ANALYSING LIPID NANOPARTICLES USING TUNABLE RESISTIVE PULSE SENSING WITH THE EXOID

APPLICATION NOTE

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1 / INTRODUCTION

What are Lipid Nanoparticles?

Lipid nanoparticles (LNPs) are, as their name suggests, a class of lipid-based nano-sized particles usually utilised as drug vectors. LNPs can trace their origins back to the discovery of the liposome in the 1960s. Since then, more complex LNPs have been designed:

- mRNA-containing LNPs: recently made headlines worldwide as the vectors of the Moderna and Pfizer-BioNTech SARS-CoV-2 vaccines
- Solid lipid nanoparticles (SLNs): a solid lipid core surrounded by a surfactant layer
- Nanostructured lipid carriers (NLCs) – a mixed solid and liquid lipid core surrounded by a surfactant layer

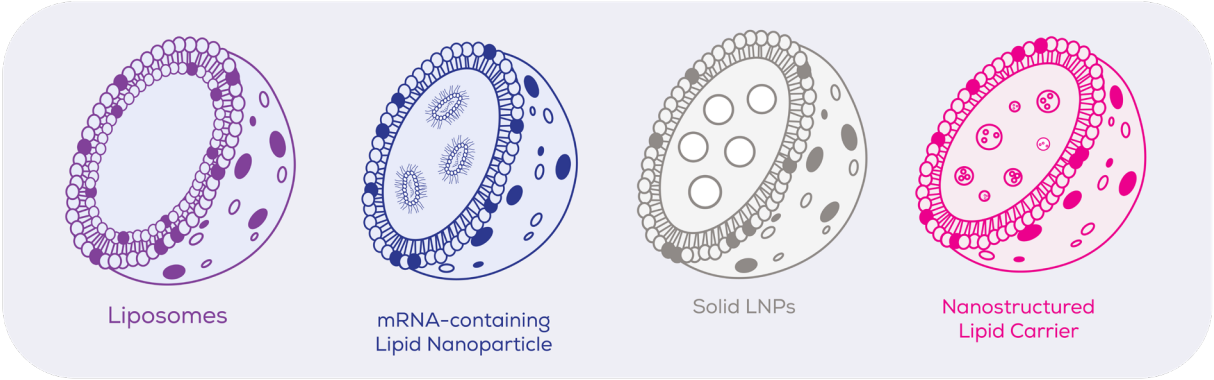

Figure 1. Types of lipid nanoparticles.

The rapid, widespread use of LNPs during the pandemic has spawned numerous potential therapies that contain mRNA and use LNPs as a delivery system, making this one of the largest growing areas of nanomedicine. With this comes an urgent need for high-resolution particle measurement techniques for LNPs.

What is Tunable Resistive Pulse Sensing (TRPS)?

The Exoid is Izon Science's most advanced Tunable Resistive Pulse Sensing (TRPS) instrument. It uses the Coulter principle to simultaneously measure size and concentration or size and zeta potential in a particle-by-particle manner. The principles of TRPS are explained in Figure 2. With two measurements you can measure the size and concentration of particles in your sample, and collect concurrent size and zeta potential measurements on individual particles.

The Exoid has been shown to have superior resolution as compared to ensemble techniques such as Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). With its capabilities of measuring particles from 40 nm all the way up to 11 µm in diameter, the Exoid is well suited to a variety of particle measurement applications. One such application is the measurement of nanomedicine vectors such as LNPs.

