Published in final edited form as:

Methods Enzymol. 2009 ; 464: 167–191. doi:10.1016/S0076-6879(09)64009-X.

Nanoliposomal Dry Powder Formulations

Gaurang Patel^{*}, Mahavir Chougule[†], Mandip Singh[†], and Ambikanandan Misra^{*}

^{*}TIFAC-CORE in NDDS, Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Kalabhavan, Vadodara, Gujarat, India

[†]Pharmaceutics Department, College of Pharmacy and Pharmaceutical Science, Florida A&M University, Tallahassee, Florida, USA

Abstract

Liposomal dry powder formulations (DPFs) have proven their superiority over conventional DPFs due to favorably improved pharmacokinetics and pharmacodynamics of entrapped drugs, and thus, reduced local and systemic toxicities. Nanoliposomal DPFs (NLDPFs) provide stable, high aerosolization efficiency to deep lung, prolonged drug release, slow systemic dilution, and avoid macrophage uptake of encapsulated drug by carrier-based delivery of nano-range liposomes. This chapter describes methods of preparation of nanoliposomes (NLs) and NLDPFs, using various techniques, and their characterization with respect to size distribution, flow behavior, *in vitro* drug release profile, lung deposition, cellular uptake and cytotoxicity, and *in vivo* pharmacokinetics and pharmacodynamics. Some examples have been detailed for better understanding of the methods of preparation and evaluation of NLDPFs by investigators.

1. Introduction

To conquer limitations of pressurized meter dose inhalers (pMDIs) and nebulizers, dry powder inhalers (DPIs) have been developed. DPIs are devices for delivering a dry powder formulation (DPF) of an active drug, including macromolecules and biotechnologicals, for local or systemic therapies (Martin and Anthony, 2005). DPFs are stable, one-phase solid blends, easy to process, very portable, patient-friendly, easy to use, do not require spacers, and have no requirement for coordination of inhalation and actuation (Ashurst *et al.*, 2000; Geller, 2005; Hickey *et al.*, 1994). Other benefits include the capacity to carry a high drug dose, high lung deposition (from 50% to 70%), minimal extra-pulmonary loss of drug due to low oropharyngeal deposition, low device retention, and low exhaled loss. Pulmonary disposition of drugs is associated with lung clearance by alveolar macrophages of the reticuloendothelial system (RES), irrespective of the method used for delivery. This drawback may lead to high drug dosing frequency, causing patient noncompliance (four to six times in some diseases) and increase in dose with time.

Liposomal drug delivery to the respiratory tract is particularly attractive, because drugs accumulate locally in lung as the target organ. It is biocompatible (Kimelberg and Mayhew, 1978) as more than 85% of lung surfactant is composed of phospholipids. It provides controlled release of drugs for local or systemic action (Taylor and Newton, 1992), has reduced local/ systemic side effects, and thus improved therapeutic index of the drug and reduced dosing frequency, total dose, and probably cost of therapy (Courrier *et al.*, 2002; Kimelberg and Mayhew, 1978; Konduri *et al.*, 2005; Newman, 2004). The delivery of drugs encapsulated in liposomes in suspension form to the lung using a nebulizer or pMDIs are already under clinical investigation. In suspension form, liposomes have problems such as lipid degradation, aggregation, and fusion of liposomes resulting, in drug leakage during storage and aerosolization to lung (Hu and Rhodes, 1999). If a drug is delivered at the right site in the lung, at the required dose, at optimum frequency, and it stays for the required

period of time, we can expect optimum therapeutic response of the drug with minimum side effects (Oku and Namba, 2005). Conventional drug DPFs fulfill the first three requisites, but fail in the fourth one. Liposomal drugs enhance the drug residence time in the lungs, prevents enzymatic degradation of the drug, and the nano-size prevents its rapid removal through the clearance mechanism, alleviating the limitations of plain drug or conventional liposomal drug. Drug encapsulated nanoliposomes (NLs) can be processed into DPF form using freeze-drying, spray-drying, and spray freeze-drying to achieve long-term stability, and overcome problems associated with the suspension form of liposomes (Courrier *et al.*, 2002; Niven, 1995).

2. Preparation of Nanoliposomal DPFs

Conventional DPFs composed of saccharide carriers containing surface adsorbed micronized drug and having particle diameter in between 3 and 5 μm (Hersey, 1975). The particle size is restricted to 3–5 μm as below 3 μm particles are engulfed by macrophages, and above 5 μm particles show extensive oropharyngeal deposition, which is often associated with widespread systemic side effects (Bisgaard, 1996; Byron and Patton, 1994; Hickey, 1992; Newman and Clarke, 1983). Desirable characteristics of ideal DPFs include enhanced powder performance, higher lung deposition, good flow properties, and efficient delivery of a wide variety of therapeutic agents.

Because of including controlled drug release, biocompatibility, biodegradability, and nontoxic nature, liposomes have been extensively researched in pulmonary drug delivery (Shek and Barber, 1986). Formulation flexibility makes liposomal drug delivery more versatile than other lipid-based delivery systems. Methods such as the Bangham method, the organic solvent injection method, and the reverse-phase evaporation method (Betagiri *et al.*, 1993a; New, 1990a) have been commonly used for the preparation of liposomes. Liposomes having a nano-size range can be obtained by passing the liposomal suspension through a high-pressure homogenizer (10,000–25,000 psi) or extrusion through polycarbonate membranes having a pore size of less than about 100 nm at 300–400 bars pressure, and 2–10 cycles above the glass transition temperature of respective lipids of liposomal membrane. Liposomes, lipid- and polymer-based nanoparticles and nanosuspensions can also be effectively formulated using the supercritical fluid technique (SCF) (Kompella and Koushik, 2001; Sunkara and Kompella, 1999). SCF technology offers advantages over the other techniques, such as narrow size distribution, uniform morphology, and assurance of complete removal of organic solvent (Kadimi *et al.*, 2007; Misra *et al.*, 2008). Fixed amounts of drug, phospholipid, and cholesterol are dissolved in an organic solvent; the solution thus formed is loaded into a high-pressure reactor maintained below the glass transition temperature and optimized pressure (2200 psi) of supercritical fluid. The sample solution is atomized in the reaction vessel, allowing the supercritical fluid to extract the organic solvent and leave behind nano-sized liposomes (Otake *et al.*, 2001). According to the physicochemical properties of the drug to be encapsulated into liposomes, the method of preparing liposomes is selected and the formulation and process variables are optimized to maximize the percentage of drug entrapment, and to achieve the desired vesicle size and size distribution, lamellarity, and stability.

