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SPRAY-DRYING OF LIPOSOMES FOR A PULMONARY ADMINISTRATION. II. RETENTION OF ENCAPSULATED MATERIALS

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ABSTRACT

Extruded multilamellar vesicles containing atropine sulphate were spray-dried in the presence of 10 % lactose. The particle mean diameter of the spray-dried product was about 7 μm and 3.5 μm when the spray-drier was equipped with a rotary atomizer or a nozzle, respectively. The size of the vesicles was not significantly modified after rehydration of the dry residue. Atropine sulphate was entrapped in liposomes constituted of soybean phosphatidylcholine (SPC), hydrogenated SPC and SPC:Cholesterol (1:1 molar ratio) with an efficiency of 4-6 %, but an important leakage (65 to 80 %) of the incorporated drug occurred during the spray-drying process. On the other hand, α-tocopherol used as a lipophilic drug model was incorporated in SPC vesicles with an efficiency of 92 % and no

significant leakage was detected during the dehydration-rehydration cycle. Thus, spray-drying constitutes an interesting method to dehydrate liposomes (especially when lipophilic drugs are incorporated) into the form of small particles that could be delivered to the respiratory tract and reconstituted *in situ*.

INTRODUCTION

Liposomes are currently under investigation for their possible use as drug delivery systems for the respiratory tract (for review see Ref. [1]). However the long-term stability is one of the major problems in the development of a pharmaceutical liposomal formulation, since aqueous liposome dispersions undergo vesicle size alteration, chemical degradation and leakage of incorporated drugs. One possible means to stabilize lipid vesicles is the development of a dry solid composition. In a previous study we have shown that liposomes can be dehydrated in the presence of 10 % lactose by a spray-drying process into the form of small sized particles that could be inhaled (7 μm mean diameter) [2]. Moreover, the vesicle size distribution and the chemical stability of phosphatidylcholine were not significantly affected following rehydration of the dry residue.

The present report describe the effect of spray-drying upon the retention of encapsulated materials in lipid vesicles. Atropine sulphate, which has bronchodilating properties, was used as a hydrophilic drug model, while α-tocopherol was chosen as a lipophilic drug model. If the drug shows a tendency to leak to a large extent from the vesicles subjected to spray-drying, then the effectiveness of such preparations as pulmonary drug carriers would be reduced or even lost completely.

Furthermore, the particle size distribution of the dry residue produced by two different dispersion systems (rotary atomizer and nozzle) were compared.

MATERIALS AND METHODS

Preparation and characterization of Liposomes

Liposome components were soybean phosphatidylcholine (SPC) (Epikuron 200, Lukas Meyer Inc., Hambourg, FRG), hydrogenated soybean phosphatidylcholine (HSPC) (Epikuron 200 SH, Lukas Meyer Inc.), and cholesterol (Chol) (Ash-free 95-98 %, Sigma Co., St Louis, Mo, USA). Atropine sulphate (At) and α-tocopherol (α-T) were purchased from Sigma.

Liposome dispersions containing 20 mg/ml of lipids were prepared and characterized as described previously [2]. Briefly, the lipids were dissolved in methanol or a chloroform:methanol mixture (2:1 v/v) and the organic solvent was slowly removed until a thin film of dry lipids was formed into a round bottom flask. The film was hydrated at room temperature with 100 ml of 15 mM phosphate buffer (pH 6.8) containing 10 % lactose. The resultant liposome dispersion was extruded three times consecutively through a 0.2 μm polycarbonate membrane filter of 90 mm diameter (Nuclepore Corp., Pleasanton, CA, USA). For the vesicles composed of HSPC, the preparation and the extrusion steps were performed above the phase transition temperature (Tc) at 60-65°C. Atropine sulphate was added within the aqueous phase at the concentration of 20 mg/ml, while α-tocopherol was dissolved with the lipids in the organic solvent at 1 mg/ml. Non encapsulated atropine sulphate was removed by dialysis at 4°C for 48 h against 100 volumes of 15 mM phosphate buffer (pH 6.8) containing 10 % lactose and changed after 24 h.

The vesicle size distribution was determined by dynamic light scattering with a Coulter N4 SD system (Coulter Electronics Ltd., Luton, U.K.).

