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Intranasal Vaccine Formulation: Advancing Towards Nasal Dry Powder Formulation

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SUMMARY

Unlike conventional mRNA vaccines, intranasal vaccines display several advantages, including the ability to generate strong mucosal immunity. However, the formulation challenges associated with intranasal mRNA vaccines have so far hindered their extensive application and further research in the area of formulation development and optimisation are required before translation. This study aims to optimise the manufacturing conditions of inhalable dry powders by examining the impact of temperature and lipid composition on liposome particle size and the effect of spray-drying parameters on the particle size of spray-dried powders. The results indicate that liposomes generated at 20°C exhibited notably larger sizes than those produced at 55°C, irrespective of the lipid composition. Notably, dry powder formulations featuring larger particle sizes were achieved at a low gas flow rate of 25mm.

INTRODUCTION

Recently, Lipid nanoparticle-mRNA vaccines, Pfizer-BioNTech and Moderna COVID vaccines have demonstrated a remarkable ability to elicit potent immune systemic responses against SARS-CoV-2 [1,2]. However, their inability to elicit a robust mucosal immunity against lung diseases is a disadvantage [3].

Intranasal administration of lipid nanoparticle vaccines has emerged as a promising and non-invasive option for mucosal delivery for generating a strong mucosal immunity against respiratory pathogens [4]. Stabilizing mRNA with dry powder-based mucoadhesive formulations can ensure dosing accuracy, extended stability, improved nasal residence time and patients' acceptability [5]. However, the formulation challenges associated with intranasal mRNA vaccines have so far hindered their extensive application and further research in the area of formulation development and optimisation are required before translation [6].

This study aims to optimise the manufacturing conditions of inhalable dry powders by examining: 1) The impact of temperature and lipid composition on liposome particle size

2) The effect of spray-drying parameters (e.g. gas flow, inlet temperature and feed rate) on the particle size of spray-dried powders.

MATERIALS AND METHODS

1,2 distearoylphosphatidylcholine (DSPC) was received from Lipoid GmbH (Germany). Cholesterol (Ch) and egg phosphatidylcholine (PC), Dioleoyl-3-trimethylammonium propane (DOTAP), trehalose dihydrate, phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich Company Ltd. (Poole, UK). Ethanol (99.8%, analytical grade) was from Fisher Scientific UK (Loughborough, UK).

Liposomes were generated using microfluidic mixing varying formulation and processing parameters as indicated in Table 1. The microfluidic device comprised two syringe pumps connected by PEEK tubing to a Y-junction (HPLC). Selected lipids were dissolved in the organic phase (ethanol) at and injected into one inlet of the microfluidic chip, whilst the aqueous phase (PBS; pH 7.4) is injected into the other. Hydrodynamic diameters of liposomes dispersed in PBS were assessed at 25°C by dynamic light scattering at an angle of 173° using a Zeta sizer NanoZS (Malvern Instruments Ltd, UK). **Spray**

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Drying: 80mg/ml Trehalose solution was spray dried with Mini Spray Dryer B-200 (BUCHI UK Ltd) and the size, glass transition and moisture content was measured by Sympatec Helos laser diffraction, Dynamic Scattering Calorimetry and Thermogravimetric Analysis respectively.

Data analysis: 2-way Anova, Tuckey post-test

Table 1. *Liposome Preparation*

Formulation Parameters			Processing Parameters		
Lipid Composition (molar ratio)	Organic Solvent	Aqueous Solvent	Flow rate ratio	Total Flow rate (ml/min)	Temperature (°C)
DSPC:Ch:DOTAP (2:1:0.85)	Ethanol	PBS pH7.4	3:1 (Aqueous: solvent)	5	20 and 55
DSPC:Ch:PC(2:1:0.85)					
DSPC:Ch (2:1)					

RESULTS AND DISCUSSION

The three-lipid formulations showed distinct particle sizes and polydispersity at both 20°C and 55°C. At both temperatures' liposomes containing DSPC: Ch: PC had the largest size compared to DSPC: Ch DSPC: DOTAP. The liposomes produced at 20°C (Figure 1) were more heterogenous and significantly larger than the ones produced at 55°C.

These findings suggest the choice of lipid plays a vital role in determining liposome sizes which aligns with previous studies [7,8]. Lipid composition influence may be attributed to differences in lipid tail length, molecular shape and membrane fluidity, the packing arrangement and interactions between lipids [9].

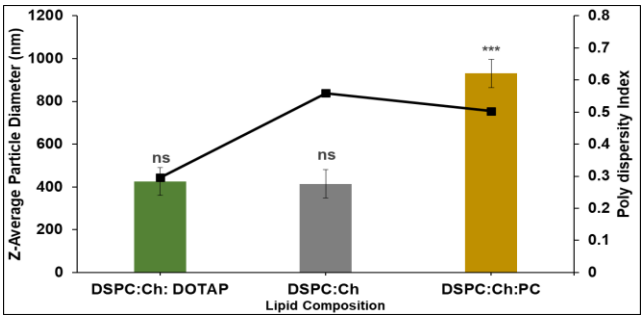

Fig. 1. *Liposome size distribution (solid block) polydispersity (solid line) of different formulations synthesised at 20°C. Results represent the mean ± standard deviation of n=3 independent experiments. ns: p>0.05***p < 0.001.*

In addition, Temperature is a crucial factor that influences lipid bilayer fluidity, leading to alterations in vesicle size. Understanding this relationship is crucial to designing suitable liposomes for a specific biomedical application [10].

Table 2 shows that gas rate has the main effect on spray-dried trehalose particle size distribution (D50). A low atomization gas flow rate produced the the largest particle size regardless of other parameters.

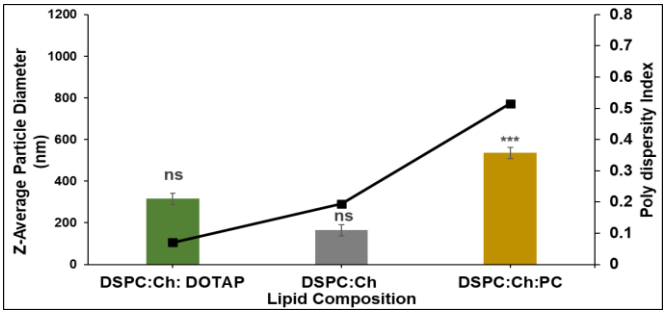

Fig. 2. *Liposome size distribution (solid block) polydispersity (solid line) of different formulations synthesised at 55°C. Results represent the mean ± standard deviation of n=3 independent experiments. ns: p>0.05***p < 0.001.*

Table 2. *Effect of Spray Parameters on Particle size*

Inlet Temperature (°C)	Feed Rate (%)	Gas Flow (mm)	D50	Tg (°C)	Percentage Yield (%)	Moisture Content (%)
160	3	25	9.27	120	43.6	2.69
160	11	55	2.10	114	72.8	2.43
160	3	55	1.60	120	79.2	1.87
110	3	55	2.09	120	81.0	3.57
110	11	25	9.09	112	87.3	3.80
135	7	40	2.67	121	77.4	3.07
110	3	25	9.77	120	56.7	2.15
160	11	25	2.50	124	56.3	2.92
110	11	55	2.82	119	77.1	5.20

CONCLUSIONS

The size of liposomes is significantly influenced by lipid composition and temperature. In the context of this study, it was observed that the particle size distribution of the spray-dried powder was primarily affected by the gas rate.

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