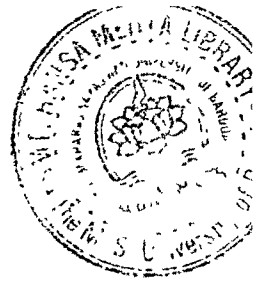

RESEARCH PUBLICATIONS

1. Development of Liposomal Amphotericin B Dry Powder Inhaler Formulation

   S.P.Shah and Ambikanandan Misra
   Drug Delivery, 11:1-7, 2004

2. Liposomal Amphotericin B dry powder inhaler: Effect of fines on in vitro performance

   S.P.Shah and A.Misra
   Pharmazie, 59 (10), 2004 (In press)

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

   Shrenik P.Shah and Ambikanandan Misra
   AAPSPharmSciTech, 2004 (Accepted for Publication – In Press)

4. Factors Affecting Development of Dry Powder Inhalers – A Review article

   S.P.Shah, S.Ganesh and A.N.Misra
   Indian Journal of Pharmaceutical Sciences, 2003, 64(4): 336-345

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Development of Liposomal Amphotericin B Dry Powder Inhaler Formulation

S. P. Shah and Ambikanandan Misra

Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, M. S. University of Baroda, Gujarat, India

The purpose of our study was to prepare and optimize liposomal Amphotericin B (AMB) dry powder inhaler (DPI) formulation for treatment of invasive lung fungal infection. Liposomes were prepared by reverse phase evaporation technique using ethyl acetate and ethanol (1:1) as organic solvents to avoid a possible risk for human health and to impart adequate stability of the vesicles. Drug lipid ratio was 1:10 with membrane composition of hydrogenated soyaphosphatidylcholine; cholesterol and either saturated soyaphosphatidylglycerol (7:3:0.5) or stearylamine (1:1.0.1) was used to prepare negatively (AMB1) and positively (AMB2) charged liposomes, respectively. Liposomes were extruded through 2 μm polycarbonate membrane, separated from unentrapped drug and subjected to lyophilization using Tris buffer containing cryoprotectants in various mass ratios. Sucrose was found to be the best cryoprotectant for liposomal AMB in a mass ratio of lipid: sucrose at 1.5 for AMB1 and AMB2, respectively. Sorbolac 400 and sieved Pharmatose 325 M (500#) in varying mass ratios were used as carriers to prepare the liposomal DPI formulations and subjected to determination of angle of repose, compressibility index, dispersibility index, water content, scanning electron microscopy, and fine particle fraction (FPF). Carrier blend of Sorbolac 400 and 10% sieved Pharmatose 325 M (liposome: carrier ratio to be 1·6) resulted in 22.6 ± 2.2% and 16.8 ± 2.2% FPF for AMB1 and AMB2, respectively with significantly different ($p > .05$) device fraction. Percent dug retention studies were conducted at different storage conditions and demonstrated a shelf life over 1 year at refrigerated storage condition (2–8°C).

Keywords Amphotericin B, Dry Powder Inhaler, Liposome, Lung Delivery

There has been a dramatic rise in the number of invasive fungal infections in immunocompromised patients in recent years. Accordingly, there is an urgent need to improve the treatments for invasive fungal infections because the overall prognosis for patients with these infections remains poor. Amphotericin B (AMB) is a broad-spectrum and potent antifungal agent, but its clinical use is sometimes limited due to adverse reactions, such as renal toxicity, hypokalemia, and anemia (Schmitt 1993, Janknegt et al. 1992). A promising approach to the treatment of invasive fungal infections is the use of liposomal AMB (Lopez-Berestein et al. 1983). Major drawbacks associated with the earlier or conventional liposomal formulation are the tendency of liposomes to leak drug while in circulation, the extensive uptake of these liposomes by tissues of reticuloendothelial systems, and inability of liposomes to extravasate into infected tissue. Liposomal dry powder inhaler (LDPI) was chosen to stabilize the liposomal system, and localized liposomal AMB delivery was considered an alternative treatment of invasive lung fungal infections. It was hypothesized that liposomal AMB will control the release rate of the drug for longer duration at localized site and is expected to reduce systemic side effects and frequency of dosing. Hence, our investigation focused on the pharmaceutical development of stable liposomal AMB DPI formulation for high pulmonary deposition.

MATERIALS AND METHODS

Amphotericin B was received as a gift sample from Ambalal Sarabhai Enterprise, Baroda, India. Hydrogenated soyaphosphatidylcholine (HSPC) and hydrogenated soyaphosphatidylglycerol (SPG-3) were gift samples from Lipoid (Germany). Nuclepore polycarbonate membrane 2 μm (Whatman, USA), cholesterol (CHOL) (S. D. Fine Chemicals, India), stearyl amine (SA) (Sigma, USA), α-tocopherol (E. Merck India Ltd., India), dextrose monohydrate, sucrose, and maltose (S. D. Fine Chemicals, India), and trehalose (Sisco Research Laboratory, India) were purchased locally. Sorbolac-400 (Meggle, Germany) and pharmatose 325 M (HMV, Netherlands) were received as gift samples and used as such without further modification. All other

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Address correspondence to Ambikanandan Misra, Pharmacy Department, Faculty of Tech. & Engg., M. S. University of Baroda, Kalabhavan, Vadodara-390001, Gujarat, India. E-mail: misraan@satyam.net.in, misraan@hotmail.com

TABLE 1
Effect of process and formulation variables

Variable	Batch no	PC Chol Charge[a] (molar ratio)	Percent drug entrapped (Mean ± SEM)[b]	Observation and inference
Choice of organic solvent (ratio of aqueous phase to organic phase was 1 3)				
Ethyl acetate	AMB1	7 3 0 5	50 2 ± 1 9	Vesicles were not properly oriented
	AMB2	1 1 0 1	43 9 ± 2 4	
Ethanol	AMB1	7 3 0 5	60 4 ± 2 5	Drug leakage from vesicles
	AMB2	1 1 0 1	58 7 ± 2 1	
Ethyl acetate Ethanol (1 1)	AMB1	7 3 0 5	78 5 ± 2 4	Good vesicles formation
	AMB2	1 1 0 1	76 9 ± 3 0	
Ratio of aqueous phase to organic phase				
1 2	AMB1	7 3 0 5	62 4 ± 2 6	Less PDE
	AMB2	1 1 0 1	55 3 ± 1 9	
1 3	AMB1	7 3 0 5	78 5 ± 2 4	Good vesicles formation
	AMB2	1 1 0 1	76 9 ± 3 0	
1 4	AMB1	7 3 0 5	86 3 ± 2 1	Increased PDE[c]
	AMB2	1 1 0 1	80 5 ± 2 3	
1 5	AMB1	7 3 0 5	95 8 ± 1 5	Good PDE and good vesicle formation
	AMB2	1 1 0 1	87 9 ± 1 3	
1 6	AMB1	7 3 0 5	95 1 ± 2 3	No major change in vesicle formation and PDE
	AMB2	1 1 0 1	87 1 ± 1 5	

[a]SPG-3 for AMB1 and SA for AMB2
[b]Mean (± SEM), $n = 5$
[c]PDE = percent dry entrapment

reagents and chemicals used were of analytical grade or pharmacopoeial grade

Preparation of Liposomes

Multilamellar vesicles (MLVs) of AMB were prepared by the modified reverse phase evaporation (REV) technique (Cortesi et al 1999) 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 percent drug entrapment (PDE) (Table 1) Drug (5 mg), HSPC, CHOL, α-tocopherol (1% of PC), and either SPG-3 or SA were mixed with ethanol–ethyl acetate solvent system (1 1) and transferred to narrow neck tube with standard B-24 joint REV cycles of 10 min at 10 in of Hg, followed by 10 min at 15 in of Hg and using 0 01 M Tris buffer pH 6 5 containing 1 mM EDTA (ratio of aqueous phase to organic phase was 1 5) were carried out with intermittent vortexing Liposomal dispersion was subjected to complete removal of last traces of organic solvent for 15 min at 20 in of Hg The formed liposomal dispersion was extruded through 2 μm polycarbonate membrane above the phase transition temperature (60°C).

