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An investigation of some of the factors influencing the jet nebulisation of liposomes

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Abstract

Multilamellar egg phosphatidylcholine liposomes with or without cholesterol have been aerosolised using four jet nebulisers. The size of aerosols generated from liposome suspensions, as measured by laser diffraction, was independent of liposome size and bilayer composition. However, increasing the phospholipid concentration caused an increase in the median size of the secondary aerosol size, although the extent of this effect was dependent on the design on the nebuliser. The total mass output of liposomal aerosols was similar for the Pari-LC and Sidestream nebulisers, though the rate of output was higher for the Sidestream. In both cases, increasing lipid concentration produced a reduced rate of aerosol output. For all the nebulisers studied, a size selective process was found, resulting in the retention of the largest liposomes.

Keywords: Aerosol; Liposome; Nebuliser; Particle size; Phospholipid

1. Introduction

Nebulisers have been extensively researched for the pulmonary administration of liposomes. They are simple to use and capable of delivering a large volume of therapeutic aerosol to sites deep within the lung. Unlike metered dose inhalers (MDI) or dry powder inhalers (DPI), liposomes may be delivered from nebulisers without further processing (Taylor and Farr, 1993).

There are a wide range of jet nebulisers available and their performance (e.g. droplet size, nebulisation time, output etc.) may vary significantly as a result of differences in design and construction (Waldrep et al., 1994; McCallion et al., 1995). The ideal aerosol characteristics depend on a number of factors, but the consensus is that nebulised droplets with a mean size $\leqslant 5$ μm are required for drug delivery to the respiratory lung regions (Nebuliser Project Group, 1997). However, targeting of specific sites within the lung may require a more precise control of droplet size. Other factors that may be important include the aerosol output rate. For nebulised liposomal systems, due consideration must also be given to the

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liposomal properties. For instance, liposome size may influence vesicle stability and thus, retention of entrapped materials (Taylor et al., 1990; Niven et al., 1991).

The aim of this study was to investigate some of the nebuliser device and formulation parameters which influence nebulisation of liposomes. The formulation variables specific to the liposome component of nebulised systems were studied, as it is known that the physicochemical properties of fluids influence their ability to form droplets (Mercer, 1973; Davis, 1978; McCallion et al., 1995). Aerosolisation of liposomes using different jet nebuliser systems was also examined.

2. Materials and methods

2.1. Materials

Unless otherwise stated, materials used in this study were AnalaR grade and obtained from BDH (UK). Egg phosphatidylcholine (EggPC, Lipoid Ltd., Italy) was refined by chromatographic purification (Bangham et al., 1974). Water was deionised (Whatman WR50 RO/Deioniser, Whatman, UK). Nebulisers were obtained from the following suppliers: Cirrus (Intersurgical, UK), Pari-LC (Pari Werk, Germany), Respirgard II (Marquest, USA), Sidestream Durable (Medic-Aid, UK).

2.2. Preparation of liposomes

Multilamellar liposomes were prepared from eggPC alone or with equimolar quantities of cholesterol (eggPC/chol), by hydration of thin films as described previously (Leung et al., 1996). Final concentrations of between 2.5 and 80 mg lipid component per millilitre of aqueous phase were prepared. The size of liposomes was reduced by repeated extrusion through polycarbonate membrane filters (Nucleopore, USA), pore size 1, 2.5 or 5 μm, held in 25 mm holders, until the volume median size, as measured by laser Fraunhofer diffraction (Malvern 2600C, Malvern Instruments, UK), approximated the pore size of the filter.

2.3. The effect of lipid concentration on droplet size

Cirrus, Pari-LC and Sidestream jet nebulisers were used to aerosolise eggPC and eggPC/chol (1:1) liposomes (1 and 5 μm median size). Each nebuliser was driven at an appropriate driving gas flow rate to produce a mean droplet volume median diameter (VMD) of ≈ 2–4 μm (McCallion et al., 1996). Nitrogen gas was delivered at a flow rate of 8 l/min for Cirrus and Pari-LC nebulisers and at 7 l/min for Sidestream. Aerosol was drawn through the laser of the Malvern 2600C diffraction sizer and the size distribution of the aerosol, prior to the sputtering phase of aerosol production, was determined using a 63 mm lens. The instrument's software expresses particle size as the VMD, i.e. the equivalent sphere diameter above and below which 50% of the volume of particles lies and the size distribution is expressed as a span value [(90% undersize − 10% undersize)/50% undersize)].

