystis carinii* pneumonia in 75% of the treated animals (Debs et al, 1987b). As in the previous study, no difference in tissue distribution of drug was observed between the two products.

Enviroxime, a lipophilic compound, was incorporated into liposomes and evaluated in vitro for rhinoviral activity and cell culture toxicity (Wyde et al, 1988). The invitro activity of the free drug and drug encapsulated in MLVs composed of egg PC were equivalent; however it was determined that the liposome preparation 10- to greater than 50- fold less toxic to strain 1A and 13 cultures cells than the free drug. Liposome encapsulated enviroxime delivered by jet nebulization produced significant levels of drug in the lungs and noses of the treated mice. Using a fluorescent marker to label the phospholipid component, it was observed that fluorescence became evident in the large and small airways within 10 min and accumulated continuously with time particularly in and around the columnar epithelium of the bronchioles. Drug levels were detected in nose washes of these animals immediately after nebulization but declined rapidly, reaching

undetectable levels by 2 hr. Levels of enviroxime in the lung were lower than levels detected in the nose and had disappeared 1 hr after cessation of nebulization. *In vitro* antiviral activity of lung lavages obtained from mice treated with liposome-encapsulated enviroxime coincided with drug levels detected in the lungs and ceased 2 hr after aerosolization. The authors stated several advantages of the liposomal drug delivery system including high drug loading of water insoluble compound for aerosolization and decreased *in vitro* toxicity. Aerosol exposure of liposome-encapsulated enviroxime to normal volunteers showed that the preparation was well-tolerated (Gilbert et al, 1988).

Investigations by Mihalko and coworkers examined the effect of liposomal encapsulation on the pharmacokinetic fate of hydrophilic and lipophilic compounds following intratracheal instillation to rats (Mihalko et al, 1988). [^{14}C] Benzylpenicillin and [^{125}I] oxytocin were entrapped in the aqueous phase while [^{14}C] nitroglycerin was incorporated into the bilayers of MLVs composed of Egg PC/PG/CHOL (5:1:4 molar ratio) extruded through a 0.4 μm polycarbonate filter. Although the concentration of benzyl penicillin in plasma following administration in either solution or liposomal form showed a gradual increase and no peak levels after 3 hr, the level produced by free drug at this time point was approximately two-fold greater than that provided by the encapsulated drug. There was a three-fold decrease in the area under the plasma curve for the liposomal product compared to the free drug; however, absorption rate constants from the lung were similar for both dosage forms. A higher percentage of encapsulated drugs remained in the lung 3hr post-instillations.

A pharmacokinetic model that describes the release of drug from carriers such as liposomes following administration directly into the lung was reported (Gonda, 1988). It

stresses the distinction between the free and “unreleased” drug in relationship to regional deposition and mechanics of clearance within those regions. In the pulmonary region, both free and encapsulated drug can be equally removed by mucociliary transport mechanisms, whereas in the alveolar region absorption of drug into the systemic circulation or lymph tissue is only significant for free drug molecules. Based on these assumptions the effect of control of release rate from vesicles on the duration of residence of drug in the lung can be predicted. Drugs with high rates of absorption in the alveolar region will display very low levels of activity if the rate of release from vesicles is slow.

2.3.4 Development of a liposomal dosage form

2.3.4.1 Delivery as an aerosol

Many of the pharmacological and pharmacokinetic studies that have explored the potential of liposomes to act as a selective and prolonged carrier for pulmonary use have administered the formulation by endotracheal instillation (Kamarei et al, 1989). Although endotracheal administration may be practical to define distribution and elimination of liposome-encapsulated drugs and test the efficacy of the product, it is obviously unacceptable as a routine method for drug delivery. Nebulization is probably the simplest and most effective way to deliver liposomes to the respiratory tract; however, one of the first questions to consider is whether or not vesicles are stable during nebulization. Vesicles must remain intact without the loss of entrapped solutes to exert sustained release of the compound. The energy required by jet and ultrasonic nebulizers to reduce aqueous solutions to a respirable aerosol may have a detrimental effect in this regard.

Investigations involving the stability of liposomes during nebulization have been limited; however, a few studies were recently reported. Using a jet nebulizer, the loss of

entrapped drug was related to vesicle size (Taylor et al, 1990). MLVs containing sodium chromoglycate lost 51% of the entrapped drug during nebulization. This was associated with a significant release in mean vesicle diameter from 5.4 to 2.7 μm indicating that the process of nebulization fragmented vesicles. REVs showed a decrease in size from 3.4 to 2.5 μm during nebulization and lost 32% of the encapsulated drug. Reducing the size of the reverse phase evaporation vesicles to 1.2 μm by extrusion reduced drug losses during nebulization to 17% without any significant change in the vesicle size. The type of liposome formulation used had no effect on the MMAD of the aerosol produced, which was approximately 2.7 μm for all preparations. This observation supports a previous study demonstrating that deposition of nebulized liposomes was dependent on the droplet size of the aerosol and not on vesicle size.

Niven et al. (1991) also reported that the loss of entrapped solute was dependent on vesicle size. Following an 80 min period of nebulization, the loss of encapsulated carboxyfluorescein (CF) ranged from 8% for vesicles extruded through a 0.2 μm filter to 76% for vesicles approximately 5 μm in size. Release of CF over a 7 hr period fit a two compartment kinetic model consisting of a fast initial phase and a slow terminal phase. A cutoff in release was observed at a size near the MMAD of the aerosol produced by the nebulizer suggesting that vesicles smaller than the aerosol droplets are likely to be less exposed to the fragmentation forces produced by nebulization.

Niven et al. (1990) Performed additional studies that investigated the effects of lipid composition on the stability of liposomes during nebulization. Although the lipid concentration had no effect on the rate or percentage of CF released, lipid concentration of the vesicles dramatically affected their ability to retain CF during nebulization.

Vesicles composed of hydrogenated soya phosphatidylcholine (HSPC) and 30 mol % cholesterol had the greatest stability while soy phosphatidylcholine (SPC) with 30 mol % dipalmitoylphosphatidylglycerol (DPPG) had the least. The presence of DPPG had little effect on the release of CF from HSPC liposomes but had significant effect on vesicles composed of unsaturated SPC. Cholesterol effects were concentration-dependent. For vesicles containing only 10 % cholesterol or less, there were marked increases in the release of encapsulated CF. Cholesterol content and the degree of saturation have previously been shown to be important factor in determining vesicle permeability and appear to influence the rate and extent the loss of entrapped solutes during nebulization (Szoka et al, 1980). In formulating liposome preparations intended for nebulization, lipid composition and vesicle size have been identified as two important factors. Fragmentation of the vesicles is the most plausible cause for the release of water-soluble compounds. It has not been determined if hydrophobic compounds that are incorporated into vesicle bilayers exhibit similar losses, although it has been suggested that maintaining vesicle integrity may be less important for intercalated compounds (Taylor et al, 1990).