For separation of unentrapped drug, the liposomal dispersion was centrifuged at $4\ 38 \times 10^3$ g for 90 sec to sediment the crystallized free drug The liposomal AMB was estimated in supernatant after dissolving it in DMSO methanol mixture (1 1) (v/v) by ultraviolet spectrophotometer (Hitachi U– 2000 spectrophotometer, Shimadzu, Japan) at 410 nm (Ruijgrok, Vulto, and Vaneheo 2000) The liposomal dispersion of AMB thus obtained was filled in amber-colored vials under nitrogen atmosphere, sealed, and stored in refrigerator until required for further experiments

Lyophilization of Liposomes

To achieve high percent drug reduction (PDR), lyophilization was carried out for 48 hr (Heto Drywinner model DW1 060E Holten, Denmark) using different cryoprotectants like maltose dextrose, trehalose, lactose, and sucrose In these studies, the liposomal pellet obtained after centrifuging liposomal dispersion was suspended in 10 mM Tris buffer pH 6 5 containing 1 mM EDTA and containing lactose, maltose, trehalose, sucrose, or dextrose in mass ratio of lipid sugar (1 2) PDR of liposomes following dehydration-rehydration cycle was determined and the influence of sequence of cryoprotectant addition and mass ratio of lipid sucrose on PDR also was studied (Table 2)

Development of LDPI Formulations

To prepare LDPI formulations, the porous cake of liposomes obtained after lyophilization was sieved (200# and 240#) and

TABLE 2
Optimization of lyophilization

Variable studied	Percentage drug retained AMB1[a]	Percentage drug retained AMB2[a]
Selection of cryoprotectant		
Maltose	45 8 ± 1.9	42 2 ± 2 5
Trehalose	62 4 ± 1 9	58 4 ± 2 0
Dextrose	38 8 ± 2 2	35 7 ± 2 3
Lactose	48 6 ± 2 2	45 6 ± 2 0
Sucrose	60 3 ± 2.3	56 0 ± 2 1
Phase of cryoprotectant addition		
External	60 3 ± 2 3	56 0 ± 2 1
Internal	48 6 ± 2 6	42 4 ± 1 9
Both	70 3 ± 2 1	66 2 ± 2 2
Mass ratio of sugar (lipid sugar)		
1 2	70.3 ± 2 1	66 2 ± 2 2
1 4	83 4 ± 1 9	80 2 ± 2 1
1 5	96 6 ± 1 8	94 7 ± 2 4
1 6	96.4 ± 2 0	95 1 ± 1 9
1 8	97 0 ± 1 6	94 6 ± 2 3

[a]Mean (± SEM), $n = 5$

mixed with Sorbolac 400 containing sieved 5% to 15% Pharmatose 325M (500#) in different mass ratio of liposome lactose (1 2 to 1 8) as recorded in Table 3 Capsules (size "2") were filled with individually weighed powder containing 250 μg of AMB and packed under nitrogen atmosphere in HDPE bottles containing silica bags as dehumactant The bottles were stored in a desiccator at refrigeration temperature (2–8°C) until further use

Characterization of Liposomes

Vesicle Size

The vesicle size of extruded liposomes was determined by laser light scattering technique using Mastersizer (Malvern Instruments Ltd , UK) operating at a beam length of 2 40 mm Range of lens at 300 mm Results of volume mean diameter of vesicles are recorded in Table 4

TABLE 3
Optimization of liposomal DPI formulation

Variable studied	Percentage FPF for AMB1[a]	Percentage FPF for AMB2[a]
Effect of liposome lactose ratio (blend containing 5% sieved* lactose)		
1 2	12 3 ± 2 2	8.5 ± 2 4
1 4	15 1 ± 3 0	11 6 ± 2 2
1 6	17 5 ± 2 4	13 2 ± 3 1
1 8	16 4 ± 2 7	11 9 ± 2 8
Effect of percentage of sieved* lactose (liposome lactose ratio was 1.6)		
5%	19 2 ± 2 6	14 9 ± 2 5
10%	22 5 ± 2 2	16 8 ± 2 2
15%	20 1 ± 1 9	14 6 ± 2 3

[a]$n = 5$ (±SEM) *500#

TABLE 4
Comparative characterization of potential batches of liposomal AMB

Variable studied	Potential liposomal batches AMB1	AMB2
Mean size (μm)[a]	1 8 ± 0 2	2 0 ± 0 3
Angle of repose[b] (θ)	28 3 ± 0 6	29 7 ± 0 4
Dispersibility index[b]	20 8 ± 1 0	21 4 ± 0 6
Compressibility index[b]	23 5 ± 1.8	22 3 ± 2 4
Moisture content[b] (%)	1 4 ± 2 0	1 5 ± 2 4
Device fraction[b] (%)	10 8 ± 2 0	15 8 ± 1 8
Respirable fraction (FPF)[b]	22 5 ± 2 2	16 8 ± 2 2
Effective index (EI)[b]	44 8 ± 1.6	37 6 ± 1 9

Control Ashthalin (Cipla Ltd , India), delivery device Rotahaler (Cipla Ltd , India)
FPF = 27 1 ± 2 0, EI = 48 6 ± 1 7
[a]Mean (± SEM), $n = 3$
[b]Mean (± SEM) $n = 5$

Photomicrography

All the batches of prepared liposomes were viewed under Olympus (BX 40F4, Japan) with polarizing attachment to study their shape, and lamellarity of AMB1 is shown in Figure 1a and 1b

