AIR-JET AND VIBRATING-MESH NEBULISERS FOR THE DELIVERY OF LIPOSOMES GENERATED FROM ETHANOL-BASED PROLIPOSOemes

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Introduction

• Definition of liposomes

Liposomes are carrier phospholipid vesicles of size range between approximately 0.05 and 10 µm, normally dispersed in hydrophilic solvent, typically water.

• Types of liposomes (Fig.1)

1- Multilamellar liposomes (MLVs)
2- Large unilamellar liposomes (LUVs)
3- Small unilamellar liposomes (SUVs)
Preparation of liposomes by ethanol-based proliposome method

• Definition of ethanol-based proliposomes
These are ethanolic phospholipid preparations which generate liposomes by addition of an aqueous phase (Perrett et al., 1991).

• Advantages of using proliposome method
1- High entrapment of hydrophilic materials
2- Relatively cheap
3- Convenient to prepare
4- Time saving
• Liposomes as carriers for pulmonary drug delivery

1- Localise the drug action within the lungs for prolonged periods of time (Farr et al., 1985 and Taylor et al., 1989).

2- Consequent minimisation of systemic adverse effects.

• Inhalation devices

1- Metered-dose inhalers

2- Dry powder inhalers

3- Nebulisers
• **Types of nebulisers**

• **1- Air-jet nebulisers** (Fig. 2)

These utilise Compressed gas (air or oxygen) converts aqueous solutions or suspensions into a spray suitable for inhalation.

• **2- Ultrasonic nebulisers**

The energy for atomising liquids comes from a piezoelectric crystal vibrating at high frequency.

• **3- Vibrating-mesh nebulisers** (Figs. 3-5)

Liquid is aerosolised by using a vibrating mesh or plate with multiple apertures.
Aim of the study

This work aims to investigate the deliverability of liposomes generated from ethanol-based proliposomes to a model *in vitro* pulmonary system (Two-stage Impinger), and to study the influence of nebuliser design and technology on the formulation mass output and phospholipid output and distribution in the impinger. Two nebulisers were used, a model air-jet nebuliser (Pari LC Plus) and a vibrating-mesh nebuliser (Omron Micro Air NE U22).
Materials and methods

• Setting the Two-stage Impinger

The impinger was set up at 60 L/min vacuum (Fig.6). The 1\textsuperscript{st} stage (upper) Impinger represents the upper airways (mouth, throat, and main bronchus), and the 2\textsuperscript{nd} stage (Lower) Impinger represents the deep lung (bronchioles and alveoli). Sodium chloride (0.9% w/v) was used as a collection medium with 7 ml in the 1\textsuperscript{st} stage and 30 ml in the 2\textsuperscript{nd} stage Impinger.
• **Generation of liposomes from ethanol-based proliposomes**

A clear ethanolic proliposome solution was made by dissolving soya phosphatidylcholine and cholesterol (1:1 mole ratio) in absolute ethanol (phospholipid: ethanol, 100:120 w/w) and heating at >60°C for 1 min. Isotonic sucrose solution (5 ml) was added at ambient temperature with manual shaking for 1 min.

• **Nebulisation of liposomes and microscopy study**

Nebulisation commenced to ‘dryness’ and samples were collected from the nebuliser reservoirs, and both stages of the impinger for microscopy.
• Determination of nebuliser mass and phospholipid output

Mass of formulation emitted from nebulisers was determined by difference. Stewart assay (Stewart, 1980) was used to determine phospholipid output and phospholipid distribution between nebulisers and Impinger.

• Measurement of aerosols droplet size using laser diffraction

Using laser diffraction, aerosols droplet size was measured.
Results and discussions

• Transmission Electron Microscopy (TEM) showed the formation of MLVs in nebuliser reservoirs and Impinger stages (Fig. 7).

• High mass and phospholipid outputs were found when both nebulisers were used with a higher mass output from Omron NE U22 vibrating-mesh nebuliser and higher phospholipid output from Pari LC Plus jet nebuliser (Fig. 8).

• Phospholipid distribution study showed that more phospholipid deposited in the 2nd stage Impinger when Pari LC Plus was used (Fig. 9).
The bar chart shows the output (%) comparison between Pari Plus and Omron nebulisers for Total and Phospholipid.

- **Pari Plus**
  - Total: A significant output percentage, indicated by the bar height.
  - Phospholipid: A notable output percentage, also indicated by the bar height.

- **Omron**
  - Total: A very high output percentage, shown by the taller bar.
  - Phospholipid: A high output percentage, shown by the shorter bar.

The chart highlights the percentage output for both Total and Phospholipid across the two nebuliser types, with error bars indicating the variability.
• The impinger findings were consolidated by droplet size measurement using laser diffraction since the volume median diameter of droplets emitted from Pari LC Plus nebuliser was smaller than that of droplets emitted from Omron NE U22 nebuliser (Fig.10).
Pari Plus Omron Nebuliser type

droplet median size, µm

- Pari Plus
- Omron

Nebuliser type
Conclusions

• Liposomes generated from ethanol-based proliposomes were multilamellar when samples from nebuliser reservoirs or impinger were taken.

• Proliposome method provided a convenient means of delivering liposomes to an in vitro pulmonary model when air-jet or vibrating-mesh nebulisers were used with a high mass and phospholipid outputs, and high phospholipid mass deposited in the 2nd stage Impinger.
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