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Delivery of liposomes in dry powder form: aerodynamic dispersion properties

Tejas R. Desai [a], Robert E.W. Hancock [b], Warren H. Finlay [a,*]

[a] *Department of Mechanical Engineering, Aerosol Research Laboratory of Alberta, University of Alberta, Edmonton, Alta., Canada T6G 2G8*
[b] *Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada V6T 1Z3*

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Abstract

Objective: In our previous study, we reported a novel approach of delivering liposomes in dry powder form that relies on spontaneous formation of liposomes upon dispersion of micronised phospholipid(s) based powders in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs [J. Pharm. Sci. 91 (2002) 482]. In this paper, we demonstrate the in vitro generation of aerosols from these novel powders. *Methodology:* Various formulations comprising different phospholipid(s) exhibiting different physico-chemical properties were prepared. Aerosol was generated using a deagglomeration rig wherein the powder was entrained at a flow rate of 60 l/min and high turbulence was generated using air-jets. Two antimicrobial agents (ciprofloxacin and CM3, a novel peptide) and a bronchodilator, salbutamol sulfate, were used as model drugs to examine the powder dispersion properties. *Results:* The deagglomeration rig used in this study was able to disperse 87–95% of the total loaded powder into the cascade impactor. Amongst the various formulations comprising different phospholipid(s), DMPG and (DMPC + DMPG) based formulations exhibited excellent aerodynamic dispersion properties. Fine particle fractions (FPF) of more than 50% were achieved for these formulations for three model drugs. Encapsulation of the model drugs in the FPF, obtained upon dispersion of these novel powders, is also discussed in this paper. An encapsulation of approximately 35, 40 and 25% was achieved in the FPF for ciprofloxacin, CM3 peptide and salbutamol sulfate, respectively.

Keywords: Liposomes; Liposome aerosol; Powder aerosol; Ciprofloxacin; Salbutamol sulfate; Cationic peptide

1. Introduction

Pharmaceutical aerosols provide an excellent mode of delivering drugs directly to the lungs for treating disease (Newman and Pavia, 1985; Finlay, 2001; Crowder et al., 2001). It is known that colloidal carriers, such as liposomes, promote an increase in the drug retention time and reduce the toxicity of drugs after administration (Weinstein and Leserman, 1984; Gregoriadis, 1988), while the aerosol mode of delivery offers the advantage of uniform and higher levels of deposition for locally active drugs (Gilbert et al., 1991; Parthasarathy et al., 1999). Liposome aerosols are commonly delivered either in aqueous form via nebulization (Finlay and Wong, 1998; Lange et al., 2001) or in dry powder form (Ho, 1995; Schreier et al., 1994). However, the aqueous dispersions of liposomes required for delivery via nebulization are often associated with stability problems (Niven et al., 1992). To overcome this problem, Schreier et al. (1994) demonstrated an alternative approach of delivery using lyophilization, micronization and aerosolization of micronised liposome powders. Among the different modes of aerosol delivery, dry powder inhalers offer additional advantages that include ease of use, portability and patient acceptance. Based on these advantages, research on the delivery of biologically active agents encapsulated in liposomes via aerosol in dry powder form has advanced rapidly in the last few years.

Dry powder liposome formulations are generally prepared by freeze-drying (lyophilization) of the aqueous liposome dispersions, followed by micronization to achieve particles in the range of 1–5 μm by jet-milling (Ho, 1995; Schreier et al., 1994). However, recent studies have reported that lyophilization followed by micronization by jet-milling could cause deleterious effects on liposome integrity, thereby causing leakage of the entrapped drug

* Corresponding author. Tel.: +1-780-492-4707; fax: +1-780-492-2200.
E-mail address: warren.finlay@ualberta.ca (W.H. Finlay).

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(Mobley, 1998; Desai et al., 2002). These studies have raised concerns regarding the potential use of lyophilized liposomes for inhalation therapy.

In a recent study we reported a novel approach for delivering liposomes in dry powder form that circumvents the potentially negative effects of lyophilization and jet-milling (Desai et al., 2002). This approach relies on formulating phospholipids-based powders that result in spontaneous formation of liposomes in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs. Two potent antimicrobial agents (ciprofloxacin and a cationic peptide) as well as a bronchodialator (salbutamol sulfate) were selected as model drugs to demonstrate this approach. This approach of delivering liposomes in dry powder form was found to be advantageous in avoiding the detrimental effects of lyophilization and jet-milling on encapsulation efficiency. In addition, the process cost of lyophilization can be eliminated with this approach.

In this study, we examine the aerodynamic dispersion properties of these novel powders. In order to identify promising candidates, various phospholipid formulations exhibiting different physico-chemical properties were investigated in terms of their aerodynamic particle size properties, and their ability to encapsulate drug in fine particle fraction (FPF) upon dispersion.

