Formulation and characterisation of liposomes loaded with a model small nucleic acid for nasal delivery

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Key Message

A liposomal suspension loaded with a model small DNA was formulated for nasal delivery. In vitro assessment showed suitable characteristics for nasal delivery: pH, osmolality and droplet distribution when loaded into Aptar CPS pump. Finally, liposomal structure and size was preserved after spraying.

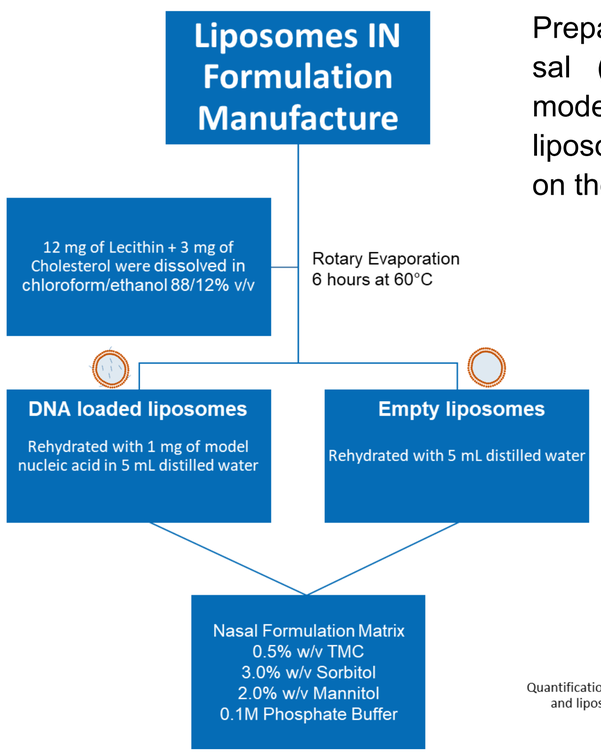
Introduction

The COVID-19 outbreak resulted in a large interest and development of techniques that enable nucleic acid delivery as vaccines. Using nucleic acids as therapeutics proved to be challenging: hence the use of delivery systems, such as liposomes. Liposomes contain an aqueous volume enclosed in a lipid bilayer composed of phospholipids and additional structure stabilising components, like cholesterol. In addition, they can be applied to a variety of therapeutic areas, which have been successfully marketed [1].

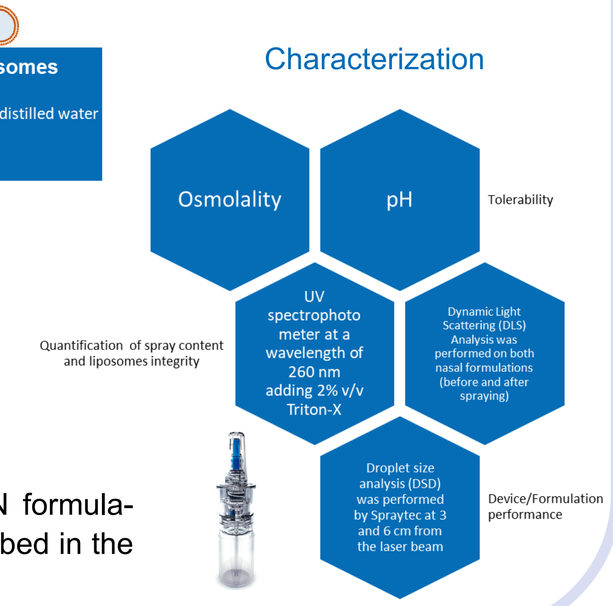
The incorporation of mucoadhesive polymers in the intranasal formulation can help increasing formulation retention and immunogenicity [2]. Trimethyl chitosan (TMC), a derivative of chitosan, for example, has been used promisingly as adjuvant for mucosal immunisation in combination with large molecules [3].

The aim of this study was to formulate a liposome system loaded with a model of small nucleic acid for intranasal delivery. A comparison of the *in vitro* performance was then carried out between the nasal formulation of deoxyribonucleic acid (DNA) loaded liposomes versus the same formulation prepared with empty liposomes.

Experimental Methods



Preparation process of the two intranasal (IN) formulation comprising the model DNA loaded liposomes or empty liposomes is illustrated in the scheme on the left.



Characterization of the two IN formulations was carried out as described in the scheme on the right.

