Micropore Technologies

e-Book

Micropore Technologies

Essential Guide to Nanoparticle Engineering for drug delivery

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Introduction

Thank you for downloading the latest edition of Essential Guide for Nanoparticle Engineering for drug delivery systems, a comprehensive collection of tips and best practices that aims to help you select the right technology to develop an optimised formulation and ensure high reproducibility with desired characteristics.

The guide will help you understand the strength and weaknesses of some popular nanoparticle production processes and outline what an ideal platform for nanoparticle production looks like. The benefits of Micropore Technologies platform will be discussed. All articles in this eBook contain practical guidance from subject matter specialists and have already proven to be invaluable resources.

Topics covered in the following pages include steamlining LNP manufacturing, key areas for success, overcoming roadblocks, Lessons learnt after COVID, a comprehensive guide to LNP manufacturing and understanding future needs and developments.

Once you are done perusing this edition, we encourage you to check out our website or contact us for a technology demonstration.

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CHAPTER ONE

The Era of Streamlining LNP manufacturing is here

This section discusses the need for a single, fit-for purpose LNP manufacturing technology

The Era of Streamlining LNP manufacturing is here

Introduction

The need for scalable production of LNP from lab to GMP has never been greater. This shift has become more intense due to demand created by mRNA-LNP based vaccines following the COVID-19 pandemic. Further compounded by research developments in cell and gene therapies in response to the need for novel treatment options the technology offers new opportunities to combat cancer, infectious disease and other currently untreatable medical conditions.

LNPs hold great promise as they not only enable the efficient delivery of therapeutic payloads such as RNA, but unlike other delivery systems like viral vectors, LNPs show low cytotoxicity and immunogenicity and can be manufactured using cell free production processes with the potential for rapid scaling. Despite clear benefits, LNP therapies can be challenging to develop and manufacture due to:

- **Complex structure:** LNPs are multi component particles, consisting of ionisable cationic lipids, phospholipids, cholesterol, and PEG lipids, with a high degree of intrinsic structural complexity which can influence their activity (Figure 1).
- **Stability:** Developers must accurately measure and carefully control a range of key attributes to ensure stability and guide production process optimisation and release specification.

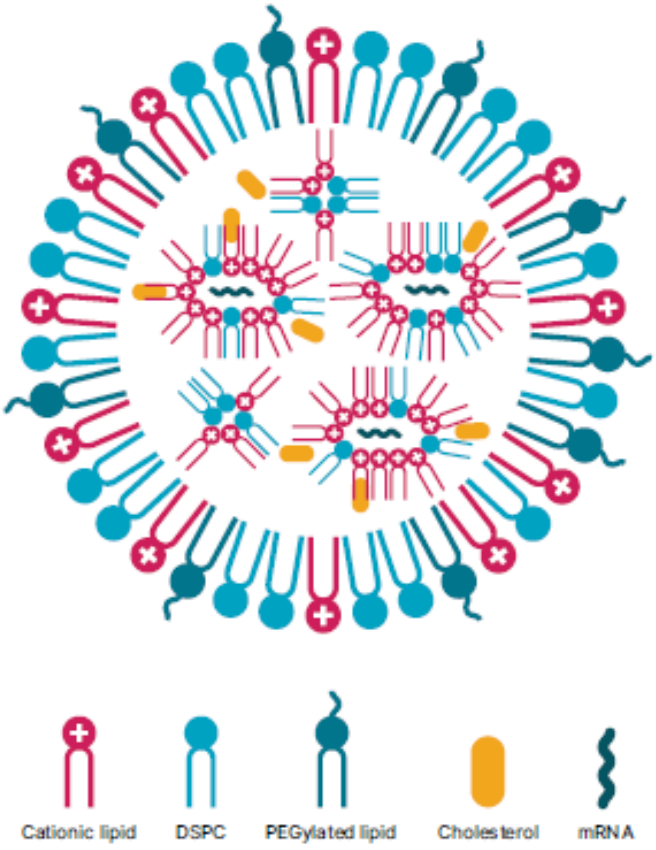

Figure 1: Illustration of a typical messenger RNA (mRNA)-LNP complex (DSPC = distearoylphosphatidylcholine).

The time for change is now

Traditional techniques used for formulating and manufacturing more established therapeutic modalities, such as monoclonal antibodies, are often not suitable for the complexity or pace of development when it comes to novel LNP-based therapies.

