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A facile method of delivery of liposomes by nebulization

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Abstract

In this study, we have demonstrated a facile approach to the aerosol delivery of liposomes by nebulization. The approach involves mere dispersion of the physical mixture of phospholipid(s) and drug in saline, which results in spontaneous formation of liposomes thereby creating reservoirs for the encapsulation of drugs. Various phospholipids exhibiting different physico-chemical properties were investigated here. Two antimicrobial agents (ciprofloxacin and CM3, a novel peptide) and a bronchodilator, salbutamol sulfate, were used as model drugs to examine the nebulization properties. Nebulization properties were found to be dependent upon the nature of the phospholipids and drug. Among various phospholipids investigated, dimyristoyl phosphatidyl glycerol (DMPG), a combination of egg phosphatidylcholine (EPC) plus DMPG (i.e., EPC+DMPG) and dimyristoyl phosphatidylcholine (DMPC) plus DMPG (DMPC+DMPG) (molar ratios 1:1) showed encouraging results in terms of higher nebulization efficiency and lower leakage of drug after nebulization. The generated aerosols were characterized by an Andersen cascade impactor operated at 28.3 l/min. The mass median aerodynamic diameter (MMAD) values of the aerosol droplets obtained by nebulization of all the preparations containing DMPG reveal that these preparations are suitable for aerosol delivery by nebulization. This facile approach is expected to overcome problems associated with stability upon storage and high production costs.

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

1. Introduction

Aerosol technology for pulmonary drug delivery enables the delivery of therapeutic agents in immune-mediated pulmonary diseases [1,2]. The development of liposomal formulations for aerosol delivery has expanded the potential for more effective utilization with an array of potent and effective drugs [3–5]. Liposomal aerosols in pulmonary therapy have many advantages including carrier suitability for lipophilic drugs, sustained release, prevention of local irritation, increased potency, reduced toxicity and uniform deposition of locally active drugs [6,7]. Amongst various aerosol delivery technologies, nebulizers have been extensively researched for the delivery of liposomes, as nebulizers offer the simplest delivery devices for liposomes and unlike metered dose inhalers or dry powder inhalers, liposomes may be delivered from nebulizers without further processing [8,9]. Aerosol characteristics by

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nebulization will depend upon a number of factors such as design of the nebulizer, operating conditions, local environment and aerosol output rate [10,11]. For nebulization of liposomal systems, the nature of the phospholipid used for preparing the liposomal dispersion and its method of preparation will also play important roles in determining the nebulization efficiency [12]. In the last few years, many studies have been reported evaluating these various parameters for liposomal systems encapsulating therapeutic agents such as anti-inflammatories, antibiotics and bronchodilators.

However, liposomal dispersions are often associated with long-term stability problems [11]. During storage, liposome dispersions can undergo physical and chemical changes that may lead to chemical degradation and leakage of the encapsulated drug. The aerosol delivery of liposomes by nebulization is hampered by such stability problems. In an attempt to improve the stability of liposomes, Darwis and Kellaway [13] reported the delivery of reconstituted freeze-dried liposomes for aerosol delivery by nebulization. However, in a few cases, the process of lyophilization is reported to cause leakage of the encapsulated drug [14,15]. In addition, the additional process cost of freeze-drying is involved, which may not be feasible from a commercial viewpoint.

In a previous study [14], we demonstrated a facile approach of delivering liposomes in dry powder form that relies on spontaneous formation of liposomes upon dispersion of micronized phospholipid(s)-based powders in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs. This approach was demonstrated with antimicrobial agents (ciprofloxacin and CM3 novel peptide) and a bronchodilator (salbutamol sulfate) as model drugs, and the powder formulations mainly contained micronized mixture of phospholipid(s), lactose and drug. It may be assumed that these powder compositions are not only suitable for liposome delivery in dry powder form, but may also be suitable for aqueous liposomal delivery by nebulization. Indeed this assumption was investigated in this study. This was done by merely dispersing phospholipid(s)-based powders in saline, and delivering the aqueous liposomal dispersion by nebulization. This facile approach is expected to circumvent the stability problems associated with aqueous liposomal dispersions prepared using conventional techniques. Various formulations derived from different phospholipids exhibiting different physico-chemical properties were evaluated to investigate the feasibility of delivering liposomes using this facile approach. Aerosol properties were determined in terms of the aerodynamic particle size, nebulization efficiency and leakage upon nebulization.

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 325M, was a generous gift from DMV International (Veghel, The Netherlands). Sodium chloride and salbutamol sulfate were purchased from Sigma (St. Louis, MO, USA). Ciprofloxacin hydrochloride (>99%) was purchased from Serologicals (Kankakee, IL, USA). CM3 peptide was synthesized by Fmoc (9-fluorenylmethoxycarbonyl) 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 earlier [16]. All materials were used as received.

