Could drying of RNA containing lipid nanoparticles make dialysis obsolete? A closer look at buffer changes

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Triggered by the successful application of RNA lipid nanoparticles (LNPs) as Covid vaccines, LNP became a focal point of research for RNA delivery. To avoid current challenges due to storage and transportation at ultra-low temperatures, drying of RNA-loaded LNP with reconstitution just before application is a promising approach. Especially freeze drying and spray drying have been tested in recent studies [1].

Even though the main purpose of lyophilization is the removal of water, other volatile components of the product such as solvents or buffers could evaporate as well. Ethanol and acetic acid are the two main components, which are essential for LNP production and need to be removed quickly prior to storage and application. Usually, this is done by dialysis, which also changes the buffered pH from acidic to neutral. But due to their rather low evaporating temperature, it is worth evaluating if they can be removed during freeze-drying. By this approach, a process intensification and acceleration would be possible, removing the dialysis step and assembling the final product just upon reconstitution in WFI. However, this represents only an alternative if two perquisites are fulfilled: properties of the LNPs remain unaltered and a neutral pH of 7 is achieved after reconstitution for parenteral application.

In our study, an acetate-phosphate buffer was applied because it changes its pH during freeze-drying from pH 4 to pH 7. The addition of sugars as commonly used lyoprotectants caused the main challenge as they impeded the pH change while sugar alcohols led to differences in the formation of a lyocake. Pure D-mannitol showed the best results, not affecting the pH change and still forming a high qualitative lyocake. To apply this to the LNP formation process, RNA-loaded LNP were produced in this buffer, frozen immediately after formation, and dried. Although the pH changed as intended, particle sizes increased mayorly. One likely cause could be the presence of ethanol during freeze-drying, which may cause structural instabilities during the freeze process and boiling of ethanol during water sublimation. As next steps we will test this formulation with spray drying and variation of freeze-drying parameters.

This project was funded by the zukunft.niedersachsen project “RNApp”.

1 Arte, Kinnari Santosh; Chen, Manlin; Patil, Chanakya D.; Huang, Yijing; Qu, Li; Zhou, Qi (2025): Recent advances in drying and development of solid formulations for stable mRNA and siRNA lipid nanoparticles. In *Journal of pharmaceutical sciences* 114 (2), pp. 805–815. DOI: 10.1016/j.xphs.2024.12.013.