As such, researchers and the biopharma industry urgently need a single, fit-for purpose LNP manufacturing technology that can be used throughout the formulation development and manufacturing process enabling more confident decision-making for faster delivery of vaccines and genetic medicines globally to patients in need.

Create LNPs Today

The massive success of the COVID-19 vaccines has brought global attention to the potential of mRNA-LNPs as medicines. . LNPs, generally with a diameter of 50–100 nm, are formed by controlled nanoprecipitation of the lipids around the RNA molecules.

A

Early phase

The ionisable lipid first surrounds the RNA by electrostatic interaction with the anionic phosphate groups.

B

Mid phase

Subsequently, the cholesterol and phospholipids contribute as structural components.

C

Final Phase

PEGylated lipid inserts into the LNP surface, with the PEG group facing outwards, providing a hydration layer, and making the LNPs less prone to early elimination by the immune system,

Your payload can be a crucial structural component

In addition to their pharmacological function, mRNA is a crucial structural component of the LNPs and together form a non-covalent complex. With no RNA present, the LNPs are kept intact mainly by hydrophobic inter-lipid interactions, and their stability is severely reduced.

mRNA molecules are large (>600 kDa) which are condensed when encapsulated into LNPs. The size, and PDI help indicate the success of particle formation. Empty LNPs are not stable over time and always show higher PDI. The final size and the success of encapsulation of the mRNA is influenced by the buffer composition. Thus understanding the structure and environment of LNPs is very important to optimise stability.

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confirm particle properties

ATA Scientific supports a range of characterisation technologies that can accurately measure and allow careful control of a range of key attributes to determine their stability and guide and inform product design, production process optimisation and release specification.

Some key attributes of LNPs include size, polydispersity index (PDI), concentration, surface charge, therapeutic payload/ encapsulation efficiency and thermal stability.

Notably, particle size measuring analytical techniques are used throughout the nanoparticle development journey, from research to industry and can include key methods such as Dynamic light scattering (DLS), Multi-Angle light scattering (MADLS) and Nanoparticle tracking analysis (NTA).

See chapter 4 “Essential analytical technologies for developing & manufacturing NPs” for a comparison of each technique.

Lessons Learnt

After COVID-19

" So I think what we could be doing in Australia is really focusing to some degree in developing that early-phase, small-scale manufacture that can go into phase 1 clinical trials " Paul Young

Looking back at our experiences during the pandemic brings into sharp focus our reliance on the rest of the world to survive. The supply chains we had become so accustomed to had fallen over. Whilst there was a global scientific collaboration, it was difficult to get the required consumables for critical pieces of equipment, as scientists clamoured to secure sufficient volumes for their research.

Roadblocks appeared related to insufficient equipment and high cost, not only in the early stages of vaccine development but also in the small-scale manufacture for early phase 1 clinical trials. The "*extraordinarily complex process and expertise needed*" to navigate the regulatory environment and GMP manufacturing only added to the hurdles..

The pandemic has highlighted the importance of developing local manufacturing capacity that can move quickly at times of need, not only for vaccines but for a range of diagnostics and therapeutics. The choice of equipment should be made with broader engagement with industry to ensure a fit-for-purpose approach.

A recent article in Lab and Life Scientist "COVID-19, vaccines and pandemic preparedness: lessons learnt" three prominent vaccinologists look back at the challenges they faced early in the pandemic. There is a common thread that runs through this article, the importance of being ready.

"So that so-called pandemic preparedness, and having those sort of platforms available, is really the most important insurance that we can do in the research sector to be prepared for whatever might unexpectedly turn up in the future" Professor Terry Nolan stated.

Reference: "COVID-19, vaccines and pandemic preparedness: lessons learnt" Lauren Davis https://bit.ly/48VyydV

Discovery, Clinical Or Commercial Batches?

Does Scale Determine What Equipment You Need?

Batch Mode

Many popular nanoparticle manufacturing technologies are scaled to provide either very small volumes for research and development (R&D) or are designed for large-scale use in accordance with good manufacturing practice (GMP) and the equipment type selected can also impact the type of NP produced. Until recently manufacturers have had to transfer technology between the preclinical, clinical, and commercial phases, but not anymore – Introducing Micropore Technologies with advanced cross flow (AXF) mixing technology.