A potential alternative approach to the delivery of liposomes to the respiratory tract was described by Farr and coworkers (1987). Phospholipids were dissolved in trichlorotetrafluoroethane and pressurized with dichlorodifluoromethane or dichlorotetrafluoroethane, commonly used chlorofluoro-hydrocarbons for MDI formulation. Phospholipid aerosols produced by pressurized pack were shown *in vitro* to form liposomes spontaneously upon release and complete evaporation of propellant in a water-rich environment. Phospholipid concentration, vapor pressure, and the diameter of

the actuator orifice were influential in determining the fraction of aerosol in the respirable range. Using salbutamol and hydrocortisone 21-octanoate as model compounds it was found that solute partitioning into liposomes derived from MDI aerosols was comparable to those prepared by the conventional solvent evaporation technique.

Another possible dosage form that may also overcome the problems of long-term stability of liposomes was described (McGurk et al, 1985). The system consists of two compartments. The non-aqueous compartment, comprising a pressurized bottle filled with a valve, contains a mixture of propellant and phospholipid solution. The aqueous compartment comprises a pressured container fitted with a metered valve and a mixing chamber. Depending on its solubility, the compound can be added to the either phase. Actuation of the valve system allows mixing of the two phases to form an emulsion that is released as a metered dose aerosol.

2.3.4.2 Pharmaceutical considerations

The potential of liposomes as a pulmonary drug delivery system may depend on whether a suitable pharmaceutical product can be developed (Ostro et al, 1988). Present evidence indicates that the production of liposomal aerosols is feasible; however, several questions pertaining to product stability remain. Retention and chemical stability of the entrapped species is an obvious pharmaceutical requirement. An acceptable shelf life for most pharmaceutical products is 2 years, a requirement unlikely to be met if liposome preparations are stored in aqueous form. However, there are alternatives. It has been demonstrated that dehydrated liposomes maintain their original chemical and physical properties and retain greater than 90% of the entrapped species upon rehydration (Madden et al, 1985). In the dehydrated state the stability of the product not only may be enhanced

but effectively reduces the aqueous stability problems to the period of use. Polymerized vesicles, composed of synthetic phospholipids capable of producing membranes substantially more stable than conventional bilayers, exhibit increased resistance to leakage; however, their biodegradability and toxicity remain uncertain (Freeman et al, 1988). Oxidation of phospholipids can be minimized by the addition of antioxidants such as tocopherol to the formulation (Yamamoto et al, 1985).

Sterility of the preparation may also be difficult because most methods of sterilization are unsuitable for liposomal drug product. Temperatures required for autoclaving can cause irreparable damage to liposomes. Filtration can guarantee sterility but reduces vesicle size to an average of 0.2 μm and thus is not applicable for MLV preparations. Components of the liposome-drug delivery system, i.e., lipid, drug, and buffer, can be sterilized separated and then combined in a sterile environment; however, it is highly impractical from a manufacturing point of view. The problems of liposome formulation are not insurmountable as illustrated by the market entry of Pevaryl Lipogel, a topical formulation containing 1% econazole.

2.4 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, plastics 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 ($<5\text{ }\mu\text{m}$). It has been demonstrated that powder adhesion, mediated in part by Vander Walls forces, is directly related to the presence of particles $<10\text{ }\mu\text{m}$. 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 ($<5\ \mu\text{m}$) 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 are also useful, particularly for assessing potential physical changes.

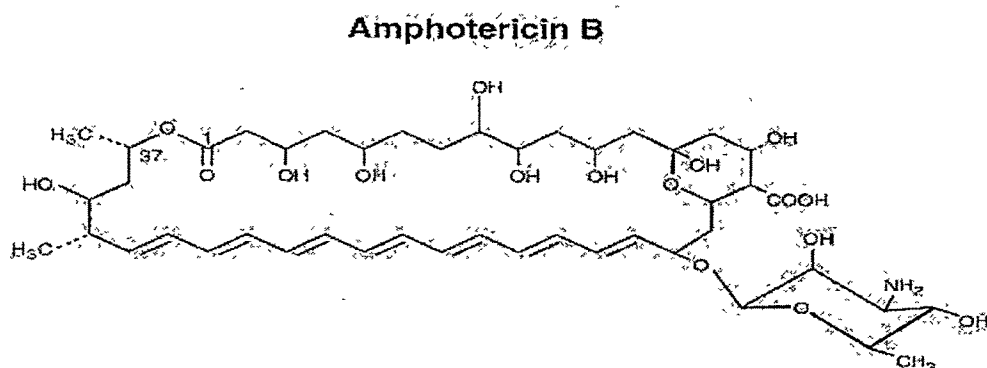
Chemical degradation after stress storage is assessed using an appropriate stability-indicating assay. In addition, physical changes are evaluated using an array of techniques available to the preformulation scientist, including polarized light microscopy (aggregation, crystal growth), differential scanning calorimetry, infrared spectroscopy, X-ray diffractometry, solution calorimetry, 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.

2.5 Drug profiles

2.5.1 AMPHOTERICIN B (Asher, 1982)

It is an amphoteric, macrocyclic heptaene with a mycosamine sugar head group.

$C_{47}H_{73}NO_{17} = 923.62$



2.5.1.1 Physical properties

Bright yellow powder. Amphotericin B is particularly difficult antibiotic to characterize analytically. It is insoluble in many solvents. Vibration grinding dramatically affects X-ray powder diffraction pattern and infrared absorption spectra. Amphotericin B is amphoteric with both polar and non polar portions. It thus dissolves poorly in most pure solvents except dimethyl sulfoxide (DMSO) and dimethylformamide (DMF).

2.5.1.2 Pharmacokinetics

An initial IV infusion of 1 to 5 mg/day, gradually increased to 0.4 to 0.6 mg/kg/day, produces peak plasma concentrations of ≈ 0.5 to 2 mcg/ml. Amphotericin B is highly protein bound (>90%) and is poorly dialyzable. Penetration of the drug into inflamed pleural cavities and joints is good; penetration is poor into the parotid gland, bronchial secretions, cerebrospinal fluid, aqueous humor, brain, pancreas, muscle and bones. Metabolic pathways of amphotericin B are not known. It has a relatively short initial serum half-life of 24 hours, followed by a second elimination phase with a half life of about 15 days. Liposomal amphotericin B has mean terminal elimination half life of 173.4 hours. The drug is slowly excreted by the kidneys with 2% to 5% as biologically active form.