Prepared NLs are separated from untrapped drug by centrifugation or dialysis. Untrapped hydrophobic drug is separated at low speed (4000 rpm), and for hydrophilic drug, liposomes are separated by centrifugation at high speed (35,000 rpm). NL pellets are redispersed in aqueous medium. Required quantities of carriers (lactose, sucrose, mannitol, etc.), cryoprotectants (trehalose, mannitol, etc.), and antiadherents (glycine, L-leucine, serine, etc.) are added into drug incorporated nanoliposomal suspension and water is removed by either spray-drying, freeze-drying, or spray freeze-drying to obtain

nanoliposomal DPFs (NLDPFs) (Fig. 9.1). Details of the methods for converting NL suspensions in DPF suitable for inhalation are elaborated with examples in this chapter.

2.1. Ternary mixture

Freeze-dried NLDPFs are either subjected to jet milling or sieved successively through sieves # 100, 200, 350, and 500. The micronized powder is mixed with small quantity of coarse carrier such as lactose, antiadherent, and lubricant as a ternary component to get NLDPFs having improved flow behavior and enhanced lung deposition.

Amphotericin B liposomes are prepared from a mixture of hydrogenated soyphosphatidylcholine; cholesterol and either saturated soyphosphatidylglycerol (7:3:0.5) for negative charge or stearylamine (1:1:0.1) using reverse-phase evaporation technique. The soyphosphatidylglycerol and stearylamine are used to prepare negatively and positively charge liposomes, respectively. Liposomes are extruded from 2 μ m polycarbonate membrane to reduce size, separated from untrapped drug and are lyophilized using sucrose as cryoprotectant. Coarse lactose (Sorbolac 400) in varying mass ratio, with or without addition of fines (500# sieved Pharmatose 325M), is used in different mixing sequence to formulate amphotericin B NLDPFs. We observed that the 10% fine with coarse lactose is found to be optimum blend at 1:6 mass ratio of liposome:lactose. The mixing sequence of the three components, that is, liposome powder, coarse carrier, and fines are also found to influence the fine particle fraction. The maximum fine particle fraction in the range of 17–28% is observed using a Rotahaler (Cipla, India) as a delivery device at 30, 60, and 90 l/min. Although amphotericin NLDPFs are prepared from micron size liposomes and same process can be applied for NLs to process into NLDPFs (Shah and Misra, 2004b).

2.2. Spray-drying

The spray-drying method to prepare NLDPFs offers many advantages, including better control over particle formation, and hence can be easily translated to large-scale production (Woolfe *et al.*, 2001). During spray-drying, materials come into contact with hot air or gas for a fraction of a second, otherwise actual drying takes place at ambient temperature, and that is why the spray-drying technique is used for the preparation of NLDPFs composed of less temperature-sensitive materials (Bosquillon *et al.*, 2004). Respirable properties of spray-dried NLDPFs containing superoxide dismutase (Briscoe *et al.*, 1995), ciprofloxacin (Sweeney *et al.*, 2005), and antiasthmatic drugs (Patel and Misra, 2007; Sunkara and Kompella, 1999) have been evaluated. In our laboratory, we have developed spray-dried liposomal powder formulations of tacrolimus (Chougule *et al.*, 2007), dapsone (Chougule *et al.*, 2008), and amiloride hydrochloride (AH) (Chougule *et al.*, 2006) with good inhalation properties. Physical properties such as particle size and size distribution, surface energy, surface rugosity, particle density, surface area and microviscosity greatly depend on processing and formulation variables (Chougule *et al.*, 2006, 2008; Parmar, 2006). By controlling such parameters we can get DPI formulation having desired properties for different classes of drugs and widen the application window of pulmonary delivery.

AH-incorporated multilamellar vesicles are prepared by thin film hydration. Two hundred milligrams AH, hydrogenated soyaphosphatidyl-choline and cholesterol (8:2) are dissolved in $\text{CHCl}_3:\text{CH}_3\text{OH}$ (1:1) and dried as a thin film under 400 mmHg vacuum and at 65 °C temperature. The film is then hydrated at 65 °C for 1 h with 50 ml of phosphate-buffered saline (PBS), pH 7.4. Hydration is followed by size reduction by passing through a high-pressure homogenizer (Emulsiflex-C5, Avestin Inc., Ottawa, Canada) preheated to 65 °C using a thermostat for three cycles at 10,000 psi. Resultant NLs are subjected to centrifugation at 35,000 rpm, 20 °C for 20 min using ultracentrifuge (Sigma Laboratory centrifuge, 3 K 30, Osterode, GmbH). Liposomal pellets are separated, resuspended, and

characterized for vesicle size and percent drug entrapment. The liposome pellets equivalent to 50 mg of AH are dispersed in 200 ml PBS containing 15 mg/ml additive (lactose/sucrose/mannitol), and 10% of glycine, based on the weight of the powder at room temperature. For spray-drying, a laboratory spray-dryer (LSD-48, JISL, Mumbai, India) is used. Inlet and outlet temperature are kept at 120 ± 5 and $65\text{--}70$ °C, respectively. The yield of the product is found to be $60 \pm 5\%$. The spray-dried product is filled in capsules (size 2) individually by weight of powder equivalent to 500 µg of AH. HDPE bottles containing capsules and silica bags are placed in a desiccator kept in a refrigerator, after purging with nitrogen and covering with PVC-coated aluminum foil until evaluation (Chougule *et al.*, 2006).

2.3. Freeze-drying

NLDPFs of highly heat-sensitive materials such as proteins, peptides, and enzymes can be prepared using freeze-drying method. NLDPFs with different aerodynamic properties can be prepared using different proportions of carriers, cryoprotectants, and antiadherents (Lu and Hickey, 2005; Shah and Misra, 2004a; Shahiwala and Misra, 2005). Freeze-dried LDPFs for pulmonary administration of therapeutic molecules such as budesonide, ketotifen, amphotericin B, leuprolide acetate (LA), and levonorgestral have been formulated (Joshi and Misra, 2001a, 2003; Shah and Misra, 2004a; Shahiwala and Misra, 2004, 2005). The method is explained using leuprolide DPF as an example.

The reverse-phase evaporation method is employed to prepare LA-loaded liposomes. LA, hydrogenated soyaphosphatidylcholine, and cholesterol, at 2:4:1 molar ratio, are dissolved in chloroform:methanol:acetate buffer, pH 5.2 (2:4:1) in a quick fit glass tube. After vortexing the tube for 5 min, it is attached to a rotary evaporator (Superfit Continental Pvt Ltd, Mumbai, India) to dry the contents at 55 °C under vacuum (400 mmHg) until a gel formation. Again the tube is agitated on vortex mixture for 5 min to collapse the gel into fluid. Ten minutes rotary drying and 5 min vortexing is again repeated twice. The liposomal dispersion is extruded by passing through polycarbonate membranes (below 100 nm). After addition of sucrose in liposomal dispersion, it is sufficiently diluted to obtain a lipid:sugar (sucrose) ratio of 1:6. The dispersion is frozen at -40 °C overnight and dried for 24 h under negative displacement pressure (model DW1 0–60E; Heto Drywinner, Birkerød, Denmark). The obtained porous cake is passed through #200 and #240 sieves. To get the final strength of 250 µg LA/10 mg of powder, equivalent amount of Sorbolac 400 is geometrically added. The DPF is filled in capsules (size 2) individually by weight of powder equivalent to 250 µg of LA. HDPE bottles containing capsules and silica bags are placed in a desiccator kept in a refrigerator after purging with nitrogen and covering with PVC-coated aluminum foil until evaluation (Shahiwala and Misra, 2005).