Controlled Continuous Mode

The Micropore AXF Pathfinder series system uses the one, single, identical mixing device to deliver 200 µl sample to 5 to 10 litres. If a closed system is required for GMP production, the very same mixer on the Pathfinder (AXF-Mini) can be used in the Horizon™ m capable of production volumes of 0.5 – 2000 Liters / hr. Whilst this is impressive, consider it is the smallest version of the AXF. This is without a doubt revolutionary, owing not only to its flexible design which enables novel formulations to be created that may otherwise not be possible, but also the ability to produce large quantities without the need for additional equipment and investment in more technology. Formulations are absolutely scalable without tweaks and changes, which for those working to meet stringent regulatory requirements, this is highly desirable.

CHAPTER TWO

Manufacturing LNPs: Current mRNA vaccine production technologies

In the next section, we explore a few popular methods currently being used to create LNPs and discuss some of the strengths and weaknesses of each technology.

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The production of technologies, like most things, is not immune to imperfection, and sure as eggs, as soon as someone makes a product that enjoys popularity, another tries to compete with a new improved variant attempting to cash in on the success without the massive development cost – virtually reverse engineering. The simplest of techniques still has a mountain of scientific theory and engineering to support the design, protected with patents.

What technologies are available now and how do they rate?

This document will discuss microfluidics, impinging jet mixers, and advanced cross flow mixing, three broad techniques that all have uses and some with very real limitations.

The context is the encapsulation of a treatment where the modality is nanoprecipitation, and the consensus of opinion is the method enables mixing rate to be faster than assembly rate that must be achieved in laminar flow.

The key to laminar flow is to allow a predictable, repeatable formulation. These techniques were also selected as they all claim to create at scale. Whilst not covered in this discussion, homogenisation, extrusion, and thin film hydration are useful tools worth considering for applications they are suited.

Microfluidics

There has been a veritable explosion of technologies in this area with a range of offerings from simple devices used in research labs to massive companies with global appeal supplying instruments spanning from low volume formulation through to litres for GMP applications. Arguably one of the earliest microfluidic mixer technologies was the Staggered Herringbone Mixer (SHM), a microfluidic channel with a repeating pattern of grooves. Kwak et al[1] noted 'Convex Grooves in Staggered Herringbone Mixer Improve Mixing Efficiency of Laminar Flow in Microchannel' detailing how the convex pattern from a negative flow pattern was less efficient than the positive pattern that has a concave SHM structure on the bottom of the microchannel. This work was built on a body of research over decades, Aubin et al. [2] demonstrated the grooves that are 30% deeper than the channel height have a higher mixing efficiency, given that it promotes spatial homogenisation without increasing the pressure in the mixer.

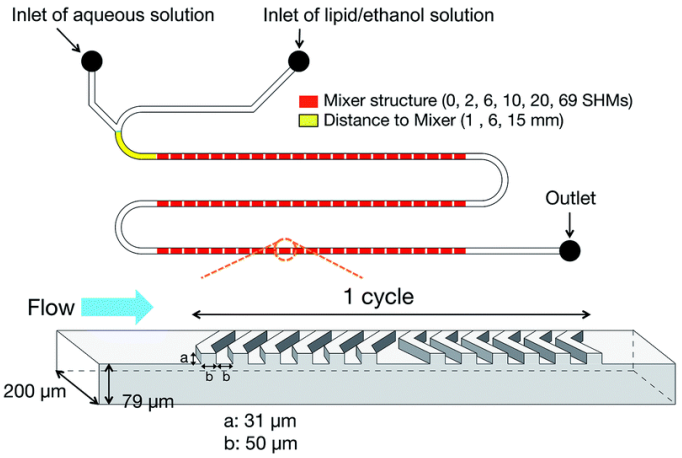

Fig. 1 Schematic illustration of microfluidic device with 69 cycle numbers of staggered herringbone micromixers (SHM)[3].

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Taking this basic design and controlling the inputs seems quite straight forward and indeed it is. Numerous commercial entities have developed systems to various degrees, some as simple as a syringe pump delivering the ingredients and a vessel to catch the formulated product, others are computer-controlled delivery in a dedicated box including ancillary support for drug development.