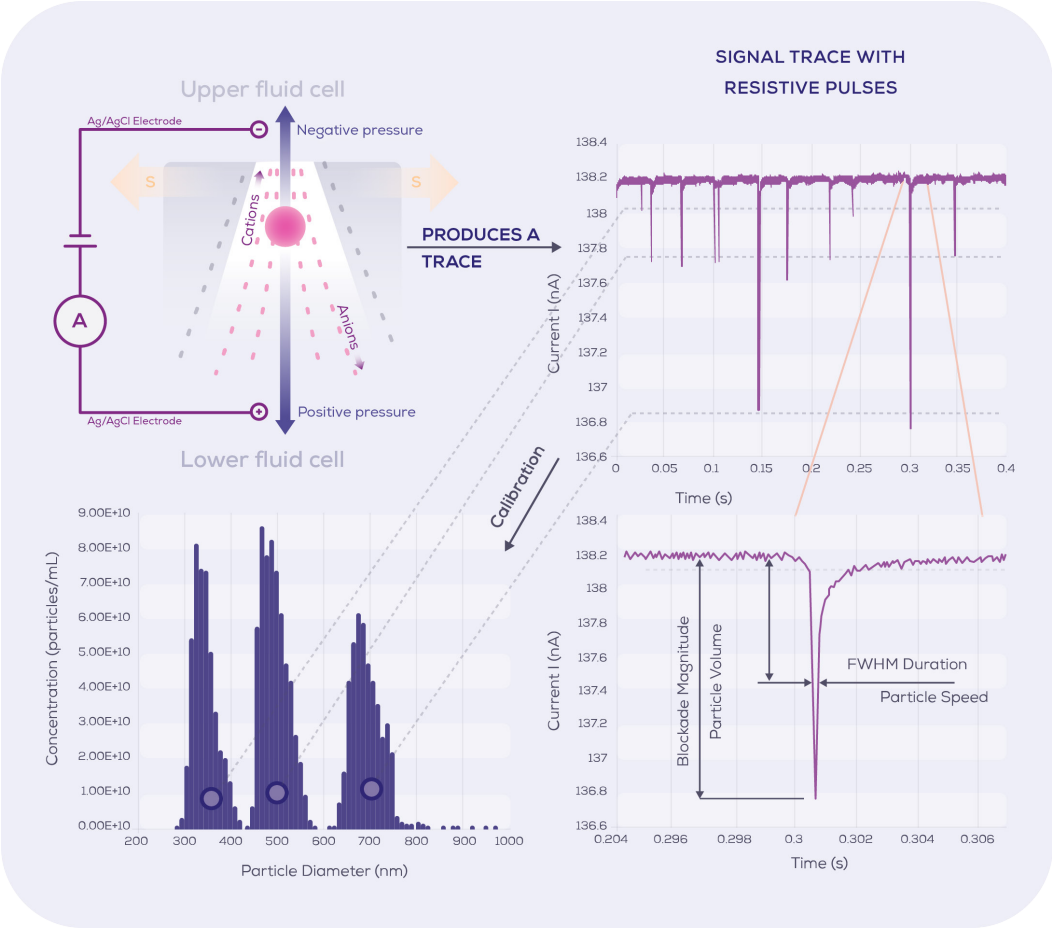

Figure 2. Schematic diagram of Tunable Resistive Pulse Sensing (TRPS). Voltage is applied across a fluid cell via two electrodes, causing particles in solution to pass through a tunable nanopore. The nanopore stretch (S), pressure, and voltage can be tuned to optimise the detection of your particles. From the resulting trace, the blockade parameters are determined, giving quantitative particle measurements on a particle-by-particle basis. From each blockade, the size (via blockade magnitude) and zeta potential (via blockade duration) of the particle can be measured. Either size and zeta potential or size and concentration (blockade rate) of particles can be measured concurrently.

Why Generate In-House Data on LNPs Using the Exoid?

Whilst we and our customers have been using the Exoid and TRPS more generally to analyse liposomes for many years, the more complex LNPs had only been used by a minority of customers and not yet by ourselves. Therefore, we set out to generate data on the capabilities of the Exoid to determine the size, concentration, and zeta potential of LNPs which are similar to those used in some SARS-CoV-2 vaccines. As such, we aimed to generate data and protocol insights to enable customers to make an informed choice on whether the Exoid is suitable for their LNP analytics.

2 / MEASURING LNP SIZE, CONCENTRATION AND STABILITY WITH THE EXOID

Size is one of the most fundamental and important parameters in nanomedicine, especially when it comes to the characterisation of formulations and their stability.

Particle Size Measurement and Size Distribution

The first trial that we put the Exoid through was a test of whether we could detect tiny differences in particle size between two populations of LNPs. For this, we used unloaded LNPs and compared them to those loaded with mRNA, hoping to see a small size difference which we theorised may arise from the reorganisation of the lipid structure around the negatively charged mRNA molecules.