2.4. Spray freeze-drying

Spray freeze-drying involves spraying the drug solution into a freezing medium (cryogen) followed by lyophilization of the suspension. Advantages of spray freeze-drying involves almost 100% yield, production of light and porous particles with enhanced aerosol performance, and minimum chances of aggregation of particles. Besides these advantages, disadvantages of the spray freeze-drying process include the expense and the time required (Sweeney *et al.*, 2005).

Dimyristoylphosphatidylglycerol, lactose, and ciprofloxacin in a weight percent ratio 5:17:1, respectively, form a smooth suspension upon vortexing (2×30 s to 4×30 s in 1 h), and the suspension remains stable at 4 °C for several days. Spray freeze-drying is performed with a two-fluid nozzle, in which compressed nitrogen and a peristaltic pump are used to drive the suspension in fine atomized form into a flask containing liquid nitrogen. The cryogen is then allowed to evaporate, and the resulting powder is dried for 48 h in a lyophilizer. The

collector is held at -52°C while the vacuum is 0.004 mbar. The powder is subsequently collected and stored in a sealed vial at 4°C (Sweeney *et al.*, 2005).

3. Physicochemical Characterization of NLDPFs

Prepared liposomes are characterized for percent drug entrapment, size and size distribution, zeta potential, entrapped volume, morphology, oxidative index, and differential scanning calorimetry (DSC) (Akwete and Hsu, 1993; Betagiri *et al.*, 1993b; Chougule *et al.*, 2006, 2007; Goel, 1988; Hyung *et al.*, 2008; Joshi and Misra, 2001b; Kaszuba, 1999; Kent *et al.*, 2007; Maria *et al.*, 2004; Markus *et al.*, 2004; Mina *et al.*, 2002; New, 1990b; Shah and Misra, 2004a; Vyas *et al.*, 2005), and NLDPFs are evaluated for following parameters.

3.1. Assay

The amount of drug present in a fixed quantity of DPF can be obtained by dissolving the required quantity of DPF in methanol or a methanol–chloroform mixture. The resultant solution is filtered to remove the sugar part. The amount of drug in the filtrate is estimated by a suitable analytical method (Chougule *et al.*, 2006).

3.2. Flow behavior

The angle of repose is measured to determine the flow properties of a powder. The compressibility index reflects the percent voids in the powder along with the flow and dispersibility properties of formulation. Bulk and tapped density of the powder is determined for the calculation of the compressibility index and the theoretical mean mass aerodynamic diameter (MMAD) of the powder. Dispersibility index is the dispersing ability of agglomerated particles on application of pressure equivalent to inhalation air pressure. A higher dispersibility index is expected to get a higher lung deposition (Carr, 1965; Chougule *et al.*, 2006).

3.3. Moisture content determination

Stability and flow properties mainly depend on the amount of moisture present in the NLDPFs. A higher amount of moisture causes powder aggregation, leading to poor lung deposition. The moisture content of LDPF is accurately determined by the Karl Fischer titration method for abrupt idea loss on drying (LOD) moisture balance can be used (Chougule *et al.*, 2006). Solid-state characterization and residual water content data for AH NLDPFs are tabulated in Table 9.1.

3.4. Reconstitution time and volume

Time and volume of phosphate buffer (pH 7.4) required to reconstitute a fixed quantity of NLDPF is measured (Chougule *et al.*, 2007).

3.5. Particle size of NLDPFs

Instruments based on laser light scattering and the photon correlation principle are mainly used to determine the particle size of NLDPFs (Chougule *et al.*, 2006; Goel, 1988; Kaszuba, 1999). Figure 9.2 shows the particle size distribution graph of AH NLDPFs.

3.6. Drug retention and stability studies

As per ICH guidelines, comparative drug retention (PDR) studies are performed on the NLDPFs for long-term stability at $5 \pm 3^{\circ}\text{C}$ for 12 months, and accelerated testing at $25 \pm 2^{\circ}\text{C}/60\% \text{RH} \pm 5\% \text{RH}$ for 6 months (ICH Guidelines, 2003; Singh, 1999). The product in its final packing is stored at the above-mentioned storage conditions. Samples are withdrawn at definite time intervals and evaluated for assay, water content, PDR, fine particulate fraction,

and emission and physical changes (Chougule *et al.*, 2007; Singh, 1999). Data of drug retention and stability of tacrolimus NLDPFs are recorded in Table 9.2.

3.7. Scanning electron microscopy (photomicrographs and image analysis)

Prepared NLDPFs are evaluated for surface morphology, size, shape, and aggregation behavior by using scanning electron microscopy (SEM) (Chougule *et al.*, 2008). Scanning electron microphotograph and b surface texture analysis of dapsone NLDPF are depicted in Fig. 9.3. Topographical features of dapsone NLDPFs measured by SEM image analysis are tabulated in Table 9.3.

3.8. Differential scanning calorimetry

Prepared NLDPFs are evaluated for thermal properties using DSC (Chougule *et al.*, 2006). DSC curves of AH NLDPFs are contained in Fig. 9.4.

3.9. *In vitro* lung deposition studies

Anderson cascade impactor (official in USP) and multiple-stage liquid impinger (official in BP and EP) resembles human respiratory track and thus used to evaluate target region within the lung and deposition of inhaled NLDPFs in each region of lung. From that data the emitted dose, fine particle dose, fine particle fraction, MMAD, and geometric standard deviation (GSD) can be calculated according to the procedure given in USP (USP 30 NF 25, 2007). The other parameters to be estimated include recoverable dose, emitted dose, percent emission, and percent dispersibility.

NLDPF density and geometric diameter are the key parameter which decides MMAD of NLDPF and thus lung deposition and effectiveness of the therapeutic delivery system (Chougule *et al.*, 2007). Tacrolimus NLDPFs were evaluated for *in vitro* pulmonary deposition using an eight stage Andersen cascade impactor with a preseparator (Graseby-Andersen, Atlanta, GA, USA) and data obtained are tabulated in Table 9.4.

3.10 *In vitro* drug release study

In vitro drug release study is a tool that provides an estimate of the *in vivo* pharmacokinetic and pharmacodynamic performance of the formulation (Fielding and Abra, 1992). Therefore an *in vitro* release technique is proposed, validated, and utilized for drug release studies from optimized NLDPFs. To examine the drug release kinetics and mechanism, the percent drug diffused (Shah *et al.*, 1993), the kinetics of release, the mean steady-state flux (Chien, 1992; Robinson and Lee, 1987), and the diffusion coefficient (Chougule *et al.*, 2006) are measured. The curve of percent drug released versus time for AH NLDPFs is graphically presented in Fig. 9.5.