2.4. The effect of lipid concentration on aerosol output

EggPC and eggPC/chol (1:1) liposomes, mean size 1 and 5 μm, were aerosolised using Pari-LC and Sidestream nebulisers, at a driving gas flow rate of 5 l/min. The time taken for each nebuliser to reach the sputtering phase and to cease producing aerosol entirely, was recorded for 10 mg/ml eggPC/chol (1:1) liposomes. Aerosol output from each nebuliser was determined at 50 and 100% of the time to sputtering and also following 2 min of sputtering, by mass measurements.

2.5. Change in concentration of liposomes during nebulisation

A 30 mg/ml aqueous solution of sodium chloride was prepared and 5 ml nebulised with the Pari-LC, Cirrus, Sidestream, and Respirgard II nebulisers, operated at 7 l/min. A 1 ml sample of the residual sodium chloride solution was withdrawn from each of the nebuliser chambers at the sputtering time. The sodium chloride content was determined by weight and compared with non-

nebulised samples. These experiments were then repeated using variously sized eggPC/chol (1:1) liposomes with a lipid concentration of 10 mg/ml.

2.6. Examination of nebuliser component orifices

Various internal components from each nebuliser were removed, mounted on microscope stubs and vacuum coated with a 30 nm thick layer of gold. Scanning electron micrographs were then taken at an accelerating voltage of 10–15 kV (Phillips XL20, Phillips, UK) and dimensions of the components measured.

3. Results and discussion

3.1. The effects of lipid concentration on droplet size

There was a direct correlation between droplet size and lipid concentration for all the formulations tested, with increasing concentrations associated with an increase in emitted droplet size (Fig. 1). Droplet formation in a jet nebuliser is aided by the shear energy of gas flow, employed to overcome the viscous and surface tension forces of the fluid. The viscosity and surface tension of a liquid

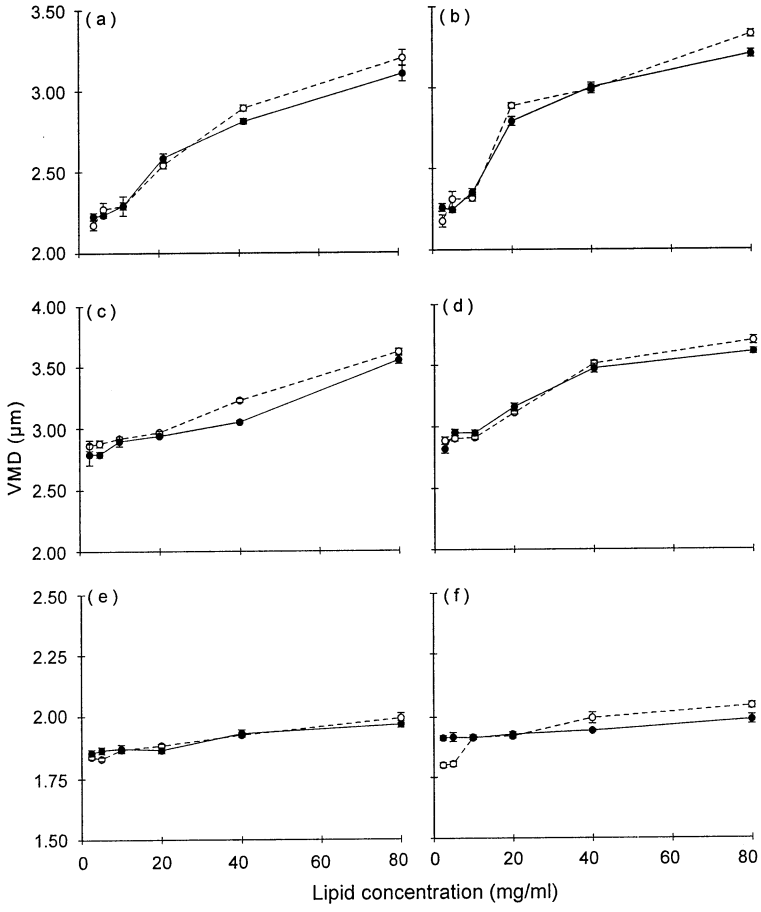

Fig. 1. Effect of lipid concentration on mean VMD of aerosols produced from the nebulisation of (○) eggPC and (●) eggPC/chol liposomes. (a) Cirrus, 5 μm liposomes; (b) Cirrus, 1 μm liposomes; (c) Pari-LC, 5 μm liposomes; (d) Pari-LC, 1 μm liposomes; (e) Sidestream, 5 μm liposomes; (f) Sidestream, 1 μm liposomes ($n = 3$; ± S.D.).