Spray-drying of Liposomes

Liposome dispersions were dehydrated with a Minor Mobile spray-drier (Niro Atomizer, Copenhagen, Denmark). Two kinds of dispersion systems were used: a rotary atomizer and a nozzle.

TABLE 1
Operating Conditions of the Spray-drying Process

	Rotary atomizer	Nozzle
Flow rate (ml/min)	10	3
Pressure (Bars)	6	6
Inlet temperature (°C)	110	140
Outlet temperature (°C)	75-80	75-85

The operating conditions are reported in table 1. The aspect of the spray-dried product was examined by optical microscopy and the size of about 100 particles was measured for the determination of the mean diameter (MD). The liposome dispersions were reconstituted by hydrating 1.228 g of the dry residue with enough demineralized water to give a final volume of 10 ml.

Quantification of Atropine Sulphate and α-Tocopherol

Atropine sulphate was quantified by high performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) on a reverse phase C18 column (Nova-Pak 3.9 mm x 15 cm; 5 μm, Waters) with monitoring at 258 nm. The eluting solvent was a mixture of 25 % acetonitrile and 75 % of a phosphate buffer (15 mM, pH 2.8) containing 5 mM sodium pentanesulfonic acid. The flow rate was set at 1 ml/min. α-Tocopherol was also quantified using a C18 HPLC column with monitoring at 296 nm. The eluting solvent was composed of methanol : tetrahydrofuran : water (90:3:7 V/V) and the flow rate was set at 1 ml/min.

Release of Encapsulated Atropine Sulphate during Spray-drying

Liposomes composed of SPC, HSPC and SPC:Chol (1:1 molar ratio) were prepared in the presence of 10 % lactose and 20 mg/ml

atropine sulphate. After removal of the non encapsulated material by dialysis, the vesicles were spray-dried using a rotary atomizer or a nozzle as dispersion systems. The liposome dispersions were analyzed for both free (At_{free}) and total (At_{tot}) atropine sulphate concentrations before and after dehydration. Free drug was separated from the vesicles by gel chromatography over a Sephadex G-50 column (14 X 1 cm I.D.) equilibrated with 15 mM phosphate buffer (pH 6.8) containing 10 % lactose. The eluted fractions containing the free drug were injected into the HPLC system. Total atropine was determined by diluting ten fold the liposome dispersion with methanol and injecting this solution directly. The percentage of encapsulated atropine sulphate was calculated by the expression:

$$\% \text{ Encaps} = (1 - At_{free}/At_{tot}) \times 100 \quad (1)$$

The percentage of entrapped drug was determined before (% $\text{Encaps}_{(o)}$) and after (% Encaps) spray-drying so that the leakage of the drug was expressed as the percentage of release:

$$\% \text{ Release} = \frac{(\% \text{ Encaps}_{(o)} - \% \text{ Encaps})}{(\% \text{ Encaps}_{(o)})} \quad (2)$$

Retention of Incorporated α-Tocopherol during Dehydration

SPC vesicles containing 1 mg/ml α-tocopherol were prepared in the presence of 10 % lactose and spray-dried with a rotary atomizer. The percentage of incorporated α-tocopherol was determined after separation of the vesicles from the free drug by a Ficoll flotation method [3]. Briefly, 2 ml of liposome dispersion were mixed with 2 ml of 10 % (w/v) Ficoll (Type 400, Sigma) prepared in 15 mM phosphate buffer (pH 6.8) containing 10 % lactose; then 4 ml of 5 % (w/v) Ficoll were layered on top of the liposome suspension, and finally the upper Ficoll layer was covered with 2 ml of phosphate buffer. After centrifugation (5000 rpm, 15

min), the vesicles were recovered and analyzed for both α-tocopherol and phospholipids content. α-Tocopherol was quantified by HPLC after dilution with methanol and the phospholipids by performing the Stewart assay [4]. The percentage of encapsulated α-tocopherol was determined before and after spray-drying so that it was possible to calculate the percentage of drug release.

RESULTS AND DISCUSSION

Release of Encapsulated Atropine Sulphate from Liposomes

Different formulations of atropine-containing liposomes were spray-dried with a rotary atomizer or a nozzle after removal of non encapsulated material by dialysis.

