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# Liposomal Amikacin Dry Powder Inhaler: Effect of Fines on In Vitro Performance

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## ABSTRACT

The aim of the present investigation was to prepare and evaluate the influence of adding fines on the in vitro performance of liposomal amikacin dry powder inhaler (AMK LDPI) formulations. Liposomes composed of hydrogenated soyaphosphatidylcholine, cholesterol and saturated soyaphosphatidylglycerol (AMK 1), or stearylamine (AMK 2) were prepared by a reverse phase evaporation technique, extruded to reduce size and separated from unentrapped drug. Purified liposomal dispersion was subjected to lyophilization using optimized cryoprotectant to achieve maximum percentage drug retention (PDR). Lactose carrier in varying mass ratios with or without addition of fines in different mixing sequences was used to formulate AMK LDPI formulations. AMK LDPI formulations were characterized for angle of repose, compressibility index, dispersibility index, scanning electron microscopy, and fine particle fraction (FPF). PDR was found to be 97.6% ± 2.2% for AMK1 and 98.5% ± 1.9% for AMK2 using sucrose as optimized cryoprotectant in lipid:sucrose ratio of 1:4. Lactose carrier containing 10% fines (wt/wt) was found to be the optimum blend at 1:5 mass ratio of liposome:lactose. The addition of fines and the order of mixing of fines were found to influence the FPF with significantly different device fractions. FPF of AMK LDPI formulations using Rotahaler as the delivery device at 30, 60, and 90 L/min were found to be 21.85% ± 2.2% and 24.6% ± 2.4%, 25.9% ± 1.8% and 29.2% ± 2.1%, and 29.5% ± 2.6% and 34.2% ± 2.0% for AMK1 and AMK2, respectively. From the studies performed in this investigation, it was observed that liposomal charge, addition of fines and order of mixing fines, has a significant effect ($P < .05$) on in vitro deposition of drug from LDPI formulation.

**KEYWORDS**: liposomes, dry powder inhalers, amikacin, lactose, fines, fine particle fraction

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## INTRODUCTION

Amikacin sulfate (AMK) is a broad-spectrum and potent aminoglycoside with limited clinical use owing to a high dose requirement and renal and audio-vestibular apparatus toxicity.[1-2] Major drawbacks associated with the use of earlier or conventional liposomal formulations are the tendency of liposomes to leak drug while in circulation, the extensive uptake of these liposomes by tissues of reticuloendothelial systems (RES), and the inability of liposomes to extravasate into infected tissue.[3-4] Therefore, localized liposomal AMK delivery was considered for the treatment of cystic fibrosis infections in the lungs. Liposomal encapsulation of AMK will give the required release of drug for a longer time duration at the localized site, thereby reducing both the chances of systemic side effects and the frequency of dosing.

Improving drug delivery to the lungs from a dry powder inhaler (DPI) formulation is possible by various techniques such as smoothing the carrier surface,[5] reducing the particle size of the carrier,[6-7] and using a ternary powder mix formulation.[8] Addition of micronized lactose to coarse lactose carrier was found to improve the dispersion and deaggregation of salbutamol sulfate and spray-dried bovine serum albumin.[9-10] Also, techniques such as spray drying the drug with phospholipid composites in a suitable range for pulmonary delivery[11] or the dissolution of lecithin in chlorofluorohydrocarbon and the formation of liposomes in situ[12] or nebulization of the preformed liposomes[13] can be attempted for liposomal drug delivery to lungs. Recently, many microparticle systems have been reported to be used for pulmonary drug delivery such as oligosaccharide ester derivative (OED),[14-15] biodegradable ether-anhydride polymer,[16] sodium hyaluronate,[17] and poly(lactic-co-glycolic acid) (PLGA).[18-19] We have studied the delivery of liposomal ketotifen and liposomal budesonide DPI by blending the lactose carrier with preformed liposomes as described previously and found the fine particle fraction (FPF) to be not more than 21%.[20-21] The aim of the present investigation was to prepare and evaluate in vitro performance of AMK LDPI. The study focuses on the pharmaceutical development of liposomal drug formulations for DPI by lyophilization of preformed liposomal dispersion with varying ratios of lactose carrier, the effect of the addition of fines, and the effect of the addition sequence of fine carrier on dispersion characteristics of the formulations using twin-stage impinger (TSI) at different flow rates.