2. Materials and methods

2.1. Materials

Dipalmitoyl phosphatidylcholine (DPPC) (>99%), dimyristoyl phosphatidylcholine (DMPC) (>99%) and dimyristoyl phosphatidylglycerol (DMPG) (sodium salt) (>99%) were purchased from Genzyme Pharmaceuticals (Cambridge, MA, USA). Egg phosphatidylcholine (EPC) (>99%) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Inhalation grade lactose, Pharmatose 325 M, was a generous gift from DMV International (Veghel, The Netherlands). Sodium chloride and salbutamol sulfate were purchased from Sigma (St. Louise, MO, USA). Ciprofloxacin hydrochloride (>99%) was purchased from Serologicals Corporation (Kankakee, IL, USA). CM3 peptide was synthesized by 9-fluorenylmethoxycarbonyl (Fmoc) chemistry at the Nucleic Acid/Protein Service Laboratory, University of British Columbia, Vancouver, BC, Canada. The amino acid sequence of CM3 is KWKKFIKSLTKSAAKTVVKTAKKPLIV, as described in Scott et al. (1999). All materials were used as received.

2.2. Methods

2.2.1. Preparation of phospholipid(s)-based powder formulations

Powder formulations comprising different phospholipid(s) were prepared by mixing phospholipid(s), Pharmatose 325 M and the drug at appropriate concentrations. The method has been described in more detail in our previous study (Desai et al., 2002). In brief, the method is as follows.

Table 1
Composition of various powder formulations used here for studying dispersion properties

Phospholipid(s)	Weight ratios of phospholipid(s):lactose:drug		
	Preparations with ciprofloxacin	Preparations with CM3 peptide	Preparations with salbutamol sulfate
DPPC	10:25:1	22:52:1	34:87:1
DMPC	10:25:1	22:52:1	34:87:1
DMPG	5:12.5:1	11:26:1	34:87:1
DMPC + DMPG[a]	5:12.5:1	11:26:1	34:87:1
EPC + DMPG[a]	5:12.5:1	11:26:1	34:87:1

[a] Weight ratio of individual phospholipid is 1:1.

Lipid concentrations were adjusted depending upon the nature of the drug to be encapsulated and the nature of the lipid. Compositions of various powder formulations used here for studying dispersion properties, containing different active are expressed in terms of the weight ratios in Table 1. After mixing appropriately weighed phospholipid(s), pharmatose 325 M, and drug, the powder formulations were micronised with a Trost Impact Pulverizer (Garlock Inc., Plastomer Products, Newton, USA) at a grinding nozzle pressure and pusher nozzle pressure of 90 psi. The jet-milled powders were collected from the collection vessel. Powder samples were stored in a dry box at low relative humidity and −20 °C for further dispersion studies. To study the effect of addition of carrier particles on the aerodynamic dispersion of these formulations, micronised phospholipid based powder was mixed with carrier, Pharmatose 325 M (particle size approx. 65 μm), in a weight ratio of 1:2 (micronised phospholipid powder: carrier). Five different formulations comprising DPPC, DMPC, DMPG and combinations of (EPC + DMPG) and (DMPC + DMPG) (weight ratio 1:1) were investigated in this study.

2.2.2. Aerodynamic particle size determination of freshly prepared powders

The collection of powder particles by impaction through the exit of the jet-mill in the collection container may cause agglomeration, which leads to spurious size distribution measurements. In order to avoid this problem, the experimental set up described by Voss (2001) was used to determine the particle size distribution. The schematic of the experimental set up is shown in Fig. 1. To allow particle rich flow from the collection device, an outlet was fabricated, which is directly connected to the Andersen cascade impactor (Andersen Mark II, Graseby Andersen, Smyrna, GA). A two-position (open/bypass) valve was designed to control whether powder flowed from the jet-mill into the cascade impactor. The impaction surfaces were coated with