Figure 1 shows micrographs of the dehydrated particles obtained with both of the dispersion systems. The particle mean diameter was about 7 μm and 3.5 μm when the liposomes were dehydrated with the rotary atomizer or the nozzle, respectively (Fig. 2). Thus, the particle size distribution obtained with the nozzle seems to be very interesting for a pulmonary delivery since most of the particles are smaller than 5 μm. Nevertheless, the mass median aerodynamic diameter (MMAD) should be determined to investigate the aerodynamic properties of these particles.

The vesicle mean diameter was near to 200 nm for all the preparations, as well as before than after spray-drying (Table 2). Even when the liposomes were constituted of HSPC that only forms vesicles above its phase transition temperature (56°C), the vesicle size distribution was the same after rehydration at room temperature.

The data relating to the entrapment of atropine sulphate before and after spray-drying are given in table 3. The trapping efficiency of the liposomes ranged from 3.5 to 6 %. After removal of the non entrapped material by dialysis, approximately 90 % of the drug still present in the preparation was associated to the vesicles. For all the formulations tested about 65 to 80 % of the entrapped material was

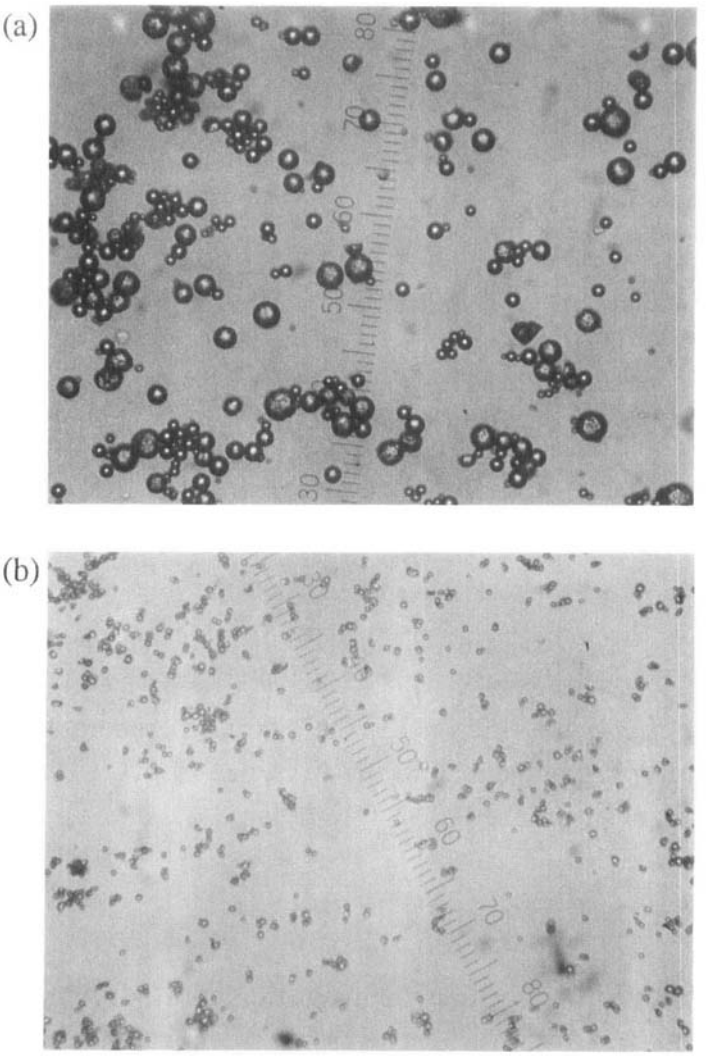

FIGURE 1

Optical micrograph (200x) of particles produced by spray-drying of atropine liposomes with a rotary atomizer (a) or a nozzle (b). The vesicles were composed of SPC and prepared in a solution of lactose at 10 %. One graduation on the scale corresponds to 6.8 μm.

