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Dry powder inhalation of liposomal Ketotifen fumarate: formulation and characterization

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Abstract

The purpose of the experiment was to formulate and characterize the dry powder inhalation (DPI) formulation of liposomally entrapped anti-asthmatic drug, Ketotifen fumarate (KF). Liposomes composed of saturated egg phosphatidyl choline (EPC) and cholesterol (CHOL) were prepared by lipid film hydration and sonicated to have the desired size (< 5 μm). Process variable such as vacuum, presonication hydration, postsonication hydration, purification and lamellae composition, were optimized for encapsulation efficiency of KF. Liposomal dispersion was blended with cryoprotectant (sugar) in varying bulk and mass ratios and assessed for its influence on retention of encapsulated drug on lyophilization. Characterization of liposomal dispersion was done for size, lamellarity, entrapped volume and oxidation index. DPI formulation was characterized for angle of repose, compressibility index, dispersibility and respirable fraction (British Pharmacopoeia, apparatus A). Process optimization revealed that a vacuum, 20 in.; presonication hydration, 60 min; postsonication hydration, 2 h and purification by dialysis gave maximum encapsulation efficiency. Sucrose was found to be the most suitable cryoprotectant at bulk strength of 500 mM and mass ratio of lipid/sugar, 1/12. Blending of sorbolac before lyophilization showed better retention of encapsulated KF ($97.92 \pm 0.54\%$). In the preparation of sonicated MLVs, the presence of nitrogen atmosphere, α-tocopherol and EDTA could not totally eliminate EPC oxidation, expressed as the change in oxidation index from 0.427 ± 0.01 to 1.510 ± 0.01. The respirable fraction of the developed formulation ($21.59 \pm 1.53\%$) is comparable with the control ($26.49 \pm 1.52\%$). From studies, it may be concluded that an optimal bulk and mass ratio of sucrose, relative to the size of liposomes is necessary for effective cryoprotection. In this investigation, DPI of liposomal KF was successfully prepared and delivered to the required site in the lungs.

Keywords: Lyophilization; Ketotifen fumarate; Liposomes; Pulmonary; Dry powder inhalation

1. Introduction

Delivery of the therapeutic compounds directly to the respiratory tract is more sophisticated today. The advantages of inhalation therapy have essentially remained the same such as the very

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rapid onset of action and very small dose (Newman and Pavia, 1985; Zeng et al., 1995). An oral dose of bronchodilator may take 2–3 h to be fully effective while an inhaled dose usually takes a minimum of 15–30 min. A few hundred micrograms of inhaled bronchodilator is as effective as an oral dose of 5–10 mg. This reduces exposure of drug to the systemic circulation and potentially minimizes adverse effects. Lower dosage regimens may provide considerable cost saving especially with expensive therapeutic agents. Despite these advantages and the widespread use of therapeutic aerosols, there are several shortcomings associated with drug delivery to the respiratory tract. Although the onset of action is very rapid, the duration is often short-lived as the drug can be quickly removed from the lung through various clearance mechanisms (Shenfield et al., 1976; Byron, 1986). Regardless of the type of aerosol device employed, i.e., metered dose inhalers (MDI), nebulizer, or insufflation device, most patients require dosing every 6–8 h and more frequently in some instances. Improving the drug delivery to the pulmonary system has been an area of increasing interest among several disciplines. Particulate carriers such as liposomes have many attractive features as pulmonary drug delivery systems particularly with respect to the controlled delivery. One of the perceived benefits of liposomes as a drug carrier is based on their ability to alter favorably the pharmacokinetic profile of the encapsulated species and thus provide selective and prolonged pharmacological effects at the site of administration. Administration of liposomes to the respiratory tract is particularly attractive because of the accessibility of the lung as a target organ, the compatibility of liposomes and lung surfactant components (85% phospholipid), and the need for sustained local therapy following inhalation (Taylor and Newton, 1992). Liposomal drug delivery to lungs have been attempted by two techniques, one involves the dissolution of lecithin in chlorofluorohydrocarbon and the formation of liposomes in situ (Farr et al., 1987) and the second is by nebulization of the preformed liposomes (McCallion et al., 1996).

Pulmonary delivery of drug using dry powder inhalation systems (DPIs) has almost became compulsion for a number of reasons including: (1) the mandated phase out of chlorofluorocarbons in MDI; (2) the problem of global warming associated with hydrofluorocarbons; (3) enhanced formulation flexibility; (4) the invent of electromechanically actuated DPIs, e.g., SPIROS™ (DURA Pharmaceuticals, San Diego; US FDA approval awaited); and (5) patient acceptability. There has been extensive effort to define the factors that influence the deposition of DPI within the respiratory tract, clearance mechanisms from the lung, circulation in the airways, and absorption and metabolism of compounds by the lung (Brown, 1974). With this information, strategies to improve drug delivery to the respiratory system have developed. Both patent and technical literature has focused primarily on drug's conventional formulation and device aspects of dry powder delivery.

The study focuses on the pharmaceutical development of liposomal drug formulations for DPI and the evaluation, optimization, and control of flow and dispersion characteristics of the developed formulations. In this study, the efforts were made to find out ideal liposomal formulation, as far as encapsulation efficiency is concerned and lyophilization of the liposomes of Ketotifen fumarate (KF) after incorporating it into an appropriate cryoprotectant at an optimal strength. KF has primarily been selected as a model drug for the purpose of study. KF is a mast cell stabilizer used in prophylaxis and management of asthma.