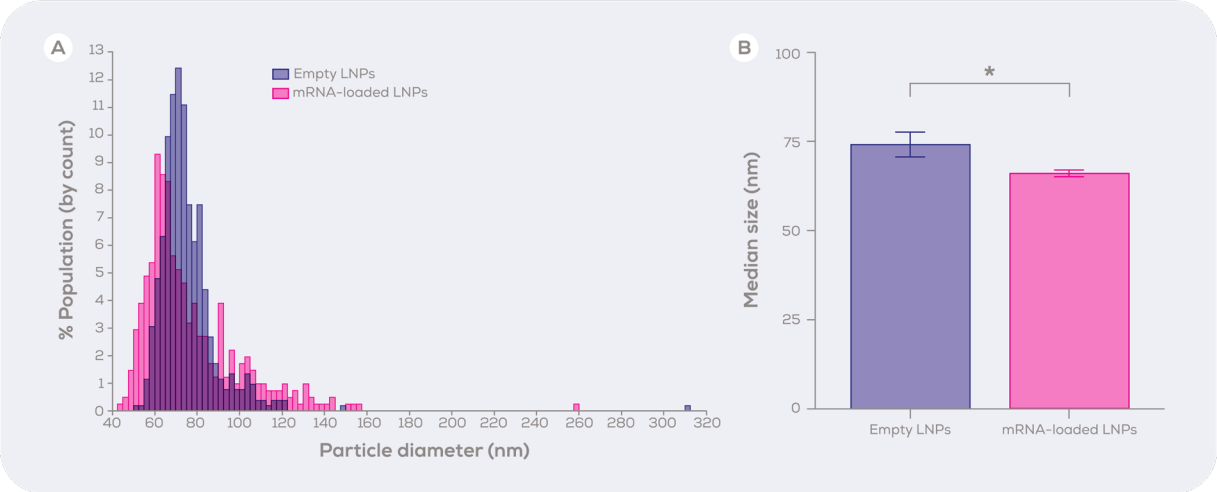

Figure 3. Size of empty LNPs and mRNA-loaded LNPs. (A) Representative size distribution graph generated using the Exoid. (B) Median size ± interquartile range of empty LNPs (n=4) and mRNA-loaded LNPs (n=4), compared using the Mann-Whitney U test, *p<0.05.

As shown in Figure 3, this was a success. In Figure 3A, you can see a representative size distribution chart comparing the empty and mRNA-loaded LNPs. The median size (and interquartile range, IQR) of the empty LNPs was 74 nm (IQR: 7 nm), while the median size of the mRNA-loaded LNPs was 66 nm (IQR: 2 nm), across 4 measurements of each particle type. Figure 3B shows how this small change in size was replicable between measurements and statistically significant ($p<0.05$), demonstrating just how capable the Exoid is of consistently detecting small size differences between samples.

Additionally, the Exoid allowed us to identify that the size distribution of the mRNA-loaded LNPs is wider than for the empty LNPs. This was reflected in their polydispersity index (PDI) which was 0.04 for empty LNPs and 0.09 for mRNA-containing LNPs.

Concentration

In those same samples, during those same measurements, the Exoid also calculated the sample concentrations. As you can see in Figure 4, the same data carries yet another story. While the size difference is less easy to see by eye, the difference in concentration (shown on the y-axis) is completely evident. The mRNA-loaded LNPs were 5.9x more concentrated than empty LNPs.

Note that this is the normalised concentration, not the concentration actually seen by the Exoid. The mRNA-loaded LNPs were diluted more than the empty LNPs in order to get them into the best concentration range for a good measurement. Simply entering the dilution factor into the Exoid Control Suite software whilst taking the measurement meant that we didn't have to do any maths or transformations afterwards. The Exoid Control Suite did that all for us.