2.5.1.3 Therapeutic use

Amphotericin B is used in patients with progressive potentially life-threatening fungal infections like cryptococcosis, North American blastomycosis; systemic candidiasis; the disseminated forms of moniliasis; coccidioidomycosis and histoplasmosis; zygomycosis including mucormycosis caused by species of the genera *Mucor*, *Rhizopus*, *Absidia*, *Entomophthora* and *Basidiobolus*; sporotrichosis and aspergillosis. Liposomal amphotericin B is indicated for the treatment of aspergillosis in patients refractory to or intolerant of conventional amphotericin B therapy.

2.5.1.4 Adverse reaction

General toxic reactions – Fever (sometimes with shaking chills); headache; anorexia; malaise; generalized pain including muscle and joint pains.

Respiratory – Respiratory failure; respiratory disorder; pneumonia.

Renal – Hypokalemia; azotemia; hyposthenuria; kidney failure; renal tubular acidosis.

Hematologic – Venous pain at the injection site with phlebitis and thrombophlebitis.

2.5.1.5 Method for estimation

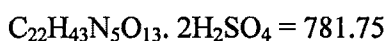
2.5.1.5.1 **Spectrophotometric analysis:** AMB is quantitatively analyzed in range using Methanol: DMSO (1:1) at 410 nm.

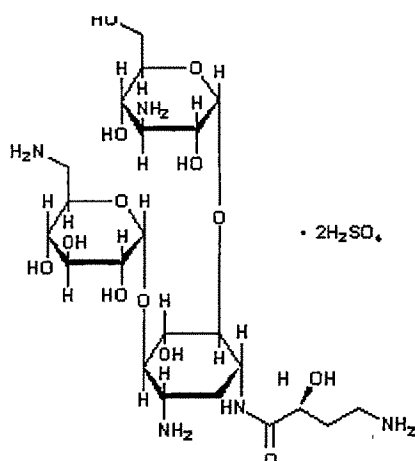
2.5.1.5.2 **High Pressure Liquid Chromatography:** Using Waters C18 columns on HPLC system are reported to separate amphotericin B and its degradation products using acidic methanol system.

2.5.1.5.3 **Microbiological assay:** Microbiological assay procedures utilizing *Saccharomyces cerevisiae*, *Candida albicans* or *Candida tropicalis* are reported. The CFR prescribes a microbiological agar diffusion assay suitable for pharmaceutical formulations using *Saccharomyces cerevisiae* (ATCC 9763). Serum and urine can be assayed by agar diffusion for Amphotericin B activity with a sensitivity of 0.01 mcg/ml using *Paecilomyces varioti* MSSC 5605 NIAID.

2.5.2 AMIKACIN SULPHATE (Monteleone et al, 1984)

Amikacin is semi synthetic aminoglycoside produced by strategic chemical alteration of kanamycin A. Amikacin sulfate is a 1:2 sulfate salt of amikacin. The following are its formula and formula weight.





2.5.2.1 Physical properties

Amikacin is white, crystalline powder with m.p. in the range of 201 – 204°C. Equilibrium solubility in water at 25°C has been reported as 185mg/ml. If the four-amine groups in amikacin are considered to be equivalent, then an apparent pKa value of 8.1 may be estimated from the half neutralization point.

2.5.2.2 Pharmacokinetics

Amikacin is usually given by intramuscular injection or intravenous infusion. Protein binding is minimal. Almost the entire dose is excreted un-metabolized in the urine. The pharmacokinetic properties of amikacin and kanamycin are similar. The reported mean half-life of 114 ± 16.7 minutes after 1-hour continuous intravenous infusion at dose of 7.5mg/kg. Peak serum concentration during infusion reached 37.5 ± 4.9 mcg/ml. Eight hours later they dropped to 1.3 ± 0.5 mcg/ml. Using a single intramuscular injection of 5 mg/kg, peak serum levels reached 21.4 ± 5.4 mcg/ml, then declined to 2.4 ± 0.9 mcg/ml by 8 hours.

2.5.2.3 Therapeutic use

It is used in short term treatment of serious infections due to susceptible strains of gram negative bacteria including *Pseudomonas* sp, *Escherichia coli*, *Proteus* sp, *klebsiella* sp,

Enterobacter sp. Effective in bacterial septicemia, in serous infections of the respiratory tract, bones and joints, CNS and skin and soft tissue infections. Also effective in serious complicated and recurrent urinary tract infections due to these organisms. It is effective in infections caused by gentamicin or tobramycin resistant strains of gram-negative organisms particularly *P. rettgeri*, *P. stuartii*, *S. marcescens* and *P. aeruginosa*.

2.5.2.4 Adverse reaction

All aminoglycosides have the potential to produce reversible and irreversible vestibular, cochlear and renal toxicity. These side effects complicate the use of these compounds and make their proper administration difficult. Rare hypersensitivity reactions including skin rashes, eosinophilia, fever, blood dyscrasias, angiodema, exfoliative dermatitis, stomatitis and anaphylactic shock have been reported.

2.5.2.5 Method for estimation

2.5.2.5.1 Thin Layer Chromatography (TLC): Solvent systems like chloroform: methanol: 28% ammonium hydroxide and water (1:4:2:1) is used to separate the related compounds of amikacin using silica gel plate. Also, solvent system of methanol: ammonium hydroxide: chloroform (60:30:25) is used to separate amikacin and related compounds using ninhydrin reagent on silicagel F 254 plates.

2.5.2.5.2 Gas-Liquid Chromatography: GLC method in determinations in serum is reported using derivatization reaction in two steps. One is using N-trimethylsilylimidazole followed by N-heptafluorobutyrylimidazole. The derivative was extracted into hexane before chromatographing. A nickel-63 detector was used. Sensitivity for amikacin was about 0.6mcg/ml of serum.

2.5.2.5.3 High Pressure Liquid Chromatography: Amikacin from its isomers was separated by forming O-phthaldehyde derivatives. Separation was carried out on C-18 column using a mobile phase of methanol/water (70:30) containing tripotassium EDTA.

2.5.2.5.4 Microbiological assay: Potency is determined by the microbiological assay for both the bulk and injection using *Staphylococcus aureus* ATCC 6538P by turbidimetric assay.

2.5.2.6 Determination in biological fluids

2.5.2.6.1 Microbiological assay

A rapid, highly specific agar diffusion method for the measurement of amikacin in body fluids using *Providencia stuartii* resistant to multiple antibiotics with the exception of amikacin is reported. Amikacin determination in blood and urine samples by 16-18 hour agar diffusion plate method using *B. subtilis* ATCC 6633 as the test organism is reported.