2. Materials and methods

2.1. Materials

KF was a gift sample from Torrent Pharmaceuticals, India. Saturated egg phosphatidyl choline (EPC) was procured from Sigma chemicals (India); cholesterol (CHOL) (S.d. Fine Chemicals, India); α-tocopherol (E. Merck India Ltd., India); Dextrose monohydrate (Roferose ST, ROQUETTE, Crow chemicals, USA); Maltose (Advantose™ 100, SPI Polyols, USA); Sorbolac –

400 (Meggle, Wasserburgs, Germany) and Sucrose (Pokkie Odcaynnlki chemicals, Gliwice, Poland).

2.2. Preparation of liposomes

Multilamellar vesicles of KF were prepared by the modified method of Bangham, reported by Juliano and Daoud (1990). Drug, EPC, CHOL and α-tocopherol (1% of EPC) were co-precipitated to a thin film, by solvent stripping ($CHCI_3$/CH_3OH, 2/1) under nitrogen atmosphere. The film was then hydrated at 40 ± 1°C for 1 h with 2 ml of 1 mM EDTA, phosphate buffer saline pH 7.4, 0.17 μ ionic strength. Liposomal dispersion was subjected to ultrasonic downsizing under nitrogen atmosphere in an icebath. Sonicated vesicles were further stabilized by hydration for 2 h and then separated from unentrapped drug by dialysis through cellophane membrane [M. W. Cutoff 12 000 Da] in an icebath. The liposomal dispersion thus obtained was filled in amber col-

Table 1
Effect of process and formulation variables

Variable	Batch Number	Drug : EPC : CHOL (molar ratio)	% Drug entrapped (Mean ± SEM)[a]	Observation and inferences
Vacuum (Inch. Hg)				
15	KF[a]	1:5:0	41.53 (0.19)	Aggregated liposomes; presence of residual organic solvent
20	KF[b]	1:5:0	49.74 (0.51)	Distinct; translucent film with microscopic striations
25	KF[c]	1:5:0	28.92 (0.22)	Poor drug entrapment; entrapment of air bubbles on lipid film surface
Pre sonication hydration (min)				
30	KF[d]	1:5:0	60.71 (0.25)	Hydration is a prerequisite for proper maturation of liposomes yet excessive hydration leads to drug leakage
60	KF[e]	1:5:0	58.98 (0.24)	
90	KF[f]	1:5:0	57.22 (0.28)	
Post sonication hydration (h)				
0.5	KF[g]	1:5:0	48.65 (0.35)	Optimum post sonication hydration time is required for regaining physical and conformational stability of the bilayer membrane.
1	KF[h]	1:5:0	49.98 (0.42)	
2	KF[i]	1:5:0	51.37 (0.64)	
Purification				
Centrifugation	KF[i]	1:5:0	51.37 (0.64)	Physical damage and poor pelletization
Sephadex G 50	KF[j]	1:5:0	32.91 (1.95)	Suffers with lack of precision and robustness
Dialysis	KF[k]	1:5:0	54.61 (0.53)	Time consuming yet gentle and efficient method
Lamellae composition (molar ratio)				
KF[1]	KF[l]	1:15:0	85.56 (0.94)	Increasing the proportion of cholesterol in lamellae competitively reduces the drug entrapment, for amphiphilic drug molecule
	KF[m]	1:11.5:3.5	76.79 (0.20)	
KF[2]	KF[n]	1:10:5	70.39 (0.82)	
	KF[o]	1:8.8:6.2	66.21 (0.45)	
KF[3]	KF[p]	1:7.5:7.5	64.19 (0.92)	
	KF[q]	1:7.2:7.8	57.28 (0.29)	

[a] Mean ± SEM ($n = 6$).

ored vials, purged with nitrogen, sealed and stored in refrigerator. Table 1 briefs about the optimization of various process variables studied during the preparation of liposomal dispersion.

2.3. Preparation of dry powder inhalation

For the preparation of DPI formulation, during preparation of liposomes, the film was hydrated with 500 mM sucrose, 1 mM EDTA, phosphate buffer saline (pH 7.4) and the formed dispersion was diluted with the required quantity of hydrating medium to obtain a lipid/sugar ratio of 1/12. Equivalent proportion of sorbolac was dispersed into it to have a final strength of 200 μg of entrapped drug per 200 mg of the formulation. The dispersion was frozen at −70°C and dried under negative displacement pressure (Heto Drywinner model DW1 0-60E, Denmark), both for 24 h. The formed porous cake was sized through #120 and #240 sieves and filled in capsules (size 2) individually by weight (200 mg) of lyophilized powder equivalent to KF 200 μg. The capsules were packed in HDPE bottles containing silica bags as dehumactant, purged with nitrogen and covered with PVC coated aluminum foil. Bottles were placed in a desiccator kept in refrigerator till further use.

2.4. Characterization of liposomes

2.4.1. Assay

Ketotifen uptake, described as percentage drug entrapped, was determined following solubilization of vesicles in 0.1% Triton X-100 in methanol, by HPLC (Zarapkar et al., 1992). EPC was quantified by ion-pair complexing as described by Stewart and Charles (1980) and CHOL by complexing with acetic acid and ferric chloride as described by Zlatkis (Goel, 1988).