2.2. Methods

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

Various phospholipid-based powder formulations were prepared by mixing phospholipids, lactose and the drug at appropriate weights to achieve the desired concentrations followed by micronization by jet-milling. Phospholipids such as DMPC, DMPG, DPPC and the combinations of EPC+DMPG and DMPC+DMPG (molar ratio 1:1) were used in this study. Lipid concentrations were adjusted depending upon

the nature of the drug to be encapsulated and the nature of the lipid. Ciprofloxacin was added at a dosage of 7 mg/ml, salbutamol sulfate at 2 mg/ml, and CM3 peptide at 1 mg/ml. The methods of formulating various compositions at appropriate concentrations have been discussed in more detail in our previous paper [14]. After mixing appropriately weighed phospholipid(s), Pharmatose 325M, and drug, the powder formulations were micronized with a Trost Impact Pulverizer (Garlock, Plastomer Products, Newton, USA) at a grinding nozzle pressure and pusher nozzle pressure of 90 p.s.i. The jet-milled powders were collected from the collection vessel. The micronized powder samples were stored in a dry box at low relative humidity and −20 °C for further nebulization studies.

2.2.2. Preparation of liposomal dispersions

Aqueous liposomal dispersions were prepared by simply dispersing the micronized phospholipid-based powders in saline and applying gentle vortex mixing for 1 min at room temperature (24±1 °C), at appropriate concentrations optimized previously [14]. The phospholipids were then allowed to hydrate by equilibrating the dispersions for 15 min at room temperature before nebulization. The encapsulation efficiencies in the spontaneously formed liposomes were determined by centrifuging the liposomal dispersions at 21 460×*g* and 4 °C for 90 min, and the amount of unencapsulated drug was determined by assaying the amount of respective drug in supernatant and pellet by UV spectrophotometry (model 8452A, Hewlett-Packard, Mississauga, ON). The method of determination of encapsulation efficiency of these drugs in spontaneously formed liposomes has been discussed in more detail in our previous paper [14]. The particle size distributions of these spontaneously formed liposomal dispersions have also been reported in our previous study [14].

2.2.3. Nebulization of liposomal dispersions

Nebulization of the spontaneously formed liposomal dispersion was done with Pari LC STAR (Pari, Starnberg, Germany) jet nebulizers, a nebulizer type that has shown superior performance in previous studies [17,18]. The nebulizers were driven by a Pulmo Aide compressor (model 5610C, DeVilbiss Sunrise Medical). Three different nebulizer units were used in this study. A volume fill of 2.5 ml was used for the nebulization. The aerosol was collected on Respigard filters (Marquest Medical Products, Englewood, CO). Usually two to three filters were connected in series for this purpose. After aerosolization, the filter contents were extracted with 0.9% saline. The nebulization efficiency of a liposomal formulation is defined as the total output of drug collected on the filters calculated as a percentage of the total submitted to nebulization. In other words, after assaying the drug content extracted from the filters, nebulization efficiency may be determined as:

$$\text{Nebulization efficiency (\%)} = \frac{\text{Aerosolized drug (i.e., collected on the filters)}}{\text{Total drug placed in nebulizer}} \times 100$$

Because nebulization can lead to the leakage of drug, it is important to also determine the nebulization efficiency of the encapsulated drug. This parameter is called encapsulated delivery and is defined as the amount of aerosolized drug that remains encapsulated after nebulization as a percentage of the total aerosol drug output of the nebulizer. A portion of the sample was then centrifuged at 21 460×*g* and 4 °C for 90 min, and the amount of leakage of drug during nebulization was determined by assaying the amount of respective drug in supernatant and pellet by UV Spectrophotometry. The percentage of the drug encapsulated was calculated as the ratio of the amount of drug in the pellet to the sum of the amounts of drug in the pellet and in the supernatant. Values of λ_{max} for ciprofloxacin, salbutamol sulfate and CM3 peptide are 278, 226 and 280 nm, respectively. The reported values are the mean of three sample replicates, i.e., three separate batches of jet-milled phospholipid-based powders. Samples of the original unnebulized preparations were submitted to the same procedure.