**Table 1.** Effect of Process and Formulation Variables for Preparation of Liposomal AMK*

| Variable | Batch No. | PDE (mean ± SEM)† | Observation and Inference |
|---|---|---|---|
| Choice of organic solvent (ratio of aqueous phase to organic phase, 1:3) | | | |
| Ethyl acetate | AMK1 | 65.4 ± 2.3 | Liposomes not properly oriented |
| | AMK2 | 62.7 ± 2.0 | |
| Ethanol | AMK1 | 64.8 ± 1.5 | Drug leakage from liposomes |
| | AMK2 | 66.1 ± 2.2 | |
| Ethyl acetate:ethanol (1:1) | AMK1 | 76.1 ± 2.0 | Good vesicle formation |
| | AMK2 | 77.9 ± 2.6 | |
| Ratio of aqueous phase to organic phase | | | |
| 1:2 | AMK1 | 60.5 ± 2.7 | Less PDE |
| | AMK2 | 58.9 ± 1.9 | |
| 1:3 | AMK1 | 76.1 ± 2.0 | Good vesicle formation |
| | AMK2 | 77.9 ± 2.6 | |
| 1:4 | AMK1 | 84.3 ± 2.8 | Increased PDE |
| | AMK2 | 86.2 ± 2.0 | |
| 1:5 | AMK1 | 96.7 ± 1.9 | Good PDE and good vesicle formation |
| | AMK2 | 98.5 ± 1.4 | |
| 1:6 | AMK1 | 96.4 ± 2.3 | No major change in vesicle formation and PDE |
| | AMK2 | 97.9 ± 1.8 | |

*AMK indicates amikacin sulfate; and PDE, percentage drug entrapment; HSPC, hydrogenated soyaphosphatidylcholine; CHOL, cholesterol; SPG-3, hydrogenated soyaphosphatidylglycerol; and SA, stearylamine.

†Mean ± SEM, n = 5. HSPC:CHOL:Charge (molar ratio) was 2:1:0.1. SPG-3 for AMK1 and SA for AMK2.

## MATERIALS AND METHODS

### *Materials*

AMK was received as a gift sample from Nicholas Piramal India Ltd (Pithampur, India). Hydrogenated soyaphosphatidylcholine (HSPC) and hydrogenated soyaphosphatidylglycerol (SPG-3) were gift samples from Lipoid (Ludwigshafen, Germany). Nuclepore polycarbonate membrane 2 μm (Whatman, Kent, UK), stearylamine (SA) (Sigma, St Louis, MO), α-tocopherol (E. Merck India Ltd, Mumbai, India), cholesterol (CHOL), dextrose monohydrate, sucrose, maltose and p-chloranil (S. D. Fine Chemicals, Baroda, India), trehalose (Sisco Research Laboratory, Mumbai, India), and Rotahaler (Cipla, Mumbai, India) were purchased locally and used as received. Sorbolac-400 (Meggle, Wasserburg, Germany) and Pharmatose 325M (HMV, Veghel, The Netherlands) were received as gift samples and used without further modification. Lactose carrier was 63-90 μm, sieved Pharmatose 325M; and fines was no. 500 sieved Sorbolac 400. High-density polyethylene (HDPE) bottles (Rexam Closures and Containers, Evansville, IN) were received as gift samples (Sun Pharmaceuticals, Baroda, India). All other reagents and chemicals used were of analytical grade or pharmacopeial grade.

### *Preparation of Liposomes*

Multilamellar vesicles (MLVs) of AMK were prepared by the modified reverse phase evaporation (REV) technique[22] by optimizing both formulation variables, such as choice of organic solvent and ratio of aqueous phase to organic phase for proper orientation of vesicles and higher percentage drug entrapment (PDE) (Table 1). HSPC, CHOL, α-tocopherol (1% of HSPC), and either SPG-3 or SA were mixed with ethanol-ethyl acetate solvent system (1:1) and transferred to a narrow neck tube with standard B-24 joint for AMK1 and AMK2, respectively. REV cycles of 10 minutes at 160 mm of Hg, followed by 10 minutes at 380 mm of Hg and using AMK (10 mg/mL) in 10 mM succinate buffer, pH 6.5, containing 1 mM EDTA (Ethylenediamine tetra acetic acid disodium salt) (ratio of aqueous phase:organic phase to be 1:5) with intermittent vortexing. Liposomal dispersions were subjected to complete removal of the last traces of organic solvent for 15 minutes at 510 mm of Hg. The formed liposomal dispersions were extruded through 2-μm polycarbonate membranes above the phase transition temperature (60°C) and separated from unentrapped drug by dialysis for both AMK1 and AMK2. The liposomal dispersions of AMK thus obtained were filled in amber-colored vials under nitrogen atmosphere, sealed, and stored in a refrigerator –2°C to 8°C until required for further experiments.