2.4.2. Photomicrography

All the batches of the liposomes prepared were viewed under Olympus (BX 40F4, Japan) with polarizing attachment to study their shape and lamellarity. Photomicrographs (1000×) before dehydration and after rehydration, each under plain and polarizing filters are shown in Fig. 1.

2.4.3. Laser light scattering measurement

The vesicle size of presonicated and postsonicated liposomes (Table 2) were determined by laser diffraction technique using Mastersizer (Malvern Instruments Ltd., UK) operating at a beam length of 2.40 mm and range of lens at 300 mm.

2.4.4. Determination of trapped volume (Betagiri et al., 1993)

Liposomes were spun at high centrifugal force ($3.6 \times 10^6 \times g$ for 30 min) to get a tight pellet. The supernatant was decanted to remove every drop of excess fluid including some liposomes if necessary. The pellets were solubilized in 0.1% Triton X-100 in methanol (10 ml). A small aliquot (0.1 ml) was removed for quantification of EPC and the remainder was used to obtain water content by Karl Fischer method (Veego, India). The trapped volume as μl per μ moles of EPC for optimized batches is reported in Table 2.

2.4.5. Oxidation index (Klein, 1970)

Liposomes were extracted with chloroform and evaporated under nitrogen atmosphere to a dried residue, pure EPC and its film was used as such. Phospholipid equivalent to 2 μ moles was solubilized to 3 ml of ethanol (95%) and spectroscopically analyzed after every relative intermediate stage.

2.5. Solid state characterization

2.5.1. Angle of repose (Carr, 1965)

The pile was carefully built up by dropping the material through a funnel till the tip of the funnel (height, 2 cm). The angle of repose (Table 2) was calculated by inversing tangentially the ratio of height and radius of the formed pile.

2.5.2. Compressibility index (Carr, 1965)

The compressibility index values reported in Table 2 were obtained by tapping the powder for 500 tap (taps sufficient to obtain the plateau condition).

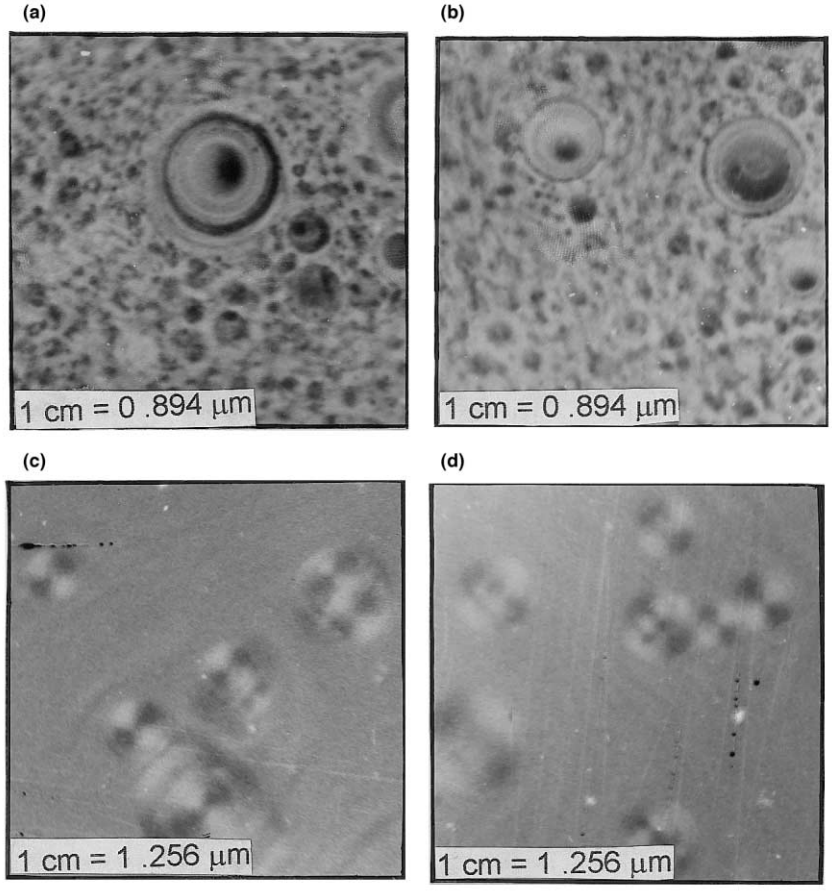

Fig. 1. Photomicrographs at 1000× magnification (a) plain before dehydration; (b) plain after dehydration-rehydration; (c) polarizing before dehydration; and (d) polarizing after dehydration-rehydration.

2.5.3. Dispersibility

The dispersibility was determined using a miniature assembly to that described by Carr (1965). Formulation (5 g) was dropped en masse through a cylinder (length, 6.5 in. and internal diameter, 2 in.) held 2 in. above a watch glass of 1 in. diameter. The dropping point was 3 in. above the cylinder, from a funnel tip. Dispersibility was calculated as the relative proportion of material lost to the material dropped.

2.5.4. Water content determination

Water content of the DPI formulation (5 g) was determined in triplicate on two consecutive days by Karl Fischer titration (Table 2).