3.11 *In vivo* studies

In vivo studies are to be performed to evaluate lung absorption and the lung deposition pattern. For this purpose, the following three approaches have been utilized.

3.11.1. Animal models (*in vivo*)—Evaluation of pharmacokinetic behavior and distribution in different sections of the lungs are carried out using different animal models, including rats and guinea pigs. Methods such as intratracheal instillation, micro spray, nebulization, and aerosol puff are used for dosing of NLDPFs in animals. Drug present in homogenate of various lobes of the lung and in bronch-oalveolar lavage is estimated at various time intervals to get the idea of amount of drug deposition as well as pattern of distribution within the lungs. The amount of drug present in blood and homogenates of other major organs is estimated to assess systemic absorption after inhalation of NLDPFs.

Validated analytical methods are developed in our laboratory to estimate the pharmacokinetic profiles of dapsone, rifampicin, amikacin, ketotifen, tacrolimus, AH, isoniazid, and amphotericin B in rats (Chougule *et al.*, 2006, 2007, 2008; Joshi and Misra, 2001a,c, 2003; Shah and Misra, 2004a,c). Serum LH levels are measured to estimate the pharmacodynamic response after intratracheal administration of leuprolide and levonorgestral liposomes (Shahiwala and Misra, 2004, 2005). However, these methods require sacrifice of animals, and hence other techniques, including *in vitro* cell lines and *ex vivo* techniques, may also be used (Chougule *et al.*, 2006).

AH-loaded NLDPFs are evaluated for *in vivo* performance following intratracheal instillation. For each time interval, six albino rats (220–240 g) of either sex are housed in individual plastic cages at a constant temperature. Rats are fasted overnight prior to each experiment. After anesthetizing rats by intraperitoneal administration of a urethane solution (1.2 gm/kg), the trachea is exposed by blunt dissection of the sternohyoideus muscle and a small midline incision is made over the trachea between the fifth and sixth tracheal rings using a 20-gauge needle. The trachea is cannulated with PE200 tubing (5–7 cm) with the tip positioned approximately at the tracheal bifurcation. The PE50 (10–15 cm) tubing connected to a glass Hamilton syringe (Waters, Bangalore, India) is inserted into the cannula and advanced to the bifurcation of the trachea. Prepared formulations equivalent to 100 µg of AH are administered, and animals are sacrificed at 2, 4, 8, 10, and 12 h after administration (Fig. 9.6).

Bronchoalveolar lavage is performed on anesthetized and recannulated animals with 12 ml PBS, prewarmed to 37 °C. To perform the lavage, the Hamilton syringe connected to the PE50 tubing is replaced with a three-way stopcock attached to two 20 ml syringes. Approximately 12 ml sterile (0.22 µm filtered) PBS is injected slowly in fractions to fill the lungs. The fluid is withdrawn by gentle aspiration; this bronchial alveolar lavage yields between 7 and 11 ml liquid, which is centrifuged at 2000 rpm for 5 min. One percent Triton X-100 is added to the supernatant and analyzed to determine unreleased AH. The lungs and portions of the trachea below the instillation site are excised and homogenized in 10 ml PBS containing 1% Triton X-100. Sulphosalicylic acid (10%) is used as a deproteinizing agent. AH released is analyzed in the supernatant after suitable dilution (Chougule *et al.*, 2006).

3.11.2. Lung epithelial cell models (*in vitro*)—*In vitro* cell line models have become more popular than *in vivo* models to evaluate prepared formulations for cell uptake, cell toxicity, and cell death kinetics studies (Anabousi *et al.*, 2006; Elbert *et al.*, 1999; Fang *et al.*, 2004; Fuchs *et al.*, 2003; Pang *et al.*, 2005; Sakagami *et al.*, 2002a,b). Intact lung studies have two limitations: (i) the contribution of different distal lung epithelial cells can not be studied separately, and (ii) the surface area for fluid absorption can only be approximated. Lung epithelial cell lines include tracheobronchial, bronchial, and alveolar cell lines and they are mainly of rat and human origin. The examples of cancer-derived cell lines include Calu-1, Calu-3, Calu-6, H441, HBE1, A427, A549, 16HBE140, and BEAS-2B. The examples of cell lines transformed from normal lung include 9HTE16o-, 16HBE14o-1HAEo-, BEAS-2BCF/T43, and AK-D. These cell lines have been tested for the change in transepithelial transport kinetics (i.e., Papp) and for predicting *in vivo* lung absorption (Tronde *et al.*, 2003).

An *in vitro* cell culture model that mimics the respiratory epithelium, in particular cells that form tight monolayers and secrete components of mucus or surfactant, would be a valuable tool for NLDPFs characterization. The air-interfaced culture (AIC) or liquid-covered culture (LCC)-grown Calu-3 monolayers can be used as an *in vitro* model to evaluate therapeutic large porous powder particles (Fiegel *et al.*, 2003).

Similar experiments can be designed for evaluation of NLDPFs. Briefly, Calu-3 cells are seeded onto Transwell clear permeable filter inserts at a density of 105 cells/cm². After seeding, cells are grown in 1500 µl (apical) and 2600 µl (basolateral) of cell culture media at 37 °C in a 5% CO₂ incubator. For LCC conditions, the media bathing the cells are removed and cells are fed with 1500 µl fresh apical and 2600 µl fresh basolateral media 1 day after seeding, and then once daily. For AIC conditions, the apical medium of cells are removed 1 day after seeding and cells are fed daily with 1400 µl fresh basolateral media only after. When the cells reach sufficient confluency, the apical fluid is removed from the LCC-grown Calu-3 monolayers, and the basolateral media is reduced to 1400 µl. AIC-grown monolayers are used as a control. Filter inserts containing cell monolayers are transferred from the 6-well plate to a cut-out single well containing prewarmed media. One well is placed directly under the second-stage nozzle of an Astra-type liquid impinger (Erweka, Heusenstamm, Germany) and the impinger is sealed, as illustrated in Fig. 9.7. Dry powder particles are aerosolized onto the monolayers for 30 s at 30 l/min from #2 gelatin capsules, using a Spinhaler device. The filter inserts are then returned to the 6-well plate and placed back into an incubator at 37 °C. Slightly wetted Transwell inserts without cells are placed in the impinger and treated as described earlier to obtain reproducible data. After particle impinging, 0.8 N NaOH is added to the apical and basolateral compartments of the filter to dissolve the dry powder. Following a 24 h incubation at 37 °C, the complete degradation of the particles on the filter inserts are verified by light microscopy. Samples are taken and analyzed for fluorescein-sodium using a fluorescence plate reader (at excitation and emission wavelengths of 485 and 530 nm, respectively; Fiegel *et al.*, 2003).

