Comparison of RNA lipid nanoparticle manufacturing techniques

Paul Gottschalk[1,2]; Heike Bunjes[1,2]

[1] Technische Universität Braunschweig, Institut für Pharmazeutische Technologie und Biopharmazie, Mendelssohnstr. 1, 38106 Braunschweig, Germany

[2] Technische Universität Braunschweig, Zentrum für Pharmaverfahrenstechnik - PVZ, Franz-Liszt-Str. 35a, 38106 Braunschweig, Germany

p.gottschalk@tu-braunschweig.de, heike.bunjes@tu-braunschweig.de

Lipid nanoparticles (LNPs) are a special type of lipid nanocarriers characterised by a continuous lipid core. These carrier systems are suitable as vectors for the systemic administration of active pharmaceutical ingredients, especially nucleic acids. The worldwide use of mRNA-based SARS-CoV-2 vaccines has impressively demonstrated the clinical benefits of this platform [1]. Commonly used techniques for RNA-LNP production are often based on microfluidics. Transfer between manufacturing methods or devices is, however, crucial in the development process as early development is often performed using technologies different from those in later production [2]. In addition to microfluidic production technology, there are also impingement jet mixing (IJM) and simple bulk mixing techniques. This work aims to identify the strengths and weaknesses of various manufacturing techniques with particular attention to the resulting particle size and RNA encapsulation efficiency.

A standard LNP formulation was produced with siRNA or mRNA using various manufacturing techniques based on particle generation by mixing an ethanolic lipid solution with siRNA or mRNA in acetate buffer (pH 4.0, 50 mM). The different manufacturing techniques were set up with settings equalized as closely as possible. Directly after mixing, the particle suspension in ethanolic acetate buffer was quenched with acetate buffer and then dialyzed against an aqueous TRIS buffer (pH 7.4, 50 mM) for 24 hours. A classic method of manufacturing RNA-LNP is microfluidic synthesis. This involves mixing the ethanolic lipid solution with the aqueous RNA solution in a microfluidic channel with specific geometries. Rapid mixing of the two solutions results in the spontaneous formation of the LNPs. The NanoAssemblr Ignite™ (Cytiva, Marlborough, USA) relies on the use of single-use plastic microchips especially for small-scale GMP production (Ignite™ NxGen™ cartridge in this study). The Sunshine device (Unchained Labs, Pleasanton, USA) employs reusable glass microchips (here Sunny 190 X chip). In impingement jet mixing (IJM), the sample is forced at high pressure into a chamber where the liquids mix very quickly. The IJM device used in this study was the Nanoscaler™ (Knauer, Berlin, Germany). A simple method of making RNA-LNPs is ethanol injection. Here, the ethanol lipid solution was continuously added to the RNA solution using a syringe pump (Fusion 200, Chemyx, Inc., Stafford, USA) under continuous magnetic stirring to ensure thorough mixing. To produce RNA-LNP by the simplest method, pipette mixing, the lipids dissolved in ethanol were pipetted up and down in the RNA solution using a 1000 µL Eppendorf pipette.

All methods yielded similar results concerning particle size and encapsulation efficiency, and all products were acceptable with the exception of that of EtOH injection which led to the formation of larger particles. Simple bulk manufacturing techniques turned out to be an option in a very early development phase, before a process is to be established using expensive microfluidic techniques. Microfluidic manufacturing offers advantages when LNPs need to be manufactured reproducibly but has limitations in terms of flow rate. For large-scale production, manufacturing using IJM is more suitable, as the flow rates are significantly higher.

1. Machado, B. A. S. et al. The importance of RNA-based vaccines in the fight against COVID-19: An overview. Vaccines, 9, 1345 (2021).
2. Xu, S., Hu, Z., Song, F., Xu, Y. and Han, X. Lipid nanoparticles: Composition, formulation, and application. Mol. Ther., Methods Clin. Dev., 33, 101463 (2025).