2.2.4. Particle size measurement of the aerosolized droplets

To determine the particle size distribution, the nebulizers were connected directly to an Andersen cascade impactor (Andersen Mark II, Graseby An-

dersen, Smyrna, GA). The nebulizers were run intermittently to avoid droplet shrinkage due to cooling in the impactor [19]. The impactor flow rate was calibrated to 28.3 l/min using a dry gas meter (DTM-115, Singer, American Motor Division). Each LC Star nebulizer was filled with 2.5 ml of liposomal preparation and connected to the Pulmo-Aide compressor. Each sample was nebulized for 30 s and then the sample was allowed to equilibrate to ambient temperature for the following 9 min 30 s, before the next cycle. Five such cycles were performed. This procedure eliminated the cooling effect that otherwise compromises particle size measurements of nebulizers with the Andersen cascade impactor [19]. Nebulization was carried out in a chamber maintained at a constant temperature of 24±1 °C and a relative humidity of 50±5%, as reported by Prokop et al. [20]. After nebulization the content of each plate was extracted and assayed for the respective drug by UV spectrophotometry, and the mass median aerodynamic diameter (MMAD) of aerosol droplets was determined from the cumulative mass distribution in the Andersen cascade impactor.

As aerosol droplets may contain different concentrations of free and encapsulated drug, it is necessary to verify the assumption that the liposomes were uniformly distributed in the aerosol droplets [21]. If the assumption is not true, and the liposomes are preferentially more concentrated in larger droplets, then assay of the drug in the extracts of the impactor plates would overestimate the droplet sizes, thereby leading to spurious results. To overcome this difficulty, particle size of the aqueous phase and liposomal phase was determined in a separate set of experiments. To accomplish this, the fractions collected on each plate were extracted with 0.9% saline. Spontaneously formed liposomes in these extracts were then centrifuged at 21 460×g and 4 °C for 90 min, and the resulting supernatant and pellet were assayed separately for the respective drugs using UV spectrophotometry. The particle size distribution of the aqueous phase and liposomal phase were then

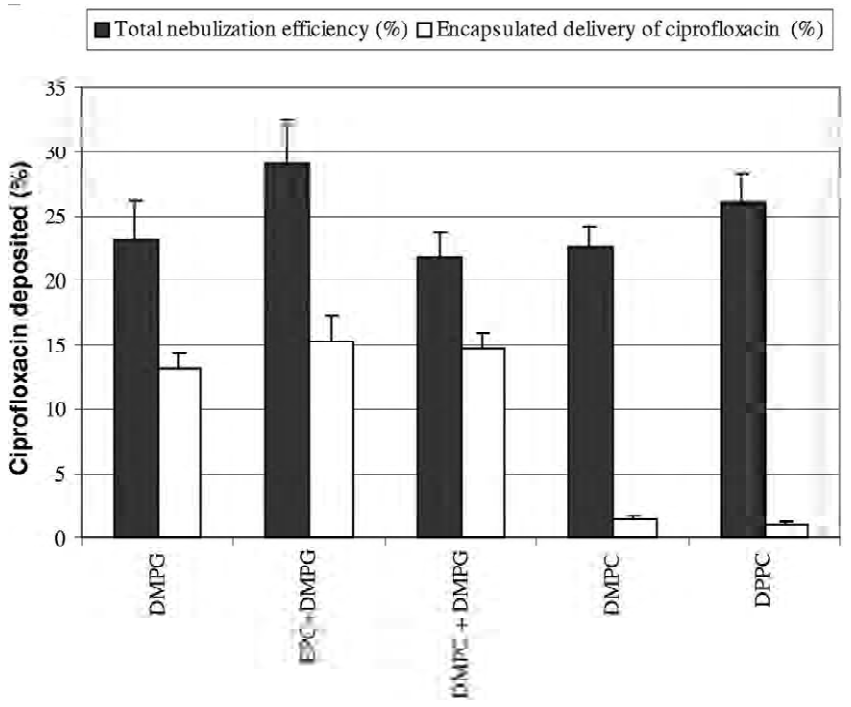

Fig. 1. Deposition of total (black bars) and encapsulated (white bars) ciprofloxacin on filters upon nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating ciprofloxacin (n=3, ±S.D.).

separately determined from the cumulative mass distribution in the Andersen cascade impactor. Statistical tests were performed using single factor analysis of variance (ANOVA) and Tukey HSD means comparisons.

3. Results and discussion

Various formulations containing different phospholipids were prepared and compared in terms of encapsulation efficiency, nebulization efficiency, encapsulated delivery and leakage during nebulization. The nebulization efficiencies of various compositions comprising different phospholipid(s) and ciprofloxacin are shown in Fig. 1. As can be seen, amongst all the preparations, the EPC + DMPG-based formulation showed maximum nebulization efficiency. It can also be seen from Fig. 1 that the EPC + DMPG-based formulation also showed maximum encapsulated delivery. In general, all the formulations comprising DMPG showed lower leakage compared to formulations without DMPG (i.e., containing DMPC and DPPC).