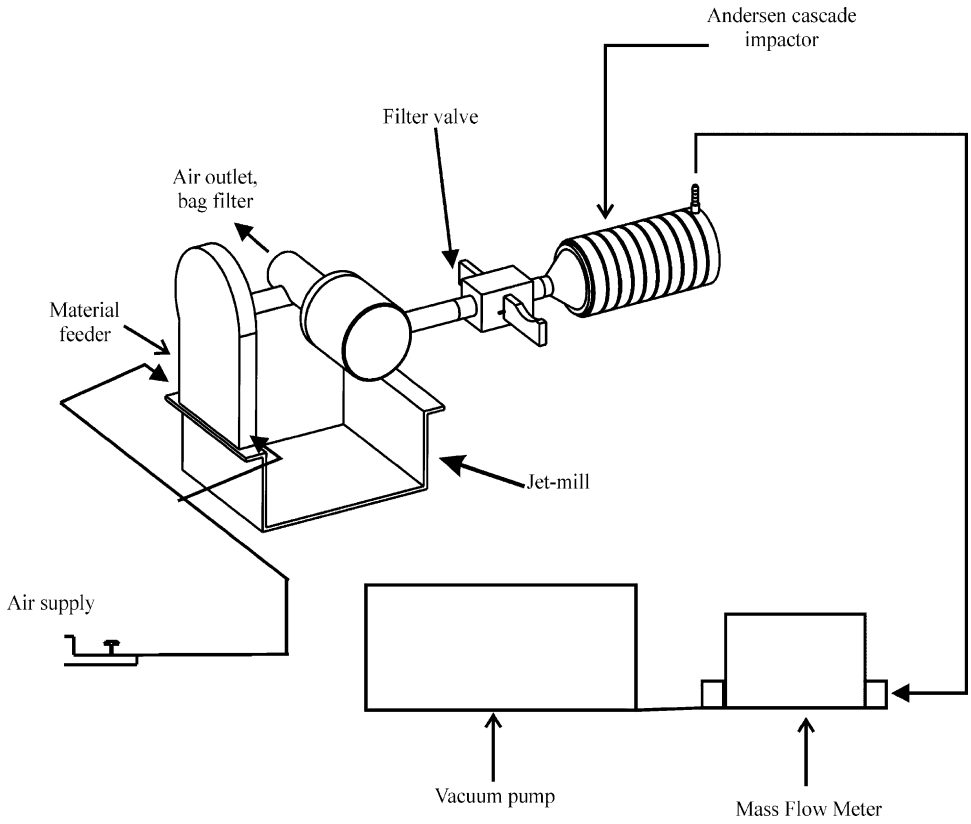

Fig. 1. Schematic of set up for determination of aerodynamic particle size distribution using cumulative mass distribution in the Andersen cascade impactor.

silicone grease to prevent inter-stage losses. The impactor flow rate was calibrated to 28.3 l/min using a dry gas meter (DTM-115, Singer, American Motor Division). As can be seen from Fig. 1, the opening of the collection vessel of the jet-mill was connected to Andersen cascade impactor through the valve, which was connected to the vacuum pump calibrated to the flow rate of 28.3 l/min. While micronising the powder under high pressure, after the powder particles begin to appear in the collection container, the vacuum pump was switched on. The valve was then opened to ensure sufficient loading of the powder in the cascade impactor (for a period of 15 s), and then the valve was closed (i.e. switched to its bypass position in which ambient air is entrained in the cascade impactor), and the vacuum pump switched off. Each plate was then assayed for the total drug deposited, and particle size was determined from the cumulative mass distribution in the Andersen cascade impactor.

2.2.3. Aerosol dispersion and aerodynamic characterization

The deagglomerating rig of Voss and Finlay (2002) was used to examine the dispersion properties of the formulations. A schematic of the rig is shown in Fig. 2. An accurately weighed powder sample (50.0 ± 2.0 mg), was placed on the tray, and then entrained in the air stream through the feeder. When entrained in the air stream, the powder is exposed to turbulence generated by the air jets. The flow rate through the jets can be controlled to give varying intensities of turbulence. Here, the air-jets were calibrated to 40 l/min, which Voss and Finlay (2002) found gave similar amounts of deagglomeration as the Ventodisk inhaler. After exposure to the turbulence, the entrained powder enters the Andersen cascade impactor. The powders were dispersed at a flow rate of 60 l/min as it simulates a typical human inspiratory flow rate for an average adult. The cut points for stages 0–7 have been recalculated and calibrated for 60 l/min, and are 5.6, 4.3, 3.4, 2.0, 1.1, 0.51, 0.29 and 0.13 μm, respectively, as reported by Nichols et al. (1998). The impaction surfaces were coated with silicone grease to prevent inter-stage losses due to particle bounce. The phospholipids based powder that deposited on each plate was extracted with 0.9% saline. The extracts were appropriately diluted with methanol (to rupture the liposomes), and the amount of drug deposited on each plate was assayed using a UV Spectrophotometer (model 8452A, Hewlett-Packard, Mississauga, ON), as described earlier (Desai et al., 2002). The total amount of drug deposited on stages 1–7 constitutes the fine particle fraction (i.e. particle size <5.6 μm). Confidence in the extraction procedure was gained by achieving mass balance in the range of 87–102% in each case.