2.5.2.6.2 Fluorescenceimmunoassay (FIA): It uses amikacin labeled by the fluorogenic enzyme substrate β -galactosylumbelliferone. The labeled amikacin is non-fluorescent; however, when it is hydrolyzed by β -galactosidase, a fluorescent product results. When labeled amikacin is bound to the antibody, the hydrolysis of its fluorescent label is inhibited. Enzymatic hydrolysis of the unbound labeled drug releases a fluorescent product, which can be related to the original concentration of amikacin in the sample. The procedure requires 2 μ l of serum. Good precision and recovery data were shown over the range of 5 to 30 mcg/ml. Other assay procedures like Radioimmunoassay, Radioenzymatic assay using ^{14}C -acetyltransferase technique, Spectrophotometric-Enzymatic assay using purified kanamycin acetyl transferase are reported in the literature.

2.5.2.6.3 HPLC assay: A HPLC method for determination in serum by derivatization reaction using O-phthaldehyde is reported in the literature. Amikacin was separated from serum on a small silica column where it was reacted with O-phthaldehyde to produce a fluorescent derivative, which was quantitated, by HPLC. Sensitivity for amikacin was about 1 mcg/ml of serum.

References

- Adjei A L, Gupta P K (1997) *Inhalation Delivery of Therapeutic Peptides and Proteins* Marcel Dekker Inc., New York.
- Akbarieh M, Besner JG, Galal A and Tawashi R (1992) *Drug Development and Industrial Pharmacy*, 18:303.
- Asher IM (1982) In, *Analytical profiles of drug substances*, ed. Florey K, Academic Press Inc., New York, 10:1-42.
- Ashurst I, Malton A, Prime D, Sumby B (2000) Latest advances in the development of dry powder inhalers, *Pharm. Sci. Technol. Today* 3: 246-256.
- Audi SH, Roerig DL, Ahlf SB, Lin W, Dawson CA (1999) Pulmonary inflammation alters the lung disposition of lipophilic amine indicators, *J Appl Physiol*, 87:1831-1842.
- Auty RM, Brown K, Neale MG, Snashall PD (1987) Respiratory tract deposition of sodium cromoglycate is highly dependent upon technique of inhalation using the Spinhaler *Brit. J. Dis. Chest* 81: 371-380.
- Bangham AD, Hill M V and Miller MGA (1974) In: Korn, E. D. (Eds.), *Methods in Membrane Biology*, Plenum Press, New York, 1974, pp. 1-68.
- Bangham AD, Standish MM, Watkins JC, (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology*, 13, 238.
- Barenholz Y, Amselem S and Lichtenberg D, *FEBS Lett.* 99 (1976) 210.
- Batzri, S, Korn ED, (1973) Single bilayer liposomes prepared without sonication. *Biochim. Biophys. Acta*, 298, 1015.
- Bell JH, Hartley PS, Cox JSG (1971) Dry powder aerosols 1 A new powder inhalation device *J. Pharm.Sci.* 60: 1559-1564.

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

Betageri GV (1993) Drug Development and Industrial Pharmacy, 19: 531.

Bhat PG, Flanagan DR, Donovan MD, (1995) The limiting role of mucus in drug absorption: drug permeation through mucus solution. Int J Pharm, 126, 179-187.

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

Brain, JD, Bloom SD, Valberg PA, Gehr P, (1984) Correlation between the behavior of magnetic iron oxide particles in the lungs of rabbits and phagocytosis. Exp Lung Res, 6, 115-131.

Braun MA, Oschmann R, Schmidt PC (1996) Influence of excipients and storage humidity on the deposition of Disodium cromolglycate (DSCG) in the twin impinger Int. J. Pharm. 135: 53-62.

Brindley A, Sumby BS, Smith IJ, Prime D, Haywood PA, Grant AC (1995) Design, manufacture and dose consistency of the Serevent Diskus inhaler Pharm. Tech. Eur. 7: 14-22.

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

Brown EAB (1974) Drug Metabolism Review, 3:33.

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

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

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

Byron PR and Phillips EM, In. *Respiratory Drug Delivery*, ed. Byron PR, CRC Press, Boca Raton, (1990) 107.

Chan HK, Gonda I (1989) *J. Pharm. Sci.* 78: 176-180.

Chawla A, Taylor KMG, Newton JM, Johnson MCR (1994) Production of spray-dried salbutamol sulphate for use in dry powder aerosol formulation *Int. J. Pharm.* 108: 233-240.

Chediak AD and Wanner A, *Advanced Drug Delivery Review*, 5 (1990) 11.

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

Clark AR (1995) Medical Aerosol Inhalers: Past, Present and Future. *Aerosol Sci. Technol.* 22:374-391.

Clark AR, Hollingworth AM (1993) The relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers *J. Aerosol Med.* 6: 99-110.

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

Clarke SW, In. *Aerosols and the Lung, Clinical and Experimental Aspects*, ed. Clarke SW and Pavia D, Butterworths, Toronto (1984) 1.

- Cohen BE, *Journal of Membrane Biology*, 20 (1975) 205.
- Collins S (1998) 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, 60-63.
- Committee for Proprietary Medicinal Products (1998) The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit. Note for Guidance on Dry powder Inhalers CPMP/QWP/158/96.
- Conley J, Yuang H, Wilson T, Blasetti K, Ninno V, Schnell G and Wong JP, (1997) *Antimicrobial Agents Chemotherapy*, 41: 1288.
- Crapo, J. D., Barry, B. E., Gehr, P., Bachofen, M., and Weibel, E. R., (1982) Cell number and cell characteristics of the normal human lung, *Am Rev Respir Dis*, 125, 740-745.
- Crommelin DJA and Storm G (1987) In: *Controlled Drug Delivery*, Muller BW (Ed), Wvmbh, Stuttgart, 80.
- Crommelin, DJA, Van Bommel, EMG (1984), Stability of liposome on storage; freeze dried, frozen or as an aqueous dispersion. *Pharm. Res.*, 1, 159-164.
- Dawson ML, Clarke JG (1998) Dynamic powder flow as a predictor of powder processability In *Drug Delivery to the Lungs IX*, The Aerosol Society, UK, 68-71.
- De Boer AH, Winter HMI, Lerk CF (1996) 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.* 130: 231-244.
- Deamer, D, Bangham AD (1976) Large volume liposomes by an ether vaporization method. *Biochim. Biophys. Acta*, 443, 629-634.
- Deasy PB (Ed), *Microencapsulation and related drug Processes*, Marcel Dekker Inc., New York, (1984) 12, 273.