Fig. 2 depicts the entrapment of ciprofloxacin in spontaneously formed liposomes derived from different phospholipids and in liposomes after nebulization. As was observed previously [14], the encapsulation of ciprofloxacin in spontaneously formed liposomes depends on the nature of phospholipid(s). Again, the formulations containing DMPG showed maximum entrapment as compared to DMPC- and DPPC-based formulations. This behavior may be attributed to the negatively charged bilayers of DMPG that cause electrostatic separation of the bilayers, thereby improving the uptake of ciprofloxacin [22]. In addition, as reported earlier, encapsulation of ciprofloxacin also depends on the phase transition temperature of the phospholipid(s) [14]. It has been established from the present study that nebulization efficiency and encapsulated delivery also depend upon the phase transition tempera-

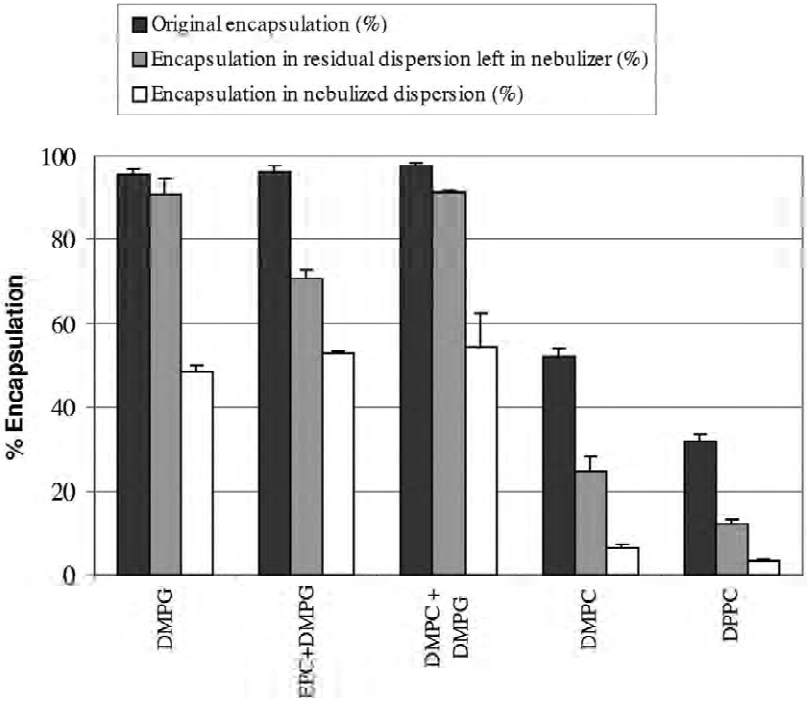

Fig. 2. Encapsulation (%) of ciprofloxacin in nebulized and residual unnebulized liposomes derived from different phospholipids ($n = 3$, ±S.D.).

Table 1
Mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) of the aerosols generated by nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating ciprofloxacin ($n=3$, ±SD)

Phospholipid	MMAD (μm)	GSD
DMPC + DMPG (0.05 M)	2.31±0.17	1.90±0.16
EPC + DMPG (0.05 M)	3.00±0.09	1.66±0.03
DMPG (0.05 M)	2.59±0.08	1.70±0.05

ture of phospholipids from which the dispersion is prepared. Similar observations were reported for liposomal dispersions prepared using conventional techniques [12,13]. As the spontaneous formation of liposomes was obtained at room temperature (24±1 °C), encapsulation of ciprofloxacin in phospholipids having phase transition temperature below 25 °C would show higher encapsulation. Fig. 2 also exhibits the leakage of ciprofloxacin during nebulization. The leakage during nebulization may be attributed to vesicle fragmentation, which may occur due to shock waves and kinematic discontinuities associated with impaction on nebulizer baffles [1, p. 209]. Another cause of leakage could be due to the dilution effect [23], since during extraction of the material deposited on the filter or Anderson impactor, the liposomes were in dilute conditions. To verify this latter effect, all the liposomal preparations were subjected to 10-fold dilution. It was observed that the preparation comprising DPPC showed maximum leakage (approximately 80%) upon 10-fold dilution, followed by DMPC>DMPG>(DMPC+DMPG) and (EPC+DMPG) showing the minimum leakage (approximately 50%) for a 10-fold dilution. A similar trend was observed for different phospholipids after nebulization, as can be seen from Figs. 1 and 2. This observation indicates that dilution is one of the causes of leakage.

