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SPRAY-DRYING OF LIPOSOMES FOR A PULMONARY ADMINISTRATION. I. CHEMICAL STABILITY OF PHOSPHOLIPIDS

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ABSTRACT

Multilamellar vesicles constituted of soybean phosphatidylcholine (SPC) and extruded through a 0.2 μm polycarbonate membrane were spray-dried in the presence of 10 % lactose. The particle mean diameter of the spray-dried product was about 7 μm when the liposomes were dispersed with a rotary atomizer. The dry residue can be resuspended in water to reconstitute liposomes without major change to the vesicle size distribution. Moreover, the chemical stability of the phospholipids was not significantly affected by this process, both in terms of hydrolysis and oxidation. It was concluded that spray-drying is a potentially interesting means to produce stable dried liposomes that could be administered to the lung by inhalation.

INTRODUCTION

The administration of drugs by inhalation is an effective means for delivering relatively small quantities of pharmacologically active compounds directly to their target site [1]. Nevertheless, most of the drugs are rapidly absorbed from the lung and pass into the systemic circulation [2,3]. This can explain the relatively short effect of inhaled drugs, requiring frequent dosing, and the occurrence of unwanted systemic side effects [4].

Recently, liposomes have gained interest as drug delivery systems for the respiratory tract [5]. Different studies have shown that the incorporation of drugs such as cytosine arabinoside [6], metaproterenol [7], sodium cromoglycate [8], or gluthathione [9] in lipid vesicles allows an increase in the drug residence time in the lung while minimizing the uptake to the systemic circulation. One major method of administering liposomes to the lung is the inhalation of an aerosol generated from an airblast nebulizer [8,10]. However, this method requires a costly device and is not suitable for ambulant treatment.

The aim of this work was to investigate the feasibility of self-contained system to deliver liposomes into the respiratory tract. The development of a dry dosage form appeared the most interesting with a view of avoiding the long-term stability problems. The dehydration of liposome dispersions can be performed by freeze-drying in the presence of cryoprotectants [11-14] or by spray-drying [15]. The latter process seemed the most adequate for our purpose since it allows to obtain small sized particles [16] which could be administered to the respiratory tract by means of a suitable device such as a breath-actuated powder-inhaler or a pressurized dose-aerosol. The inhaled particles would be rehydrated in contact of the moist surface of the airways and the vesicles reconstituted *in situ*.

The present study reports preliminary observations following the dehydration of a liposome dispersion by spray-drying. The

particle size of the spray-dried product, the vesicle size distribution and the chemical stability of the phospholipids were investigated.

MATERIALS AND METHODS

Preparation of Liposomes

Purified and highly purified soybean phosphatidylcholine (SPC) (Epikuron 200 and 200 S, respectively) were obtained from Lukas Meyer Inc. (Hambourg, FRG).

A liposome dispersion containing 20 mg/ml of Epikuron 200 S was prepared in the presence of 10 % lactose by an extrusion technique similar to that described by Olson et al. [17]. Briefly, the lipids were dissolved in methanol into a round bottom flask containing glass beads. Organic solvent was slowly removed at reduced pressure on a rotary evaporator at 30°C, until a thin film of dry lipids was formed on the beads and walls of the flask. The film was hydrated at room temperature for 30 min with 100 ml of 15 mM phosphate buffer (pH 6.8) containing 10 % lactose. The resultant liposome dispersion was maintained at room temperature for 2 h under nitrogen and then extruded three times consecutively through a 0.2 μm polycarbonate membrane filter of 90 mm diameter (Nuclepore Corp., Pleasanton, CA, USA).

Spray-drying of Liposomes

A spray-drier Minor Mobile (Niro Atomizer, Copenhagen, Denmark) equipped with a rotary atomizer was used for the dehydration of the liposome dispersion. The operating conditions were following:

- Flow rate of the feed : 10 ml/min
- Pressure at the atomizer : 6 Bars
- Inlet temperature : 110 °C
- Outlet temperature : 75-80 °C

The liposome were rehydrated in the appropriate amount of demineralized water to give the initial concentration of phospholipids.

Particle Size of the Spray-dried Product

The spray-dried product was examined by optical microscopy. The size of about 100 particles was measured for the determination of the mean diameter (MD). It was not possible to define a standard deviation as the particle size distribution did not fit a normal or log-normal distribution. The aspect of the particles was also checked by scanning electron microscopy (SEM).