- Debs RJ, Straubinger RM, Brunette EN, Lin JM, Lin EJ, Montgomery AB, Friend DS and Papahadjopoulos DP, *American Review of Respiratory Diseases*, 135 (1987) 731.
- Debs RJ, Straubinger RM, Brunette EN, Lin JM, Lin EJ, Montgomery AB, Friend DS and Papahadjopoulos DP, *Antimicrobial Agents and Chemotherapy*, 31 (1987b) 37.
- Donawa M E, Horhota S T (2002) In: Byron P R, Dalby R N, Farr S J Eds., *Respiratory Drug Delivery VIII*, Interpharm Press, IL, 371.
- Dunbar C, Hickey A J (1997) *Pharm. Technol.* 21: 116.
- Etsuo N, Bitamin, 63 (1989) 539.
- Farr SJ, Kellaway IW, Carman-Meakin B (1987) Assessing the potential of aerosol generated liposomes from pressurized pack formulations. *J. Control. Release.* 5:119-127.
- Fielding R.M, Kerr E, Wong A, Tsukamoto T and Abra R (1988) *Pharmaceutical Research*, 5 S-66.
- Folkesson HG, Westrom BR, Pierzynowski SG, Karlsson BW, (1991) Lung to blood passage of different-sized molecules during inflammation in the rat. *J Appl Physiol*, 71, 1106-1111.
- Forrest JB (1985) In. *Aerosols in Medicine*, ed. Moren F, Newhouse MT, and Dolovich MB, Elsevier, New York, 21.
- Freeman FJ, Champman D (1988) In. *Liposomes as Drug Carriers: Recent Trends and Progress*, Gregoriadis G, John Wiley and sons, Toronto, 821.
- Fresta M and Puglisi G (1997), *Journal of Controlled Release*, 44: 141.
- Fukuzawa K, Chida H, Tokumura A, Tsukatani H (1981) *Archeology of Biochemsitry and Biophysics*, 206, 173.
- Ganderton D (1992) The generation of respirable clouds from coarse powder aggregates *J. Biopharm. Sci.* 3: 101-105.

Gilbert BE and Knight V (1988) *European Journal Clinical Microbiology and Infectious Disease*, 7: 721.

Girod S, Zahm JM, Plotkowski C, Beck G, Puchelle E, (1992) Role of the physiochemical properties of mucus in the protection of the respiratory epithelium. *Eur Respir J*, 5, 477-487.

Godfrey RW, (1997) Human airway epithelial tight junctions, *Micro Res Tech*, 38, 488-499.

Goel BK (Ed) (1988) *Medical Laboratory Technology*, Vol. III, Tata McGraw Hill, New Delhi, 33, 1031.

Gonda I, *Journal of Pharmaceutical Science*, 77 (1988) 340.

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

Gregoriadis G, Davis C (1979) Stability of liposomes in vivo and in vitro is promoted by cholesterol content and the presence of blood cells. *Biochem. Biophys. Res. Commun.*, 89, 1287-1293.

Greiff L, Andersson M, Svensson J, Wollmer P, Lundin S, Persson CGA, (2002) Absorption across nasal airway mucosa in house dust mite perennial allergic rhinitis. *Clin Physiol Func Im*, 22, 55-57.

Grossman J (1994) The evolution of Inhaler technology, *J. Asthma* 31: 55-64. ✓

Gulati M, Grover M, Singh M and Singh S (1998) *Journal of Microencap.* 15 485.

Gunawardena KA, Pleace KJ, Clay MM (1994) Salmeterol delivered from a new multi-dose powder inhaler (Diskus Accuhaler inhaler) or Diskhaler inhaler in adult asthmatics *Amer. J. Respir. Crit. Care Med.* 149: A21.

Haley PJ, Muggenburg BA, Weissman DN, Bice DE, (1991) Comparative morphology and morphometry of alveolar macrophages from six species. *Am J Anatomy*, 191, 401-407.

Hashmi N, Matthews GP, Martin AB, Lansley AB, Forbes B, (1999) Effect of mucus on transepithelial drug delivery. *J Aerosol Med*, 12, 139.

Hernandez-Caselles T, Villalin JG (1990) Stability of liposomes on long term storage. *J. Pharm. Pharmacol.*, 42, 397-400.

Heyder J, Svartengren MU, (2002) Basic principles of particle behavior in the human respiratory tract. In: Bisgaard, H., O'Callaghan, C. and Smaldone, G. C. (Eds.), *Drug delivery to the lung*, Marcel Dekker, Inc., New York.

Hickey AJ (1992) *Pharmaceutical Inhalation Aerosol Technology*, Marcel Dekker Inc., ✓
New York.

Hickey AJ (1996) *Inhalation Aerosols : Physical and Biological Basis of Therapy*, Marcel Dekker Inc., New York.

Hickey AJ, Concessio N M, Van M M, Platz A M (1994) Factors influencing the dispersion of dry powders as aerosols *Pharm.Tech.* 18: 58-64.

Hickey AJ, Gonda I, Irwin WJ, Fildes FJT (1990) Factors influencing the dispersion of dry powders as aerosols *J. Pharm. Sci* 79: 1009-1014.

Hinds WC (1982) In. *Aerosols Technology: Properties, Behavior and measurement of Air born Particles*, Wiley, New York, 127, 424.

Hogg JC, (1981) Bronchial mucosal permeability and its relationship to airways hyperreactivity. *J Allergy Clin Immunol*, 67, 421-425.

Hunt CA, Tsang S (1981) α -Tocopherol retard autoxidation and prolongs the shelf-life of liposomes. *Int. J. Pharm.*, 8, 101-110.

Ilowite JS, Bennet WD, Sheetz WS, Groth ML, Nierman DM, (1989) Permeability of the bronchial mucosa to ^{99m}Tc -DTPA in asthma. *Am Rev Respir Dis*, 139, 1139-1143.

Inhalanda (1999) Preparations for inhalation *Pharm. Eur. Suppl.* O671, 984-989.

Ja SB and Hong KN (1990) *Arch. Pharmacology Research*, 31: 64.

Jeffery PK, (1995) Microscopic structure of normal lung. In: Brewis RAL, Corrin B, Geddes DM, Gibson GJ (Eds.), *Respiratory Medicine*, W.B. Saunders Company Ltd, London.