### *Lyophilization of Liposomes*

Lyophilization was performed for 48 hours (Heto Drywinner, model DW1 060E, Holten, Allerod, Denmark) using different cryoprotectants such as maltose, dextrose, trehalose, lactose, and sucrose. Liposomal pellets obtained after centrifug-

**Table 2.** Optimization of Lyophilization*

| Variable Studied | PDR AMK1† | PDR AMK2† |
|---|---|---|
| Selection of cryoprotectant | | |
| Maltose | 45.6 ± 2.5 | 43.8 ± 1.9 |
| Trehalose | 69.4 ± 1.1 | 70.9 ± 1.8 |
| Dextrose | 40.2 ± 2.4 | 39.4 ± 1.2 |
| Lactose | 41.8 ± 1.7 | 42.9 ± 2.1 |
| Sucrose | 62.3 ± 2.5 | 64.0 ± 2.3 |
| Phase of cryoprotectant addition (sucrose) | | |
| External | 62.3 ± 2.5 | 64.2 ± 2.3 |
| Internal | 54.6 ± 2.7 | 61.8 ± 1.5 |
| Both | 74.2 ± 2.4 | 77.8 ± 2.5 |
| Mass ratio of sucrose (lipid:sucrose) | | |
| 1:2 | 74.2 ± 2.4 | 77.8 ± 2.5 |
| 1:4 | 97.6 ± 2.2 | 98.5 ± 1.9 |
| 1:6 | 97.2 ± 2.5 | 98.1 ± 1.7 |
| 1:8 | 95.3 ± 1.5 | 97.2 ± 2.2 |

*PDR indicates percentage drug retention; and AMK, amikacin sulfate.
†Mean ± SEM, n = 5.

ing liposomal dispersions ($3.3 \times 10^6 g$, 2 hours) were suspended in 10 mM Tris buffer, pH 6.5, containing 1 mM EDTA and containing either lactose or maltose or trehalose or sucrose or dextrose in mass ratio of lipid:sugar (1:2) for both AMK1 and AMK2. PDR of liposomes following dehydration-rehydration cycle were determined. The influence of selection of cryoprotectant, addition sequence of cryoprotectant (sucrose), and mass ratios of lipid:sucrose on PDR for both AMK1 and AMK2 are shown in Table 2.

### ***Development of Liposomal Dry Powder Inhaler Formulations***

#### *Effect of Carrier Addition*

The liposomal dispersions containing sucrose as a cryoprotectant were frozen at –40°C and lyophilized for 48 hours. The porous cakes thus formed were sized successively through no. 120 and no. 240 sieves for both AMK1 and AMK2. Capsules (size 2) were filled with individually weighed powder containing 1000 ± 50 µg of AMK and packed under nitrogen atmosphere in HDPE bottles containing silica bags as dehumactant. Similarly, the sieved lyophilized liposomal powders were mixed with lactose carrier (63-90 µm sieved Pharmatose 325M) in varying mass ratios from 1:1 to 1:6 (Table 3). The bottles were stored in a desiccator at refrigeration temperature (2°C-8°C) until further use. The in vitro deposition studies of these formulations were determined using a TSI (Apparatus A, *British Pharmacopoeia*) after aerosolization of 5 capsules at 60 L/min via Rotahaler as the delivery device for both AMK1 and AMK2 (Table 3).