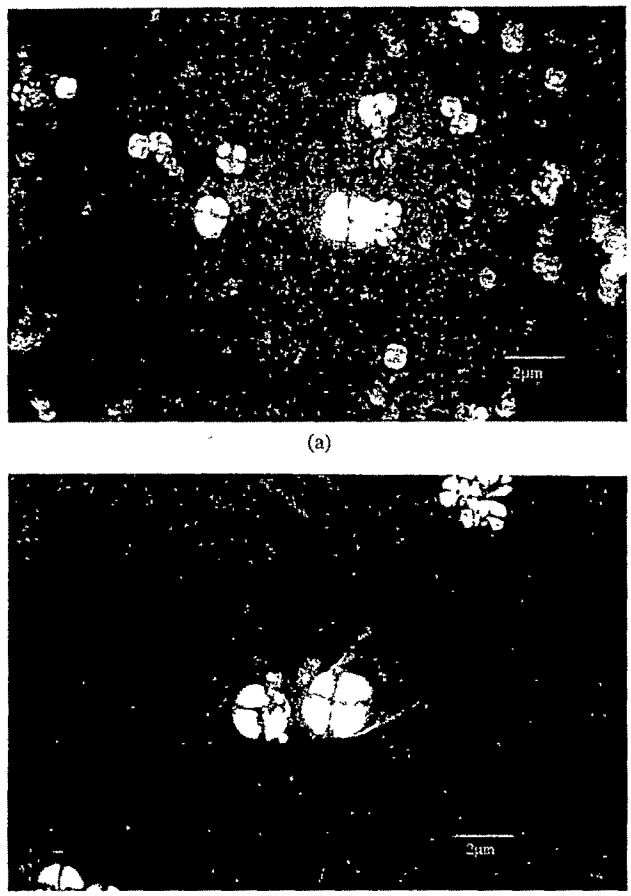
Physical Characterization of LDPI Formulation

Angle of Repose

The pile of powder was carefully built up by dropping the powder material through a funnel tip from a height of 2 cm (Carr 1965) The angle of repose was calculated by inverting tangentially the ratio of height and radius of the formed pile (Table 4)

Compressibility and Dispersibility Index

The compressibility index was determined as described by Carr (1965) by tapping the formulation to reach plateau condition The dispersibility index was determined using a miniature assembly as described by Carr (1965) Formulation (5 g) was dropped through a cylinder (length 6 5 in and internal diameter 2 in), which has been held 2 in above a watch glass (diameter 1 in) The dropping point was 3 in above the cylinder, from a funnel tip Dispersibility index was calculated as the relative proportion of material lost to the material dropped (Table 4)



(a)

(b)

4c/art

FIG 1 Photomicrographs show (a) liposomal vesicles before lyophilization and (b) liposomal vesicles after lyophilization

Water Content Determination and Fine Particle Fraction

Water content of the DPI formulation (1 g) was determined in triplicate on two consecutive days by Karl Fischer Titration (Table 4) The volume of capturing solvent (methanol) in the upper (stage 1) and lower (stage 2) were 7 ml and 30 ml, respectively in TSI (B P Apparatus A) Two capsules of DPI formulations were used for determination of fine particle fraction (FPF) using Rotahaler (Cipla, India) as a delivery device at flow rate of 60 L/min and compared with marketed preparation (Asthalin Rotacaps, Cipla Ltd , India) containing Salbutamol sulphate The Rotahaler device was rinsed with methanol to determine the device fraction and effective index (Hino et al 1998) (Table 4)

Scanning Electron Microscopy

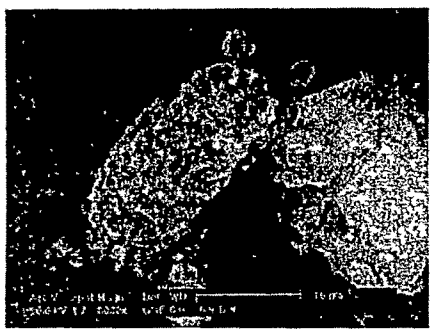
Scanning electron microscopy (Philips XL30 ESEM, Netherlands) of both DPI formulations were carried out and photomicrographs are shown in Figures 2a and 2b

Percent Drug Retention Studies

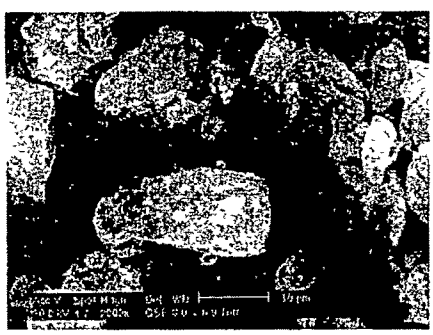
PDR studies were carried out on LDPI formulations at refrigerated (2–8°C), controlled room temperature (25°C ± 2°C 60 ± 5% RH), and accelerated (40°C ± 2°C and 75 ± 5% RH) conditions The sampling points were as per ICH guideline (Singh 1999), for countries falling under Zone III (hot, dry) and Zone IV (very hot, humid) The LDPI formulations in its final packing were stored separately at all storage conditions The samples of each batch stored at various storage conditions were withdrawn at definite time intervals, rehydrated with distilled water for 30 min, and analyzed for the drug remained entrapped in liposomes (Figure 3) The samples also were examined for the evidence of caking and discoloration

Statistical Analysis

Each batch was prepared five times and data from all experiments were expressed as mean ± SEM unless specified Process



(a)



(b)

FIG 2 (a) Scanning electron microscopic photomicrograph of AMB1 and (b) scanning electron microscopic photomicrograph of AMB2

variables were studied by comparing PDE of two batches having all other variables the same PDE is expressed as the percentage of the drug initially added Similarly, the PDR is relative to the drug initially entrapped T_{90} as specified in Figure 3 refers to

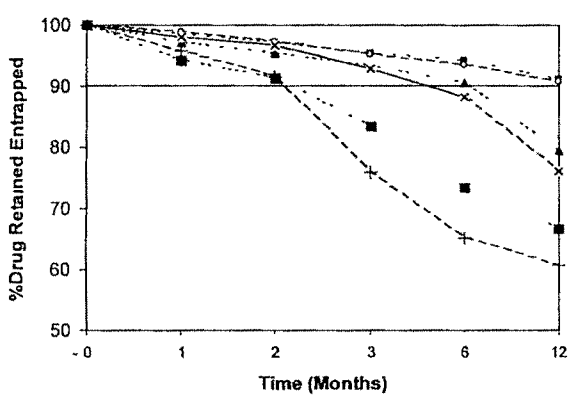


FIG 3 PDR of AMB liposomes at different storage conditions and its effect on T_{90} Refrigerator AMB1 (—□—), AMB2 (□□□□□) controlled room temperature AMB1 (—□—), AMB2 (□□×□□), accelerated AMB1 (—□—) AMB2 (□□+□□)

90 PDR within the liposomes Effective index is the geometric mean of the total emitted dose and FPF, represented by the equation (Hino et al 1998)

$$EI = \sqrt{(100 - DF) \times FPF} \quad [1]$$

where DF is the device fraction

Significant differences were calculated by ANOVA and mutual differences were detected with Student's t-test and differences greater than $p > 05$ were considered significant

RESULTS

The liposomes of AMB were prepared by REV technique using ethyl acetate and ethanol (1 1) as organic solvents and 10 mM Tris buffer pH 6 5 containing 1 mM EDTA as aqueous phase are recorded in Table 1 The prepared liposomes were extruded by passing through 2 μm polycarbonate membranes to a reproducible mean liposomal size of less than 5 μm (Martonen et al 1993) Free drug from liposomes was separated by centrifugation at 4 38 × 10^3 g and complete separation of unentrapped drug was confirmed by optical microscopy Maximum PDE estimated in AMB1 and AMB2 liposomes were 95 8 ± 1 5 and 87 9 ± 1.3, respectively (Table 1)

Microscopy with polarized light confirmed the formation of spherical and multilamellar liposomes (Figures 1a and 1b) Multilamellar vesicles also were identified by the presence of Maltese crosses, a characteristic of bilayer configuration Laser light scattering microscopy revealed mean liposomal sizes for AMB1 (1 8 ± 0 2 μm) and for AMB2 (2 0 ± 0 3 μm)

Among maltose, dextrose, trehalose, lactose, and sucrose used as cryoprotectants, sucrose and trehalose were found to be comparable cryoprotectants and sucrose was selected for the further studies (Table 2) When sucrose was added on both sides of lamellae, maximum PDR was observed The optimum lipid.sucrose mass ratio was found to be optimum at 1 5 with PDR of 96 6 ± 1 8 and 94 7 ± 2 4 for AMB1 and AMB2, respectively (Table 2).