- Johnston DS, Chapman D (1984) Preparation of liposomes, In: *Liposomal Technology*, Vol. I, Gregoriadis G (Ed), CRC Press Inc, BocaRaton, FL, (a) 123 (b) 235 (c) 139.
- Jones JG, Minty BD, Lawler P, Hulands G, Crawley JC, Veall N, (1980) Increased alveolar epithelial permeability in cigarette smokers. *Lancet*, 1, 66-68.
- Juliano RL, McCullough N, (1980) Controlled delivery of an antitumor drug: localized action of liposome encapsulated cytosine arabinoside administered via the respiratory system *J Pharmacol Exp Ther*, 214, 381-387
- Kagawa Y, Racker E, (1971) Partial resolution of the enzymes catalysing oxidative phosphorylation. XXV Reconstitution of vesicles catalysing 32Pi adenosine triphosphate exchange. *J. Biol. Chem.*, 246, 5477-5487.
- Kaye BH (1996) Measuring parameters relevant to the flow and packing of powders. In: Byron PR, Dalby RN, Farr SJ Eds. *Respiratory Drug Delivery V*, Interpharm Press, IL, 95-101.
- Keller M, Muller-Walz R, Gilchrist P, Lefrancoise G, Haeberlin B (1997) The jago dry powder inhaler prototype 10, in vitro assessment compared to Pulmocort Turbohlaer In: Byron P R, Dalby R N, Farr S J Eds., *Respiratory Drug Delivery VII*, Interpharm Press, IL, 511.
- Kimelberg HK, Mayhew EG (1978) *Critical Review Toxicology*, 6: 25.
- Kjellman NIM, Wirenstrand B (1981) *Allergy* 36: 437-438.
- Lasic DD (1993) (Ed), *Liposomes: From Physics to Applications*, Elsevier, Amsterdam.
- Law SL, Lo WY, Lin M (1994) Increase of liposome stability by incorporation of bovine serum albumin. *Drug. Dev. Ind. Pharm.*, 20(8), 1411-1423.
- Le Brun, PP H, de Boer AH, Heijerman HGM, Frijlink HW (2000) A review of the technical aspects of drug nebulization *Pharm. World Sci.* 22: 75-81.

- Leak LV, Jamuar MP, (1983) Ultrastructure of pulmonary lymphatic vessels. *Am Rev Respir Dis*, 128, 859-65.
- Lethem MI (1993) The role of tracheobronchial mucus in drug administration to the airways. *Adv Drug Deliv Rev*, 11, 271-298.
- Lucas P, Clarke KA, Tobyn MJ, Staniforth JN (1998) The role of fine particle excipients in pharmaceutical dry powder aerosols In: Byron P R, Dalby R N, Farr S J Eds, *Respiratory Drug Delivery VI*, Interpharm Press, IL, 1998, 243-250.
- Ma J, Bhat M, Rojanasakul Y (1996) Drug metabolism and enzyme kinetics in the lung. In: Mickey, A.J. (Ed.), *Inhalation Aerosols*, Marcel Dekker, Inc., New York.
- Madden TD, Balley MB, Hope MJ, Cullis PR, Schieren HP and Janoff AS (1985) *Biochem. Biophysics Acta*, 817 (1985) 67.
- Malcolmson RJ, Buckton G, Darcy P, Cox RL, Renwick CE, Embleton JK (1998) Physicochemical characterization of Passcal formulations and excipients In: Byron P R, Dalby RN, Farr SJ Eds., *Respiratory Drug Delivery VI*, Interpharm Press, IL, 365-367.
- Malcolmson RJ, Embleton JK (1998) Dry powder formulations for pulmonary delivery, *Pharm. Sci. Technol. Today* 1: 394-398.
- Malton A, Sumby BS, Smith IJ (1995) A comparison of in vitro drug delivery from two multidose powder inhalation devices *Eur. J. Clin. Res.* 7: 177-193.
- Martin FJ (1990) *Specialized Drug Delivery Systems Manufacturing and Production Technology*, Tyle P (Ed), Marcel Dekkar Inc., New York and Basel. 6, 267.
- Mason GR, Peters AM, Bagdades E, Myers MJ, Snooks D, Hughes JMB (2001) Evaluation of pulmonary alveolar epithelial integrity by the detection of restriction to diffusion of hydrophilic solutes of different molecular sizes. *Clin Sci*, 100, 231-236.
- Mason RJ, Crystal RG, (1998) Pulmonary cell biology. *Am J Respir Crit Care Med*, 157, 872-81.

Mazumder MK, Bhattacharyya D, Guo W, Hickey AJ (1998) Electrostatic effects on the flowability of pharmaceutical powders and their evaluation technique In: Byron PR, Dalby RN, Farr SJ Eds., *Respiratory Drug Delivery VI*, Interpharm Press, IL, 369-372.

McAllister SM., Alpar HO, Teitelbaum Z, Bennett DB, (1996) Do interactions with phospholipids contribute to the prolonged retention of polypeptides within the lung? *Adv Drug Deliv Rev*, 19, 89-110.

McCalden TA, (1990) Particulate systems for drug delivery to the lung. *Advanced Drug Delivery Review*, 5, 253-263.

McCullough HN, Juliano RL (1979) Organ selective action of an antitumor drugs: pharmacologic studies of liposome encapsulated beta cytosine arabinoside administered via the respiratory system of the rat. *J. Natl. Cancer Inst.* 163, 727.

McGurk JG, Ross AR and GC Gilroy (1985) *Journal of Pharmaceutics and Pharmacology*, 38, supplement 20.

Meakin BJ, Caine JM, Woodcock PM (1993) Effect of exposure to humidity on terbutaline delivery from Bricanyl Turbuhaler dry powder inhaler devices *Eur. Respir. J.* 6: 760-763.

Meakin BJ, Caine JM, Woodcock PM (1995) Drug delivery characteristics of Bricanyl Turbuhlaer dry powder inhaler *Int. J. Pharm.* 119: 91-102.

Meakin BJ, Caine JM, Woodcock PM (1995b) Simulated 'in use' and mis-use' aspects of the delivery of terbutaline sulphate from Bricanyl Turbuhaler dry powder inhalers *Int. J. Pharm.* 119: 103-108.

Meeren PV, Laethem MV, Vanderdeelen J, Baert L (1992) *Journal of Liposome Research*, 2: 23.

Meisner D (1989) Ph.D. thesis, Dalhousie University, Halifax

Mihalko PJ, Schreier H and Abra RM (1988) In. *Liposomes as Drug Carriers: Recent Trends and Progress*, ed. G. Gregoriadis, John Wiley and sons, Toronto, 679.

Monteleone PM, Muhamdd N, Brown RD, McGrory JP, Hanna SA (1984) In, *Analytical profiles of drug substances*, ed. Florey K, Academic Press Inc., New York, 12:37-71.

Moren F (1985) In. *Aerosols in Medicine: Principles, Diagnosis and Therapy*, Moren F, Newhouse MT and Dolovich MB, Elsevier, New York, 265.