2.6. Fine particle fraction

The in vitro aerosol behavior of the formulation was investigated in terms of respirable fine particle fraction (FPF). The FPF values were obtained using twin impinger (Apparatus A) official in British Pharmacopoeia (1988) using Rotahaler (Cipla, India) for powder delivery and results are shown in Fig. 5. Since the apparatus was prepared in-house, as per the specifications of BP, the FPF value of marketed preparation was also determined as a control. The flow rate and the actuation time of the twin impinger were 60 l/min and 5 s, respectively. The volume of capturing solvent (methanol) in the upper (stage 1) and

lower (stage 2) were 7 and 30 ml, respectively. FPF was calculated from the amount of drug collected in lower impingement chamber as a percentage of the dose taken for inhalation.

2.7. Statistical analysis

Each batch was prepared six times and data from all experiments are recorded as mean ± SEM. One variable was changed at a time and the results were compared using ANOVA or Dunnets *t*-test. Percent drug entrapment was expressed as the percentage of the drug initially added. Similarly, the percent drug retained is the percentage of the drug initially entrapped before lyophilization. Differences were considered significant at $P < 0.05$.

Table 2
Characterization of Ketotifen liposomes and its dry powder inhalation

Variable studied	Optimized liposomal batches[a]		
	KF[1]	KF[2]	KF[3]
Size (μm)			
Pre sonication	8.45(0.99)	6.51 (0.65)	5.52 (0.50)
Post sonication	1.56 (0.26)	1.70 (0.12)	2.05 (0.10)
Trapped volume (μl/μmole)	0.50 (0.01)	0.64 (0.01)	0.75 (0.01)
Oxidation index			
Initial	0.427 (0.01)	0.428 (0.01)	0.428 (0.01)
Dry film	0.470 (0.01)	0.464 (0.01)	0.451 (0.01)
Post hydration	0.472 (0.01)	0.465 (0.01)	0.455 (0.01)
Post sonication	1.367 (0.01)	1.296 (0.01)	1.262 (0.01)
Post dialysis	1.372 (0.01)	1.310 (0.01)	1.272 (0.01)
Post lyophilization	1.510 (0.01)	1.425 (0.01)	1.328 (0.01)
Angle of repose (θ)	27.47 (0.93)	27.19 (0.79)	28.54 (0.83)
Compressibility index	24.96 (0.70)	23.47 (0.99)	23.69 (0.46)
Dispersibility	23.60 (1.03)	24.23 (1.24)	23.58 (1.54)
Moisture content (%)	1.13 (0.16)	0.97 (0.08)	0.99 (0.10)

[a] Mean ± SEM ($n = 6$).

3. Results and discussion

For the formulation of DPI, prepared liposome should have a high drug entrapment, a high retention capacity upon dehydration–rehydration cycle, and a reproducible mean liposomal size below 5 μm (Martonen and Katz, 1993). These requirements were satisfied well when MLVs were prepared by the lipid film hydration technique used in this investigation followed by sonication. The findings of this work show few different observations when compared to previously reported work. Reasons may be differences in type, method of preparation, lamellae composition and size of the liposomes as discussed below.

3.1. Formulation variables

The variables studied, results obtained, and inferences drawn from it are recorded in Table 1. The experiments were carried out using many variables but discussion here is limited to significant differences.

The vacuum used for drying of film was raised from 15 to 25 in. of Hg. Vacuum of 15 in. of Hg was found to be insufficient for complete removal of the solvents and resulted in aggregation of the liposomes on hydration (KF(a)). The vacuum of 25 in. of Hg resulted in rapid evaporation of solvents, leading to entrapment of air bubbles on lipid film surface. Hydration of these films resulted in liposomes with poor drug entrapment (KF(c); 28.92 ± 0.22%). At an optimal vacuum of 20 in. of Hg, the lipid films were translucent and on hydration gave a better drug entrapment (KF(b); 49.74 ± 0.51%) in liposomes. The hydration time of the lipid film before sonication was increased from 30 to 90 min. At hydration time of 60 min, the formed liposomes were spherical and well hydrated having drug entrapment of 58.98 ± 0.24% (KF(e)). Presonication hydration time below and more than 60 min gave liposomes with irregular shape or drug diffusion due to hydration

beyond equilibrium. It may be concluded that proper hydration of the film for orientation of EPC molecules and intimate packing of lamellae (annealing) is necessary and hydration temperature (near Tg) and time are essential parameters influencing it. Post sonication hydration time was varied from 30 min to 2 h. Sonication mechanically breaks the liposomes and produces orientational distortion in the preformed bilayers. Postsonication hydration time of 2 h was found to be optimum and resulted in uniform sized spherical liposomes with good drug entrapment (KF(i); $51.37 \pm 0.64\%$). It was observed that an optimum post sonication hydration time is required for regaining physical and conformational stability of the bilayer membrane (Szoka and Papahadjopoulous, 1980).

Separation of unentrapped drug from liposomes was attempted either by gel filtration, centrifugation or dialysis. In this investigation, dialysis was found to be most appropriate method for fast and complete separation of unentrapped drug. To verify that the entrapment is not due to incomplete separation, pure drug (20 mg) was dialyzed through the same system and the drug was found to be emptied within 45 min into the receptor compartment. However, the results and inferences drawn from the techniques attempted are recorded in Table 1. When CHOL content was increased in the bilayer composition, the drug entrapment in liposomes reduced proportionately (KF[l] > KF[m] > KF[n] > KF[o] > KF[p] > KF[q]). This may be because CHOL molecules compete with amphilphilic drug such as KF for orientation between EPC molecules in the bilayer (Patel and Misra, 1998).