**Table 3.** Optimization of LDPI Formulation*

| Variable Studied | Percentage FPF for AMK1† | Percentage FPF for AMK2† |
|---|---|---|
| Without carrier (liposome:carrier mass ratio) | | |
| 1: 0 | 7.5 ± 2.1 | 6.8 ± 2.3 |
| Effect of liposome:lactose ratio | | |
| 1: 1 | 9.8 ± 2.4 | 8.3 ± 2.5 |
| 1: 3 | 11.6 ± 2.0 | 10.3 ± 1.5 |
| 1: 5 | 14.5 ± 1.6 | 12.9 ± 1.8 |
| 1: 6 | 14.8 ± 2.2 | 13.5 ± 2.4 |
| Effect of sieved Sorbolac 400 (liposome:lactose ratio was 1:5) | | |
| 5.0% | 19.4 ± 2.2 | 24.6 ± 2.4 |
| 10.0% | 25.9 ± 1.8 | 29.2 ± 2.1 |
| 15.0% | 22.1 ± 1.5 | 25.2 ± 2.0 |

*LDPI indicates liposomal dry powder inhaler; FPF, fine particle fraction; and AMK, amikacin sulfate.
†Mean ± SEM, n = 5, at 60 L/min.

#### *Effect of Adding Fines*

The sieved lyophilized liposomal powders were mixed with lactose carrier (63-90 µm) containing 5% to 15% sieved Sorbolac 400 (no. 500) in mass ratios of liposome:lactose at 1:5, and these formulations were evaluated using TSI for both AMK1 and AMK2 as described under "Effect of Carrier Addition."

#### *Effect of Adding Sequence of Fines*

In 1 set of experiments, the fines (5% or 10% or 15% wt/wt sieved Sorbolac 400) were first mixed with lactose carrier (63-90 µm) forming a blend of lactose and then with sieved lyophilized liposomes in a mass ratio of liposome:lactose at 1:5 (Formulation A) for both AMK1 and AMK2. In the second set of experiments, 5% or 10% or 15% wt/wt sieved Sorbolac 400 as fines were first mixed with sieved lyophilized liposomes and then with carrier at a 1:5 ratio (Formulation B) for both AMK1 and AMK2. These formulations were evaluated using TSI as described under effect of carrier addition (Table 3).

### ***Characterization of Liposomes***

#### *Assay*

AMK as PDE was determined by UV-Visible (VIS) spectrophotometer after forming a charge transfer complex using p-chloranil at 350 nm as described elsewhere.[23]

#### *Photomicrography*

All batches of the liposomes prepared were viewed under Olympus Microscope (BX 40F4, Tokyo, Japan) with polarizing attachment (×1000) to study shape and lamellarity of the liposomes.

**Table 4.** Comparative Characterization of Potential Batches of Liposomal AMK*

| Variable Studied | Potential Liposomal Batches | |
|---|---|---|
| | **AMK1** | **AMK2** |
| Mean size of liposomes (μm)† | 2.0 ± 0.2 | 1.9 ± 0.3 |
| Angle of repose (θ)‡ | 27.1 ± 0.4 | 28.7 ± 0.5 |
| FPD (μg) | 256.4 ± 8.2 | 294.9 ± 7.6 |
| FPF (%) | 25.9 ± 1.8 | 29.2 ± 2.1 |
| Mean size of LDPI formulations (μm)† | 51.7 ± 1.6 | 50.9 ± 1.7 |
| Dispersibility (%) | 29.1 ± 1.6 | 34.6 ± 1.4 |
| Emission (%) | 88.9 ± 2.0 | 84.4 ± 1.7 |
| EI‡ | 41.9 ± 1.5 | 46.1 ± 1.8 |
| Moisture content‡(%) | 1.6 ± 1.8 | 1.9 ± 2.3 |
| Control: asthalin capsules: FPF = 27.1 ± 2.0, EI = 48.6 ± 1.7 | | |

*AMK indicates amikacin sulfate; FPD, fine particle dose; FPF, fine particle fraction; LDPI, liposomal dry powder inhaler; and EI, effective index.
†Mean ± SEM, n = 3
‡Mean ± SEM, n = 5 at 60 L/min

*Laser Light Scattering Measurement*

The vesicle size of extruded liposomes was determined by the laser light scattering technique using Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) operating at a beam length of 2.4 mm and range of lens at 300 mm and results of the volume mean diameter of vesicles are recorded in Table 4.

### *Characterization of Liposomal Dry Powder Inhaler Formulations*

*Angle of Repose*

The pile of powder was carefully built up by dropping the powder material through a funnel tip from height of 2 cm.[24] The angle of repose (Table 4) was calculated by inverting tangentially the ratio of height and radius of the formed pile.

