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A preliminary assessment of the stability of 5-CF containing liposomes following aerosolisation with TouchSpray™ Technology and nebulisation with a commercial jet nebuliser

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INTRODUCTION

TouchSpray™ Technology (licensed to ODEM LIMITED) is an electronic aerosolisation generating technology, which is entirely propellant free, and capable of converting bulk fluids into a very fine stream of low velocity, precisely controlled droplets perfectly suited for inhalation. Figure 1 illustrates the **TouchSpray™** atomising head in operation in still air.

TouchSpray™ is being combined with other elements to create a complete inhaler device suitable for mass production which is small, portable and convenient to use.

In-vivo data utilising inhalation scintigraphy (see Figure 2) has shown that the core technology can target the deep lung with a very high delivery efficiency (83%)[1].

Figure 1: TouchSpray™ atomising head in operation in still air.

Figure 2: Scintigram of normal volunteer following inhalation of a labelled TouchSpray™ generated aerosol

Published data has shown that **TouchSpray™** is capable of aerosolising a wide range of liquid formulations[2]. **TouchSpray™** has also been shown to be able to aerosolise relatively fragile macromolecules whilst maintaining their molecular integrity[3].

The aims of this study were to compare the recovery of liposomes following aerosolisation with an unoptimised **TouchSpray™** atomising head and a commercial jet nebuliser.

MATERIALS AND METHODS

SLV-type liposomes (liposomes consisting of a single phospholipid bilayer) were manufactured and the hydrophilic model marker compound (5 (6) – carboxyfluorescein (5-CF)) was trapped inside the aqueous core. This marker substance is widely used to test liposomal integrity.

Fluorimetric measurements of a liposome sample diluted in phosphate buffer were compared against a 5-CF standard and the 5-CF content outside the liposomes was determined. Liposomes contained within the sample were then destroyed by incubation (in a 1% Triton X-100 solution in a phosphate buffer for 30mins at 40^0C) and fluorimetric measurements made to determine total 5-CF content inside and outside of the liposomes. Calculation of the relative amounts of 5-CF inside and outside the liposomes could then be derived.

A total of 2ml of the liposomal preparation was aerosolised with the single unoptimised **TouchSpray™** head and collected in a flask containing a phosphate buffer (20ml). Similarly, a total of 5ml of the liposomal preparation was nebulised with the jet nebuliser and collected in a flask containing 30ml of the phosphate buffer. The collected liposome samples were then reanalysed for 5-CF inside and outside the liposomes and any leakage due to aerosolisation and nebulisation was then determined.

RESULTS AND DISCUSSION

The results from this preliminary work are outlined below in Table 1.

The results presented in Table 1 show that the liposomes appear to have poor physical stability characteristics and a low resistance to shear stress. This is shown by the fact that almost 25% of the originally entrapped 5-CF had already leaked from the liposomes prior to aerosolisation/ nebulisation. This is probably due to the low transition temperature of the phospholipids in the liposomes' bilayer.

The results obtained with the unoptimised **TouchSpray™** atomising head following aerosolisation show that virtually no additional leakage of 5-CF from the liposomes had occurred. These results confirm the previously presented work with macromolecules, indicating that the **TouchSpray™** aerosolisation process appears to generate little shear or other mechanical stress on fragile formulations/molecules.

The jet nebuliser, however, caused a partial rupture of the phospholipid bilayer of the liposomes which resulted in a 49% increase in 5-CF concentration outside of the liposomes. It appears that the jet nebulisation process causes increased shear and other mechanical stress on the liposomes compared with **TouchSpray™**

CONCLUSIONS

It can be concluded from these preliminary results that **TouchSpray™** generates less shear or other mechanical stress than jet nebulisation and that **TouchSpray™** offers the potential of delivering SLV-type liposomes. Further testing is necessary to confirm these results but **TouchSpray™** offers the potential of a relatively simple, gentle and efficient inhaler device for the delivery of liposomes.

REFERENCES

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Table 1: Characterisation of 5-CF containing liposomes pre and post aerosolisation (TouchSpray™) and nebulisation (jet nebuliser)

Sample	5-CF Distribution	
	Outside liposomes	Inside liposomes
Prior to aerosolisation/nebulisation	24.8%	75.2%
Post aerosolisation (TouchSpray™)	23.9%	75.1%
Post nebulisation (jet nebuliser)	36.9%	62.8%