In the next set of experiments, the use of cryoprotectants (sugars) during lyophilization of liposomes was evaluated and compared. Liposomal suspension was diluted with 1 mM EDTA, phosphate buffer saline containing 100 mM lactose or maltose or sucrose or dextrose and the amount of KF retained by the liposomes following lyophilization and rehydration was determined (Fig. 2). The data reveals that sucrose gave significantly higher percentage KF retained ($18.84 \pm 0.42\%$) as compared to maltose ($15.11 \pm 1.41\%$), dextrose ($4.90 \pm 0.43\%$) and lactose ($9.00 \pm 0.32\%$). This is in agreement with the findings of Madden et al. (1985) who examined the effectiveness of number of sugars in maintaining structural and functional properties of microsomal membranes at low mean liposomal size and found trehalose to be the most effective one. However at higher concentrations, sucrose was found to be equally effective for liposomes of large mean size. When sucrose as a cryoprotectant was provided only on the outside or the inside of the liposomes, marked decrease in preservation of liposomal membrane permeability was observed, $18.84 \pm 0.42\%$ and $3.80 \pm 0.52\%$ KF retention was achieved respectively. When sucrose was present on both sides of the bilayers, $38.00 \pm 0.39\%$ retention of the entrapped KF was achieved (Fig. 2). Similar results were also obtained by Crowe and Crowe (1988) and others (Madden et al., 1985).

Egg phosphatidylcholine MLVs containing entrapped KF were lyophilized in the presence of varying concentrations of sucrose. The percent KF retained by the lyophilized liposomes upon rehydration was found to be dependent upon the sucrose concentration (Fig. 3). With the increase in the concentration of sucrose from 100 to 600 mM, the percent KF retained was found to be maximum at 500 mM concentration and it was $62.65 \pm 0.77\%$. When the concentration of sucrose was further increased from 500 to 600 mM, there was a significant reduction in the percent KF retained (Fig. 3). This may be due to the fact that in the process of dehydration, the liposomal vesicles get constricted and coated on the surface of internally crystallized sugar. This stabilization by coating is in addition to stabilization by the hydration of polar head groups with hydroxyl group of sucrose, which replaces the lyophilizing water molecules. If the sucrose concentration is less than optimum, the internally crystallized sugar does not provide adequate surface for the adherence of constricted bilayers. Similarly, if the sucrose concentration is high, the large sized crystals pierce out the bilayers. Based on the assumption that the vesicles once dehydrate in the presence of sucrose, retain their contents and do not re-encapsulate, it may be concluded that the bulk concentration of

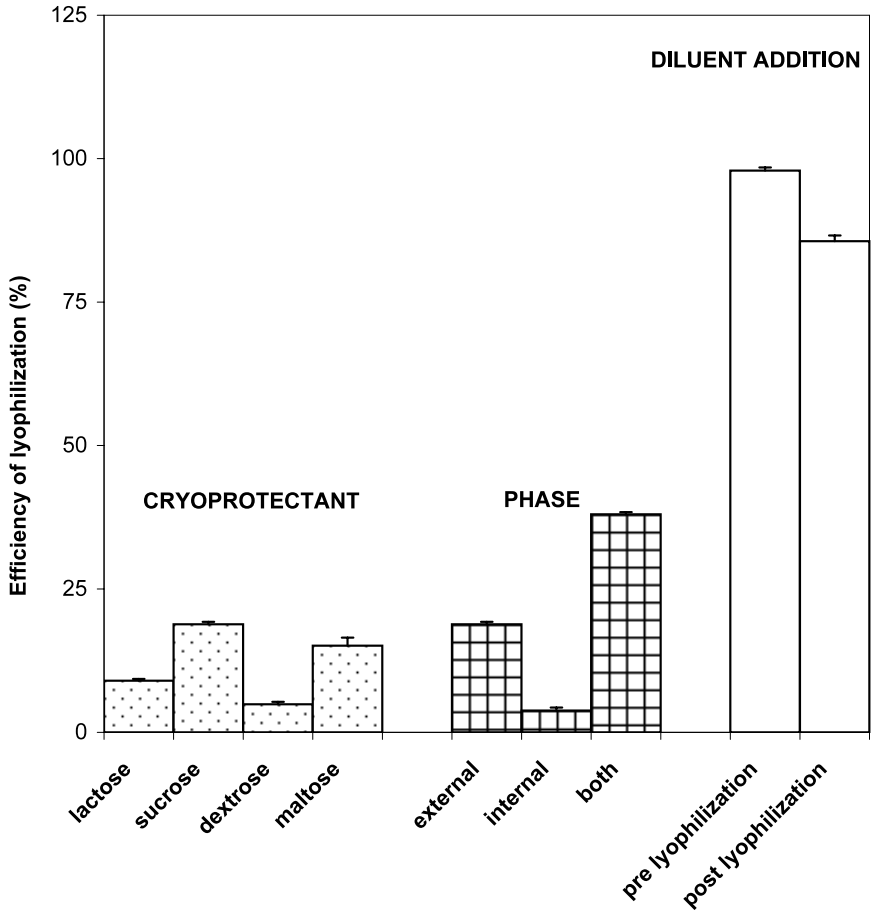

Fig. 2. Effect of type of cryoprotectant, phase with cryoprotectant and stage of diluent addition on the efficiency of lyophilisation of KF liposomes.

sugar required as cryoprotectant depends on the type of sugars selected and the saturation of the polar head groups of the bilayers by drug or other formulation components. The lamellarity and size of liposomes are expected to change these requirements.