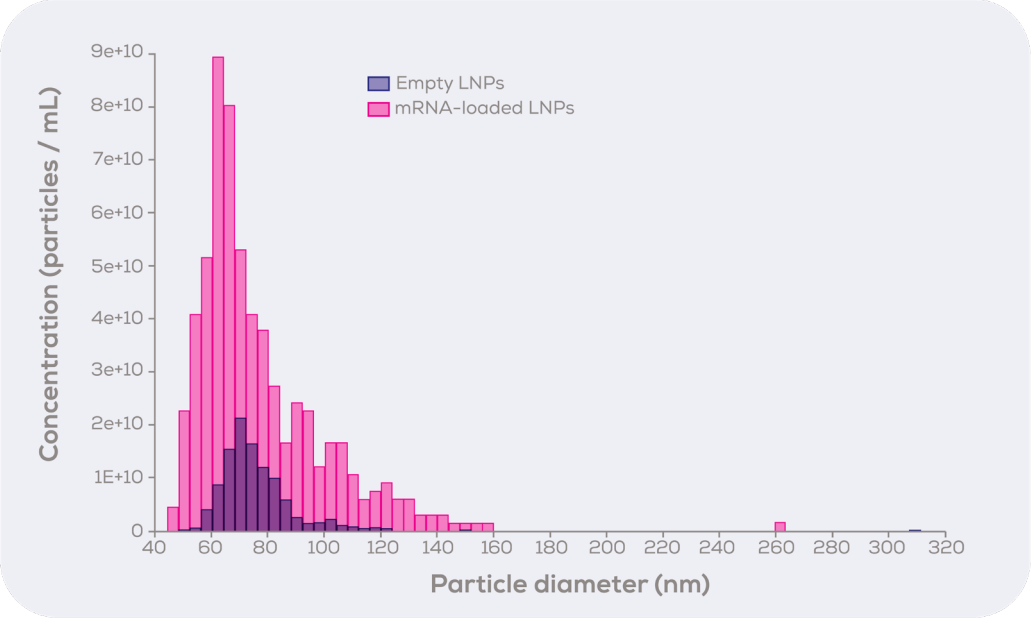

Figure 4. Size and concentration of empty and mRNA-loaded LNPs.

Stability

One of the biggest challenges for mRNA-loaded LNP vaccine rollout across the globe was the relatively short shelf-life of the particles at ambient or even refrigerator temperatures. This meant that not only did these vaccines need to be kept under strict storage conditions, but also that vast quantities of vaccine doses had to be discarded due to passing use-by dates. As such, stability is not only a key metric for LNPs, but is also likely to be a major area of research in improving formulations. Especially when it comes to vaccinating away from major population centres where strict storage condition requirements cannot always be met.

In order to stress-test LNPs and induce fusion or aggregation, we put them through two freeze-thaw cycles and compared them to their 'fresh' counterparts. Figure 5 shows what happened when we put the mRNA-loaded LNPs through this test. As expected, mRNA-loaded LNPs subject to freeze-thaw cycles were larger in size (median 93 nm; IQR 11.5 nm; n=3) than fresh particles (median 66 nm; IQR 2 nm; n=4). Figure 5B shows that this increase in size is statistically significant, highlighting the power of the Exoid to monitor LNP stability.

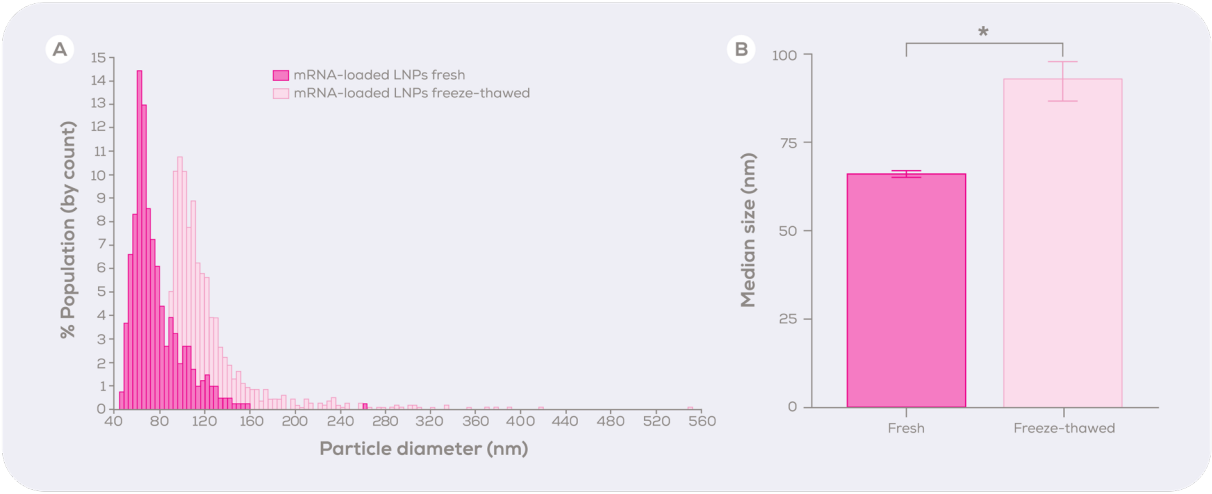

Figure 5. Comparison of fresh and freeze-thawed mRNA-loaded LNPs by size. (A) Representative size distribution graph generated using the Exoid. (B) Median size and interquartile range of fresh mRNA-loaded LNPs (n=4) and freeze-thawed mRNA-loaded LNPs (n=3), compared using the Mann-Whitney U test, *p<0.05.