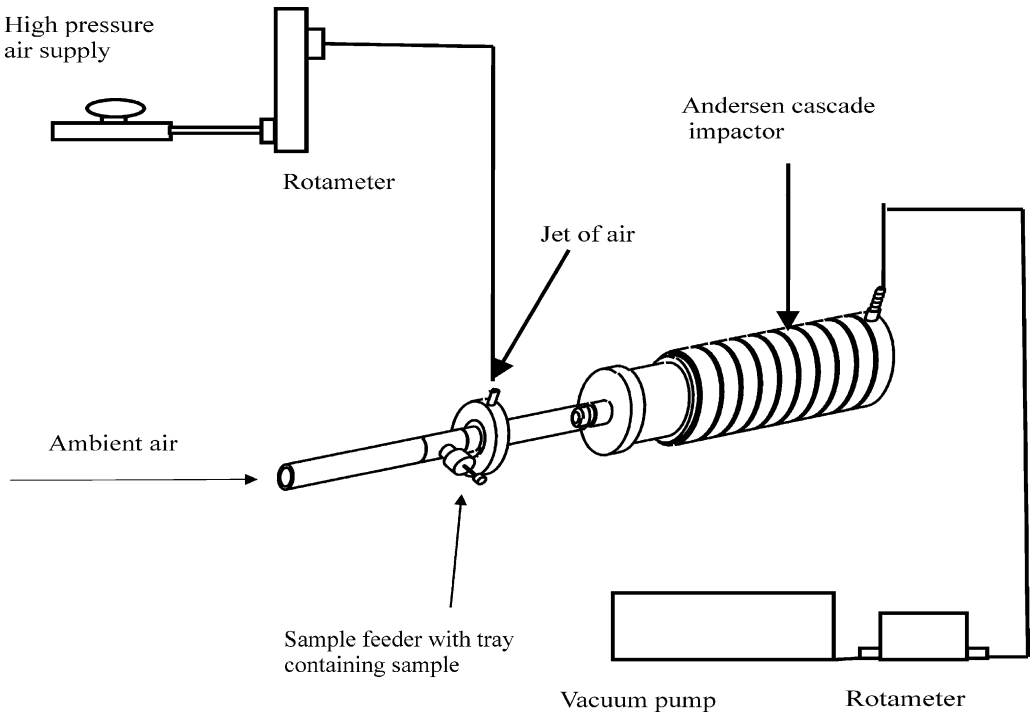

Fig. 2. Schematic of set up for in vitro aerosolization and aerodynamic characterization.

2.2.4. Determination of encapsulation efficiency in spontaneously formed liposomes in FPF

The entrapment efficiencies of drugs in liposomes spontaneously formed from the FPF powder were determined by dispersing the formulation into the cascade impactor and extracting the particles deposited on stages 1–7 (i.e. particle size <5.6 μm) with a defined amount of saline. The phospholipids were then allowed to hydrate by equilibrating the extracted dispersions at 37 °C for 15 min, before centrifugation. The powder dispersed in the preseparator and other parts of the rig was also extracted in saline to determine the amount of encapsulated drug and total drug for the determination of mass recovery. These extracts were also subjected to the same treatment, before centrifugation. Unencapsulated drug was then separated from the hydrated liposomes by centrifuging the dispersions at 21,460 × *g* and 4 °C for 90 min, and separating the supernatant and residue. After dilution with methanol, the amount of drug was determined separately in the supernatant and residue by UV Spectroscopy. The percentage of drug encapsulated was then calculated as the ratio of the drug in the pellet to the sum of the drug in the pellet and the drug in the supernatant. Values of λ^{max} for ciprofloxacin, salbutamol sulfate and CM3 peptide are 278, 226 and 280 nm, respectively. The reported values of encapsulation efficiencies are the mean of three sample replicates, i.e. three separate batches of jet-milled phospholipids based powders. Statistical tests were performed using single factor analysis of variance (ANOVA) and Tukey HSD means comparisons.

3. Results and discussion

3.1. Aerodynamic particle size distribution

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of various phospholipids-based powders containing ciprofloxacin, CM3 peptide and salbutamol sulfate, as measured by mass deposition in the Andersen cascade impactor sampled directly from the mill, are shown in Table 2. It appears that the phase transition temperature of the phospholipid plays a vital role in determining the aerodynamic size of these phospholipid-based powders. It is known that the phase transition temperature of various phospholipids used in the study increases in the order: EPC < DMPC = DMPG < DPPC. The results obtained in this study indicate that the higher the phase transition temperature of the phospholipid the lower will be the MMAD. From an inhalation and deposition standpoint, all preparations showed sizes acceptable for inhalation purposes (i.e. below 5 μm) and may be selected as candidates for further dispersion studies (Table 2).

3.2. Aerodynamic dispersion

It has been previously observed in the case of liposomal powder formulations prepared from lyophilized lipids that the addition of carrier particles improved aerodynamic dispersion (Schreier et al., 1994). The higher the concentration of carrier, the better was the dispersion. To investigate the effect of addition of carrier particles on the dispersibility

Table 2
Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of micronised phospholipid(s)-based powders containing ciprofloxacin, CM3 peptide and salbutamol sulfate, respectively, determined using cumulative mass distribution in the Andersen cascade impactor ($n = 3$, ±S.D.)

Preparation	Ciprofloxacin		CM3 peptide		Salbutamol sulfate	
	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD
DPPC	2.63 ± 0.15	2.03 ± 0.16	3.17 ± 0.17	2.15 ± 0.02	2.91 ± 0.14	1.96 ± 0.07
DMPC	2.55 ± 0.16	2.13 ± 0.25	3.26 ± 0.21	2.09 ± 0.14	3.17 ± 0.06	2.16 ± 0.03
DMPG	3.15 ± 0.37	2.01 ± 0.4	3.42 ± 0.08	1.83 ± 0.18	3.19 ± 0.34	2.05 ± 0.24
DMPC ± DMPG	3.45 ± 0.23	2.08 ± 0.2	3.68 ± 0.14	1.77 ± 0.14	3.68 ± 0.20	2.15 ± 0.19
EPC ± DMPG	4.67 ± 0.17	1.72 ± 0.11	4.80 ± 0.49	1.82 ± 0.19	4.72 ± 0.25	2.03 ± 0.11

of our phospholipid(s)-based powders, various compositions comprising different phospholipid(s) and ciprofloxacin were mixed with carrier in the weight ratio 1:2. FPFs obtained for formulations with and without carrier determined at a flow rate of 60 l/min are shown in Fig. 3. As can be seen, addition of carrier did not significantly improve the dispersion of any of the formulation. As a result, compositions without carrier were investigated in the rest of the study. It can also been seen from Fig. 3 that aerodynamic dispersion depends on the nature of the phospholipid, with the formulation comprising EPC dispersing poorly.