being nebulised may thus alter the properties of the aerosol generated. However, size-selectivity of the nebuliser design results in recycling of the majority of the primary aerosol mass, such that changes in the size distribution of the primary aerosol resulting from variations in fluid properties are not necessarily reflected in the size of the secondary aerosol that is emitted from the nebuliser (Mercer, 1981). The increase in median droplet size was dependent on the nebuliser used (Fig. 1). Aerosols produced by the Sidestream were least affected by concentration changes, showing a mean increase in droplet size of $\approx 8\%$ over the concentration range. This may be due to the highly efficient baffling systems of the Sidestream, which compensate for increases in primary aerosol size by filtering out an increased proportion of droplets. The Pari LC gave an average increase of $\approx 30\%$ and the Cirrus $\approx$ 50%. For each nebuliser, inclusion of equimolar cholesterol had a small but significant ($P < 0.05$) effect on the mean droplet size, generally producing slightly smaller aerosols. For the three nebulisers studied there was no apparent correlation between the size of the liposomes or the inclusion of cholesterol with the emitted droplet size, as previously described (Farr et al., 1985; Taylor et al., 1990; Niven et al., 1991).

Viscosity of liposome dispersions is directly proportional to lipid concentration (Bridges et al., 1995) with preparations having higher viscosity producing larger droplets. An inverse relationship between solution viscosity and droplet size, in two jet nebulisers including the Pari-LC, over a similar viscosity range has previously been demonstrated (McCallion and Patel, 1996), suggesting that properties of liposomes themselves, rather than simply viscosity, may be responsible for the observed increase in droplet size with elevated concentration. All aerosols were heterodisperse, with span values of between 1.7 and 2.6, not significantly changed with increasing liposome concentration.

Several studies have demonstrated that suspensions yield significantly different aerosol droplet sizes, when atomized, to those given by simple solutions. For instance, a change in formulation from solution to suspension yielded a 95% increase in droplet size from a MDI (Dalby and Byron, 1988). This was attributed to aggregation of the suspended particles, present as multiple inclusions within the aerosolised droplets. Although a nebuliser operates differently from an MDI, liposome aggregation in the more concentrated samples may have led to the observed increase in droplet size. Further, Marks et al. (1983) suggested that the surface active nature of phospholipids may reduce water transport from a droplet. This may be due to formation of a condensed film at the air–water interface and/or as a result of phospholipid–water hydrogen bonds in the liquid bulk. More concentrated liposomes would provide a greater barrier to water transport and thus maintain a larger droplet size when dispersed in higher concentrations.

3.2. The influence of lipid concentration on output

In clinical practice, nebulisers are generally operated to 'dryness', the point at which no further useful aerosol is generated, although some residual fluid always remains in the nebuliser reservoir. The output of fluid, which dictates the total dose delivered, depends on the nebuliser design and the physicochemical properties of the fluid nebulised. The output of fluid from the Pari-LC nebuliser ranged from 47.2 to 77.1% of the total liposome mass added (Table 1), whilst output from the Sidestream ranged from 51.2 to 84.8%. For eggPC/chol liposomes at any concentration or size, fluid output was greatest for the Sidestream nebuliser and in all cases decreased with increasing phospholipid concentration.

An increase in lipid concentration from 5 to 80 mg/ml produced approximate falls in output of 10 and 37% for the 1 and 5 μm liposomes, respectively, nebulised with the Pari-LC. A similar change in lipid concentration yielded a 14 and 37% reduction in output for these liposomes nebulised with the Sidestream. The fall in output with increasing lipid concentration proved to be significant ($P < 0.05$) for both liposome formulations nebulised with the Sidestream, but only for the 5 μm mean size liposomes nebulised with the Pari-LC.

The Sidestream nebuliser may not resist the lipid concentration-induced droplet size changes through the filtering action of its baffles alone.