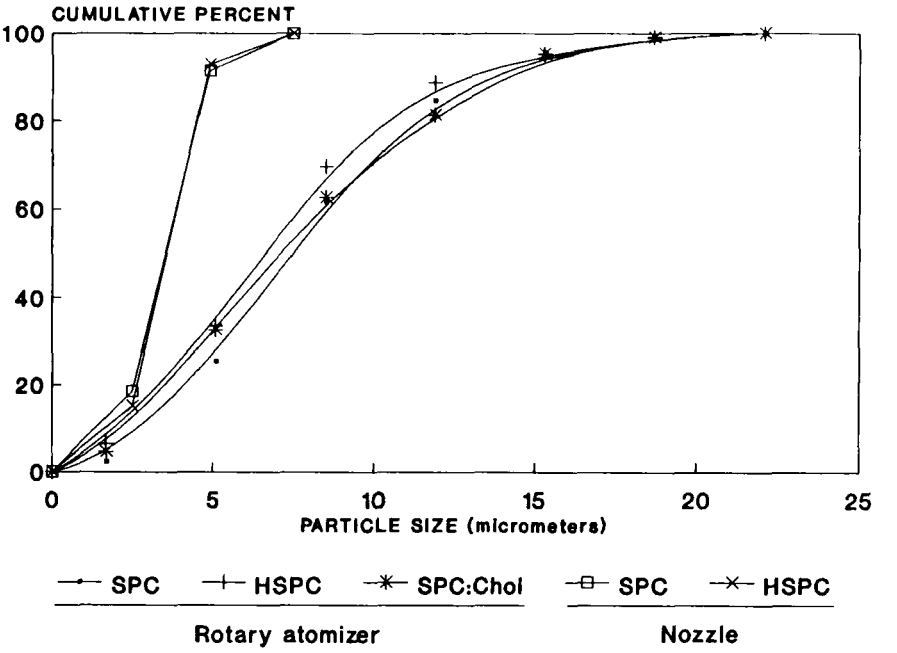

FIGURE 2

Cumulative frequency curves of the particles generated by spray-drying of atropine liposomes in the presence of 10 % lactose with a rotary atomizer or a nozzle.

released from the liposomes during the spray-drying-rehydration process (Fig. 3). Moreover, the drug retention was not influenced by the lipid composition as it was expected.

Indeed, solute permeability of phospholipid vesicles depends on the degree of disorder of the bilayers [5]. Vesicles in the gel state are less permeable to entrapped materials than when they are in the liquid crystalline state. However, the use of saturated phospholipids (HSPC) which are at the gel state at room temperature did not decrease the leakage during spray-drying. A possible reason is that

TABLE 2

Vesicle Size Distribution of Atropine Liposomes before and after Spray-drying.

Dispersion system	Rotary atomizer			Nozzle	
Lipid composition	SPC	HSPC	SPC:Chol (1:1)[a]	SPC	HSPC
Before[b] (nm)	160 (±45)	190 (±38)	185 (±30)	175 (±21)	173 (±22)
After[c] (nm)	160 (±34)	190 (±41)	177 (±21)	184 (±28)	162 (±33)

[a] Mole ratio

[b] Mean diameter (±SD) of the vesicles before spray-drying

[c] Mean diameter (±SD) after spray-drying and rehydration.

under the effect of the heating temperature of 110 °C, the lipid bilayers pass to the liquid crystalline state, the membrane fluidity increases a lot and the vesicles become very permeable to the entrapped material. Moreover, it is well known that thermotropic gel to liquid crystalline phase transitions result in an increased permeability of phospholipid membranes to the solutes [6].

The incorporation of cholesterol into liposomes is known to stabilize phospholipid bilayers and decrease their permeability to solutes [7]. However, the adjunction of cholesterol at a molar ratio of 50 percent in SPC vesicles had no influence on the drug retention upon spray-drying.

On the other hand, the leakage was about the same when the two kind of dispersion systems were used, rotary atomizer or nozzle.