Since nebulization efficiency and encapsulated delivery are both important in evaluating a formulation, it appears from Figs. 1 and 2 that formulations comprising DMPG (i.e., DMPG, EPC+DMPG and EPC+DMPG) are suitable for delivering ciprofloxacin by this facile approach. The encapsulated deliv-

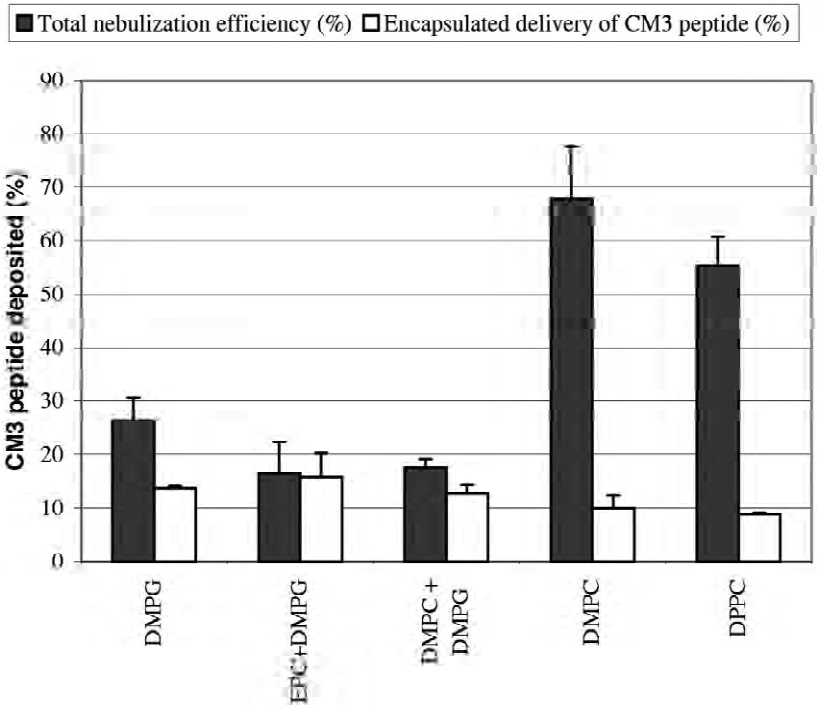

Fig. 3. Deposition of total (black bars) and encapsulated (white bars) CM3 peptide on filters upon nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating CM3 peptide ($n=3$, ±S.D.).

ery may appear lower than typically reported for liposomes prepared using conventional techniques [9,17]; however, keeping other advantages in mind, this facile approach indeed shows interesting data.

Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) values for the preparations comprising DMPG are shown in Table 1. The GSD values demonstrate the polydisperse nature of the distribution of the aerosolized particles of all the liposomal preparations.

Measurement of the free and encapsulated ciprofloxacin contained in the different particle size ranges collected on each Andersen cascade impactor plate showed that there was no statistically significant difference between the cumulative size distribution of ciprofloxacin in aqueous and liposomal phase ($P > 0.5$). These results indicate that the encapsulated ciprofloxacin was homogeneously distributed in the aerosol droplets. It should be noted that the possibility of leakage of drug from the liposomes due to impaction of aerosolized droplets on the plates may be ruled out, as the velocity of the droplets impacting the plates is much less than the velocity of the droplet impactions that occur during their creation in the nebulizer (by at least a factor of three). In addition, impaction on the plate occurs only once, while impaction in the nebulizer occurs many times before material finally leaves the exiting aerosol. Hence the effect of impaction on the plates is expected to play a negligible role in the leakage of drug.

Fig. 3 depicts the nebulization efficiency and encapsulated delivery of spontaneously formed liposomes encapsulating CM3 peptide, prepared by dispersing different phospholipid(s) in saline. As can be seen, DMPC- and DPPC-based formulations exhibit high nebulization efficiencies. However, judging a formulation merely by its nebulization efficiency may be misleading, as the formulation may be showing higher drug output because of higher leakage. The high nebulization efficiency in the case of DMPC- and DPPC-based formulations may be attributed to this fact, i.e., the higher output of drug may be mainly because of high leakage