Table 1
Output (percentage of initial total mass in nebuliser) of eggPC/chol liposomes from Pari-LC and Sidestream nebulisers[a]

Lipid concentration (mg/ml)	Output (%)			
	Pari-LC		Sidestream	
	1 μm	5 μm	1 μm	5 μm
5	74.9 (±4.5)	74.9 (±7.0)	84.8 (±1.8)	81.6 (1.1)
10	72.6 (±9.5)	72.4 (±6.4)	82.4 (±3.3)	83.8 (5.1)
20	77.1 (±12.7)	64.0 (±14.3)	83.0 (±4.8)	77.1 (5.4)
40	71.3 (±12.0)	65.6 (±12.6)	83.4 (±4.4)	79.6 (3.0)
60	71.7 (±15.3)	70.0 (±9.8)	79.7 (±8.7)	52.4 (3.9)
80	67.4 (±15.0)	47.2 (±16.0)	73.3 (±7.7)	51.2 (9.5)

[a] $n = 4 \pm$ S.D. for 1 μm and $n = 5 \pm$ S.D. for 5 μm liposomes.

Other factors, such as the diameter and geometry of capillaries, jets and other internal structures, may additionally be involved. In particular, the open vent design of the nebuliser means that supplementary gas drawn through the vent results in droplet shrinkage due to evaporation, with an associated improvement in nebuliser performance (O'Callaghan and Barry, 1997). Multiple aggregates within the droplets may inhibit evaporation and hence more become retained within the device. However, the baffles will still be responsible for filtering out the larger droplets that are so produced. Alternatively, reduced aerosol output at higher lipid concentrations may be due to a reduced tendency for the Sidestream nebuliser to damage liposome bilayers, resulting in liposomes with a mean size larger than the aerosol size being retained within the nebuliser. This is supported by the observation that the 5 μm liposomes yielded a greater reduction in output as the liposome concentration became raised, compared with the 1 μm formulation (Table 1). However, previous work suggests that there is little difference in the damage done by the Pari-LC and Sidestream nebulisers to liposomes (Bridges and Taylor, 1998).

The results suggested that a size selective process was occurring in the release of liposomes from each nebuliser. The mean droplet size of the nebulisers studied was ≈ 2 μm and thus a greater proportion of the relatively rigid 5 μm eggPC/chol liposomes failed to become aerosolised. The polydisperse nature of liposomal aerosols means that a significant fraction of large liposomes in the 5-μm mean size formulations could potentially be aerosolised. In addition, liposome size reduction may occur (Taylor et al., 1990). Despite this, the great majority of large liposomes become included in the larger droplets of the primary aerosol that are recycled within the device, reducing the total aerosol output. Reduction of the mean liposome size to 1 μm ensured that the majority of the liposomes were aerosolised and consequently, aerosol output was increased. The Pari-LC nebuliser generated liposomal aerosols containing a greater spread of droplet sizes than the Sidestream device. In these studies, span values ranged from 2.30 to 2.56 for the Pari-LC and from 1.74 to 2.27 for the Sidestream. Consequently, more of the 5 μm liposomes were emitted from the Pari-LC and so there was less of a difference in output between the two liposome sizes. Retention of larger liposomes within each nebuliser would increase their exposure to shear forces within the device and create the possibility of significant bilayer instability.

The rate of aerosol output from both the Pari-LC and Sidestream nebulisers was reduced in the latter half of nebulisation, prior to sputtering for each of the formulations studied (Figs. 2 and 3). This is a result of a reduction in residual volume and an increase in fluid viscosity (Clay et al., 1982). The difference in aerosol output between the initial and later stages of nebulisation was reduced for the formulations that had highest lipid concentration (i.e. 40, 60 and 80 mg/ml).

Concentration and temperature changes during nebulisation are likely to have a smaller relative effect for such high viscosity fluids.

3.3. Concentration of liposomes during nebulisation

Jet nebulisation increased the concentration of sodium chloride solutions that remained within the fluid reservoir of each nebuliser during operation, by 14% in the Pari-LC nebuliser, to as much as 43% in the Respirgard II. This is attributable evaporative loss of water due to flow of high velocity, unsaturated gas through the nebulisers (Ferron et al., 1976).