The FPF of various formulations with different phospholipid(s) and containing CM3 peptide and salbutamol sulfate are shown in Figs. 4 and 5, respectively.

It is interesting to note that 87–95% of the total loaded powder was deposited in the cascade impactor using the deagglomeration rig reported in this paper. This is larger than the 60% of the powder dispersed using a Spinhaler as reported in a previous study (Schreier et al., 1994). Also, previous studies indicate that a maximum FPF of 15–23% can be achieved using powders derived from lyophilized liposomes (Ho, 1995; Schreier et al., 1994; Joshi and Misra, 2001). Using our approach, a FPF of approximately 55% could be achieved, which is encouraging.

3.3. Encapsulation of drugs in FPF

Previous research articles on the aerodynamic dispersion of liposomal powders have focused on the FPF, which is determined using either Andersen cascade impactor or a twin impinger. However, to the authors' knowledge, no study has so far reported on the effect of deaggregating forces, imposed during the dispersion of the powder, on the liposome integrity and the encapsulation of drug in the FPF. Hence it is interesting to investigate the encapsulation of drugs in the aerosolized fine particle fraction obtained from

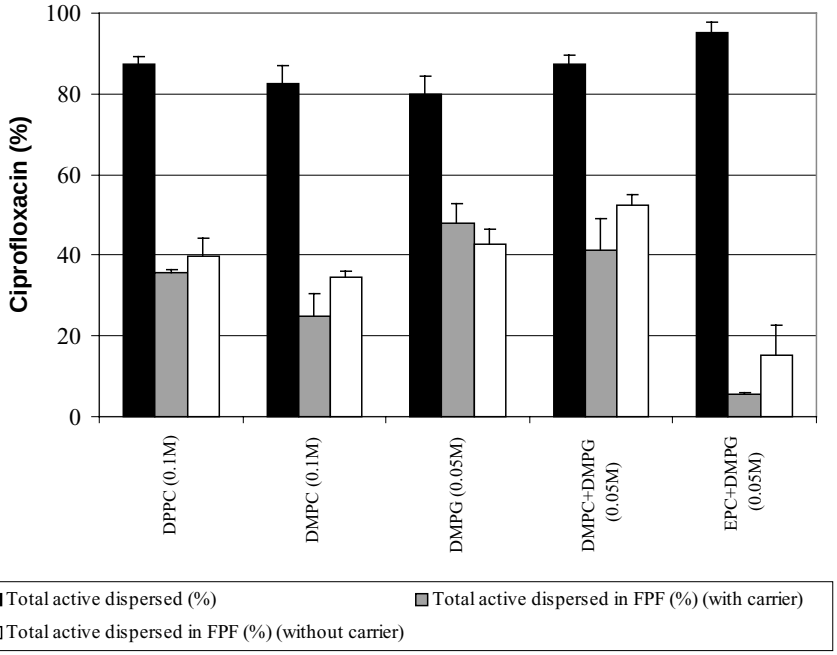

Fig. 3. Comparative FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing ciprofloxacin with and without carrier ($n = 3$ error bars indicate S.D.), given as a percentage of loaded dose (50.0 ± 2.0 mg).

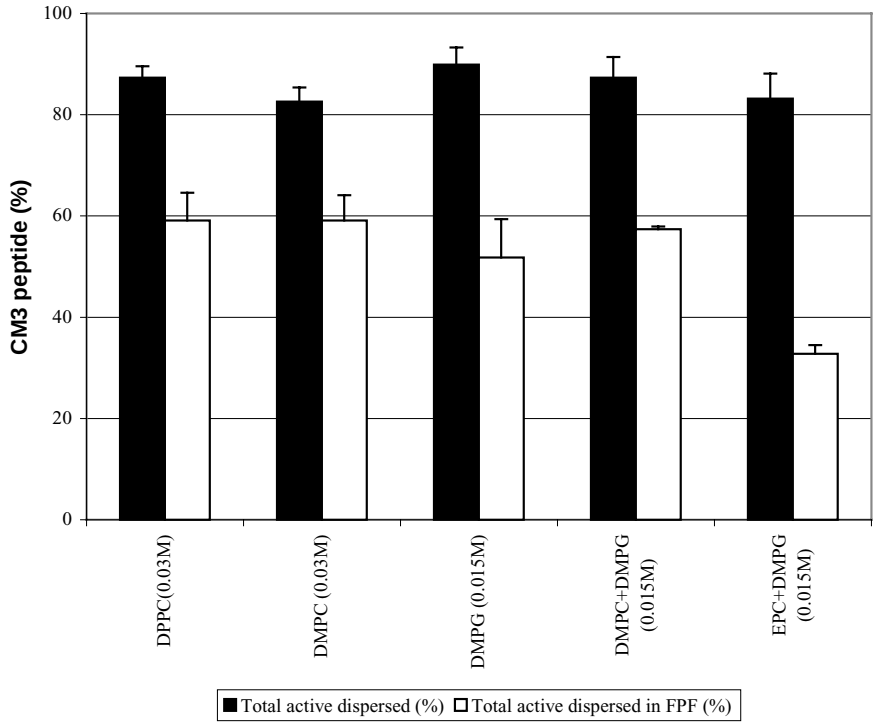

Fig. 4. Comparative FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing CM3 peptide ($n = 3$ error bars indicate S.D.), given as a percentage of loaded dose (50.0 ± 2.0 mg).