*Angle of Spatula*

The pile of 10-g formulation was built on a flat surface and a flat spatula was placed into the bottom of the mass, and then the spatula was lifted straight up and out of the material. The angle of new surface on the spatula to the horizontal was measured immediately and again after gentle tapping of the spatula. The average of 2 measurements was taken as the value of angle of the spatula.[24]

*Compressibility Index*

The compressibility index was determined by tapping the formulation for 500 taps to reach plateau condition.[24]

*Dispersibility Index*

Formulation (10 g) was dropped through a cylinder (length 16.5 cm, internal diameter 5 cm) held 5 cm above a watch glass of 2.5-cm diameter. The dropping point was 7.6 cm above the cylinder from a funnel tip. Dispersibility index was calculated as the relative proportion of material lost to the material dropped.[24]

*Water Content Determination*

Water content of the liposomal dry powder inhaler (LDPI) formulations (1 g) was determined in triplicate on 2 consecutive days by Karl Fischer Titration (Table 4).

*Fine Particle Fraction*

The volume of capturing solvent (water) in the upper (stage 1) and lower (stage 2) were 7 mL and 30 mL, respectively, in TSI (*British Pharmacopoeia,* Apparatus A).[25] Rotahaler was used as a delivery device at a flow rate of 30 ± 2 L/min, 60 ± 2 L/min, and 90 ± 2 L/min for 5 seconds for 5 capsules. The inhaler body, capsule shells, mouthpiece, stage 1, and stage 2 were washed 5 times with water and analyzed to measure the amount of drug retained as described before.[23] The fine particle dose (FPD) was denoted as the quantity (μg) of the particles per capsule that deposited in the lower stage of the TSI after aerosolization at 30 L/min, 60 L/min, and 90 L/min. Each capsule contained a powder mass of 69 ± 2 mg equivalent to nominal dose of 1000 ± 50 μg AMK. The recovered dose (RD) was taken as the total quantity of drug recovered per capsule after each actuation, while the emitted dose (ED) was that emitted from the inhaler device. Percentage emission was calculated as the percentage of emitted dose to total dose. FPF was the ratio of FPD to RD, while dispersibility was the percentage of FPD to ED (Table 4). As a control, a marketed preparation (Asthalin Rotacaps, Cipla) containing salbutamol sulfate powder was used and the FPF determined at 30 L/min, 60 L/min, and 90 L/min flow rate using Rotahaler as the delivery device (Table 4).

*Scanning Electron Microscopy Photomicrographs*

Scanning electron microscopy (SEM) (Philips XL30 ESEM, Eindhoven, The Netherlands) of the representative LDPI formulations (AMK1 and AMK2) was performed and photomicrographs are shown in Figure 1.

*Statistical Analysis*

Each batch was prepared 5 times and data from all experiments are expressed as mean ± SEM unless otherwise spec-