The mass ratio of liposomes Sorbolac 400–10% sieved (500#) Pharmatose 325M (1 6) resulted in maximum FPF of 22 5 ± 2 2 and 16 8 ± 2 2% for AMB1 and AMB2, respectively, and interestingly significantly different device fractions (Table 3) The deposition of liposomal AMB was more efficient in AMB1 than the AMB2 based on the effective index (EI) The FPF ratios of control to that of the developed LDPI formulations were 0 83 and 0 62 and EI ratios were 0 92 and 0 77 for AMB1 and AMB2, respectively

The flowability and floodability were observed to be good and floodable in point score evaluation as described by Carr (1965) for angle of repose, dispersibility index, and compressibility index (Table 4) The formulations were found to contain moisture content below 1 5% (Table 4)

T_{90} PDR was found to be between 2 to 2 5 months at accelerated storage (40°C – 75% RH), 5 to 6 months at controlled room temperature (25°C – 60% RH) and above 1 year at

refrigerated (2–8°C) conditions of storage Caking and discoloration (cream color) was observed under accelerated storage conditions after 3 months This phenomenon was not evident at long-term storage of developed LDPI formulations at refrigerated storage condition Even the flow and dispersion properties of the formulation stored at long term refrigerated conditions remained unaltered

DISCUSSION

The organic solvents such as diethyl ether or methanol used in the liposome preparation, although usually removed by evaporation, may remain as traces in the final formulation representing a possible risk for human health and can lead to inadequate stability of the vesicles (Cortesi et al 1999) Use of other organic solvents like ethyl acetate and ethanol can solve this problem Ethanol forms a monophasic system upon contact with aqueous phase whereas ethyl acetate forms biphasic system (emulsion) upon contact with aqueous phase. When ethyl acetate was used alone, it resulted in distorted spherical vesicles from formation of unstable biphasic system upon contact with aqueous phase Use of ethanol alone resulted in high PDE from formation of monophasic system upon contact with aqueous phase However, drug leakage was observed due to presence of traces of ethanol leading to disruption of bilayer

In the ethyl acetate ethanol (1.1) combination, proper spherical vesicles and high PDE were observed Combination of these organic solvents with aqueous phase forms stable emulsion, which is prerequisite for REV (Betageri, Jenkins, and Parsons 1993) When aqueous phase to organic phase ratio was raised from 1 3 to 1 5, a marked increase in the PDE was observed Further increase in the organic phase did not result in increase in PDE The prepared liposomes were found to be multilamellar and identified by Maltese crosses in liposomal photomicrographs (Figures 1a and 1b).

Among maltose, dextrose, trehalose, lactose, and sucrose used as cryoprotectants, sucrose and trehalose were found to be comparable cryoprotectants. Trehalose was found to be the most effective cryoprotectant in maintaining structural and functional properties of microsomal membranes at low mean liposomal size (Cullis et al 1985) However at higher concentrations, sucrose was found to be equally effective for multilamellar liposomes of large mean size (above 1 μm) Thus, sucrose was used as cryoprotectant on both sides of lamellae.

During the drying process of liposomes, liposomes constrict and get coated on the optimum surface of crystallized sugar and the hydration of polar head groups with 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 constricted bilayer leading to drug leakage Hence, it may be concluded that the bulk concentration of sugar required as cryoprotectant depends upon 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.

Liposome Sorbolac 400 with sieved Pharmatose 325M ratio used in formulation of DPI and percent of sieved Pharmatose 325M were found to influence FPF Effect of liposome lactose blend ratio was optimum at 1.6. Optimum concentration of carrier is required to achieve detachment of liposomal drug from carrier molecule Carrier concentration less or more than optimum resulted in low FPF or no further increase in FPF High-energy adhesion sites (HA) of lactose bind strongly to the liposomal drug particles and low-energy adhesion sites (LA) allow the formation of more reversible bonds with liposomal drug This results in efficient detachment of liposomal drug from the carrier as observed with plain DPI formulations (Staniforth 1996) Hence, 10% sieved Pharmatose 325M added to LDPI formulation occupies HA sites leaving LA sites for attachment of liposomal drug and thus results in higher FPF

Drug liposomal powder adheres to carrier particles as seen in scanning electron microscopy photographs of LDPI formulations (Figures 2a and 2b) The EI of AMB1 was found to be better than the AMB2 suggestive of more effective liposomal drug deposition into the lung It may be due to turboelectrification or charge generation in liposomal powder during dispersion via the Rotahaler The lower ratio of EI/ FPF is suggestive of efficient dispersion of AMB1 from the device, but unlike the control more proportion of the dispersed powder has been deposited in the upper respiratory tract (Hino 1998)

Evaluation and control of flow and dispersion (deaggregation) characteristics of the formulation are of critical importance in the development of DPI products Inter-particle forces that influence flow and dispersion properties are particularly dominant in micronize or microcrystalline powders required for inhalation therapy (<5 μm) (Gonda 1992, Hickey 1996) Predictions of powder rheology based on the possible relationship of a number of physicochemical properties are extremely complicated Hence, such flow and dispersion properties as angle of repose, dispersibility index, compressibility index, moisture content, and FPF are characterized and controlled (Table 4) Moisture content determination also is important for drug stability upon storage and deaggregation upon inhalation

At refrigerated and controlled room temperature storage, higher PDR of AMB1 and AMB2 were observed (Figure 3) It may be due to CHOL having an association with PC and drug at molecular level, reducing its rate of hydrolysis or oxidation in anhydrous state At accelerated storage i e., near Tg (phase transition temperature), decrease in the PDR for LDPI formulations was observed due to increased fluidity of bilayer resulting into drug leakage Higher PDR of AMB1 at accelerated storage may be due to presence of higher proportion of amphiphilic PC leading to re-encapsulation of the drug by the liposomes

CONCLUSION

In this study, the small multilamellar AMB liposomes were successfully prepared and stabilized by lyophilization into LDPI

formulations for shelf life over 1 year at refrigerated storage Findings of this investigation demonstrate delivery of liposomally entrapped AMB from trachea to terminal bronchioles in comparable doses of marketed DPI formulation. The parameters controlling the drug deposition into the lung also were established This method offers an exciting possibility of localized pulmonary liposomal AMB delivery in the anhydrous state However, the role of LDPI formulation developed in this investigation can only be settled after in vivo evaluation of the product on two species of animals followed by clinical evaluation

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SHORT COMMUNICATIONS

Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, M S University of Baroda, Baroda (Gujarat), India

Liposomal Amphotericin B dry powder inhaler: Effect of fines on *in vitro* performance

S. P. SHAH, A. MISRA

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Ambikanandan Misra, Pharmacy Department, Faculty of Technology, and Enggineering M.S University of Baroda, Kalabhavan, Vadodara 390001, Gujarat, India