our formulations. Fig. 6 shows the encapsulation obtained by dispersion of the FPF in saline for ciprofloxacin. It can be seen that the encapsulation of ciprofloxacin in the FPF is dependent upon the nature of the phospholipid(s). This was very much expected based on our previous study (Desai et al., 2002).

The lower entrapment in the case of the formulation containing EPC may be attributed to its hygroscopic nature due

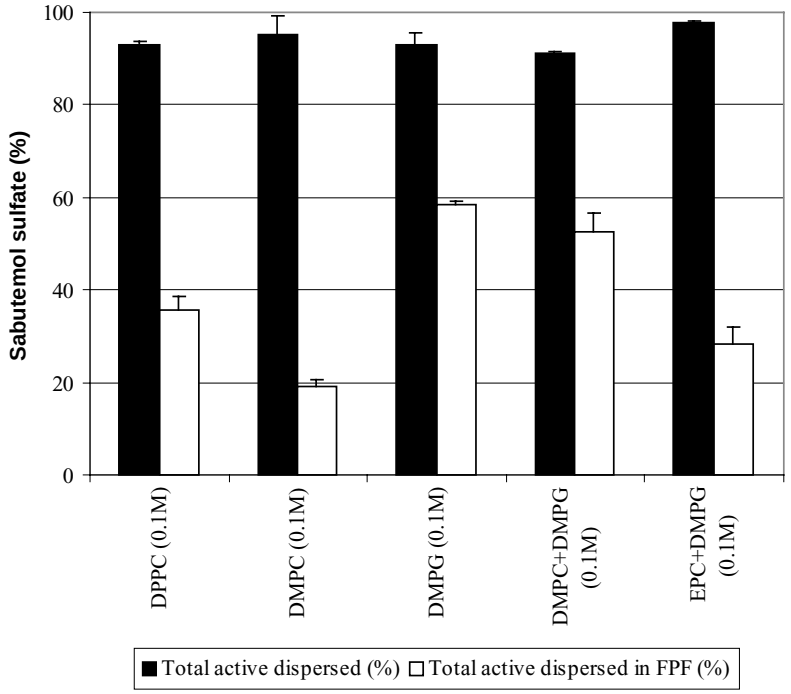

Fig. 5. Comparative FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing salbutamol sulfate ($n = 3$ error bars indicate S.D.), given as a percentage of loaded dose (50.0 ± 2.0 mg).

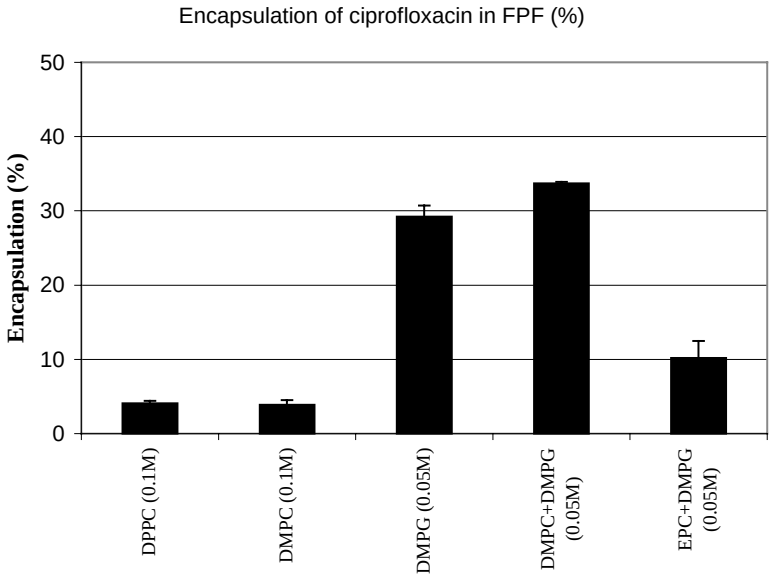

Fig. 6. Encapsulation efficiency of ciprofloxacin in FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing ciprofloxacin ($n = 3$ error bars indicate S.D.).

to the presence of double bonds in its fatty acid chains, giving it a "sticky" behavior. This behavior is expected to play a vital role in determining the aerodynamic dispersion of formulations comprising EPC. In contrast, the other phospholipids used have saturated fatty acid chains, giving it a crystalline appearance, and hence showed better aerodynamic dispersion upon aerosolization. Higher encapsulation of ciprofloxacin in formulations comprising DMPG compared to merely DPPC and DMPC based formulation may be mainly due to the presence of negative charge in DMPG. It is known that the inclusion of a charged lipid into the phospholipid bilayers causes electrostatic separation of the bilayers, and is a method by which uptake of drugs associated with aqueous volume may be improved (Johnson, 1973; Alpar et al., 1981). Higher entrapment in DMPC based liposomes as compared to DPPC based liposomes may be attributed to the difference in the phase transition temperatures of the two lipids. Due to the lower phase transition temperature of DMPC (23 °C), most of the lipid molecules are in the fluid phase upon hydration at 37 °C, thereby exhibiting higher encapsulation. Similar trends were observed in our previous study (Desai et al., 2002).