Results and Discussion

The concentration of DNA in the loaded liposomes nasal formulation was measured with a result of 121.95 µg/mL. In addition, the pH of the DNA loaded liposome formulation was slightly more acidic compared to the empty liposomes formulation (pH 6.07 and 6.20 for DNA loaded liposomes and empty liposomes, respectively). In both cases, this was suitable for nasal delivery to avoid nasal irritation, as the nasal mucosa is slightly acidic [4]. Finally, an increase in the osmolality was also noted for the DNA loaded liposomes nasal system (339.67 ± 3.06 mOsm/kg) compared to the empty liposomes one (294.00 ± 1.00 mOsm/kg). Results are presented in Table 1.

Table 1. pH, Osmolality and Quantification results for the formulations

Formulation	pH	Osmolality	DNA Concentration (µg/mL)
Empty Liposomes	6.07	294.00	-
DNA Loaded Liposomes	6.20	339.67	121.95

Table 2. DLS and concentration measurement for empty liposomes formulation and DNA loaded liposomes formulation before and after spraying

Sample	Hydrodynamic Diameter (nm)	Zeta potential (mV)	DNA Concentration (µg/mL)
Empty liposomes	404.80 ± 11.59	+ 37.40 ± 1.13	-
Empty liposomes after spraying	432.33 ± 3.59	+ 34.23 ± 0.98	-
DNA loaded liposomes	661.33 ± 27.14	+ 36.43 ± 0.97	121.95
DNA loaded liposomes after spraying	586.93 ± 11.05	+ 34.40 ± 1.07	12.93 ± 0.61

No effect of shear rate was observed (Table 2). Hydrodynamic diameter measured before and after spraying was comparable for both formulations with the DNA loaded liposomes reporting a higher size, perhaps due to DNA conjugation to the external liposomal structure and/or DNA induced liposome-liposome fusion. Zeta potential consistently showed a positive charge determined by the presence of TMC. Finally, a delivered concentration of a 106% (± 4.75%) of the theoretical expected value (12.20 µg) was obtained.

The 50th percentile of the droplet size distribution (Dv50) obtained by laser diffraction of the spray emitted from the nasal pump was slightly lower for the DNA loaded liposomes formulation at both 3 and 6 cm (Figure 1). DSD was suitable for nasal delivery (generally 30-120 µm), even though much closer to the upper limit, with a percentage of droplets below 10 µm ≤ 1% in all setting tested. A reduction of the concentration of TMC may help in generating lower DSD (Dv50 < 50 µm), particularly to ensure efficient deposition at the target posterior region [5] (i.e. NALT, and turbinates).

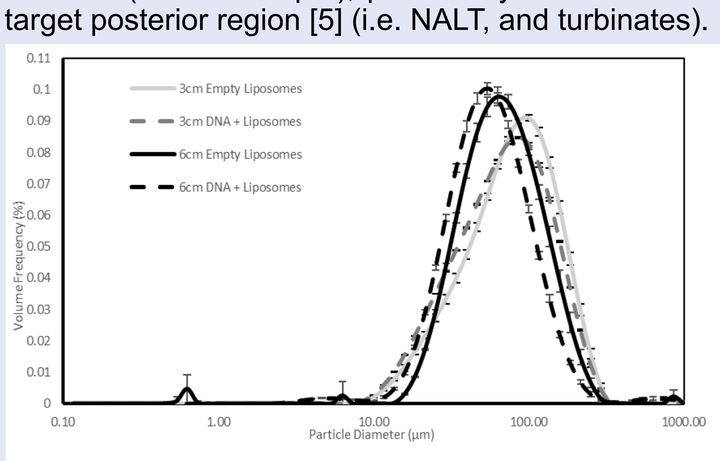


Figure 1. DSD of the empty liposomes and DNA loaded liposomes formulations sprayed with Aptar CPS pump at 3 cm and 6 cm

Conclusion

This study shows the characterisation of a liposomal suspension loaded with a model DNA for nasal delivery with potential application for vaccination.

- pH and osmolality of the resulting formulations were suitable for the nose.
- DSD of the spray emitted from Aptar CPS nasal pump was in the range for nasal deposition, even though much closer to the upper limit.
- Liposomal structure and size was preserved after spraying as reported by DLS and assessment of delivered drug.

References

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