In the other set of experiments, the stabilization achieved by the mass ratio of sugar and lipid in the dry state was studied. The samples were sequentially diluted to produce mixtures with varying lipid/sucrose mass ratio, but with the same concentration of sucrose (500 mM). The results shown in Fig. 4 reveal that the lipid/sugar mass ratio is equally important (Crowe and Crowe, 1988) for retaining the permeability barrier of liposomal vesicles. A mass ratio of 1/12 for lipid/sucrose was found to be the most effective and gave $85.61 \pm 1.00\%$ of the drug retained. Further increase in lipid/sucrose mass ratio was found to decrease percent drug retained ($82.67 \pm 1.15\%$). Hence, 12 times sugar/lipid ratio is required for better drug retention during lyophilization. The addition of sorbolac before lyophilization further increases drug retention to $97.92 \pm 0.54\%$ (Fig. 2). This significant increase in drug retention may be due to the differences in the solubility of sorbolac and sucrose that may have resulted in the formation of more porous structure causing less damage to the vesicular structure of liposomes. However, when sorbolac was incorporated internally in the

same ratio along with sucrose prior to film hydration, no significant improvement in the retention of KF ($85.61 \pm 1.00\%$) was observed.

3.2. Characterization

Estimations of different components of liposomes were carried out to obtain the mass balance and recovery of drug, EPC and CHOL and results were found to be between 98 and 100%. Polarizing microscopy confirmed the formation of multilamellar and spherical liposomes (Fig. 1). Multilamellar vesicles were also identified by the presence of Maltese crosses. By sonication, mean liposomal size was reduced to below 5 μm with narrow size distribution (Table 2). The mean liposome size prior to sonication decreased with the increased proportion of CHOL (KF1, 8.45 ± 0.99 μm; KF3, 5.52 ± 0.50 μm). However, after sonication, the mean liposome size increased with increase in the proportion of CHOL (KF1, 1.56 ± 0.26 μm; KF3, 2.05 ± 0.10 μm). CHOL imparts rigidity in the liposomal membrane and may have prevented it from equivalent downsizing by sonication. In batches KF1 to KF3 the trapped volume also increases (Table 2), which confirms its direct relationship to mean liposomal size. Trapped volume is proportionally related to the entrapped material if the drug is highly water-soluble, and is present predominantly in the inner aqueous compartment only. For KF, amphilphilic drug, it is only a parametric study before going

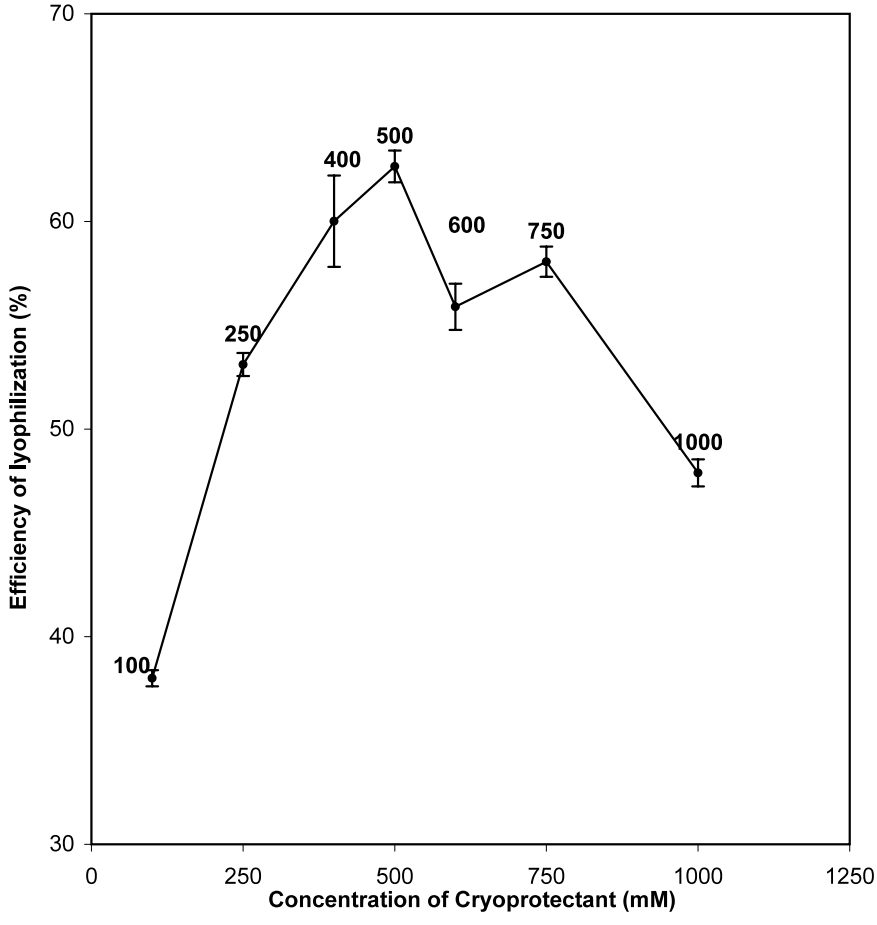

Fig. 3. Effect of mill molar concentration of cryoprotectant on the effeciency of lyophilisation of KF liposomes.