TABLE 3

Atropine Content of the Liposomes before (o) and after Spray-drying

Lipid composition (Mole ratio)	Atropine content before spray-drying (μg/ml) (a)			Atropine content after spray-drying (μg/ml) (b)			% Release (c)
	Total (o)	Free (o)	% Encapsulated (o)	Total	Free	% Encapsulated	
Rotary atomizer:							
SPC	920.11 (±2.52)	155.50 (±12.25)	87.45 (±1.37)	1018.15 (±13.42)	747.50 (±20.42)	26.56 (±2.97)	69.59 (±3.88)
HSPC	1202.68 (±4.99)	62.97 (±4.96)	94.76 (±0,39)	1261.77 (±11.71)	883.75 (±27.54)	29.96 (±1.53)	68.38 (±1.49)
SPC:Chol 1:1	786.94 (±23.02)	44.65 (±6.64)	94.34 (±0.68)	949.35 (±26.79)	747.02 (±20.79)	21.25 (±4.41)	77.49 (±4.51)
Nozzle:							
SPC	1018.08 (±14.32)	152.93 (±17.93)	84.96 (±1.97)	1235.35 (±5.62)	984.35 (±12.98)	20.32 (±0.69)	76.07 (±1.37)
HSPC	875.64 (±29.76)	83.44 (±8.19)	90.47 (±1.26)	1077.33 (±45.20)	913.94 (±11.72)	15.11 (±2.47)	83.27 (±2.97)

(a) Atropine content is expressed as μg of drug per ml of liposome suspension. "Total" refers to the concentration of atropine sulphate which is still present in the preparation after dialysis. "Free" refers to the concentration of non entrapped material determined after separation by gel chromatography. "% Encapsulated" is the percent of atropine incorporated in the vesicles. Results are a mean (±SD) of two independant experiments.
(b) Atropine content determined after spray-drying and rehydration of the liposomes.
(c) Percent of drug released during spray-drying.

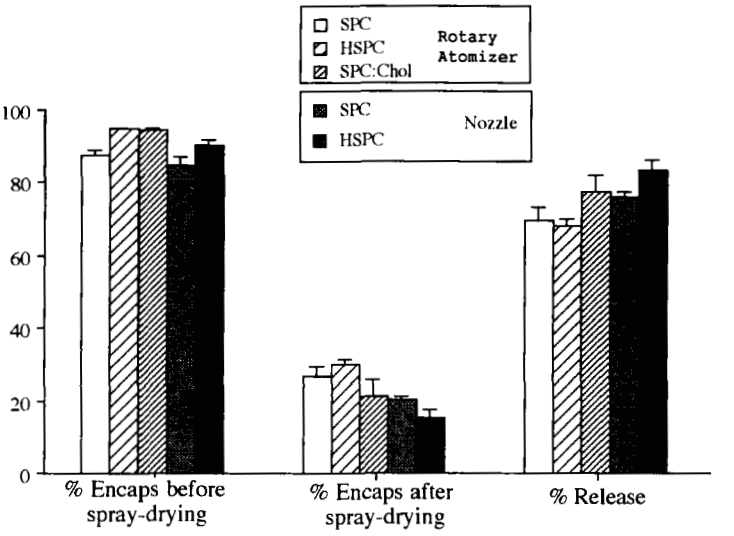

FIGURE 3

Percentage of atropine sulphate encapsulated in the vesicles before and after spray-drying with a rotary atomizer or a nozzle and percentage of drug release. Mean ± SD, n=2.

These results are not in accordance with those of Hauser et al. [8] who found that 90 % of the originally entrapped material (raffinose and $K_3Fe(CN)_6$) remained entrapped in small unilamellar vesicles (SUV) during spray-drying. A possible explanation of this leakage is that the drug crosses the lipid bilayers with the water at the time of the drying step at 110°C, the fluidity being extremely high at this temperature. The leakage could also occur during rehydration as it was hypothesized by Crowe et al. [9] in their work about liposomes subjected to freeze-drying. A mechanical bursting of the vesicles under the effect of the dispersion system is less probable since the vesicle size distribution was not affected by the process and the kind of dispersion system used had no influence. Studies investigating the influence of the drying temperature and the nature of the protective agent are currently under way.