3.11.3. Lung tissue models (ex vivo)—*Ex vivo* lung tissue models have been preferred when the information on the mechanisms involved in drug transport or lung disposition kinetics are not predictable from *in vivo* or *in vitro* models. The isolated perfused lung (IPL) model is most widely used for the purpose of establishing the mechanisms of drug absorption and deposition in the lungs. In this type of model, the lung are isolated from the body and placed in a simulated environment to perform the desired experiment. IPL prepared from small rodents have been used for drug deposition and organ toxicity studies (Pang *et al.*, 2005; Sakagami *et al.*, 2002a,b; Tronde *et al.*, 2003). The limitations of IPL models includes. The only drawbacks associated with these models are the short viable periods of (2–3 h), and the absence of blood and mucous circulation.

The IPL preparation has been employed mostly with rats and guinea pigs rather than with mice, dogs and monkeys. The preparation consists of (a) peristaltic pumps and a tubing assembly that is used to carry the perfusate to and from the lungs and (b) a double-jacketed artificial thorax to house the IPL at a certain temperature, normally at 37 °C. A flow rate of 12–15 ml/min (rat lung) is used to pump the perfusate from the reservoir to the IPL through a central porthole of the thorax lid. The opening at the bottom of the thorax is used to return the perfusate to the reservoir or to collection. The air-tight sealed thorax enables negative- or positive-pressure ventilation, or the maintenance of a certain pressure. Under anesthesia, the pulmonary circulation of the lung is cannulated via the pulmonary artery and lung perfusion is initiated and maintained. The pulmonary vein is also cannulated as an exit of the lung perfusion, or as an alternative, the bottom of the left auricle and both sides of the ventricles in the heart are amputated to allow the free flow of perfusate without any unnecessary back pressure in the IPL. The lung and heart are excised from the body *en bloc*. The trachea is also cannulated and often exteriorized out of the thorax. This setup supports the IPL suspension in the thorax, and for pulmonary biopharmaceutics research, allows airway luminal administration of test molecules. The IPL can be suspended in a horizontal or vertical fashion, as shown in Fig. 9.8. Autologous whole blood or a buffered artificial medium, such as Krebs–Ringer or Krebs–Henseleit buffer (pH 7.4) supplemented with 5 mM glucose and/or 4–5% albumin can be used as the perfusate. The perfusate medium is

equilibrated with a mixture of oxygen and carbon dioxide throughout the experiments. Generally, the perfusion is recirculated, but can also be a single-pass system. Using sufficient surgical technique, it has been shown that the IPL viability can be maintained for a maximum of 2–3 h at 37 °C, after which the lung gradually develops translucent areas, indicating edema formation (Sakagami *et al.*, 2006).

After the perfusion begins, the perfusate samples are with drawn via the tracheal port at different time intervals, enabling the characterization of the absorption profiles.

The rat IPL is used for administration of different sizes of powder aerosols with the help of the exteriorized tracheal cannula port. The IPL is suspended in a physiologically natural horizontal fashion, with the help of a stainless rod through the esophagus (Fig. 9.8A). Dry powder aerosol of fluorescein is administered to the IPL via negative pressure ventilations created in the artificial thorax. The fractional fluorescein transfer to the perfusate is correlated to the depth of aerosol penetration into the lung. However, the inhalation exposures have been limited to lower aerosol concentrations, and thus necessitate an extended time of administration (Byron *et al.*, 1986).

Most existing technologies for suspending cohesive powders are continuous generators that consume large amount of powder. Examples of such technologies are the Wright dust feeder, fluidized bed generators, venturi tube injectors, and the jet mill. Powder consumption is often increased by the requirement of having a downstream impactor stage, installed to reduce the nonrespirable fraction of larger particle sizes in the exposure atmosphere. Recently, the DustGun aerosol technology has been applied to expose the isolated and perfused rat lung to dry powder aerosols of dieselsoot and the carcinogenic solute benzo(a)pyrene.

4. Concluding Remarks

The methods and studies described in this chapter indicate that various drugs and peptides can be encapsulated stably into NLs, and successfully formulated into NLDPFs suitable for pulmonary administration. The preclinical studies show that NLDPFs are more effective than the free drug under both *in vitro* and *in vivo* conditions. The successful pulmonary delivery of therapeutic agents using NLDPFs suggests their potential use for treatment of pulmonary and systemic diseases. Ongoing research in the area of NLDPFs can improve clinical outcomes, and continue to expand therapeutic options in the treatment of many more diseases. Further developments focused on NL delivery in dry form to the lung are therefore warranted.

REFERENCES

- Aerosols, Nasal Sprays, Metered Dose Inhalers, and Dry Powder Inhalers. USP 30 NF. 2007; 25:601–607.
- Akwete, L.A.; Hsu, L. Leuprolide and other LH-RH analogues in stability and characterization of protein and peptide drugs: Case histories. In: Wang, YJ.; Pearlman, R., editors. "Formation, Characterization, and Stability of Protein Drugs,". New York: Plenum Press; 1993. p. 159-199.
- Anabousi S, Bakowsky U, Schneider M, Huwer H, Lehr CM, Ehrhardt C. *In vitro* assessment of transferrin-conjugated liposomes as drug delivery systems for inhalation therapy of lung cancer. Eur. J. Pharm. Sci. 2006; 29:367–374. [PubMed: 16952451]
- Application Note by Malvern Instruments. Size and zeta potential characterization of anionic and cationic liposomes on the Zetasizer Nano.
- Ashurst II, Malton A, Prime D, Sumby B. Latest advances in the development of dry powder inhalers. Pharm. Sci. Technol. 2000; 3:246–256.