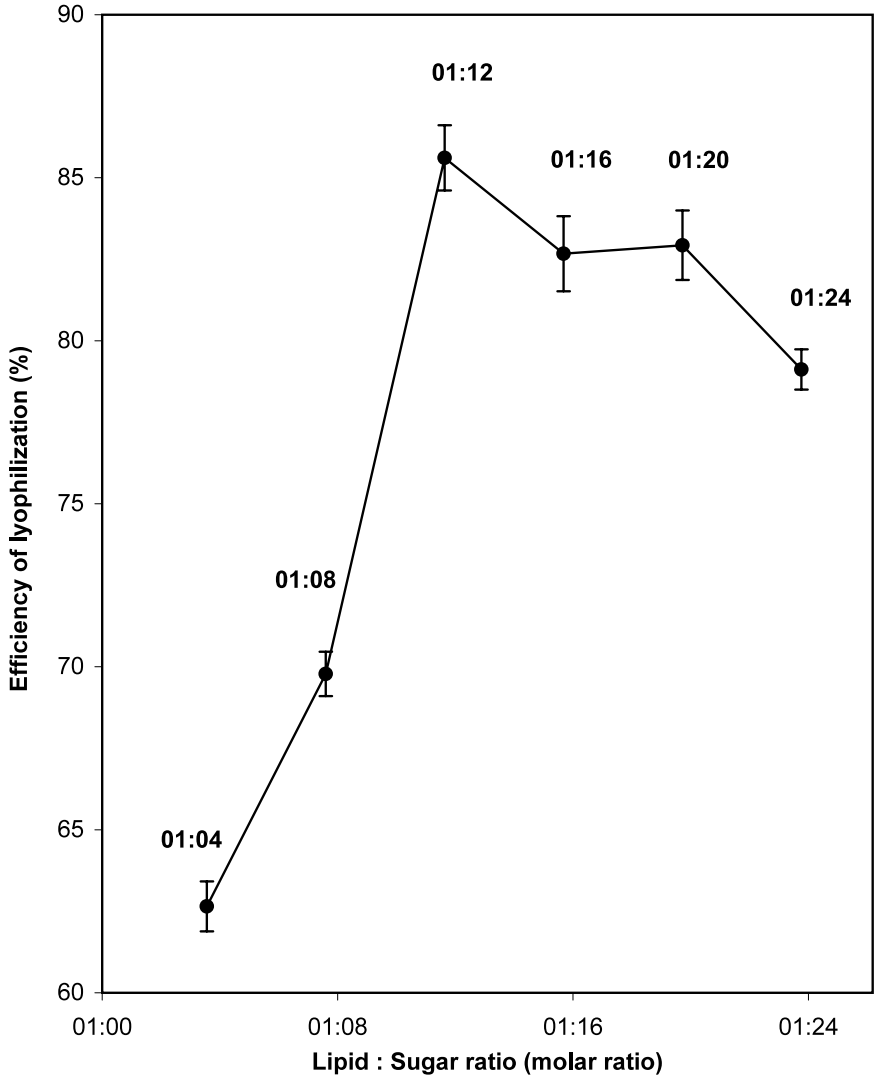

Fig. 4. Effect of sugar-vesicle preperation by the indicated amount on the effeciency of lyophilization of KF liposomes.

for further investigations. For the mean liposomal size of 1.56 ± 0.26 μm (KF1), the estimated trapped volume (if unilamellar) as described by Hope et al. (1987), may be ≈ 45 μl per μ mole. This may be compared with the measured value of 0.50 μl per μ mole, which is almost 100 times less than the estimated value. In MLVs, the presence of every lamellae would replace equivalent entrapped volume with a sphere of lamellae of 4-μm thickness (Blaurock, 1982), therefore, the entrapped volume for MLVs will be many folds less to that of a unilamellar liposomes of the same size. Hence, the large majority of liposomes produced by downsizing with sonication are still multilamellar.

Phospholipids particularly lecithin undergo degradation by two mechanisms, one by hydrolysis at β-ester linkage and other by oxidation of unsaturated hydrocarbon chains. For lyophilized DPI formulation, hydrolysis is not as important as oxidation. Hence, oxidation index (A_{233} nm/A_{215} nm) as described by Klein (1970) was used

for screening of liposomal preparations for auto-oxidation. The oxidation index of post lyophilization batches of KF1 and KF3 is 1.510 ± 0.01 and 1.328 ± 0.01, respectively, which is significantly higher than the initial oxidation index of 0.427 ± 0.01 and 0.428 ± 0.01 (Table 2). The initial ratio of oxidation index KF1/KF3 was 0.997 (0.427/0.428) which is significantly less as compared to post lyophilization batch ratio of 1.137 (1.510/1.328). It may be concluded that incorporation of EDTA, α-tocopherol and the maintenance of nitrogen atmosphere offers limited protection to oxidation in liposomal formulation. Drastic processing like sonication of liposomes to get desired particle size may limit protection against oxidation. CHOL imparts rigidity to the bilayer membrane and does not protect the liposomal vesicles against auto-oxidation.