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The aim of the present investigation was to improve *in vitro* pulmonary deposition of amphotericin B (AMB) liposomal dry powder inhaler (LDPI) formulations Liposomes with negative (AMB1) and positive (AMB2) charge were prepared by the reverse phase evaporation (REV) technique, extruded to reduce size, separated from unentrapped drug and lyophilized using an optimized cryoprotectant to achieve maximum drug retention Lactose carrier (Sorbolac 400) in varying mass ratio with or without addition of fines (500# sieved Pharmatose 325M) in different mixing sequence were used to formulate AMB LDPI formulations *In vitro* evaluation was done with twin stage impinger (TSI) for fine particle fraction. The lactose carrier containing 10% fines was found to be optimum blend at 1 : 6 mass ratio of liposome lactose The addition of fines and order of mixing fines were found to influence the fine particle fraction (FPF) significantly. FPF of LDPI formulations using a Rotahaler (Cipla, India) as delivery device at 30, 60 and 90 L/min were found to be 23 1 ± 1.5 percent and 17.3 ± 2.2 percent; 25 3 ± 1 8 percent and 19.6 ± 1.5 percent and 28.4 ± 2.1 percent and 22.9 ± 1.9 percent for AMB1 and AMB2 respectively

Improving the drug delivery to the lungs from a DPI formulation can be made possible by various techniques like smoothing the carrier surface (Ganderton, 1992), reducing the particle size of the carrier (Steckel et al 1997) and use of a ternary powder mix formulation (Staniforth 1996 a). Addition of micronized lactose to coarse lactose carrier was found to improve the dispersion and deaggregation of salbutamol sulphate and spray dried bovine serum albumin (Lucas et al 1998) Also, techniques like spray drying the drug with phospholipid composites in a suitable range for pulmonary delivery (Kim et al 2001) or the dissolution of lecithin in chlorofluorohydrocarbon and the formation of liposomes *in situ* (Farr et al 1987) or nebulization of the preformed liposomes (McCallion et al 1996) can be attempted for liposomal drug delivery to lungs 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) not more than 21% (Joshi et al 2001 a, 2001 b) The aim of the present investigation was to study the effects of addition of fines and the addition sequence of fine carrier on *in vitro* deposition of the formulations using TSI at different flow rates.

Multilamellar vesicles (MLVs) composed of drug (5mg), HSPC, cholesterol, α-tocopherol (1% of PC) and either soyaphosphatidylglycerol (SPG-3) (AMB1) or stearylamine (AMB2) of AMB were prepared by the modified reverse phase evaporation technique (Cortesi et al. 1999) by using 0.01M Tris buffer pH 6.5 containing 1mM EDTA (ratio of aqueous phase : organic phase was 1 5) with intermittent vortexing. The formed liposomal dispersion was extruded through a 2 μm polycarbonate membrane above the phase transition temperature and separated from unentrapped drug by controlled centrifugation. To achieve high PDR, lyophilization was carried out for 48 h using sucrose as cryoprotectant in a mass ratio of 1 5.

To prepare LDPI formulations, the porous cake of liposomes obtained after lyophilization was sieved (200# and 240#) and filled in capsule size "2" containing 250 μg of AMB Similarly the sieved lyophilized powder was mixed with Sorbolac 400 in different mass ratio (1 . 2 to 1 : 8). The addition of fines in the range of 5%–15% and addition sequence of fines were investigated i.e., first fines were mixed with carrier and then mixed with lyophilized liposomes or fines were mixed with lyophilized liposomes and then mixed with the carrier (Table 1).

The volume of capturing solvent (methanol) in the upper (stage 1) and lower (stage 2) were 7 and 30 ml respectively in TSI (B.P Apparatus A) (British Pharmacopoeia 1993) Rotahaler (Cipla, India) was used as delivery device at flow rates of 30 ± 2 L/min, 60 ± 2 L/min and 90 ± 2 L/min for 5 s for 5 capsules The inhaler body, capsule shells, mouthpiece, stage 1 and stage 2 were washed five times with methanol and analyzed to measure the amount of drug retained as described before (Ruijgrok et al 2000) 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 84 8 ± 2 mg (for AMB1) and 71.0 ± 2 mg (for AMB2) equivalent to nominal dose of 250 ± 7 μg AMB. 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 Percent emission was calculated as the percentage of emitted dose to total dose FPF was the ratio of FPD to

Table 1: Optimization of LDPI formulation

Variable studied	Percentage FPF for AMB1[a]	Percentage FPF for AMB[a]
Effect of liposome lactose ratio (Sorbolac 400)		
1 2	12 3 ± 2 2	8 5 ± 2 4
1 4	15 1 ± 3 0	11 6 ± 2 2
1 6	17 5 ± 2 4	13 2 ± 3 1
1 8	16 4 ± 2 7	11 9 ± 2 8
Effect of percentage of carrier (liposome lactose ratio was 1 : 6)		
5%	19 2 ± 2 5	14 9 ± 2 5
10%	22 5 ± 2 2	16 8 ± 2 2
15%	20 1 ± 1 9	14 6 ± 2 3
Effect of sequence of addition of fines (10%, 500# sieved Pharmatose 325M)		
Fines + carrier + lyophilized liposomes	25 3 ± 1 8	19 6 ± 1 5
Fines + lyophilized liposomes + carrier	22 1 ± 1 6	18 1 ± 1 9

[a] n = 5 (± SEM)

Table 2: Comparative characterization of potential batches of AMB LDPI formulations

Parameters	AMB1						AMB2					
	30 L/min		60 L/min		90 L/min		30 L/min		60 L/min		90 L/min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FPD (µg)	56 6	1 8	64 5	1 5	73 9	2 0	40.7	1.7	47 0	1 4	56 1	1.8
FPF (%)	23 1	1 5	25.3	1.8	28 4	2 1	17 3	2 2	19 6	1.5	22.9	1.9
Dispersibility (%)	26 1	1 5	28 4	2 2	31.8	2.1	20.8	2.4	23 4	1.8	27 1	1.3
Effective Index	45 2	1.2	47.5	1.9	50.4	1.4	37 9	2 0	40.5	1.6	44 0	1.7

Control Asthalin (Cipla Ltd , India)
Delivery device Rotahaler (Cipla Ltd , India)
FPF = 27 1 ± 2 0, EI = 48 6 ± 1 7 at 60 L/min

RD, while dispersibility was the percentage of FPD to ED (Table 2). As a control, a marketed preparation (Asthalin Rotacaps, Cipla Ltd , India) containing salbutamol sulphate 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 2). The Rotahaler device was rinsed with methanol to determine the device fraction and Effective Index (EI) (Hino et al 1998)

Effective index is the geometric mean of the total emitted dose and FPF, represented by the equation (Hino et al. 1998).