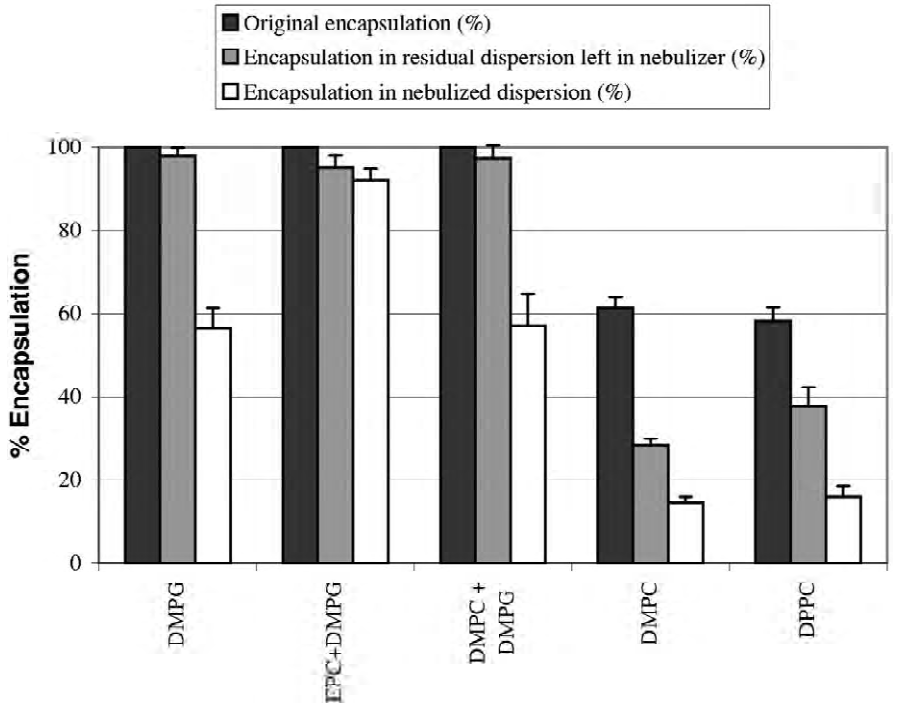

Fig. 4. Encapsulation (%) of CM3 peptide in nebulized and residual unnebulized liposomes derived from different phospholipids ($n = 3$, ±S.D.).

Table 2
Mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) of the aerosols generated by nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating CM3 peptide ($n=3$, ±S.D.)

Phospholipid	MMAD (μm)	GSD
DMPC + DMPG (0.05 M)	2.41±0.15	1.82±0.18
EPC + DMPG (0.05 M)	3.43±0.11	1.90±0.21
DMPG (0.05 M)	3.16±0.1	1.80±0.24

during nebulization. Indeed these formulations showed high amount of leakage upon nebulization as seen from Fig. 3. Fig. 4 shows the leakage of CM3 peptide from spontaneously formed liposomes upon nebulization. As observed in the case of ciprofloxacin, all the formulations containing DMPG show maximum encapsulations, with the (EPC + DMPG)-based formulation showing over 92% encapsulation of the peptide in nebulized liposomes. On the other hand, formulations containing DPPC and DMPC showed high amounts of leakage upon nebulization. Again, to check the effect of dilution on the leakage of CM3 peptide from liposomes, all the liposomal preparations were subjected to 10-fold dilutions. A similar trend to that observed in case of ciprofloxacin occurred, with DPPC-based formulations showing the highest leakage (approximately 70%) and the (EPC + DMPG)-based formulation showing the least leakage (approximately 10%). The particle size distribution and the GSD values of the aerosols generated by nebulization of various liposomal formulations encapsulating CM3 peptide is shown in Table 2. Measurement of the free and encapsulated CM3 peptide contained in the different particle size ranges collected on each Andersen cascade impactor plate showed that there was no statistically significant difference between the cumulative size distribution of CM3 peptide in aqueous and liposomal phase ($P>0.5$), indicating the homogeneous distribution of liposomal CM3 peptide in the aerosol droplets.

Nebulization efficiency and encapsulated delivery upon aerosolization of spontaneously formed liposomes derived from various phospholipids encapsulating salbutamol sulfate is shown in Fig. 5. As can be seen from the figure, all the preparations con-

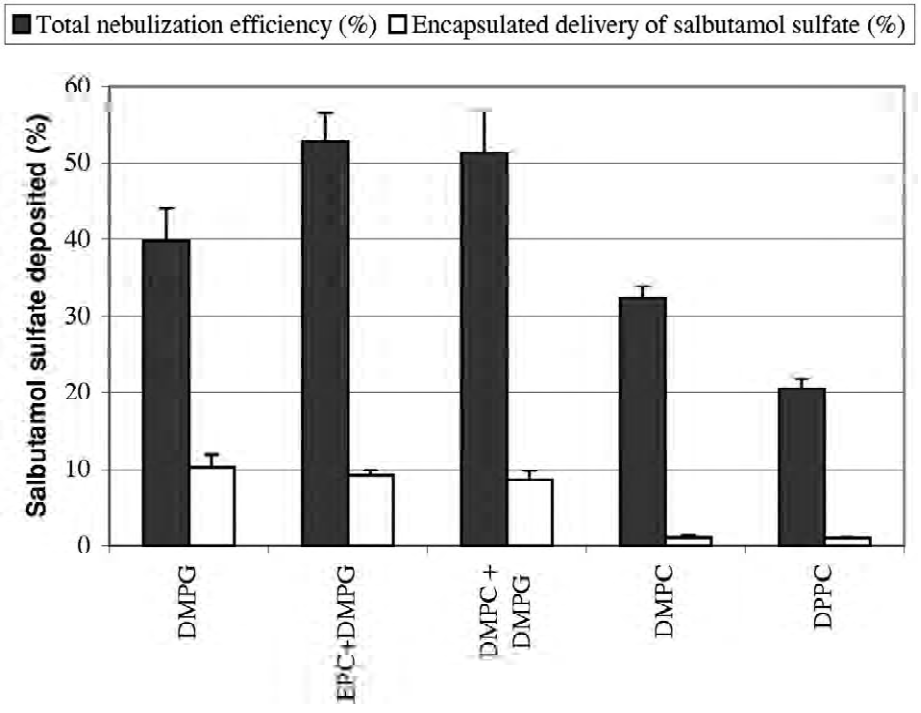

Fig. 5. Deposition of total (black bars) and encapsulated (white bars) salbutamol sulfate on filters upon nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating CM3 peptide ($n=3$, ±S.D.).