Characterization of the Vesicles

The vesicle size was determined by dynamic light scattering, performed using a laser particle sizer (Model N4 SD, Coulter Electronics Ltd., Luton, U.K.). This apparatus provides a mean diameter (MD) of the vesicles in combination with a standard deviation (±SD). Liposomes were diluted in 15 mM phosphate buffer (pH 6.8) containing 10 % lactose. The viscosity was set at 1.3 centipoises on the apparatus because of the presence of lactose in liposome suspension and phosphate buffer.

Phospholipid Content

Phospholipid concentrations were determined by first extracting the lipids according to the method of Bligh-Dyer [18] and then performing the Stewart assay [19], where the ability of phospholipids to form a complex with ammonium ferrothiocyanate in organic solution is utilized.

Chemical Stability of Phospholipids

The chemical stability of phosphatidylcholine during dehydration was investigated by subjecting the liposomes to a spray-drying-rehydration cycle.

The formation of lysolecithins was visualized by thin layer chromatography (TLC) using a silica gel plate (Kieselgel 60 F254, Merk AG, Darmstadt, FRG) [20]. The eluting solvent was a mixture of chloroform : methanol : water : ammonium hydroxide (65 : 35 : 2.5 : 2.5 V/V). Standard dispersions of phospholipids (20 mg/ml) were prepared by dispersing Epikuron 200 S (containing 0.3 % lysolecithins) or Epikuron 200 (containing 1.8 % lysolecithins) in 15 mM phosphate buffer (pH 6.8) containing 10 % lactose. The lipids of the standard dispersions, as well as those of the fresh and dehydrated liposomes, were extracted by chloroform according to the Bligh-Dyer method. Samples of the chloroformic solutions were deposited on the plate and after 45 min of migration the lipids were visualized with the molybdenum blue spray (Sigma).

Oxidation of the phospholipids was estimated by spectrophotometry [21]. The fresh and spray-dried liposomes, as well as a standard dispersion of Epikuron 200 S, were diluted in absolute ethanol; the absorbance spectra were recorded in the ultra-violet range (UV) from 400 to 200 nm using a Shimadzu UV-2101 PC spectrophotometer (Shimadzu Corp., Kyoto, Japan). The absorption was measured at 215 and 233 nm and an "oxidation index" was defined as the ratio $A_{233\ nm}/A_{215\ nm}$.

RESULTS AND DISCUSSION

The liposome dispersion was prepared and spray-dried in the presence of 10 % lactose. This disaccharide has been used extensively in the literature on freeze-drying of liposomes [11,13]. Lactose was used here as bulking agent to facilitate spray-drying, both by drawing water from the liposomes in the terminal stages of drying, and by reducing the tendency of the vesicles to aggregate.

Particle Size of the Spray-dried Product

A typical micrograph of the dry residue is shown on figure 1. The particles have a spherical shape and are relatively small sized.

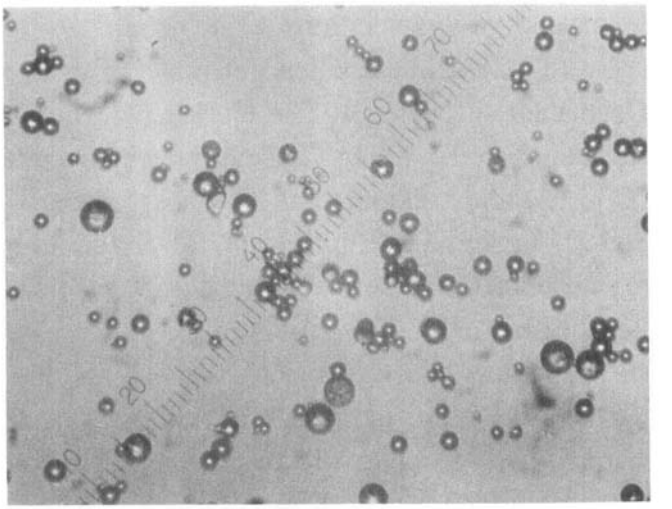

FIGURE 1

Optical micrograph (200x) of particles obtained by spray-drying of liposomes composed of SPC in the presence of 10 % lactose. The spray-drier was equipped with a rotary atomizer as dispersion system. One graduation on the scale corresponds to 6.8 μm.

The particle mean diameter is 7.1 μm according to the cumulative frequency curve (Fig. 2). Since it is generally accepted that the optimum size for inhaled drug particle ranges between 0.5 and 5 μm [22], only a relatively small fraction of the spray-dried particles would reach the lower airways. These results could certainly be improved by using a nozzle as dispersion system to produce smaller particles.

The scanning electron microscopy analysis shows particles which are globally spherical, but the lipid vesicles, which are certainly included in the lactose matrix, are not visible (Fig.3).