Control of flow and dispersion (deaggregation) characteristics of the formulation is of critical importance in the development and evaluation of DPI products. Inter particle forces, which influence flow and dispersion properties are particularly dominant in micronized or microcrystalline powders required for inhalation therapy (< 5 μm). It has been demonstrated that powder adhesion, mediated by Van der Waal forces, is directly related to particles < 10 μm (Atkins et al., 1992). It is apparent that prediction of powder rheology based on the potential interplay of a number of physicochemical properties would be extremely complicated. Instead, flow and dispersion properties are generally characterized such as angle of repose, compressibility, dispersibility, moisture content and FPF. These properties were determined for the optimized formulations (KF1, KF2 and KF3) and are recorded in Table 2. To ensure optimum and consistent product performance, critical parameters, both fundamental and derived are important to be identified. The flowability and floodability expressed by angle of repose (27.19–28.54 θ), compressibility index (23.47–24.96) and dispersibility falls in the category of good and floodable, in the point score evaluation expressed

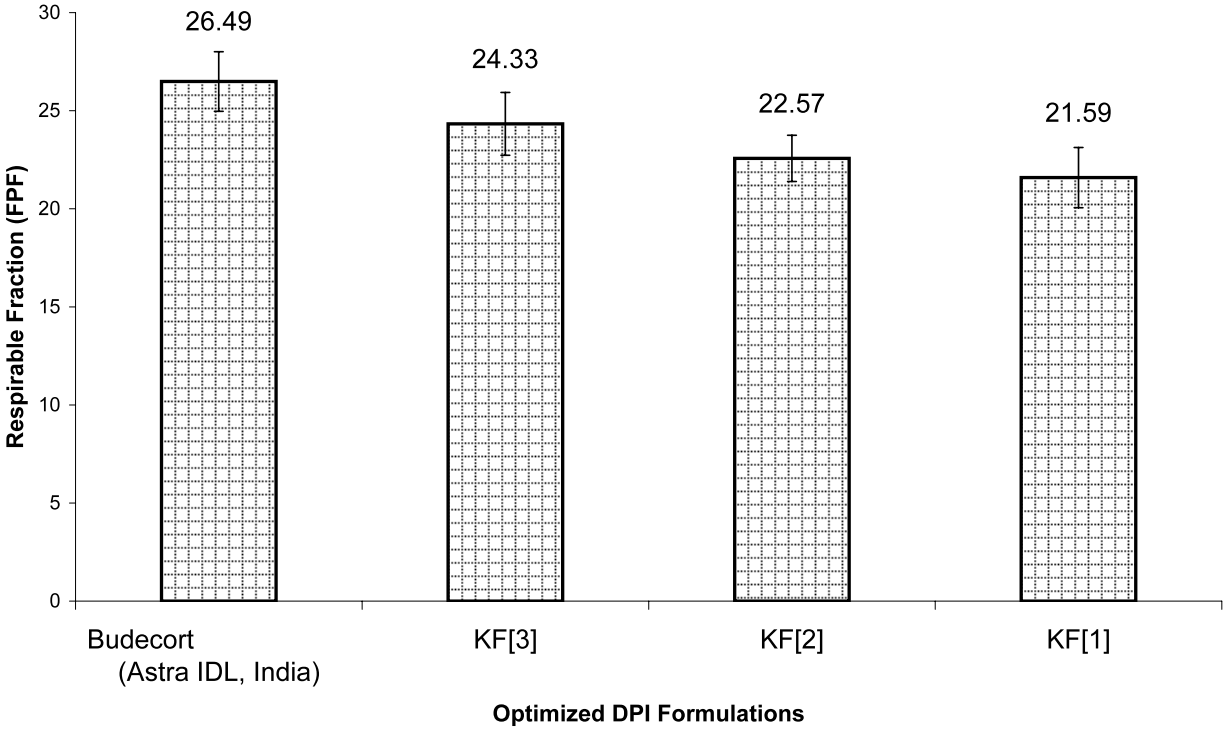

Fig. 5. Comparative respirable fraction (FPF) of DPI formulations.

by Carr (1965). Moisture content determination is important for stability on storage and deaggregation upon inhalation. The formulation had a low moisture content ($< 1.13\%$), which confirms its low aggregation tendency. Liquid impinger was used for particle characterization of respirable fraction as described (British Pharmacopoeia, 1988). The data derived from the device reflects the fraction of a pressurized inhalation likely to result in its deposition in the central and peripheral airways. The particles passing to the lower portion of the device are considered respirable (FPF). The mean FPF value for the optimized formulation ($21.59 \pm 1.53\%$) is comparable with that of marketed control ($26.49 \pm 1.52\%$), suggestive of substantial deposition of the developed liposomal DPI formulation in the required region of lung (Fig. 5).

Recently, considerable interest has developed in the use of liposomal system in the delivery of drugs and other biologically active molecules through lungs (Sachetelli et al., 1999; Ruijgrok et al., 2000). The ability of liposomes to encapsulate a drug within multilamellar vesicles and storage of liposomes conveniently in a dehydrated form using a cryoprotectant offer a number of advantages over conventional drug delivery techniques. This aim can be commercially realized, when the liposomally entrapped drug is obtained in the form that can be conveniently and selectively delivers it at required site in the lungs. The findings of this investigation show that the small multilamellar liposomes loaded with KF can be dried in the presence of sucrose and then can be delivered as DPI to the desired sites in the lung. Hence, developed liposomal DPI of KF offers exciting possibilities of liposomal delivery in the anhydrous state.

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