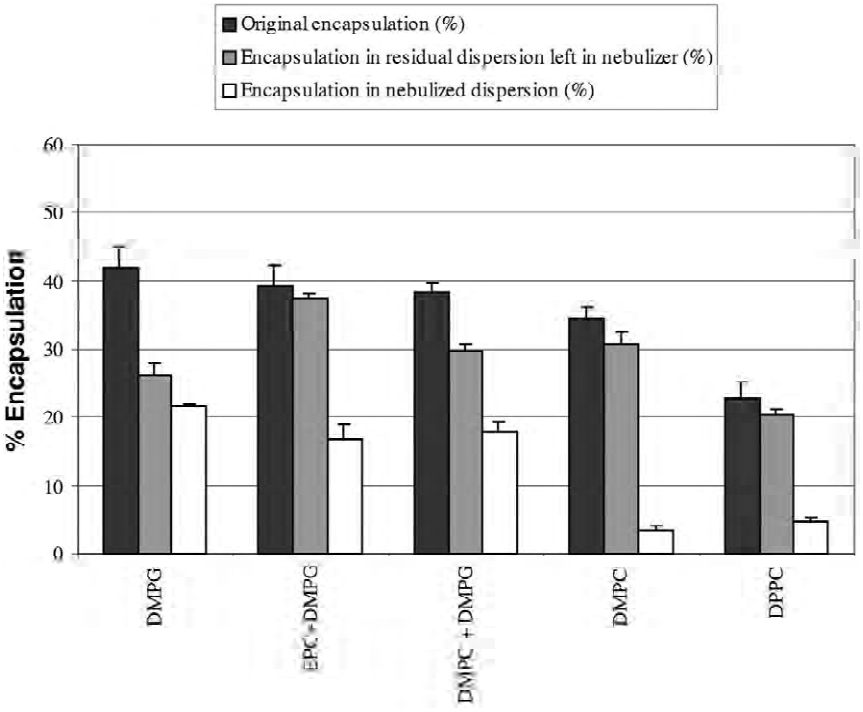

Fig. 6. Encapsulation (%) of salbutamol sulfate in nebulized and residual unnebulized liposomes derived from different phospholipids ($n=3$, ±S.D.).

taining DMPG showed high nebulization efficiency, whereas DMPC- and DPPC-based formulations showed poor nebulization efficiencies. Fig. 6 shows the leakage of drug on filters upon nebulization for these formulations. As expected, preparations containing DMPG showed less leakage compared to DMPC- and DPPC-based preparations. Liposomes when subjected to 10-fold dilutions revealed that the formulations containing DMPG showed lower leakage compared to preparations containing DPPC and DMPG, indicating that dilution of liposomes is the main cause of leakage. Table 3 reveals the particle size distribution and GSD values for the aerosols generated by spontaneously formed liposomes from various phospholipids encapsulating salbutamol sulfate.

Table 3
Mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) of the aerosols generated by nebulization of spontaneously formed liposomes derived from various phospholipid(s) encapsulating salbutamol sulfate ($n=3$, ±S.D.)

Phospholipid	MMAD (μm)	GSD
DMPC+DMPG (0.05 M)	2.16±0.17	1.72±0.06
EPC+DMPG (0.05 M)	2.59±0.08	1.69±0.07
DMPG (0.05 M)	2.18±0.2	1.76±0.03

Overall, for all three drugs studied here, the formulations containing DMPG (i.e., DMPG, EPC+DMPG and DMPC+DMPG) showed good nebulization efficiency, encapsulated delivery and lower leakage upon nebulization.

4. Conclusions

This study presents a facile approach of delivering liposomes, that relies on nebulization of drugs encapsulated in spontaneously formed liposomes obtained by mere dispersion of micronized phospholipid(s)-based powders in saline. Amongst the various phospholipids studied here, the formulations containing DMPG, EPC+DMPG and DMPC+DMPG showed good nebulization efficiency, encapsulated delivery and lower leakage upon nebulization for all the drugs. Output of the encapsulated drug on nebulization was also found to be dependent

on the phase transition temperature of the phospholipids. This study also reveals that selection of appropriate phospholipid(s) for a particular drug is important. Overall, this facile approach showed encouraging results and may overcome several problems such as stability upon storage and high production cost. The approach suggests a new direction for the respiratory delivery of liposomes by nebulization and opens new doors for future in vivo testing for the treatment of pulmonary diseases.