3 / MEASURING LNP ZETA POTENTIAL WITH THE EXOID

Comparing the Zeta Potential of Empty and mRNA-Containing LNPs

Finally, we put the Exoid to the test of discerning any difference in the zeta potential – i.e., the surface charge – of the empty and mRNA-loaded LNPs. If you take a look at Figure 6, you can see that zeta potential differs between the two particle types; the empty LNPs had a zeta potential (mode ± SD) of -10 ± 1.1 mV, whilst the mRNA-containing LNPs had a lower zeta potential of -17 ± 1.1 mV (Figure 6), potentially reflecting the loading of negatively charged mRNA.

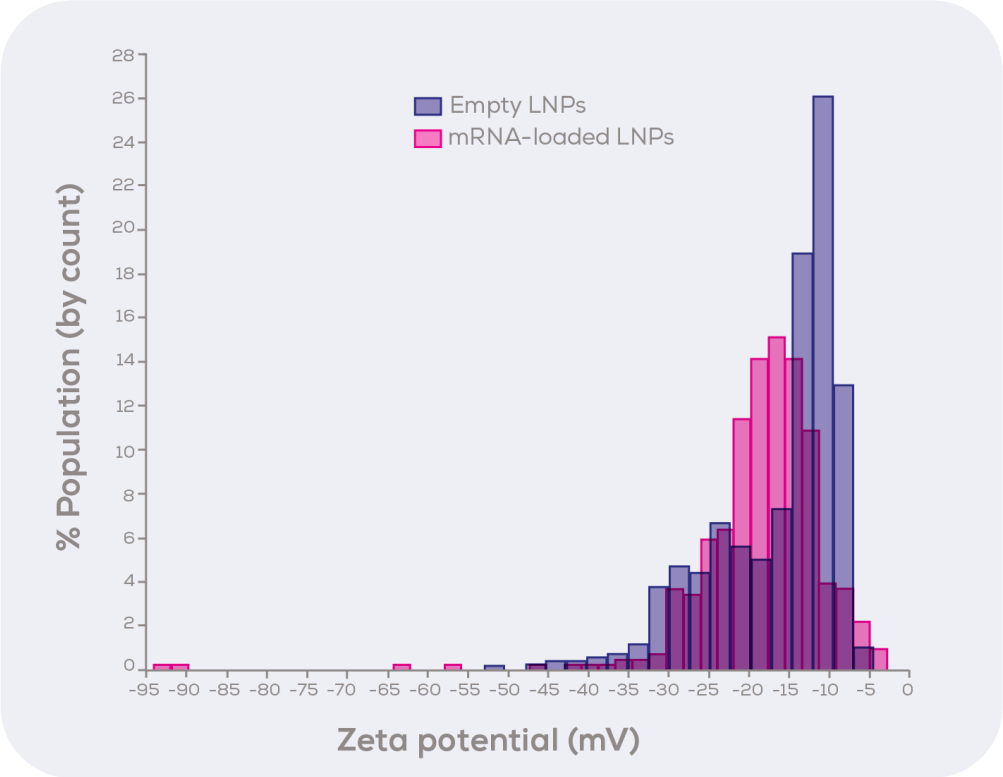

Figure 6. Zeta potential of empty and mRNA-loaded LNPs.

Considerations for Measuring LNP Zeta Potential Using the Exoid

It's worth pointing out here that some LNPs, perhaps even many LNPs, are positively charged. You can get some idea of whether a particle is positively or negatively charged based upon the voltage needed to induce the particles to pass from the upper fluid chamber of the Exoid to the lower fluid chamber.

In a regular set-up where a positive voltage is applied, the lower electrode is positive and would therefore have repelled the LNPs if they were of positive charge. In this case, we would have had to reverse polarity of the system, which is as easy typing a '–' in front of the voltage. Currently, the Exoid Control Suite software is unable to analyse this data (a patch for this is in the works!) but in the meantime we are more than happy to play the part of the software and do the analysis for you. Should this option be of interest, be sure to reach out so we can discuss this in more detail.

4 / THE EXOID BRINGS SINGLE-PARTICLE CHARACTERISATION TO NANOMEDICINE

In our hands, the Exoid performed excellently when it came to the analysis of LNP size, concentration and zeta potential. Unlike other technologies, TRPS measures particles individually, which reduces the chances of overlooking small yet potentially crucial differences between samples. In the field of nanomedicine, this is incredibly important as these differences could reflect quality issues that could cause harm and derail your therapeutic. Consequently, the Exoid is a wise choice for nanomedicine in general, and specifically for LNP development.

To learn more about how the Exoid could transform your nanoparticle analysis, reach out to us via our website at www.izon.com


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