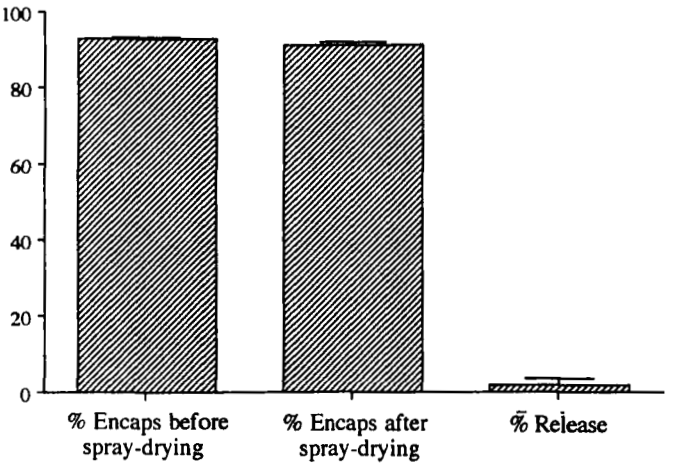

FIGURE 4

Percentage of α-tocopherol incorporated in the vesicles before and after spray-drying with a rotary atomizer and percentage of drug release. Mean ± SD, n=2.

Retention of Incorporated α-Tocopherol

Liposomes containing 1 mg/ml α-tocopherol were spray-dried with a rotary atomizer. α-Tocopherol is usually included in liposome formulations as anti-oxidizing agent [10].

The particle mean diameter of the spray-dried product was 7.4 μm. The mean diameter of the vesicles was 191 (±49) nm before spray-drying and 170 (±28) nm after rehydration.

The incorporation of α-tocopherol in the liposomes was quantified before and after spray-drying, following separation of the non entrapped material by a Ficoll flotation method. It was found that 92 % of the drug was localized in the vesicles after preparation of the liposomes (Figure 4). These data hardly changed after spray-drying, as 91 % of the drug was still incorporated in the vesicles after dehydration; the percentage of drug release was determined to

be 1-2 %. Thus, spray-drying is an effective means to dehydrate liposomes incorporating lipophilic compounds such as α-tocopherol without loss of the entrapped material.

CONCLUSION

Stable dried liposomes can be produced by a spray-drying process in the presence of 10 % lactose. The particle size distribution of the spray-dried product obtained with a nozzle seems to be acceptable for a pulmonary application since the particle mean diameter was 3.5 μm. As shown previously [2], the vesicle size distribution was about the same before and after dehydration.

When atropine sulphate was used as a hydrophilic drug model, about 65 to 80 % of the entrapped material was released during spray-drying. The lipid composition of the vesicles and the dispersion system used had no influence on drug retention. These results could probably be improved by varying the drying temperature and/or by using protective agents other than lactose.

On the other hand, it was shown that α-tocopherol used as a lipophilic drug model was effectively incorporated in the liposomes with a trapping efficiency greater than 90 %. Furthermore, the spray-drying-rehydration cycle did not induce any significant leakage of the entrapped material.

Thus, spray-drying constitutes an interesting alternative to the more frequently reported freeze-drying process, especially when lipophilic drugs are incorporated in the vesicles. Our approach is very new in the sense that it allows the production of small sized, spray-dried liposomes which could be inhaled and delivered to the respiratory tract by means of a suitable device. In contact with the moist surface of the airways, the vesicles would be rehydrated *in situ* and release their content at a controlled rate in the lung.

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REFERENCES

1. I. Gonda, Crit. Rev. Ther. Drug. Car. Syst, 6, 273 (1990).
2. P.Goldbach, H. Brochart, and A. Stamm, submitted.
3. R.R.C. New, "Liposomes: a practical approach", IRL Press, Oxford, 1990.
4. J.C.M. Stewart, Anal. Biochem., 104, 10 (1980).
5. K.M.G. Taylor, G. Taylor, I.W. Kellaway, and J. Stewens, Int. J. Pharm., 58, 49 (1990).
6. D. March, A. Watts, and P.F. Knowles, Biochimistry, 15, 3570 (1976).
7. B. De Gier, J.G. Manderslott, and L.L.M. Van Deenen, Biochim. Biophys. Acta., 219, 245 (1970).
8. H. Hauser and G. Strauss, Biochim. Biophys. Acta, 897, 331 (1987).
9. L.M. Crowe, J.H. Crowe, A. Rudolph, C. Womersley, and L. Appel, Arch. Biochem. Biophys., 242, 240 (1985).
10. G.S. Wu, R.A. Stein, and J.F. Mead, Lipids, 17, 403 (1986).