In the context of laboratory R&D, the staggered herringbone Mixer is an interesting option. There is a shortfall though, it has few prospects for scaling up. Some have strung a pile of the SHM in parallel in an attempt to achieve volume – not very useful in a GMP environment to produce thousands of litres. While microfluidic technologies have many advantages, one key disadvantage is the limited solvent compatibility for devices made of polydimethylsiloxane (PDMS). While these materials are common for devices fabricated by soft lithography, they can interact with organic solvents by swelling and deforming the intended structures, making them unsuitable for many formulations 7. They also give problems because of leachables / extractables with certain solvents. This is generally regarded as not being a problem with ethanol, but there seems to be increased regulatory scrutiny as data emerges.

In 2004, a mixer, based on the Dean Vortex, was fabricated, and tested in an on-chip format – although it was not overly novel at this time, there were many versions before. Howell[4] described the action inside a Dean Vortex; when fluid is directed around a curve under pressure driven flow, the high velocity streams in the centre of the channel experience a greater centripetal force and so are deflected outward.

This creates a pair of counter-rotating vortices moving fluid toward the inner wall at the top and bottom of the channel and toward the outer wall in the centre.

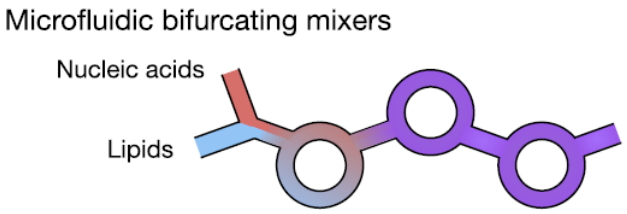

Fig. 2 Cartoon design of the Microfluidic Bifurcating Mixer[7]

This work was built on by Chen et al[5] in 2011 by optimising the geometry of design using the effects of various Reynolds numbers and channel configurations. The paper noted “The results indicate that for low Reynolds numbers (<5) diffusion is the primary mechanism by which mixing occurs. At Reynolds numbers greater than 10, secondary flows come into play and the lamellar formation contributes to increased levels of mixing.

There has been a litany of papers around the world with various versions of this style of mixer, all seeming to build on the previous works. The thesis by Ms Mathilde Enot, from Grenoble University – Pharmacy [6] focuses on the Dean Vortex Bifurcating Toroidal Mixer patented by Precision Nanosystems Inc. This comprehensive work defines the narrow ‘sweet spot’ this mixer has, not only with flow rates, but lipid concentrations to ensure the mixing conditions are maintained. To maintain formulation integrity across platforms, Enot describes the necessity to match the Reynolds numbers and Dean numbers of one system to another ensuring formulations are similar.

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Additionally, as she moved a formulation from the low volume system to the next volume device there was a significant increase in mechanical loss of the pDNA payload.

Most commercial players attempt to sell the 'journey' from research up through to GMP, it is clear this may not be as seamless as they purport given the need to manufacture greater volumes means the mixing architecture must change to accommodate the change in flow, but still be able to maintain the Reynolds number, Dean number, and laminar flow conditions, despite the use of a Dean Vortex Bifurcating Toroidal Mixer design throughout the scaling steps.

Impinging Jets Mixers (IJM)

Opposed IJMs/reactors are generally divided into two types, depending on the geometry: Confined impinging jets mixers with cylindrical chamber and injectors and T-jets mixers with rectangular cross-section chamber and injectors[10].

T- mixers

By absolute definition the T mixer isn't a microfluidic process as the mixer dimensions are over 1mm.

As the name suggests, two streams are forced toward each other with a perpendicular output. To be efficient in the mix, both streams need to be of equivalent force and at quite high flow rates. This can be a limitation given it would be difficult to scale it down for low volume applications. Low flow rates minimise the effectiveness of the turbulence required, and it is likely side-by-side diffusion.

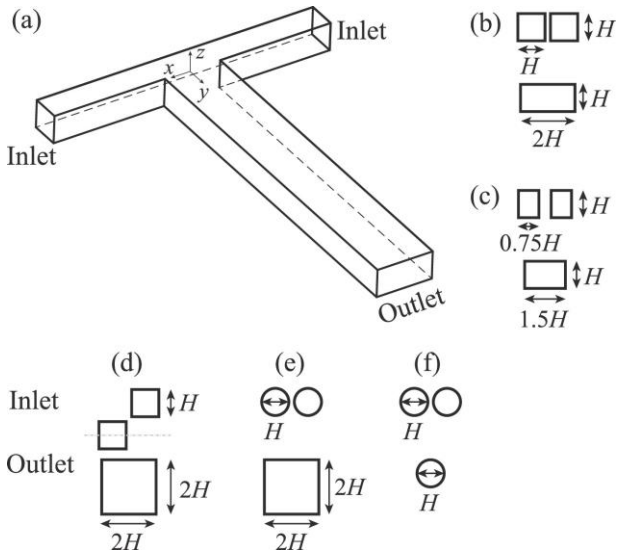

Fig. 3. (a) A schematic illustration of a T-junction section, where a Cartesian coordinate is set with the origin located at the Centrepoint of the junction. (b–f) Geometries of the inlets and the outlet[8].