- Betagiri, JV.; Jenkins, SA.; Parsons, DL. "Liposomes Drug Delivery System,". Lanchaster, Basel, Switzerland: Technomic Publishing Co. Inc; 1993a. p. 7-20.
- Betagiri, JV.; Jenkins, SA.; Parsons, DL. "Liposomes Drug Delivery System,". Lanchaster, Basel, Switzerland: Technomic Publishing Co. Inc; 1993b. p. 21-24.
- Bisgaard H. Drug delivery from inhaler devices. Lung deposition, clinical effect and cost effectiveness vary. *Br. Med. J.* 1996; 313:895–896. [PubMed: 8876085]
- Bosquillona C, Rouxhetb PG, Ahimoub F, Simonc D, Culotd C, Preata V, Vanbever R. Aerosolization properties, surface composition and physical state of spray-dried protein powders. *J. Control. Release.* 2004; 99:357–367. [PubMed: 15451594]
- Briscoe P, Caniggia I, Graves A, Benson B, Huang L, Tanswell AK, Freeman BA. Delivery of superoxide dismutase to pulmonary epithelium via pH-sensitive liposomes. *Am. J. Physiol.* 1995; 268:L374–L380. [PubMed: 7900818]
- Byron PR, Patton JS. Drug delivery via the respiratory tract. *J. Aerosol Med.* 1994; 7(1):49–75. [PubMed: 10147058]
- Byron PR, Roberts NSR, Clark AR. An isolated perfused rat lung preparation for the study of aerosolized drug deposition and absorption. *J. Pharm. Sci.* 1986; 75:168–171. [PubMed: 3958927]
- Carr RL. Evaluating flow properties of solids. *Chem. Eng.* 1965; 72:163–168.
- Chien, YW. "Novel Drug Delivery Systems, Drugs and the Pharmaceutical Sciences,". Vol. 50. New York: Marcel Dekker; 1992. p. 797
- Chougule M, Padhi B, Misra A. Nano-liposomal dry powder inhaler of amiloride hydrochloride. *J. Nanosci. Nanotechnol.* 2006; 6(9–10):3001–3009. [PubMed: 17048511]
- Chougule M, Padhi B, Misra A. Nano-liposomal dry powder inhaler of tacrolimus: Preparation, characterization, and pulmonary pharmacokinetics. *Int. J. Nanomed.* 2007; 2(4):675–688.
- Chougule M, Padhi B, Misra A. Development of spray dried liposomal dry powder inhaler of dapsone. *AAPS PharmSciTech.* 2008; 9(1):47–53. [PubMed: 18446460]
- Courrier HM, Butz N, Andamme F. Pulmonary drug delivery systems: Developments and prospects. *Crit. Rev. Ther. Drug Carrier Syst.* 2002; 19(4–5):425–498. [PubMed: 12661699]
- Elbert KJ, Schafer UF, Schäfers HJ, Kim KJ, Lee VH, Lehr CM. Monolayers of human alveolar epithelial cells in primary culture for pulmonary absorption and transport studies. *Pharm. Res.* 1999; 16:601–608. [PubMed: 10349999]
- Fang X, Song Y, Zemans R, Hirsch J, Matthay MA. Transport across cultured rat alveolar epithelial cells: A novel *in vitro* system. *Am. J. Physiol. (Lung Cell Mol. Physiol.)*. 2004; 27:L104–L110. [PubMed: 14990396]
- Fiegel J, Ehrhardt C, Schaefer UF, Lehr CM, Hanes J. Large porous particle impingement on lung epithelial cell monolayers—Toward improved particle characterization in the lung. *Pharm. Res.* 2003; 20(5):788–796. [PubMed: 12751635]
- Fielding RM, Abra RM. Factors affecting the release rate of terbutaline from liposome formulations after intratracheal instillation in the guinea pig. *Pharm. Res.* 1992; 9:220–222. [PubMed: 1553346]
- Fuchs S, Hollins AJ, Lehr CM, et al. Differentiation of human alveolar epithelial cells in primary culture: Morphological characterization and synthesis of caveolin-1 and surfactant protein-C. *Cell Tissue Res.* 2003; 311:31–45. [PubMed: 12483282]
- Geller DE. Comparing clinical features of the nebulizer, metered-dose inhaler, and dry powder inhaler. *Respir. Care.* 2005; 50(10):1313–1322. [PubMed: 16185367]
- Goel, BK. "Medical Laboratory Technology,". Vol. III. New Delhi, India: Tata McGraw-Hill; 1031. p. 1031
- Hersey JA. Ordered mixing: A new concept in powder mixing practice. *Powder Technol.* 1975; 11:41–44.
- Hickey, AJ. "Summary of Common Approaches to Pharmaceutical Aerosol Administration; Pharmaceutical Inhalation Aerosol Technology,". New York: Marcel Dekker; 1992. p. 255-288.
- Hickey AJ, Concessio NM, Platz RM, et al. Factors influencing the dispersion of dry powders as aerosols. *Pharm. Technol.* 1994; 8:58–84.
- Hu C, Rhodes DG. Proniosomes: A novel drug carrier preparation. *Int. J. Pharm.* 1999; 185:23–35. [PubMed: 10425362]

- Hyung JL, Eun CC, Jongwon S, Do-Hoon K, Eun JA, Junoh K. Polymer-associated liposomes as a novel delivery system for cyclodextrin-bound drugs. *J. Colloid Interface Sci.* 2008; 320:460–468. [PubMed: 18261738]
- ICH Guidelines. Stability testing of new drug substances and products. ICH Topic Q 1 A (R2). 2003 Page 7 of 20 August 2003.
- Joshi MR, Misra A. Liposomal budesonide for dry powder inhaler: Preparation and stabilization. *AAPS PharmSciTech.* 2001a; 2(4):25–31. [PubMed: 14727862]
- Joshi M, Misra A. Dry powder inhalation of liposomal ketotifen fumarate: Formulation and characterization. *Int. J. Pharm.* 2001b; 223(1–2):15–27. [PubMed: 11451628]
- Joshi M, Misra A. Pulmonary disposition of budesonide from liposomal dry powder inhaler. *Meth. Find Exp. Clin. Pharm.* 2001c; 23(10):531.
- Joshi M, Misra A. Disposition kinetics of ketotifen from liposomal dry powder for inhalation in rat lung. *Clin. Exp. Pharmacol. Physiol.* 2003; 30:153–156. [PubMed: 12603343]
- Kadimi US, Balasubramanian DR, Ganni UR, Balaraman M, Govindarajulu V. In vitro studies on liposomal amphotericin B obtained by supercritical carbon dioxide-mediated process. *Nanomedicine.* 2007; 3(4):273–280. [PubMed: 17962084]
- Kaszuba M. The measurement of nanoparticles using photon correlation spectroscopy and avalanche photo diodes. *J. Nano Res.* 1999; 1(3):405–409.
- Otake K, Imura T, Sakai H, Abe M. Method of liposomes using supercritical carbon dioxide. *Langmuir.* 2001; 17:3898–3901.
- Kent J, Thomas LA, Anders FV, Huiling M, Xuebing X. Oxidative stability of liposomes composed of docosahexaenoic acid-containing phospholipids. *J. Am. Oil Chem. Soc.* 2007; 84(7):631–637.
- Kimelberg HK, Mayhew EG. Properties and biological effects of liposomes and their uses in pharmacology and toxicology. *Crit. Rev. Toxicol.* 1978; 6(1):25–79.
- Kompella U, Koushik K. Preparation of drug delivery systems using supercritical fluid technology. *Crit. Rev. Ther. Drug Carrier Syst.* 2001; 18(2):173–199. [PubMed: 11325031]
- Konduri K, Nandedkar S, Rickaby DA, Du'zgu'nes, N, Gangadharam PRJ. The use of sterically stabilized liposomes to treat asthma. *Methods Enzymol.* 2005; 391:413–427. [PubMed: 15721394]
- Lu D, Hickey A. Liposomal dry powders as aerosols for pulmonary delivery of proteins. *AAPS PharmSciTech.* 2005; 6(4):E641–E648. [PubMed: 16408866]
- Maria LI, Paola B, Flavio R, Silvia A, Maurizio C, Luigi C. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing lipophilic gemcitabine prodrugs. *J. Control. Release.* 2004; 100:331–346. [PubMed: 15567500]
- Markus M, Stefan M, Christel C, Müller G. Physicochemical characterisation of liposomes with encapsulated local anaesthetics. *Int. J. Pharm.* 2004; 274:139–148. [PubMed: 15072790]
- Martin JT, Anthony JH. Dry powder inhaler formulation. *Respir. Care.* 2005; 50(9):1209–1227. [PubMed: 16122404]
- Mina A, Kanako Y, Kazuo M. Oxidative stability of polyunsaturated fatty acid in phosphatidylcholine liposomes. *Biosci. Biotechnol. Biochem.* 2002; 66(12):2573–2577. [PubMed: 12596850]
- Misra A, Parmar N, Naik S, Patel G. Preparation of Amphotericin B liposomes by supercritical fluid technology. 2008 IN391/MUM/2008.
- New, RRC. “Liposomes—A Practical Approach,”. New York: Oxford University Press; 1990a. p. 33–103.
- New, RRC. “Liposomes—A Practical Approach,”. New York: Oxford University Press; 1990b. p. 105–160.
- Newman SP. Dry powder inhalers for optimal drug delivery. *Expert Opin. Biol. Ther.* 2004; 4(1):23–33. [PubMed: 14680466]
- Newman SP, Clarke SW. Therapeutic aerosols I—Physical and practical considerations. *Thorax.* 1983; 38:881–886. [PubMed: 6364439]
- Niven RW. Delivery of biotherapeutics by inhalation aerosol. *Crit. Rev. Ther. Drug Carrier Syst.* 1995; 12:151–231. [PubMed: 9501969]
- Oku N, Namba Y. Glucuronate-modified, long-circulating liposomes for the delivery of anticancer agents. *Methods Enzymol.* 2005; 391:145–162. [PubMed: 15721379]