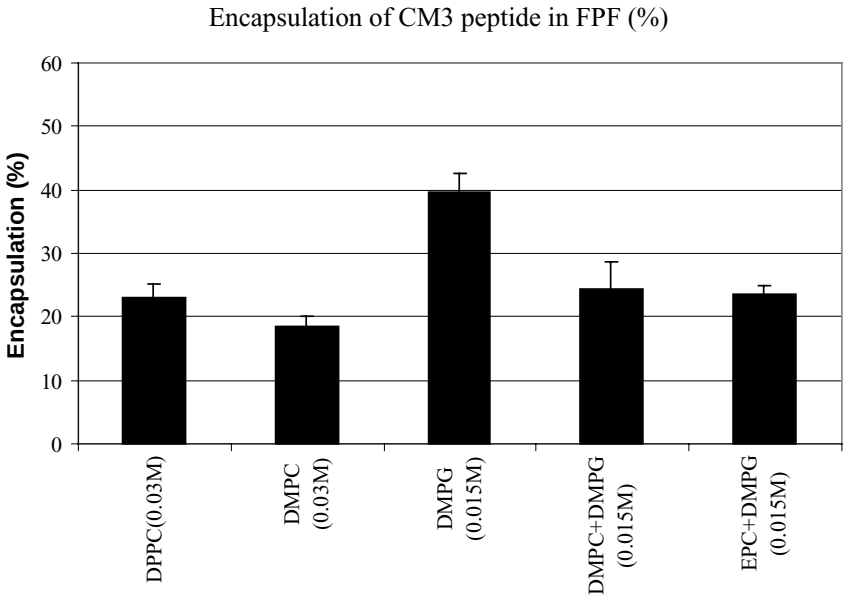

Fig. 7. Encapsulation efficiency of CM3 peptide in FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing CM3 peptide ($n = 3$ error bars indicate S.D.).

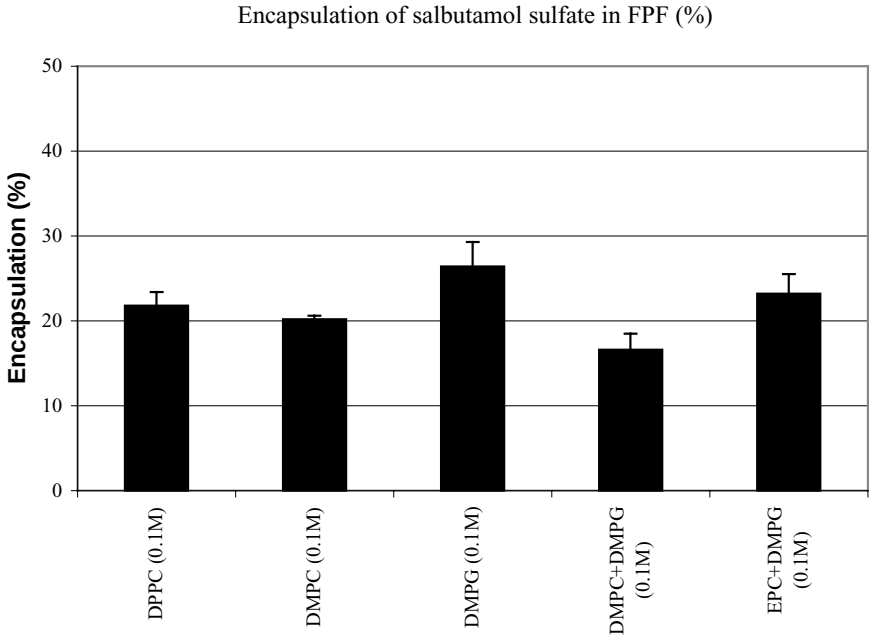

Fig. 8. Encapsulation efficiency of salbutamol sulfate in FPF (particle size <5.6 μm) obtained by dispersing various phospholipid-based powders containing salbutamol sulfate ($n = 3$ error bars indicate S.D.).

Fig. 7 shows the encapsulation of CM3 peptide in the FPF upon dispersion in saline. As observed in the case of ciprofloxacin, CM3 peptide shows higher encapsulation in formulations comprising DMPG. This may again be attributed to the presence of positively charged moieties in the peptide and negatively charged moieties in DMPG.

Similarly, the encapsulation of salbutamol sulfate in FPF for various phospholipid(s) based powder formulations is shown in Fig. 8.