Morgenroth K, Bolz J, (1985) Morphological features of the interaction between mucus and surfactant on the bronchial mucosa. *Respiration*, 47, 225-231.

Nantel NP (1996) Paediatric inspiratory flows through a novel multidose DPI In: Byron P R, Dalby RN, Farr SJ Eds., *Respiratory Drug Delivery V*, Interpharm Press, IL, 386-388.

New RRC (1990) (Ed), *Liposomes : A Practical Approach*, Oxford University Press, UK.

Newman SP (1995) A comparison of lung deposition patterns between different asthma inhalers *J. Aerosol. Med.* 8: S21-S27.

Newmen SP and Pavia D (1985) In. *Aerosols in medicines*, ed Moren F, Newhouse MT and Dolovich MB, Elsevier, New York, 193.

Nicod LP (1999) Pulmonary defence mechanisms. *Respiration*, 66, 2-11.

Niven RW, Schreier H (1990) *Pharmaceutical Research*, 7: 1127.

Niven RW, Speer M, Schreier H, (1991) Nebulization of liposomes, II: the effects of size and modelling of solute release profiles. *Pharm Res.*,8:2, 217-221.

O'Byrne PM, Dolovich M, Dirks R, Roberts RS, Newhouse MT, (1984) Lung epithelial permeability: relation to non-specific airway responsiveness. *J Appl Physiol*, 57, 77-84.

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

Ostro MJ (1988) In. *Liposomes as Drug Carriers: Recent Trends and Progress*, ed. Gregoriadis G, John Wiley and sons, Toronto, 855.

Ostro MJ, Cullis PR, (1989) Use of liposomes as injectable-drug delivery systems. *American Journal of Hospital Pharmacy*, 46, 1576-1587.

Padmanabhan RV, Gudapathy R, Liener IE, Schwartz BA, Hoidal JR, (1985) Protection against pulmonary oxygen toxicity in rats by the intratracheal administration of liposome-encapsulated superoxide dismutase or catalase. *Am. Rev. Respir. Dis.*, 132, 164-7.

Pang JA, Butland RJA, Brooks N, Cattell M, Geddes DM, (1982) Impaired lung uptake of propranolol in human pulmonary emphysema. *Am Rev Respir Dis*, 125, 194-198.

Papahadjopoulos D, Jacobson K, Nir S and Isac T (1973) *Biochemi. Biophys. Acta*, 311: 330.

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

Patton JS (1996) Mechanisms of macromolecule absorption by the lungs. *Adv Drug Deliv Rev*, 19, 3-36.

Pearce JO (1989) Dispensers for powdered medication EP patent No. 0333334A2.

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

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

Pedersen S, Steffensen G (1986) Fenoterol powder inhaler technique in children: Influence of inspiratory flow rate and breathholding *Eur. J. Respir. Dis.* 68: 207-214.

Pergini P, Pavanetto F, *Journal of Microencapsulation*, 15 (1998) 473.

- Persson CGA, Erjefalt JS, (1997) Airway epithelial restitution after shedding and denudation. In: Crystal, R. G., and West, J. B. (Eds.), *The lung*, Lippincott-Raven Publishers, Philadelphia, pp. 2611-2627.
- Phillips EM, Byron PR, Naini V (1996) Characterizing variable amorphous content in powders for inhalation In: Byron P R, Dalby R N, Farr S J Eds., *Respiratory Drug Delivery V*, Interpharm Press, IL, 253-261.
- Phillips NC, Moras ML, Chedid L, LeFrancier P, Bernard JM (1985) *Cancer Research*, 45: 128.
- Pitcairn G, Lunghetti G, Venture P, Newman S (1994) A comparison of the lung deposition of salbutamol inhaled from a new dry powder inhaler at two inhaled flow rates *Int. J. Pharm.* 102: 11-18.
- Plopper CG (1996) Structure and function of the lung. In: *Respiratory system*, Jones TC, Dungworth DL, Mohr U, Eds. Berlin, Springer Verlag, pp. 135-150.
- Podczek F (1996) Relationship between centrifugal adhesion measurement and aerosol performance of dry powder inhalations In: *Drug Delivery to the Lungs VII*, The Aerosol Society, UK, 70-73.
- Prime D, Slater A L, Haywood P A, Smith I J (1996) Assessing dose delivery from the Flixotide Diskus inhaler – A multi-dose powder inhaler *Pharm. Tech. Eur.* March, 23-26.
- Puchelle E, Girod de Bentzmann S, Higenbottam T, (1995) Airway secretions and lung liquids. In: Brewis RAL, Corrin B, Geddes DM, Gibson GJ (Eds.), *Respiratory Medicine*, W.B. Saunders Company Ltd, London, pp. 97-111.
- Ranade VV (1989) *Journal of Clinical Pharmacology*, 29: 685.
- Redington AE (2001) Airway remodelling in asthma. *CME Bull Resp Med*, 3, 37-40.

Rennard SL, Beckmann JD, Robbins RA, (1991) Biology of airway epithelial cells. In: Crystal RG, West JB (Eds.), *The Lung: Scientific Foundations*, Raven Press Ltd., New York, pp. 157-167.

Riaz M, Weiner N, Martin F (1988) *Pharmaceutical Dosage Forms : disperse Systems* (vol. 2), Leiberman HA, Reiger MA and Banker GS (Ed), Marcel Dekker Inc., New York, 16, 567.

Ringdorf H, Schlarb B, Venzmer J (1988) *Journal of Angew Chemical Institute of Educational Engineering*, 27: 113.

Rubin BK, (1996) Therapeutic aerosols and airway secretions. *J Aerosol Med*, 9, 123-130.

Samet JM, Cheng PW (1994) The role of airway mucus in pulmonary toxicology. *Environ Health Perspect*, 102, 89-103.

Scherphof G, Dame J, Wilschut J (1984) Interactions of Liposome with Plasma Proteins In: *Liposome Technology*, Vol. III, Gregoriadis G (Ed), CRC Press Inc., BocaRaton, FL, 205.

Scherphof G, Damen J and Hoekstra D (1981) In: *Liposomes : From Physical structure to Therapeutic Applications*, Knight CG (Ed), Elsevier /North Holland Biomedical Press, Amsterdam, 310.

Schlesinger RB (1985) Comparative deposition of inhaled aerosols in experimental animals and humans: A review. *J Toxicol Environ Health*, 15, 197-214.

Schmekel B, Borgstrom L, Wollmer P, (1991) Difference in pulmonary absorption of inhaled terbutaline in healthy smokers and non-smokers. *Thorax*, 46, 225-228.