- Pang Y, Sakagami M, Byron PR. The pharmacokinetics of pulmonary insulin in the isolated perfused rat lung: Implications of metabolism and regional deposition. *Eur. J. Pharm. Sci.* 2005; 25:369–378. [PubMed: 15979534]
- Parmar M. Formulation of insoluble small molecule therapeutics in lipid-based carriers. 2006 US20060051406A1.
- Patel, G.; Misra, A. Pulmonary delivery of liposomal dry powder inhaler of formoterol for effective treatment of asthma. 2007. http://www.aapsj.org/abstracts/AM_2007/AAPS2007001080.PDF
- Robinson, JR.; Lee, VHL. *Controlled Drug Delivery: Fundamentals and Applications*. 2nd edn.. New York: Marcel Dekker; 1987.
- Sakagami M, Byron PR, Rypacek F. Biochemical evidence for transcytotic absorption of polyaspartamide from the rat lung: Effects of temperature and metabolic inhibitors. *J. Pharm. Sci.* 2002a; 91:1958–1968. [PubMed: 12210043]
- Sakagami M, Byron PR, Venitz J, Rypacek F. Solute disposition in the rat lung *in vivo* and *in vitro*: Determining regional absorption kinetics in the presence of mucociliary clearance. *J. Pharm. Sci.* 2002b; 91:594–694. [PubMed: 11835216]
- Sakagami M, Byron PR, Venitz J, Rypacek F. *In vivo*, *in vitro*, and *ex vivo* models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. *Adv. Drug Deliv. Rev.* 2006; 50:9–10. 1030–1060.
- Shah SP, Misra A. Development of liposomal Amphotericin B dry powder inhaler formulation. *Drug Deliv.* 2004a; 11(4):247–253. [PubMed: 15371106]
- Shah SP, Misra A. Liposomal amikacin dry powder inhaler: Effect of fines on *in vitro* performance. *AAPS PharmSciTech.* 2004b; 5(4):E65. [PubMed: 15760062]
- Shah SP, Misra A. Liposomal amphotericin B dry powder inhaler: Effect of fines on *in vitro* performance. *Pharmazie.* 2004c; 59(10):812–813. [PubMed: 15544064]
- Shah VP, Elkins JS, Williams RL. *In vitro* drug release measurement of topical glucocorticoid creams. *Pharmaceutics Forum.* 1993; 19:5048–5059.
- Shahiwala A, Misra A. Pulmonary absorption of liposomal levonorgestrel. *AAPS PharmSciTech.* 2004; 5(1):E13. [PubMed: 15198534]
- Shahiwala A, Misra A. A preliminary pharmacokinetic study of liposomal leuprolide dry powder inhaler: A technical note. *AAPS PharmSciTech.* 2005; 6(3):E482–E486. [PubMed: 16354008]
- Shek PN, Barber RF. Liposomes: A new generation of drug and vaccine carriers. *Mod. Med.* 1986; 41:314–326.
- Singh S. Drug stability testing and shelf life determination according to international guidelines. *Pharm. Technol.* 1999; 23:68–86.
- Sunkara G, Kompella U. Drug delivery applications of supercritical fluid technology. *Drug Deliv. Technol.* 1999; 2:33–34.
- Sweeney L, Wang Z, Loebenberg R, Wong JP, Lange CF, Finlay W. Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery. *Int. J. Pharm.* 2005; 305(1–2):180–185. [PubMed: 16242277]
- Taylor KMG, Newton JM. Liposomes for controlled delivery of drugs to the lung. *Thorax.* 1992; 47:257–259. [PubMed: 1585287]
- Tronde A, Norden B, Jeppsson AB, Brunmark P, Nilsson E, Lennernas H, Bengtsson UH. Drug absorption from the isolated perfused rat lung—Correlations with drug physicochemical properties and epithelial permeability. *J. Drug Target.* 2003; 11(1):61–74. [PubMed: 12852442]
- Vyas SP, Quraishi S, Gupta S, Jaganathan KS. Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. *Int. J. Pharm.* 2005; 296:12–25. [PubMed: 15885451]
- Woolfe, AJ.; Zheng, XM.; Langford, A. *Methods to produce powders for pulmonary or nasal administration*. 2001. WO0113885

Figure 9.1.

(A) Detailed flow chart describing formulation optimization of nanoliposomes and (B) detailed flow chart describing formulation optimization of NLDPFs.

Figure 9.2.

Particle size distribution of amiloride hydrochloride NLDPFs. (Reprinted with permission from Chougule *et al.*(2006). Copyright © American Scientific Publishers, www.aspbs.com.)

Figure 9.3. Scanning electron micrograph (A) and surface texture analysis (B) of dapsone NLDPF. (With kind permission from Springer Science + Business Media: Chougule *et al.* (2008).

Figure 9.4. Differential scanning calorimetric graphs of developed amiloride hydrochloride NLDPF (a), plain drug (b), and physical mixture (c). (Reprinted with permission from Chougule *et al.* (2006). Copyright © American Scientific Publishers, www.aspbs.com.)