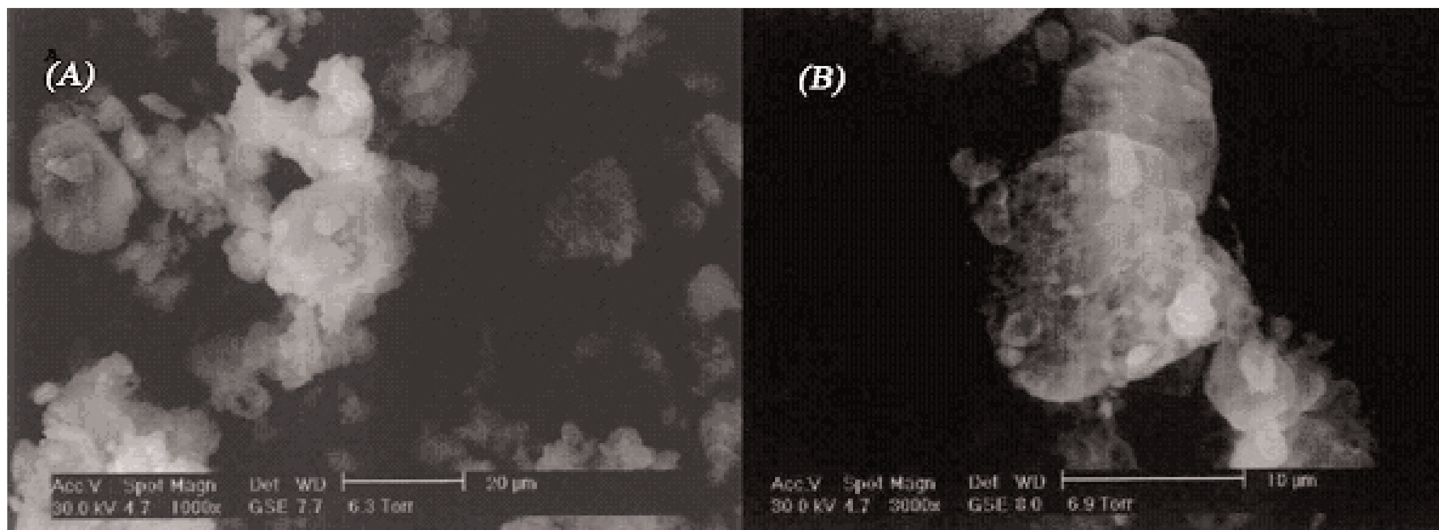


**Figure 1.** SEM photomicrographs of AMK1 (1000X) (A) and AMK2 (3000X) (B).

ified. Process variables were studied by comparing PDE of 2 batches having all other variables the same. PDE was expressed as the percentage of the drug initially added. Effective index (EI) is the geometric mean of the total ED and FPF, represented by the equation[26]:

$$EI = \sqrt{(100 - DF) \times FPF} \qquad (1)$$

where DF is the device fraction.

Significant differences were calculated by analysis of variance (ANOVA); mutual differences were detected with Student *t* test and differences at $P < .05$ were considered as significant.

## Results and Discussion

The liposomal AMK were prepared using modified REV technique using alternative organic solvents such as ethyl acetate and ethanol (1:1). The organic solvents such as diethyl ether or methanol employed in the liposome preparation (although usually removed by evaporation) may remain as traces in the final formulation and can lead to a possible risk for human health and inadequate stability of the vesicles.[22] Use of other organic solvents such as ethyl acetate and ethanol can solve this problem. Ethanol forms a monophasic system upon contact with aqueous phase, while ethyl acetate forms a biphasic system (emulsion) upon contact with aqueous phase. When ethyl acetate was used alone, it resulted in distorted spherical vesicles, which may be due to formation of an unstable biphasic system upon contact with the aqueous phase. Use of ethanol alone resulted in high PDE, which may be due to formation of a monophasic system upon contact with the aqueous phase. However, drug leakage was observed due to the presence of traces of ethanol leading to disruption of the bilayer. In the case of ethyl acetate:ethanol (1:1) combination, proper spherical vesicles and high PDE were observed. Combination of these organic solvents with aqueous phase forms a stable emulsion, which is a prerequisite for REV.[27] When the aqueous phase to organic phase ratio was raised from 1:3 to 1:5, marked increase in the PDE was observed. Further increase in the organic phase did not result in increase in PDE (Table 1). The prepared liposomes were found to be multilamellar and were identified by the presence of Maltese crosses in liposomes. The prepared liposomes were extruded by passing through 2-µm polycarbonate membranes to a reproducible mean liposomal size below 5 µm.[28] The liposomal dispersion was dialyzed and free drug was removed. PDE in liposomes was 96.7 ± 1.9 and 98.5 ± 1.4 for AMK1 and AMK2, respectively (Table 1). Laser light scattering microscopy revealed mean liposomal sizes for AMK1 (2.0 ± 0.2 µm) and for AMK2 (1.9 ± 0.3 µm). Difference in liposomal size may be due to the presence of a different charge present on the liposomal surface.

The prepared liposomes were lyophilized using appropriate cryoprotectant and optimized for the drug retained in lyophilized liposomal AMK as described previously.[16] Liposomes were best preserved in their structure with PDR using sucrose as a cryoprotectant in mass ratio of lipid:sucrose at 1:4 (PDR of AMK1, 97.6 ± 2.2 and AMK2, 98.5 ± 1.9). During the freeze-drying process of liposomes, liposomes constrict and get coated on the optimum surface of crystallized sugar. Hydration of polar head groups with the hydroxyl group of sucrose leads to stabilization of liposomes. If the sucrose concentration is less than optimum, the crystallized sugar does not provide adequate surface for the adherence of the constricted bilayer leading to drug leakage. Hence, it may be concluded that the bulk concentration of sugar required as cryoprotectant depends on the type of sugar selected and saturation of the polar head groups of the bilayer by drug or other formulation components. The lamellarity and size of liposomes are expected to change these requirements.