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

where, DF is the device fraction

Significant differences were calculated by ANOVA and mutual differences were detected with Students t-test and differences at $P < 0.05$ were considered as significant

The liposomes of AMB were prepared by REV technique using ethyl acetate and ethanol (1 : 1) as organic solvents and 10 mM Tris buffer pH 6.5 containing 1 mM EDTA as aqueous phase. Liposomes were extruded to reduce size, separated from unentrapped drug and lyophilized using optimized cryoprotectant to achieve maximum percent drug retention Maximum PDE estimated in AMB1 and AMB2 liposomes were 95.8 + 1.5 and 87.9 + 1 3 respectively. The optimum lipid sucrose mass ratio was found to be optimum at 1 5 with PDR of 96 6 + 1.8 and 94.7 + 2 4 for AMB1 and AMB2 respectively

The mass ratio of liposomes Sorbolac 400 at 1 6 resulted in FPF of 17 5 + 2 4 and 13 2 + 3 1 percent for AMB1 and AMB2 respectively Optimum concentration of carrier is required to achieve detachment of liposomal drug from carrier molecule. Carrier concentration is less or more than optimum resulted in too low FPF or no further increase in FPF. Further the effect of increasing fines from 5% to 10% resulted in higher FPF of 22.5 + 2.2 and 16 8 + 2 2 percent respectively Furthermore the addition sequence of fines such as fines first mixed with carrier and then mixed with lyophilized liposomes resulted in FPF of 25 3 + 1.8 and 19 6 + 1 5% with significantly different EI. High-energy adhesion sites (HA) of lactose bind strongly to the liposomal drug particles and low-energy adhesion sites (LA) allow the formation of more reversible bonds with liposomal drug This results in efficient detachment of liposomal drug from the carrier as observed with plain DPI formulations (Staniforth 1996b) Hence, 10% sieved Pharmatose 325 M added to LDPI formulation occupies HA sites leaving LA sites for attachment of liposomal drug and thus resulted in higher FPF. Based on EI, the deposition of liposomal AMB was more efficient in case of AMB1 than the AMB2 The FPF ratios of control to that of the developed LDPI formulations were 0 93 and 0 72 and EI ratios were 0 97 and 0 83 for AMB1 and AMB2 respectively The EI of AMB1 was found to be better than the AMB2 suggestive of more effective liposomal drug deposition in to lung It may be due to triboelectrification or charge generation in liposomal powder during dispersion via the Rotahaler. The lower ratio of EI/FPF is suggestive of efficient dispersion of AMB1 from the device but unlike the control more proportion of the dispersed powder has been deposited in the upper respiratory tract (Hino, 1998).

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Liposomal Amikacin Dry Powder Inhaler: Effect of fines on In vitro Performance

Shrenik P. Shah[1] and Ambikanandan Misra[1]

[1]Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, P.O.Box No.51, M.S.University of Baroda, Vadodara – 390001 (Gujarat), India.

Corresponding author: Ambikanandan Misra, Pharmacy Department, Faculty of Technology and Engineering, M.S. University of Baroda, Kalabhavan; Vadodara – 390001. Gujarat, India. Tel.: (O) 91-265-2434187 (R) 91-265-2785284, Fax 91-265-2423898.

E-mail: misraan@hotmail.com

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.

REVIEW ARTICLE

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Factors Affecting Development of Dry Powder Inhalers

S P SHAH, S GANESH AND A N MISRA*
Pharmacy Department, Faculty of Technology and Engineering
M S University of Baroda, Kalabhavan, Vadodara-390001

The search for alternatives to metered-dose inhalers has driven impetus for finding effective products that do not use chlorofluorocarbon propellants The purpose of this paper review is to address the factors to be considered in developing dry powder inhalers; particularly the formulation, metering design and flow path in the device and importance of various regulatory requirements are discussed The advantages and disadvantages of current dry powder inhalers and future approaches for pulmonary drug delivery are also discussed.

Inhalation drug delivery has been used for many years for the delivery of pharmacologically active agents to treat respiratory tract disease Traditional asthma therapy with bronchodilators, steroids, mast cell stabilizers and anticholinergic drugs has primarily used the pressurized metered dose inhaler (pMDI) however, there is increasing threat because of the environment concerns regarding chlorofluorocarbon (CFC) propellants In 1989 when the Montreal Protocol was implemented (an International convention that restricts the use of substances that deplete the ozone layer), it defined the need to replace CFC propellants in all pMDIs The alternative hydrofluoroalkane (HFA) propellants have been difficult to formulate with inhalation drugs because of crucial differences in densities and solubilities of drugs and excipients The pMDI market now includes both CFC and HFA aerosols with suspension and solution formulations of commonly used drugs such as salbutamol and beclomethasone dipropionate[1] Large difference in particle size distribution of the emitted doses has also been demonstrated[2] The problem on beclomethasone dipropionate prescribing is compounded by other CFC free formulations having same nominal dose as the original suspension[3] Therefore there is a pressing need to clarify the clinical equivalence of newly formulated pMDI products and reduce the problems of HFA reformulation The dry powder inhaler (DPI), being propellant-free, is an increasingly attractive and less confusing alternative for pMDI as drug delivery devices There has been tremendous activity in the development of DPI devices over recent years with many innovative systems now at various stages of development[4,5] 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

The DPIs does not contain CFC propellants to disperse the drug, so they can be regarded as ozone-friendly delivery systems However, they can not totally replace pMDIs due to limitations of dose delivered and flow rates achieved through the devices for severely diseased patients are probably valid[6], based on the capabilities of currently available powder inhalers Vidgren *et al* have shown different deposition patterns in healthy volunteers from the same formulation in four single-dose DPIs[7] Newman *et al* have also shown different *in vivo* deposition patterns in healthy volunteers using Turbuhaler inhalers operated at optimal and sub-optimal peak inspiratory flow rates[8] Clearly, some current designs of DPIs are subject to variations in performance due to differences in inhalation flow rates. Future designs should be independent of patient inhalation for the disper-

*For correspondence
E-mail misraan@satyam net in, misraan@hotmail.com

sion of the powder dose

FACTORS INFLUENCING DPI FORMULATION DESIGN

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[9] 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)[10] 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 reviewed the factors influencing the dispersion of dry powders as aerosols[11] 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 reviewed particle surface characteristics and several other studies have measured the adhesion forces in inhalation powders[12,13] Peart and co-workers measured electrostatic charge interactions from Turbohalers and drug powders and the results suggest that the inhaler itself and the deaggregation mechanisms influenced the charging phenomena[14] Electrostatic effects in DPIs have been extensively studies by others[15] and powder flow properties have also been studied[16] Further particle characteristics have been studied such as the crystallization and amorphous content of inhalation powders[17,18] and the measurement of their surface properties by inverse gas chromatography[19] and computer aided image analysis to plot a Facet Signature[20]

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[21] Blending the drug with a carrier has a number of potential advantages, such as increasing the bulk of the formulation This allows easier metering of small quantities (typically <100μg) of potent drugs, either at the manufacturing stage (if the doses are pre-metered) or within the device itself for a reservoir device Provided the content uniformity of the blend is well controlled, this approach can improve the subsequent dosing consistency of the inhaler The presence of the carrier material, in separating the very fine drug particles, can also improve processing (e g flow characteristics) of the formulation The carrier properties (particle size distribution, particle surface characteristics) can be used to influence/control fine particle mass