Figure 9.5.

In vitro release profile of developed amiloride hydrochloride NLDPFs and plan drug DPFs (SAHL = plain drug formulation, SLDPIM = NLDPF containing mannitol, SLDPIL = NLDPF containing lactose, and SLDPFs = NLDPF containing sucrose). (Reprinted with permission from Chougule *et al.* (2006). Copyright © American Scientific Publishers, www.aspbs.com.)

Figure 9.6.
Intratracheal instillations and bronchoalveolar lavage on rat.

Figure 9.7.
Astra-type liquid cascade impinger used with a Transwell under the second-stage nozzle.
(With kind permission from Springer Science + Business Media: Fiegel *et al.* (2003)).

Figure 9.8.

Isolated perfused rat lung (IPRL) preparations of (A) Horizontally positioned IPRL with a scheme of forced solution instillation and (B) vertically positioned IPRL with a scheme of nebulization catheter dosing. (Reprinted from Sakagami *et al.* (2006). Copyright 2006, with permission from Elsevier.)

Table 9.1

Solid-state characterization and residual water content of amiloride hydrochloride nanoliposomal dry powder formulations

Formulations	Tapped density (g/ml)	Angle of repose (°)	Carr's compressibility index	Residual water content (%)
AHL	0.9 ± 0.1	45.6 ± 2.4	25.8 ± 1.4	5.9 ± 0.6
AHNLDPF-lactose	0.3 ± 0.05	31.1 ± 1.9	35.8 ± 2.1	4.4 ± 0.5
AHNLDPF-mannitol	0.2 ± 0.01	27.3 ± 1.5	39.8 ± 1.5	2.5 ± 0.4
AHNLDPF-sucrose	0.36 ± 0.04	30.8 ± 1.5	33.4 ± 1.9	5.7 ± 0.8

Reprinted with permission from Chougule *et al.* (2006). Copyright © American Scientific Publishers, www.aspbs.com.

Table 9.2

Stability data of tacrolimus nanoliposomal dry powder formulations

Stability conditions	Description	Assay (%)	Water content	Percent drug retained	Liposomal size (VMD) (nm)	Emission (%)	Fine particle fraction (FPF)
Initial	White free flowing powder	101.16 ± 1.86	2.8 ± 0.3	101.38 ± 2.47	0.140 ± 0.02	82 ± 3.0	71.1 ± 2.5
40 ± 2 °C and 75 ± 5% RH							
1M	White free flowing powder	101.08 ± 3.24	2.7 ± 0.5	95.92 ± 3.51	0.145 ± 0.03	80.6 ± 2.2	70.45 ± 2.7
2M	White free flowing powder	99.43 ± 2.97	3.3 ± 0.5	91.45 ± 3.95	0.152 ± 0.03	78.4 ± 3.0	67.13 ± 2.5
3M	White free flowing powder	98.09 ± 3.95	3.4 ± 0.6	87.43 ± 3.91	0.158 ± 0.02	77.2 ± 2.9	62.91 ± 1.9
6M	White free flowing powder	99.72 ± 3.54	3.0 ± 0.7	85.71 ± 3.98	0.161 ± 0.03	75.4 ± 2.6	60.11 ± 3.0
30 ± 2°C and 65 ± 5% RH							
1M 2M	White free flowing powder	103.46 ± 2.72	2.8 ± 0.4	100.26 ± 3.29	0.144 ± 0.03	81.87 ± 3.7	70.93 ± 2.1
	White free flowing powder	102.16 ± 2.91	2.9 ± 0.5	101.49 ± 3.96	0.138 ± 0.03	81.46 ± 3.4	71.08 ± 2.4
3M	White free flowing powder	101.13 ± 2.58	2.9 ± 0.4	100.17 ± 3.21	0.148 ± 0.02	81.4 ± 3.9	70.84 ± 2.3
6M	White free flowing powder	100.18 ± 3.1	3.0 ± 0.4	102.41 ± 4.23	0.151 ± 0.03	80.19 ± 3.2	68.42 ± 2.9
9M	White free flowing powder	99.42 ± 2.9	2.9 ± 0.5	99.59 ± 3.91	0.153 ± 0.04	80.07 ± 3.0	67.82 ± 2.8
12 M	White free flowing powder	100.51 ± 3.59	2.9 ± 0.4	99.14 ± 4.1	0.152 ± 0.03	78.76 ± 3.5	65.19 ± 2.8
25 ± 2 °C and 60 ± 5% RH							
3M	White free flowing powder	102.19 ± 2.53	2.8 ± 0.3	101.80 ± 2.95	0.138 ± 0.03	82.4 ± 2.6	71.20 ± 2.7
6M	White free flowing powder	101.17 ± 3.5	2.8 ± 0.4	102.11 ± 3.44	0.143 ± 0.02	82.1 ± 3.1	70.12 ± 2.5
9M	White free flowing powder	101.83 ± 3.92	2.9 ± 0.5	101.74 ± 4.28	0.148 ± 0.02	80.74 ± 2.7	68.17 ± 2.9
12 M	White free flowing powder	102.81 ± 4.59	2.9 ± 0.6	101.62 ± 4.38	0.143 ± 0.02	79.13 ± 3.4	68.28 ± 2.8

Reprinted with permission from Chougule *et al.* (2008). Copyright © Dove Medical Press.

Table 9.3

Topographical features of dapson NLDPFs measured by SEM image analysis

Formulations	Roundness	Aspect ratio	Fractal dimension	Heterogeneity	Clumpiness
NLDPF-lactose	1.106 ± 0.076	1.356 ± 0.210	1.129 ± 0.064	0.492 ± 0.057	0.194 ± 0.057
NLDPF-trehalose	1.015 ± 0.065	1.163 ± 0.196	0.856 ± 0.052	0.351 ± 0.051	0.109 ± 0.047
NLDPF-sucrose	1.376 ± 0.092	1.453 ± 0.209	1.341 ± 0.064	0.674 ± 0.153	0.274 ± 0.095

With kind permission from Springer Science + Business Media: Chougule *et al.* (2008).

Table 9.4*In vitro* aerosol deposition data of tacrolimus nanoliposomal dry powder formulations

Formulations	Emitted dose (%)	Fine particle fraction (%)	Mean median aerodynamic diameter (μm)	Geometric standard deviation
NLDPF-trehalose	82 ± 3.0	71.1 ± 2.5	2.2 ± 0.1	1.7 ± 0.2
NLDPF-sucrose	63 ± 5.5	53.7 ± 3.6	2.8 ± 0.2	2.3 ± 0.1
NLDPF-lactose	74 ± 4	62.8 ± 3.1	2.6 ± 0.2	2.2 ± 0.1

Reprinted with permission from Chougule *et al.* (2008). Copyright © Dove Medical Press.