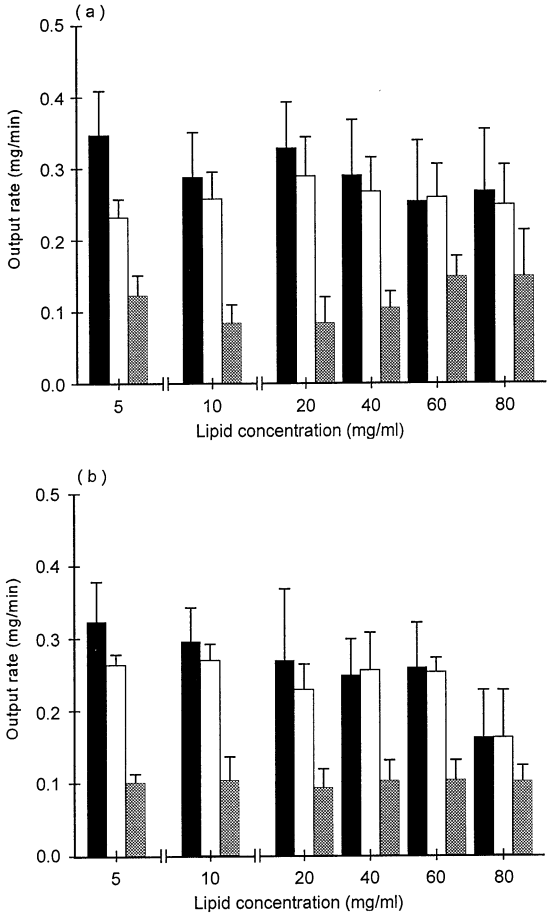

Fig. 2. Rate of fluid output from Pari-LC nebuliser during nebulisation of (a) 1 μm and (b) 5 μm eggPC/chol (1:1) liposomes, at (■) 0–50% of sputtering time, (□) 50–100% of sputtering time, (⊠) initial 2 min of sputtering ($n = 4$; ± S.D.).

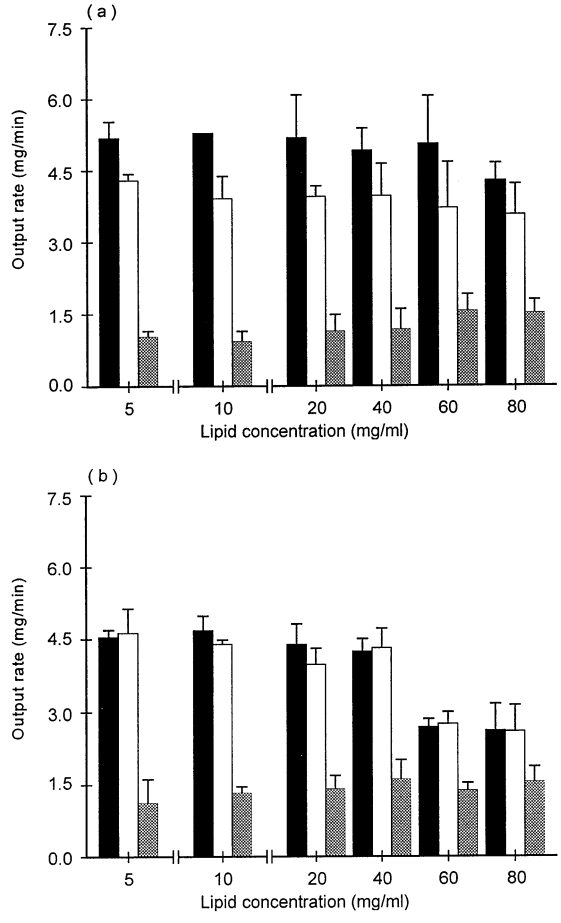

Fig. 3. Rate of fluid output from Sidestream nebuliser during nebulisation of (a) 1 μm and (b) 5 μm eggPC/chol (1:1) liposomes, at (■) 0–50% of sputtering time, (□) 50–100% of sputtering time, (⊠) initial 2 min of sputtering ($n = 4$; ± S.D.).

The results of the increase in residual lipid concentration, following nebulisation of liposomes, are shown in Fig. 4 for Cirrus and Pari-LC nebulisers and Fig. 5 for Respirgard II and Sidestream. Nebulisation of liposomes yielded a significantly greater percentage rise in residual dry mass than for nebulised sodium chloride solutions. The increase in residual phospholipid concentration ranged from 23 to 97%, compared with 14–43% for sodium chloride, which could be adjusted for evaporative losses, with reference to the increase in concentration of sodium chloride solutions.

There was a direct relationship between residual lipid concentration and the mean liposome size (Figs. 4 and 5). Reducing liposome size below 1 μm did not significantly improve liposome output. However, in each nebuliser there was an increase in residual lipid concentration with an increase in the median liposome size to 2.5 μm. Increasing the liposome size limits the number of liposomes that may be included in aerosolised droplets with a mean size less than that of the liposomes themselves. The heterodisperse nature of the aerosols and the shearing of the liposomes within the nebuliser, allows a certain proportion of the larger liposomes to be aerosolised. However, because the median droplet size produced by the Pari-LC, Respirgard II and Sidestream nebulisers was $<$ 2.5 μm (mean: 2.15, 1.70 and 1.99 μm, respectively) a greater proportion of liposomes with a size of 2.5 μm or greater are excluded from the aerosol.