An additional benefit that may be gained by the use of a carrier such as lactose is the taste/sensation on inhaling, which can assure the patient that a dose has been delivered Clearly, the influence of the carrier material on product stability must be carefully assessed, and the range of materials available for use as carriers in inhaled products is limited for toxicological reasons Lactose and other sugars have been studied and used and modification of these materials may allow further formulation optimization Modifications to the lactose surface have been proposed that would improve the surface characteristics (reduce the rugosity) of the material Ganderton claims that reducing the rugosity increases the percentage of respirable particles in conventional powder inhalers[22] Zeng and coworkers 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[23] They suggested that this may be because of the fine particles occupy possible drug binding sites on the larger lactose particles Lucas *et al* demonstrated a similar performance modifying effect with a model protein, albumin and a high-dose agglomerated preparation of nedocromil sodium[24] Other studies have looked at similar effects of lactose size fractions and agglomerates[25] The properties of lactose such as particle size and surface morphology[26] had a profound effect on the fine particle fraction

of the generated aerosol Other excipients, like sugars, have also been studies to establish their preformulation characteristics Braun *et al*[27] used two grades each of α-lactose monohydrate and dextrose monohydrate with disodium cromoglycate and generated aerosols using a unit-dose device, the Microhaler[28]

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[29], spray-dried salbutamol sulfate was seen to perform as well as micronized material In the case of sodium cromoglycate, several approaches have been successfully employed to improve flow and dispersion characteristics, including controlled adherent flocs[30,31] This approach takes advantage of the inherent cohesiveness of the particles

In a review, Staniforth has outlined the development of improved performance dry powder inhalation systems by preformulation characterization of drug-carrier combinations[32] Staniforth described 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[32,33] 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[10] In addition, crystals of the parent acid and the effect of aspect ratios (longest and shortest dimensions) have been studies[34] Vidgren *et al* 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[35]

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[36] have evaluated the SEDS (Solution enhanced dispersion by supercritical fluids) technique that enables a drug solution to be processed into a micrometer sized particulate product in a singles step operation

METERING DESIGN

DPIs can be divided into two classes passive and active devices Passive devices rely solely upon the patient's inhalatory 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[37] 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[38] 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* who had developed this device for the administration of powdered sodium cromoglycate[30] 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[39] Other devices dispense drug loaded into hard gelatin capsules like the Berotec (Boehringer Ingelheim) used for fenoterol[40]

These devices have performed well in clinical use for almost 25 years Their primary disadvantage is the cumbersome nature of loading the capsules, which may not be easily feasible if a patient is undergoing an asthma attack and requires immediate relief

The development of multi-does DPI has been pioneered by A B Draco (a division of Astra) with their Turbuhaler[41] and

by Glaxo Wellcome with the introduction of the Diskhaler[42] and recently the Diskus[43] 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[44]

The Diskhaler (Glaxo Wellcome) has been introduced for the delivery of both the short-acting β-agonist, salbutamol, as well as longer-acting, salmeterol[45] Also, the steroids like beclomethasone dipropionate and fluticasone propionate are available as disks These devices have a circular 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 and fig 1 illustrates this point The graph shows the frequency distribution of doses delivered at 60 l/min from a terbutaline Turbuhaler and a salmeterol Diskus[46] 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 60 l/min from the salmeterol Diskus device is unaffected by storage at high humidity[45] 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[47,48]

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[49]

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/deaggreagate 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[50] 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[51] and the resultant flow rates were compared New DPIs such as the Chiesi inhaler[52] (Chiesi Farmaceutici, Italy) and the Innovata Biomed Inhaler[53] (Innovata Biomed Ltd UK) are evaluated for dosing performance at a range of flow rates

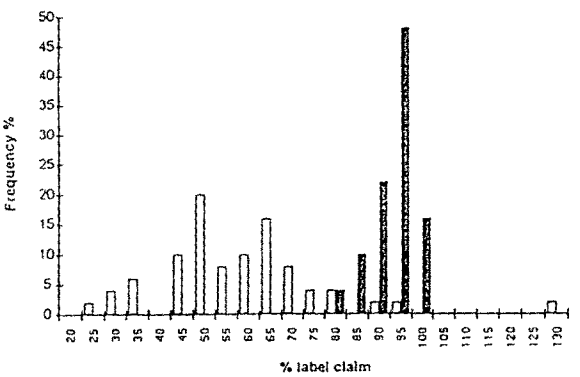

Fig 1 Frequency distribution of doses delivered at 60 l/min

Percentage Frequency distribution of doses delivered at 60 l/min for salmeterol Diskus (-■-) and terbutaline Turbohaler (-□-) n=50 [Ref 45]

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[54] 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[44] At 60 l/min, the Turbuhaler can produce up to 50% of the emitted dose as respirable particles (<5 μm), although the percentage is considerably reduced at lower flow rates[55]

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[56] The flow rate achieved may be important in determining the performance of the device[57] 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[58] Device resistance can also affect the patient's comfort in using the inhaler De Boer *et al* 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[59] Fig 2 compares the dose delivered from the Diskus and Turbuhaler inhalers at a range of flow rates The inhaler resistances at each flow rate are also shown in the figure and indicate that the Turbuhaler has a higher resistance than the Diskus inhaler The graph also shows that the Turbuhaler delivers a smaller production of each dose than the Diskus and is more dependent on flow rate

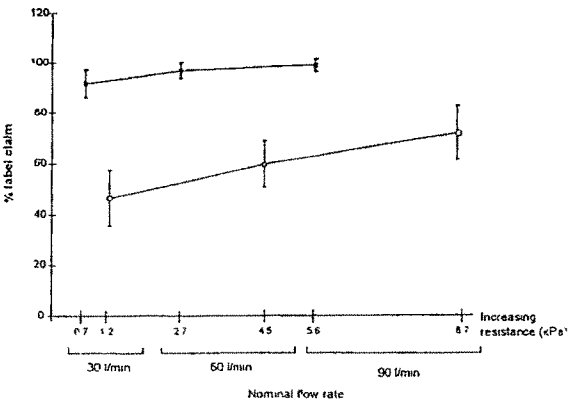

Fig 2 Measurement of emitted dose from salmeterol Diskus and terbutaline Turbohaler at varying flow rates

Measurement of emitted dose from salmeterol Diskus (-■-) and terbutaline Turbohaler (-□-) at varying flow rates like 30, 60 and 90 l/min with median and interqratile range for 5 devices each, n=50 [Ref 43]

REGULATORY AND PHARMACOPOEIAL REQUIREMENTS

The late 1990s have seen the published agreements from the FDA (US Food and Drug Administration)[60] and the European Inhalanda group[61] 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[60]

A presentation of FDA Guideline for Product Development Strategy[62] 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 This would create significant problems both to prescriber and patient as different labeled (metered) doses could be therapeutically equivalent The new European Pharmacopoeial Monograph defines the fine particle dose as that fraction of the delivered dose that is <5 μm However, a new DPI should establish some measure of therapeutic equivalence as part of its marketing information to reduce the prescribers confusion

The European Pharmacopoeial Monograph also 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

TABLE 1 COMMERCIALLY AVAILABLE DPIS AND NEW DPIS CURRENTLY UNDER DEVELOPMENT AND ITS DISPERSION MECHANISM

Type of the Device and 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)	-
Miat-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 (Volois)	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