Vesicle Size Distribution and Lipid Content

The vesicle size distribution was determined before and after spray-drying (Table 1). The mean diameter and the standard

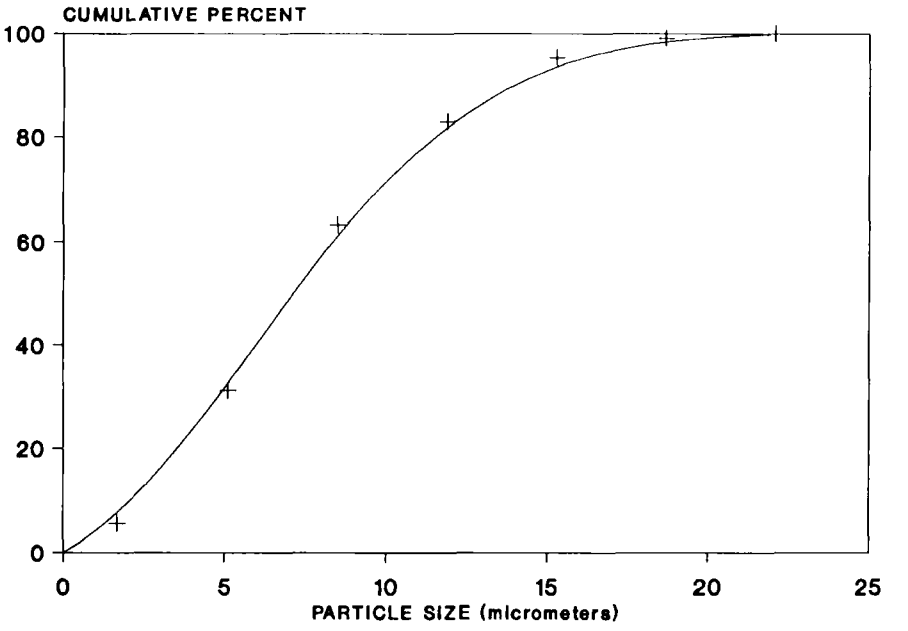

FIGURE 2

Cumulative frequency curve of the spray-dried particles obtained with a rotary atomizer.

deviation were not significantly modified by the dehydration-rehydration cycle, showing that the spray-drying process does not lead to an important aggregation and/or fusion of the vesicles. These results are in accordance with those of Hauser et al. [15] who found that the size of small unilamellar vesicles (SUV) was about the same before and after spray-drying.

The lipid content, determined in the fresh and reconstituted dispersion, was close to a standard dispersion containing 20 mg/ml of Epikuron 200 S, as shown in table 1.

Chemical Stability of Phospholipids during Spray-drying

From the point of view of stability, two main decomposition pathways for phospholipids were investigated: hydrolysis and oxidation.

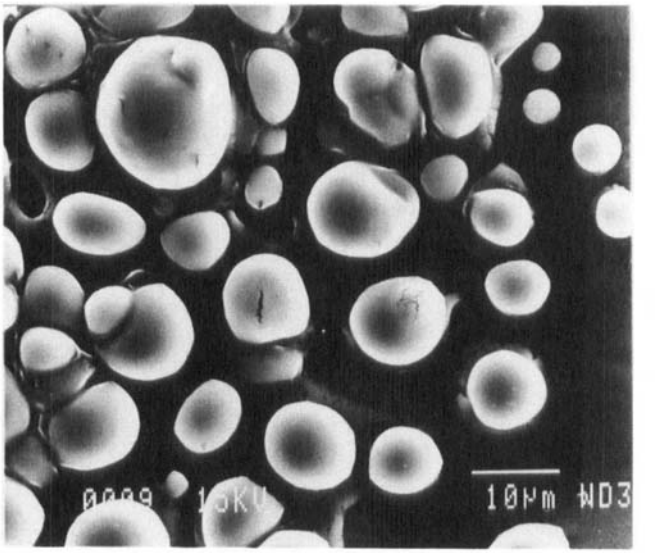

FIGURE 3

Aspect of the spray-dried product by scanning electron microscopy. The liposomes were composed of SPC and spray-dried in the presence of 10 % lactose with a rotary atomizer.

TABLE 1
Vesicle Size Distribution and Lipid Content of the Liposome Dispersion before and after Spray-drying.