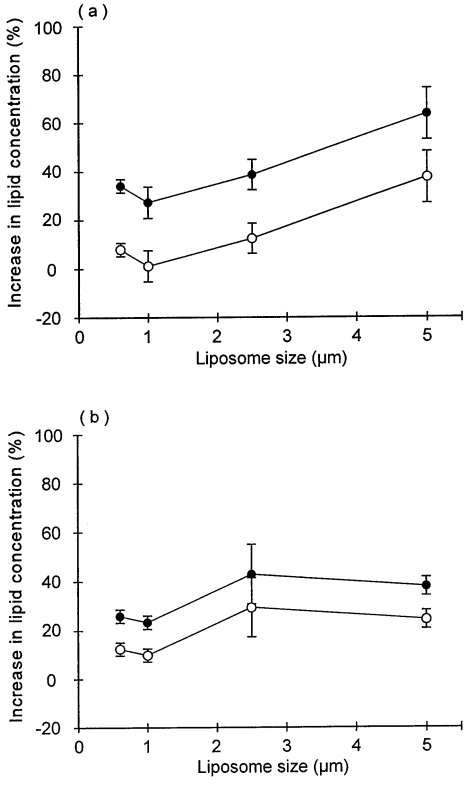

Fig. 4. Increase in the residual lipid concentration of eggPC/chol (1:1) liposomes following nebulisation with Cirrus (a) and Pari-LC (b) nebulisers. Data plotted unadjusted (●) and adjusted (○) for evaporative losses during nebulisation ($n = 3$; $\pm$ S.D.).

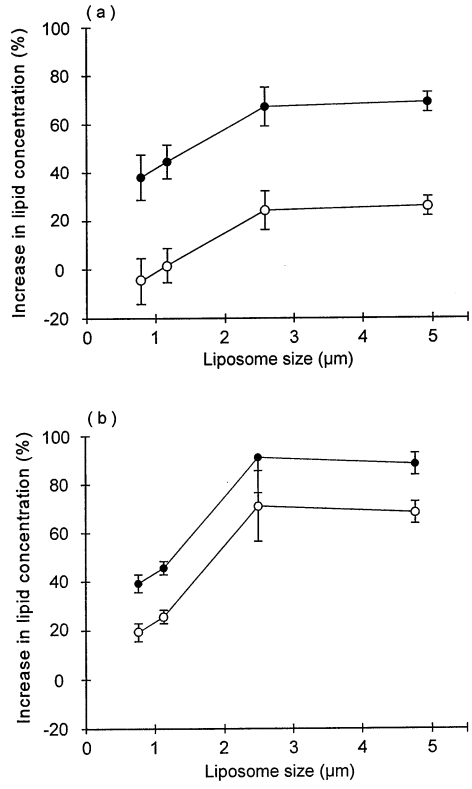

Fig. 5. Increase in the residual lipid concentration of eggPC/chol (1:1) liposomes following nebulisation with Respirgard II (a) and Sidestream (b) nebulisers. Data plotted unadjusted (●) and adjusted (○) for evaporative losses during nebulisation ($n = 3$; $\pm$ S.D.).

For smaller liposomes, the increase in concentration of phospholipid in the residual fluid was comparable to the increase in sodium chloride concentration. The small liposomes were effectively being treated as a solute (Figs. 4 and 5). However, a size selective process appeared to operate for liposomes with a median size ≥ 2.5 μm. The extent to which larger liposomes were retained within the device varied between nebulisers. In particular, the design of the Sidestream nebuliser encouraged the retention of liposomes.

If data for all nebulisers are combined there is a correlation between liposome retention as indicated by the increase in lipid concentration and

vesicle size (Fig. 6). The greatest variability and deviation from the linear correlation occurs with liposomes of 2.5 μm that approximates to the mean size of the aerosols produced by most of these nebulisers. This is in accordance with the hypothesis proposed by Dahlbäck (1994), that suspended drugs must be micronised to < 2 μm if they are to be efficiently delivered from nebulisers producing 3 μm droplets. This would seem to be applicable to liposomes, even though, unlike drug particles they may deform and fracture under stress.