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

TABLE 2 US FDA REQUIREMENTS FOR TESTING DRY POWDER INHALERS

Drug Product
This includes the device with all of its parts, any protective packaging and the formulation
Components
Composition
Specifications for the formulation components like active ingredients and excipients
Manufacturers
Method of manufacturing and packaging
Specifications for the drug product
Container and closure system
Drug product stability
Drug product characterization studies
Determination of appropriate storage conditions
Stability of primary (unprotected) package
Effect of varying flow rates
Effect of storage on the particle size distribution
Dose build-up and flow resistance
Effect of orientation
In vitro dose proportionality
Effect of patient use
Effect of moisture
Photostability
Profiling of doses near device exhaustion
Priming
Fill weight
Device ruggedness
Cleaning instructions
Labeling considerations
Defines information to be included on the device label and packaging insert

Several studies have demonstrated improvements in the designs of cascade impactors[63] and emitted-dose-measurements apparatus[64] used for the evaluation of the performance of DPIs A new impactor is being developed by an industry consortium, the Next Generation Impactor group[65] phase I of the project is an evaluation of new designs

The requirements form the Medicines Control Agency (MCA)[66] also include stricter controls on the uniformity of the delivered dose than the Pharmacopoeial limits and states that the applicant should be able to attain a mean of ± 20% or better from the nominal content per dose In addition, the MCA requires each multi-dose unit to have the following two safety features 1 A counter device or other indicator to give the patient some indication of when it is becoming exhausted and 2 A system to prevent inadvertent multiple dosing because of multiple actuations of the dose measuring device

The new SkyePharma powder inhaler (SkyePharma AG, Switzerland) containing a reservoir of 300 doses[67] and the Bulkhaler device (Astra Medica AG, Germany) incorporating a refillable cartridge[68] fulfill these MCA requirements The committee for proprietary medicinal products (CPMP) has published guidelines on DPIs in 1998[69]

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

NOVEL INHALATION DELIVERY SYSTEMS

Interest in the design of more compact portable inhalation delivery systems is increasing The patent literature offers numerous examples of applications for novel delivery systems that purport to be potential replacement for the pMDIs, and much is being published in this field[64,65] Consideration is being given to delivery of biotherapeutic materials, such as some proteins and peptides, by inhalation aerosol[67]

Initial research into the production of microspheres using substances such as poly (D, L- lactide-co-glycide) (PLGA) and poly(L-lactic acid) (PLA)[70] has demonstrated that the administration of microspheres to the pulmonary airways could be a route for sustained-release drug therapy in respiratory disease, although the toxicity of this type of formulation has yet to be established Another group of workers has studies the pharmacokinetics of mucoadhesive budesonide microspheres administered to guinea pigs, dem-

onstrating an increase in duration of drug action from 6 to 24 hours after lung administration[71]

In recent years, the development of inhalation simulation machines has enabled the measurements of *in vitro* DPI performance using patient's inhalation profiles[72] The dose dispersion process is driven by a pre-programmed theoretical inhalation profile or a previously recorded patient inhalation profile with varying flow rates and flow accelerations, and the resultant aerosol could is subsequently analyzed for dose and particle size using an impactor or impinger at a fixed flow rate These machines have increased the understanding of the complex relationship between acceleration of inhalation flow rate and the dose output of DPIs[73] These machines also facilitate the *in vitro* evaluation of dosing performance of new DPI designs for a range of simulated patient conditions, and thus they are becoming established as part of the ongoing testing of DPIs

A number of potential new devices are emerging in the powder area, ranging from simple unit-dose devices to more complex multidose systems[74] In addition, true breath-activated systems, coupled with an auxiliary means for dispersion of the metered powder[75] hold much promise for the future, if they can pass the trials of converting a sound laboratory principle into a commercially successful device This, of course, will take several years and may well be driven by patients' needs and the acceptability of alternatives to the widely used pMDI

Recently we have developed liposomal DPI formulation of budesonide[76 77], ketotifen fumarate[78] and terbutaline sulphate[79 80] Liposomal budesonide was stabilized by lyophilization, delivered as an aerosolized DPI and evaluated by twin stage impinger gave the fine particle fraction of 20% The developed liposomal budesonide DPI was found to provide desired drug levels in the lungs for a prolonged period of time, which is expected to enhance the therapeutic index of the drug and probably reduce the dose and cost of therapy as well[77]

Liposome aerosols are promising vehicles for respiratory delivery of therapeutic drugs and have attracted the attention of many researches, especially in the area of DPIs[81 82] Liposomal delivery by dry powders has been considered mainly based on the fact that liposomes can be more stable when dried by lyophilization[83 84] With liposome powders as drug carriers for inhalation therapies, the lyophilized precursor should be micronized to particles of 1-6 μm in diameter for efficient delivery to the lung Micronization has normally achieved by jet-milling[81 85], which causes particles to break apart on colliding in a high-velocity air-stream As a measure of circumventing the potentially negative effects of lyophilization and jet-milling advantage might be made of the fact that phospholipids are known to orient into liposomal configuration through a spontaneous, entropic process in a water-rich environment Such conditions exist in the airways of the respiratory tract, so that it is feasible to postulate that spontaneous liposome formation would occur following pulmonary disposition of microfine phospholipids-based aerosols This was demonstrated by Desai *et al* for three model drugs[86] (viz , ciprofloxacin, CM3 peptide and salbutamol sulphate) The effects of several parameters, including lactose concentration, lipid composition and lipid concentration on the encapsulation efficiency of these model drugs were investigated

Inhalation aerosol characterized by particles of small mass density and large size, permitted the highly efficient delivery of inhaled therapeutics into the systemic circulation Particles with mass densities less than 0 4 per cubic centimeter and mean diameters exceeding 5 μm were inspired deep into the lungs and escaped the lungs natural clearance mechanisms until the inhaled particles delivered their therapeutic payload Inhalation of large porous insulin particles resulted in elevated systemic levels of insulin and suppressed systemic glucose levels for 96 hours, whereas small nonporous insulin particles had this effect for only 4 hours High systemic bioavailability of testosterone was also achieved by inhalation delivery of porous particles with a mean diameter of 20 μm approximately 10 times that of conventional inhaled therapeutic particles[87] Porous particles comprising therapeutics and pharmaceutical excipients can easily be formed by spry-drying[88], rapid expansion of supercritical fluids[89] and other particle formation technologies Hence, they can immediately address a variety of needs as therapeutic carriers for inhalation therapies Their potential for high aerosolization efficiency, long-term drug release and increased systemic bioavailability makes large porous particles especially attractive for systemic inhalation therapies

CONCLUSIONS

Common to all inhalation dosage forms and delivery systems is the need to generate the optimum 'respirable dose' (particles with aerodynamic diameter <5 0 μm) of a therapeutic agent consistently and reliably This is a key performance feature in the rational design and selection of a delivery system Moreover, this performance, in terms of

aerosol quality, should be demonstrated throughout the product's shelf life In addition to the more usual chemical and physical stability criteria, when considering these delivery systems, it is important that the device design and formulation work have been integrated in the overall design and development of the product Frequently, therefore, such inhalation delivery systems tend to be compound or company specific

In summary, in the short term, suitable replacements for pMDIs (be they powder or liquid based) are unlikely, but if some of the systems that are currently being developed are able to achieve the convenience and compactness of the pMDI and have similar (or improved) pharmaceutical performance, they might be in widespread use in the later part of the decade

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