Multiscale Modelling for the Manufacturing of Lipid Nanoparticles for the Delivery of mRNA Pharmaceuticals

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Abstract

Messenger RNA–based therapeutics have emerged as a transformative class of pharmaceuticals, enabling applications ranging from infectious disease vaccines to cancer immunotherapy and protein replacement therapies. Their rapid development, high flexibility, and favourable safety profile compared to viral vectors make them particularly attractive for personalised medicine. However, the clinical translation of RNA therapeutics is strongly limited by the intrinsic instability of RNA and its poor cellular uptake. Lipid nanoparticles have therefore become the most widely adopted delivery platform, as they protect RNA from degradation and promote its intracellular delivery. Despite their success, the formulation and manufacturing of lipid nanoparticles remain largely empirical, as particle properties are highly sensitive to lipid chemistry, solvent composition, and processing conditions. Microfluidic synthesis has emerged as a powerful technique to produce lipid nanoparticles with controlled size, low polydispersity, and high reproducibility, yet a mechanistic understanding of the underlying self-assembly process is still incomplete.

In this context, mathematical modelling represents a valuable tool to complement experimental approaches, offering insight into phenomena that are difficult to observe directly and enabling a rational optimisation of both formulation and synthesis. This thesis adopts a multiscale modelling framework to investigate the formation of mRNA-loaded lipid nanoparticles, bridging molecular-scale interactions and process-scale transport phenomena. Molecular dynamics simulations were combined with computational fluid dynamics and population balance modelling to provide a coherent description of lipid nanoparticle self-assembly under conditions relevant to microfluidic production.

At the molecular scale, coarse-grained molecular dynamics simulations were employed to investigate the spontaneous self-assembly of lipid nanoparticles starting from lipids and RNA dispersed in a mixture of ethanol and water. The simulations revealed that self-assembly proceeds through three main stages: the formation of small lipidic clusters driven by their insolubility in water, their progressive interaction with RNA leading to surface coverage and encapsulation, and a final rearrangement step in which lipids reorganise according to their affinity with the surrounding environment. The resulting nanoparticles exhibited an electron-dense core, with RNA confined in disordered cylindrical cavities surrounded by lipids arranged in an inverted hexagonal-like structure. Ionisable lipids were found to be the dominant species throughout the particle and to interact preferentially with RNA, while neutral and PEGylated

lipids mainly occupied interfacial regions. Cholesterol filled voids between lipid tails, contributing to structural stability.

The effect of solvent composition was investigated by varying the water–ethanol ratio to mimic conditions imposed by different flow rate ratios in microfluidic devices. Increasing ethanol content led to less compact and more disordered particles, in addition to a moderate increase in particle size. Furthermore, this impacted the lipid organisation in the lipid nanoparticle, since the PEGylated lipids migrated towards internal aqueous cavities at higher ethanol concentrations. This behaviour can potentially reduce their steric stabilisation at the particle surface. These findings may provide a molecular interpretation of experimentally observed increases in particle size and polydispersity at lower aqueous fractions.

The role of lipid chemistry was further explored by comparing different ionisable lipid structures. Lipids with more branched hydrophobic tails disrupted lipid packing and promoted the formation of larger particles, while the presence of hydroxyl groups in the headgroup enhanced RNA encapsulation through additional interactions with nucleobases. Simulations of buffer exchange, mimicking the transition from acidic to physiological pH and the removal of ethanol, showed significant morphological rearrangements. In particular, the loss of ionisable lipid charge reduced electrostatic interactions with RNA, leading to lipid migration towards bilayer-like structures. Ionisable lipids containing hydroxyl groups exhibited stronger RNA retention upon neutralisation, highlighting key chemical features governing encapsulation efficiency and long-term stability.

Insights from the effect of the ethanol content on the particle size were used to establish a multiscale model for the synthesis of mRNA-loaded lipid nanoparticles. At the process scale, computational fluid dynamics coupled with population balance modelling was employed to investigate the influence of microfluidic fluid dynamics on particle formation. Simulations suggested that the flow regime within the device lies in a transitional region, where laminar transport dominates most of the channel while localised regions of enhanced mixing arise at geometric disruptions. These features were essential to promote efficient mass transfer between solvent streams and trigger the formation of nanoparticles. Population balance modelling revealed that, under the investigated conditions, aggregation was predominantly governed by Brownian mechanisms, while turbulent fluctuations had a negligible effect.

The effect of operating parameters was systematically analysed. Increasing the flow rate ratio resulted in a sharp reduction in particle size and polydispersity, consistent with experimental observations. This effect was associated with the lower lipid content in the system, which formed a lower number of particles containing fewer molecules each. Similarly, increasing the total flow rate enhanced mixing efficiency, increased particle number density, and reduced both particle size and variability. These results demonstrate that changes in nanoparticle size arise not only from solvent-induced effects, but also from fundamental variations in the aggregation dynamics driven by fluid dynamics.

Overall, this thesis demonstrates that a multiscale modelling approach provides a robust and physically consistent description of lipid nanoparticle formation, capturing key trends observed experimentally and offering mechanistic insight into the role of formulation and processing

parameters. The results highlight the strong coupling between molecular interactions, solvent environment, and microfluidic transport phenomena. As such, the modelling framework developed in this work represents a valuable foundation for the rational optimisation of both lipid formulations and microfluidic synthesis strategies, with significant implications for the development and scalable manufacture of RNA-based therapeutics.