3.4. Examination of nebuliser component orifices

One explanation for the size selectivity exhibited by the nebulisers is that structures within the nebuliser impede the transport of larger liposomes to the site(s) of droplet formation. Such a mechanism was proposed by McCallion et al. (1996) who determined that retention of latex spheres was size dependent. However, the smallest orifice (determined by electron microscopy) through which the liposomes must flow in each device varied between 510 and 960 μm, making blockage unlikely.

The Cirrus nebuliser (Fig. 7a) operates by forcing high velocity gas through a central jet with a typical diameter of 770 μm. The Cirrus differs from each of the other devices in that it lacks true fluid feed capillaries. Instead, fluid is 'sandwiched' between the wall of the central gas jet and an outer 'cap' which contains the fluid/gas orifice. Fluid is drawn up the walls of these structures and ejected through a 510-μm orifice.

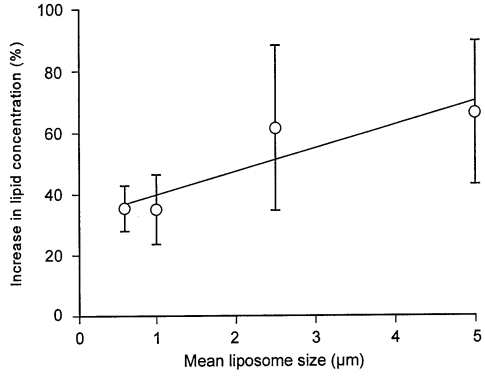

Fig. 6. Increase in residual lipid concentration of eggPC/chol (1:1) liposomes following nebulisation as a function of the mean liposome size (mean of all nebulisers studied). Equation of line of best fit shown: $y = 7.576x + 32.54$, $r = 0.910$ ($n = 3$; $\pm$ S.D.).

The Pari-LC nebuliser (Fig. 7b) operates in a similar manner to the Cirrus, except the device has two capillary feed channels, ≈ 960 μm in diameter, etched into the wall of the outer 'cap'. Fluid is drawn up these channels in response to the flow of gas through the 420 μm diameter gas jet orifice in a cone-like structure over which the outer cap fits. Fluid and gas are mixed internally and exit through a fluid/gas orifice of dimensions $\approx 1.3 \times 3.0$ mm.

The Respirgard II nebuliser (Fig. 7c) has four 1.40 mm fluid feed capillaries in the cap, which fits over a central structure bearing a 450 μm gas jet orifice. Fluid and gas are mixed internally before leaving a 1 mm fluid/gas orifice.

Fluid in the Sidestream (Fig. 7d) is drawn up the gap formed between the central structure in the reservoir and the baffle cap. The fluid is drawn through two 840 μm diameter fluid feed capillaries, adjacent to a central 530 μm gas jet orifice. The Sidestream nebuliser is the only device to mix the fluid and gas externally from the nozzle.

The Sidestream nebuliser was found to give a significantly reduced output of aerosol during the nebulisation of 5 μm liposomes. This has been shown to be at least partially attributable to failure of larger liposomes to become included within the smaller aerosol droplets that leave the device. However, retention of liposomal lipid cannot be solely attributed to the droplet size, as the Sidestream yielded a significantly greater retention of liposomes compared with the Respirgard II, despite producing larger droplet sizes.

This study suggests that there is a direct correlation between the size of the gas jet orifice and the size of the aerosol droplets. The Cirrus has the largest gas jet orifice of the nebulisers studied and accordingly produces the largest droplet size. A larger jet orifice means that driving gas is forced