Overall, for all three drugs studied here, formulations comprising DMPG and (DMPC + DMPG) show excellent aerodynamic dispersion, and good encapsulation of drugs in FPF.

4. Conclusions

This study presents supplementary data to our previous study where a novel approach of delivering liposomes in dry powder form was described, that relies on spontaneous formation of liposomes upon dispersion of phospholipid(s)-based powder formulations in saline. The present data allows selection of suitable phospholipid(s) to achieve optimum aerodynamic dispersion and encapsulation of drug in FPF upon aerosolization. Amongst all the formulations comprising various phospholipid(s) studied, DMPG and (DMPC + DMPG) based formulations showed excellent dispersion, with FPF of more than 50% for all the drugs, and FPF encapsulation of approximately 35% for ciprofloxacin, 40% for CM3 peptide and 25% for salbutamol sulfate. Overall, the approach seems promising to circumvent the deleterious effects of lyophilization and jet-milling, which not only leads to leakage of drug but also shows poor dispersion properties. This novel approach opens the door for future in vivo testing of the respiratory delivery of liposomes in dry powder form for the treatment of pulmonary diseases.

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References

Alpar, O.H., Bamford, J.B., Walters, V., 1981. In vitro incorporation of release of hydroxycobalamin by liposomes. Int. J. Pharm. 7, 349–351.

Crowder, T.M., Louey, M.D., Sethuraman, V.V., Smyth, H.D.C., Hickey, A.J., 2001. An odyssey in inhaler formulation and design. Pharm. Technol. 25 (7), 99–113.

Desai, T.R., Wong, J.P., Hancock, R.E.W., Finlay, W.H., 2002. A novel approach to the pulmonary delivery of liposomes in dry powder form to eliminate the deleterious effects of milling. J. Pharm. Sci. 91, 482–491.

Finlay, W.H., 2001. The Mechanics of Inhaled Pharmaceutical Aerosols: An Introduction. Academic Press, UK.

Finlay, W.H., Wong, J.P., 1998. Regional lung deposition of nebulized liposome encapsulated ciprofloxacin. Int. J. Pharm. 167, 121–127.

Gilbert, B.E., Wyde, P.R., Wilson, S.Z., Robins, R.K., 1991. Aerosol and intraperitoneal administration of ribavarin and ribavarin triacetate: pharamcokinetics and protection of mice against intracerebral infection

with influenza A/WSN virus. Antimicrob. Agents Chemother. 35 (7), 1448–1453.
Gregoriadis, G. (Ed.), 1988. Liposomes as Drug Carriers. Wiley, London.
Ho, J., 1995. Generation and analysis of liposome aerosols. In: Shek, P.N. (Ed.), Liposomes in Biomedical Applications. Harwood Academic Publishers, GmBH, pp. 199–208.
Johnson, S.M., 1973. The effect of charge and cholesterol on the size and thickness of phospholipid vesicles. Biochim. Biophys. Acta 307, 27–41.
Joshi, M., Misra, A., 2001. Dry powder inhalation of liposomal ketotifen fumarate: formulation and characterization. Int. J. Pharm. 223, 15–27.
Lange, C.F., Hancock, R.E.W., Samuel, J., Finlay, W.H., 2001. In vitro delivery and regional airway surface liquid concentration of a liposomal cationic peptide. J. Pharm. Sci. 90, 1647–1657.
Mobley, W.C., 1998. The effect of jet-milling on lyophilized liposomes. Pharm. Res. 15, 149–152.
Newman, S.P., Pavia, D., 1985. In: Moren, F., Newhouse, M.T., Dolovich, M.B. (Eds.), Aerosols in Medicines. Elsevier, New York, p. 193.
Nichols, S.C., Brown, D.R., Smurthwaite, M., 1998. New concepts for the variable flow rate Andersen cascade impactor and calibration data. J. Aerosol Med. 11 (Supplement 1), S133–S138.
Niven, R.W., Carvajal, M.A., Schreier, H., 1992. Nebulization of liposomes. III. The effects of operating conditions and local environment. Pharm. Res. 9, 515–520.
Parthasarathy, R., Gilbert, B., Mehta, K., 1999. Aerosol delivery of liposomal all-trans retinoic acid to the lungs. Cancer Chemother. Pharmacol. 43, 277–283.
Schreier, H., Mobley, W.C., Concessio, N., Hickey, A.J., Niven, R.W., 1994. Formulation and in vitro performance of liposome powder aerosol. STP Pharm. Sci. 4, 38–44.
Scott, M.G., Yan, H., Hancock, R.E.W., 1999. Biological properties of structurally related α-helical cationic antimicrobial peptide. Infect. Immun. 67, 2005–2009.
Voss, A.P., 2001. Deaggregation of dry powder pharmaceutical aerosols. Master of Science Dissertation, University of Alberta, 2001.
Voss, A.P., Finlay, W.H., 2002. Deagglomeration of dry powder pharmaceutical aerosols. Int. J. Pharm. 248, 39–50.
Weinstein, J.N., Leserman, L.D., 1984. Liposomes as drug carriers in cancer chemotherapy. Pharmacol. Ther. 24, 207–233.