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References

[1] W.H. Finlay, in: The Mechanics of Inhaled Pharmaceutical Aerosols: An Introduction, Academic Press, London, 2001.
[2] J.C. Waldrep, New aerosol drug delivery systems for the treatment of immune-mediated pulmonary diseases, Drugs Today 34 (6) (1998) 549–561.
[3] K.M.G. Taylor, S.J. Farr, Liposomes for drug delivery to the respiratory tract, Drug Dev. Indust. Pharm. 19 (1993) 123–142.
[4] J.N. Weinstein, L.D. Leserman, Liposomes as drug carriers in cancer chemotherapy, Pharmacol. Ther. 24 (1984) 207–233.
[5] H. Schreier, Liposome aerosols, J. Liposome Res. 2 (1992) 145–184.
[6] B.E. Gilbert, P.R. Wyde, S.Z. Wilson, R.K. Robins, 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) (1991) 1448–1453.
[7] R. Parthasarathy, B. Gilbert, K. Mehta, Aerosol delivery of liposomal all-trans retinoic acid to the lungs, Cancer Chemother. Pharmacol. 43 (1999) 277–283.
[8] M. Saari, M.T. Vidgren, M.O. Koskinen, V.H.M. Turjanmaa, M.M. Neiminen, Pulmonary distribution and clearance of two beclomethasone liposome formulations in healthy volunteers, Int. J. Pharm. 188 (1999) 1–9.
[9] C.F. Lange, R.E.W. Hancock, J. Samuel, W.H. Finlay, In vitro delivery and regional airway surface liquid concentration of a liposomal cationic peptide, J. Pharm. Sci. 90 (2001) 1647–1657.
[10] R.W. Niven, M. Speer, H. Schreier, Nebulization of liposomes. II. The effects of size and modeling of solute release profiles, Pharm. Res. 8 (1991) 217–221.
[11] R.W. Niven, M.A. Carvajal, H. Schreier, Nebulization of liposomes. III. The effects of operating conditions and local environment, Pharm. Res. 9 (1992) 515–520.
[12] R.W. Niven, H. Schreier, Nebulization of liposomes. I. Effects of lipid compositions, Pharm. Res. 7 (1990) 1127–1133.
[13] Y. Darwis, I.W. Kellaway, Nebulization of rehydrated freeze-dried beclomethasone dipropionate liposomes, Int. J. Pharm. 215 (2001) 113–121.
[14] T.R. Desai, J.P. Wong, R.E.W. Hancock, W.H. Finlay, A novel approach to the pulmonary delivery of liposomes in dry powder form to eliminate the deleterious effects of milling, J. Pharm. Sci. 91 (2002) 482–491.
[15] W.C. Mobley, The effect of jet-milling on lyophilized liposomes, Pharm. Res. 15 (1998) 149–152.
[16] M.G. Scott, H. Yan, R.E.W. Hancock, Biological properties of structurally related α-helical cationic antimicrobial peptide, Infect. Immun. 67 (1999) 2005–2009.
[17] W.H. Finlay, J.P. Wong, Regional lung deposition of nebulized liposome encapsulated ciprofloxacin, Int. J. Pharm. 167 (1998) 121–127.
[18] W.H. Finlay, C.F. Lange, M. King, D.P. Spreet, Lung delivery of aerosolized dextran, Am. J. Respir. Crit. Care Med. 161 (2000) 91–97.
[19] W.H. Finlay, K.W. Stapleton, Undersizing of droplets from a vented nebulizer caused by aerosol heating during transit through an Anderson impactor, J. Aerosol Sci. 30 (1999) 105–109.
[20] R.M. Prokop, W.H. Finlay, K.W. Stapleton, P. Zuberbuhler, The effect of ambient relative humidity on regional dosages delivered by a jet nebulizer, J. Aerosol Med. 8 (1995) 363–372.
[21] W.H. Finlay, K.W. Stapleton, P. Zuberbuhler, Predicting regional lung dosage of a nebulized suspension: Pulmicort (budesonide), Part. Sci. Technol. 15 (1997) 243–251.
[22] S.M. Johnson, The effect of charge and cholesterol on the size and thickness of phospholipid vesicles, Biochim. Biophys. Acta 307 (1973) 27–41.
[23] K.M.G. Taylor, G. Taylor, I.W. Kellaway, J. Stevens, The stability of liposomes to nebulization, Int. J. Pharm. 58 (1990) 57–61.