To formulate AMK LDPI formulation, a series of experiments was conducted. Lyophilized liposomes when formulated as an LDPI formulation without using any carrier molecule resulted in low FPF value. This observation describes the importance of the addition of lactose carrier in formulating the LDPI formulation. Pharmatose 325M was sieved to get 63- to 90-µm size range fraction as a carrier to formulate LDPI formulations. The lyophilized liposomes were mixed with sieved Pharmatose 325M (63-90 µm) in the range of liposome:lactose mass ratio from 1:1 to 1:6, and Pharmatose 325M's effect on FPF was studied (Table 3). The data revealed the optimum liposome:lactose mass ratio of 1:5. Optimum concentration of carrier is required to achieve detachment of liposomal drug from carrier molecule. Carrier concentration of less or more than optimum resulted in low FPF or no further increase in FPF. The effects of fines (sieved Sorbolac-400 through no. 500) in 5%, 10%, and 15% proportion (wt/wt) and mixing sequence with carrier (63-90 µm) and sieved lyophilized liposomes keeping the final liposome:lactose mass ratio of 1:5 (Table 3) were evaluated. Data revealed an optimum concentration of 10% wt/wt fines (sieved Sorbolac 400) and mixing sequence of fines with carrier and then with sieved lyophilized liposomes. At the 10% level of fines, high-energy adhesion sites (HA) of lactose may bind strongly to the carrier and low-energy adhesion sites (LA) may allow the formation of more reversible bonds with liposomal drug. This action results in efficient detachment of liposomal drug from the carrier as observed with plain DPI formulations.[28] Hence, 10% sieved Sorbolac 400 (wt/wt) added to AMK LDPI formulation occupies HA sites leaving LA sites for the attachment of liposomal drug and thus resulting in higher FPF. This observation was also confirmed by observing the effect of adding sequence of fines to the liposomal formulation. Blending the fines (10% wt/wt sieved Sorbolac 400) with carrier (63-90 µm) resulted in higher FPF with interestingly different device fraction. Liposomal drug powder adheres to carrier particles as seen in SEM photomicrographs of AMK LDPI formulations (Figure 1). The FPD (µg), FPF (%), dispersibility (%), and emission (%) at 30, 60, and 90 L/min flow rate using Rotahaler as dispersing device are shown in Table 4. The EI of AMK2 was found to be better than the AMK1, suggesting more effective liposomal drug deposition into lung. This finding may be due to turbo-electrification or charge generation in liposomal powder during dispersion via the Rotahaler. The lower ratio of EI/FPF is suggestive of efficient dispersion of AMK1 from the device, but unlike the control more proportion of the dispersed powder has been deposited in the upper respiratory tract.[26]

Evaluation and control of flow and dispersion (deaggregation) characteristics of the formulation are of critical importance in the development of DPI products. Interparticle forces that influence flow and dispersion properties are particularly dominant in micronized or microcrystalline powders required for inhalation therapy (<5 µm).[29-30] It has been demonstrated that powder adhesion, mediated in part by Van der Waal forces, is directly related to particles <10 µm.[31] Predictions of powder rheology based on the possible relationship of several physicochemical properties are extremely complicated. Hence, flow and dispersion properties such as angle of repose, dispersibility index, compressibility index, moisture content, and FPF are characterized and controlled (Table 4). The flowability and floodability expressed by angle of repose (27.1 ± 0.4 and 28.7 ± 0.5), dispersibility index (22.4 ± 0.2 and 20.8 ± 0.6), and compressibility index (23.8 ± 2.4 and 21.9 ± 2.0) for AMK1 and AMK2, respectively, falls under the category of good and floodable.[24] Moisture content determination is also important for drug stability upon storage and deaggregation upon inhalation. The formulations are found to contain moisture content below 2.0% confirming low aggregation tendency (Table 4).

## Conclusion

Liposomal charge, addition of fines, and order of mixing fines can have a significant effect on in vitro deposition of AMK from LDPI formulations.

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