	Mean diameter nm (±SD)	Lipid content mg/ml
Before spray-drying	197 (±46)	19.82
After spray-drying	188 (±36)	20.70

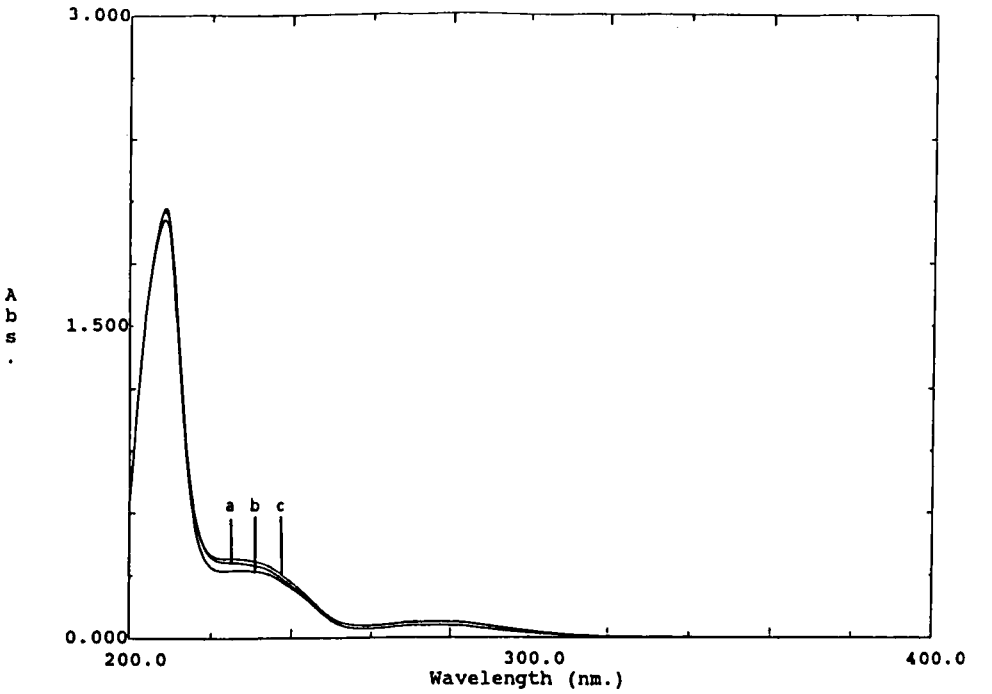

FIGURE 4

Ultra-violet absorption spectra of SPC dissolved in absolute ethanol. Spectra of standard Epikuron 200 S (a) and liposomes before (b) and after (c) spray-drying.

Lysolecithin is the major hydrolysis product of lecithin, in which one fatty acid chain is lost by de-esterification [23]. Lysophospholipids can be separated and visualized by TLC. Two standard lecithins (containing 0.3 % and 1.8 % lysolecithins) as well as the fresh and rehydrated liposome dispersions (containing 0.3 % lysolecithins) were chromatographied after extraction of the lipids. A secondary spot corres-ponding to the degradation product was clearly apparent for the standard lecithin containing 1.8 % lysolecithins, whereas it was barely visible for the standard lecithin containing 0.3 % of lysoproduct. Furthermore, the intensity of the

secondary spots was not increased for both the fresh and spray-dried liposomes, showing that phosphatidylcholine was not significantly hydrolyzed during the manufacturing and the spray-drying of the lipid vesicles.

The apparition of oxidative reactions resulting from the formation of conjugated dienes was also examined [21]. Indeed, natural phospholipids contain only double bonds which are non-conjugated, and thus have an ultra-violet absorbance peak at a very short wavelength (200-205 nm). The initial step in oxidation of acyl chains is supposed to involve a free radical chain mechanism which leads to bond migration and diene conjugation. Since conjugated dienes absorb at 233 nm, the occurrence of oxidative reactions can be monitored by measuring of the change in absorbance at this wavelength. The ultra-violet spectrum of a standard dispersion of Epikuron 200 S shows that the lipids were already moderately oxidized by the time of use (Figure 4). Nevertheless, the spectra of the liposome dispersions were about the same before and after spray-drying. The oxidation index ($A_{233\ nm}/A_{215\ nm}$) was found to be 0.476 for the standard lecithin, 0.483 for the fresh liposomes and 0.489 after spray-drying. Thus, the lipids were not significantly oxidized during the dehydration process.

CONCLUSION

This study shows that liposome dispersions can be dehydrated into the form of small sized particles by a spray-drying process. Particles ranging from 1 to 20 μm (7 μm mean diameter) were generated when a rotary atomizer was used as dispersion system.

The vesicle size distribution was about the same in the fresh dispersion and after rehydration of the dry residue with the appropriate amount of demineralized water. Moreover, phosphatidylcholine was not significantly hydrolyzed or oxidized by the drying process at 110°C, probably by virtue of the shortness of the exposure to the heat.

Studies investigating the retention of encapsulated materials in liposomes during spray-drying and the use of a nozzle as dispersion system are now under way.

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