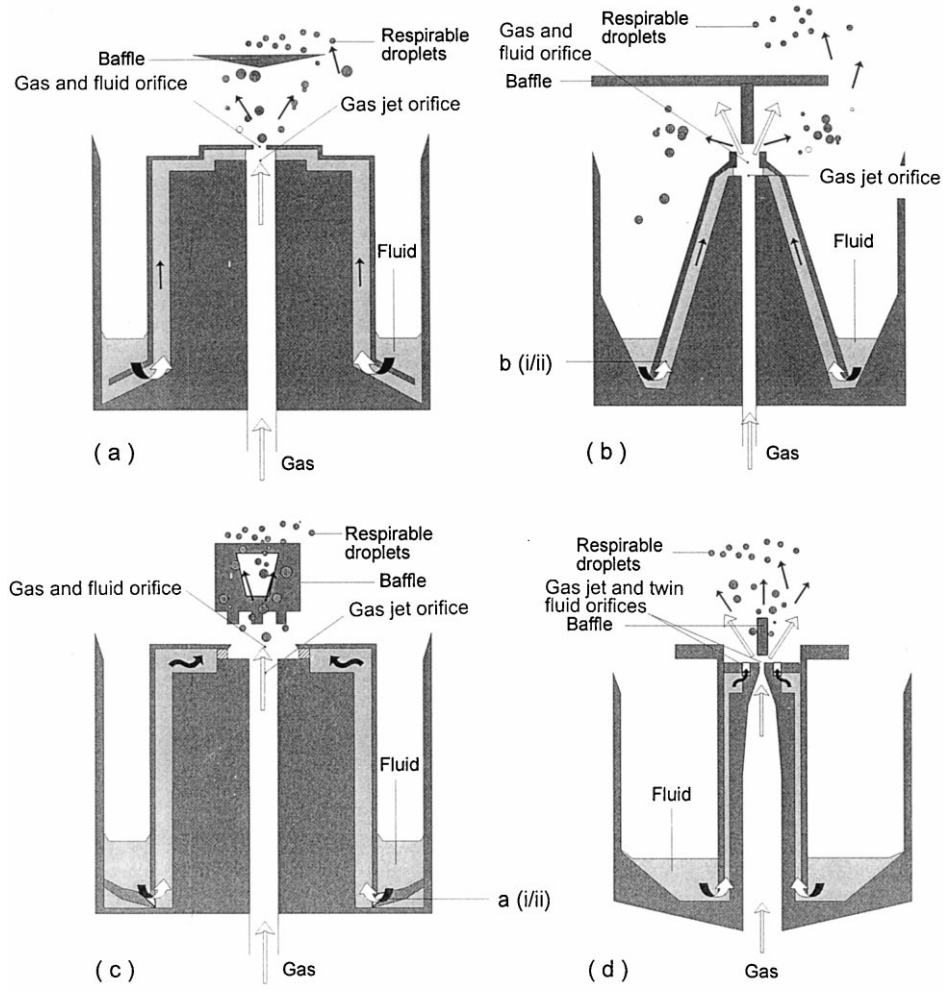

Fig. 7. Schematic diagrams of the fluid and gas orifices within each jet nebuliser, with unit top/mouthpiece removed (not to scale). (a) Cirrus; (b) Pari-LC; (c) Respirgard II; and (d) Sidestream.

through the Venturi at a reduced pressure. This provides less shear energy for droplet formation and larger droplets are generated. The droplets are also aspirated at a reduced velocity and hence, are less likely to impact on the baffles. The similarity between the droplet size produced by the Pari-LC and the Sidestream, despite the Sidestream's larger jet, is a consequence of the more efficient baffling system of the Sidestream. It may also be due to the Sidestream's open vent design, which results in greater droplet evaporation, producing a smaller droplet size at a relatively low shear energy.

4. Conclusions

The results demonstrate that selection of both nebuliser and liposome components of a nebuliser-liposome system are important.

There is a relationship between the size of the liposomes and the extent to which they are delivered from or retained in the nebulisers, which is determined by the design of the individual nebuliser. Aerosol droplet size is independent of liposome size and bilayer composition, although it may be critically influenced by lipid concentration. Increases in lipid concentration cause a rise

in viscosity and an increase in the number of liposome bilayers within the system. This produces an associated rise in the secondary aerosol size, which may be attributed to various mechanisms, including an increased fluid viscosity, bilayer fracture, vesicle aggregation or an inhibition of droplet evaporation. The extent of the droplet size increase is dependent upon the efficiency of the nebuliser baffles and other nebuliser design factors. As a result, a nebuliser such as the Sidestream, is capable of minimising lipid concentration effects, although this may be at the expense of reduced output rate.

The output of aerosol from a nebuliser may be as critical in determining clinical response as the droplet size (Nebuliser Project Group, 1997). The results demonstrate that the Pari-LC and Sidestream nebulisers produce similar outputs of aerosol, despite significant differences in their design. The effect of an increased lipid concentration in both nebulisers is to reduce aerosol output rate. This is due to increases in the median droplet size that result from increased lipid concentration. Larger droplets result in an increase in the fraction of the primary aerosol retained. However, as the mean liposome size is increased, so the residual masses become elevated. It can, therefore, be concluded that the nebulisation of large liposomes is associated with a reduced total liposomal aerosol output. This is in addition to the recognised fact that larger liposomes are more unstable during nebulisation (Taylor et al., 1990; Niven et al., 1991).

Acknowledgements

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