Schneeberger EE (1978) Structural basis for some permeability properties of the air-blood barrier. *Fed Proc*, 37, 2471-2478.

Schneeberger EE (1980) Heterogeneity of tight junction morphology in extrapulmonary and intrapulmonary airways in the rat. *AnatRec*, 198, 193-208.

Schulz H, Brand P, Heyder J (2000) Particle deposition in the respiratory tract. In: Gehr P, Heyder J (Eds.), Particle-lung interactions, Marcel Dekker, Inc., New York, pp. 229-290.

Shew RL, Deamer D, (1985) A novel method for encapsulation of macromolecules in liposomes. *Biochem. Biophys. Acta*, 816, 1-8.

Simionescu M (1991) Lung endothelium: Structure-function correlates. In: Crystal RG, West JB (Eds.), *The Lung:Scientific foundations*, Raven Press Ltd., New York, pp. 301-312.

Sone S, Mutsuura S, Ogawara M, Utsungi T, Tsubura E (1984b) *Cancer Immunology and Immunotherapeutics*, 18:169.

Sone S, Tachibana K, Shono M, Ogushi F, Tsubura E (1984) *Journal Biology Respiratory Model*, 3: 185.

Stamp D, Juliano RL (1979) *Canadian Journal Physiology Pharmacology*, 57: 535.

Staniforth JN (1996) Pre-formulation aspects of dry powder aerosols In: Byron PR, Dalby RN, Farr SJ Eds., *Respiratory Drug Delivery V*, Interpharm Press, IL, 65-73.

Stewart JCM (1980) Colorimetric determination of phospholipids with ammonium ferrothiocyanate *Analytical Biochemistry*, 104: 10.

Storm G, Nassander UK, Roerdink FH, Steerenberg PA, de Jong, wH, Crommelin DJA (1989) Studies on the mode of action of doxorubicin-liposome, In: Lopez-Berestein G, Fidler IJ (Eds.), *Liposome in the therapy of infectious diseases and cancer*. Alan R. Liss, N.Y., pp 105-116.

Sugarman SM, Perez-Solar R (1992) *Critical Reviews on Oncology and Hematology*, 12 231.

Sumby BS, Churcher KM, Smith IJ, Grant AC, Truman KG, Marriott RJ, Booth SJ (1993) Dose reliability of the Servent Diskhaler systems *Pharm. Tech. Int.* June 20-27.

- Summers MP (1996) The dry powder inhaler: regulatory issue, In Recent Advances in Dry Powder Inhalers, Management Forum Ltd. UK, 142.
- Summers QA (1991) Inhaled drugs and the lung. *Clin Exp Allergy*, 21, 259-268.
- Sveinsson SJ, Holbrook WP (1993) *Int. J. Pharm.* 95 : 105.
- Szoka F, Papahadjopoulos D (1978) *Biochemistry* 75: 4199.
- Szoka F, Papahadjopoulos D (1980) *Annual Review of Biophysics and Bioengineering*, 9: 467.
- Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect*, 109, 547-551
- Talsma H, Crommelin DJ (1993) Liposome as drug delivery system part-III Stabilization. *Pharm. Tech.*, 16, 48-59.
- Talsma H, Crommelin DJA (1992) *Pharmaceutical Technology*, 96.
- Taylor G (1990) The absorption and metabolism of xenobiotics in the lung, *Adv Drug Deliv Rev* 5, 37-61.
- Taylor KMG, Newton JM (1994) *Journal of Applied Medicine*, 20 (1994) 735.
- Taylor KMG, Taylor G, Kellaway IW, Stevens J (1990) *Int. J. Pharm.* 58 :57.
- Thiel CG (1996) From Susie's question to CFC free: an inventor's perspective on forty years of MDI development and regulation, *Resp. Drug Deliv.* V, Arizona, 115-123.
- Thielmann F, Levoguer C, Domingue J (2002) In: Byron P R, Dalby R N, Farr S J Eds., *Respiratory Drug Delivery VIII*, Interpharm Press, IL, 611.
- Tolstoshev P (1993) *Annual Reviews on Pharmacology Toxicology*, 33: 573.
- Trafny EA, Antos-Bielska M, Grzybowski J (1999) *J. of Microencap.* 16 : 419.
- Twigg HL (1998) Pulmonary host defenses. *J Thoracic Imaging* ,13, 221-233.

US FDA (CDER) (1998) Draft Guidance for Industry Metered Dose Inhalers (MDI) and Dry Powder Inhalers (DPI) Drug Products. CMC Documentation.

Van Oort M, Downey B (1996) 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, 22, 2204-2210.

Van't Veen A, Mouton JW, Gommers D, Kluytmans JAJ, Dekkers P, Lachmann B (1995) Influence of pulmonary surfactant on in vitro bactericidal activities of amoxicillin, ceftazidime, and tobramycin. *Antimicrob Agents Chemother*, 39, 329-333.

Velpandian T, Gupta SK, Gupta YK, Biswas NR and Agarwal HC (1999) *J. of Microencap.* 16 : 243.

Venkataram S, Awni WM, Jorden K, Pahman YE (1990) *J. Pharm. Sci.* 79 : 216.

Vidgren M, Vidgren P, Uotila J, Paronen P (1988) *Acta Pharm. Fennica* 97: 187-195.

Villalain J, Aranda FJ, Gomez-Fernandez JC (1986) *Eur. J. Biochem.* 158: 141.

Weibel ER (1963) *Morphometry of the Human Lung*. Springer Verlag, Berlin, 151.

Weibel ER (1991) Design of airways and blood vessels considered as branching trees. In: Crystal RG, West JB (Eds.), *The Lung: Scientific Foundations*, Raven Press Ltd., New York, pp. 711-720.

Wetterlin K (1988) Turbuhaler : A new powder inhaler for administration of drugs to the airways *Pharm. Res.* 5: 506-508.

Weibel ER (1991) In. *The Lung: Scientific Foundations*, ed. Crystal RG and West JB, Raven Press, New York, 712.

Wiedmann TS, Bhatia R, Wattenberg LW (2000) Drug solubilization in lung surfactant. *J Control Release*, 65, 43-47.

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

Wyde PR, Six HR, Wilson SZ, Gilbert BE, Knight V (1988) Antimicrob. Agents Chemother. 32 : 890.

Yamamoto Y, Niki E, Eguchi J, Kamiya Y, Shimasaki H (1985) Biochem. Biophys. Acta, 819: 29.

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

Yoshioka S, Carstensen JT (1990) J. Pharm. Sci. 79: 943.

Zeng XM, Martin GP, Tee S, Ghoush AA, Marriott C (1999) Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation. Int. J. Pharm., 182, 133-144.