Huixin Li[8] explored the numerical and experimental simulations elucidating the elementary fundamentals of fluids mixing in a T-Mixer. This manuscript discusses at length the correlation of mathematical models such as Reynolds Number (Re), Schmidt Number, the Navier-Stokes (NS) equations used to describe the flows. Particle Image Velocimetry (PIV) is an optical method used to measure instantaneous velocity of flows. Li used this method amongst others to determine the validity of the mathematical simulations. Decades of flow research and Li concedes "Overall, more efforts are necessary and greatly favoured to advance the knowledge of (turbulent) mixing in T-mixers."[8]

Clearly the unpredictable nature of turbulent flows would question the suitability of this method to be useful when developing a medical treatment for injection into a human patient given such variability.

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Confined Impinging Jet Mixers

A Confined Impinging Jet Mixer (CIJM) has two impinging jets with equal momenta. The liquid solutions, typically a solvent and a non-solvent, are injected into the CIJM and deflect off each other, creating extreme turbulence and rapid mixing. Nanoprecipitation occurs in an order of milliseconds, thus mixing must occur within this short window of time. Due to the speed and chaotic nature of this mixing processes, it is difficult to make predictions without a posteriori knowledge[9].

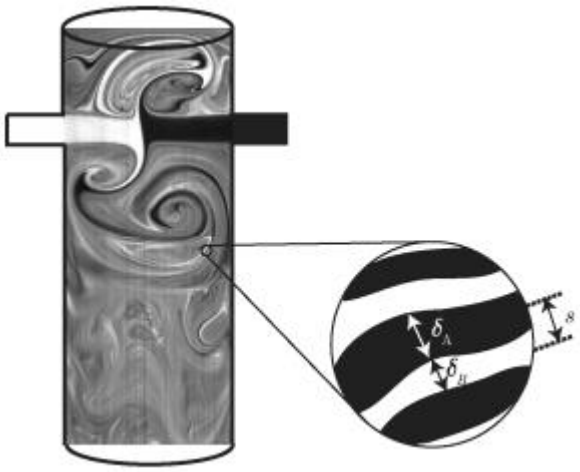

Fig. 4 Planar Laser Induced Fluorescence (PLIF) technique image of impingement mixing of a binary mixture. [10]

Pereira da Fonte's[10] dissertation focused on high viscosity monomers and pre- polymer mixing for Reaction Injection Moulding. Whilst not particularly suited to this topic of Lipid nanoparticle formulation, there are fundamental learnings from this work. Importantly Pereira da Fonte noted: " Unbalanced jet conditions were found to affect the flow significantly by moving the impingement point towards the chamber walls.

Under unbalanced conditions, the jets' oscillations are partially or completely damped, even when a dynamic flow regime is expected[10]". It is critical that impingement mixing occurs at the centre of the mixing chamber. Additionally, "When the Reynolds number is increased, maintaining the jets' kinetic energy rate ratio and at values different from one, the impingement point moves towards the chamber walls, closer to the lowest Reynolds number jet side. This phenomenon indicates that the impinging jets flow becomes more sensitive to small deviations in flow rates as Re is increased[10]".

Direct Number Simulation (DNS) modelling for such mixers is restricted to low Reynolds Numbers given the terrific amount of computational time, this can be enumerated for a sense of magnitude. Pope[11] "estimated that if the Re is doubled from 1,500 to 6,000 for a simulation of isotropic turbulence that is run at 1 gigaflop*, the simulation time increases from 13 days to 20 months. This is because the amount of floating-point number computations increases with Re by a cubed factor [9]".

*If computational Mathematics is not your forte – a Gigaflop is 1 billion floating point operations per second.

Understanding the predictive nature of a CIJM is seemingly more complex than a 'simple' T-Mixer, whilst not out of the question there will be a method to predict the result from the inputs, given the decades devoted to T-mixers, I feel it is unwise to hold your breath whilst waiting for this to occur.

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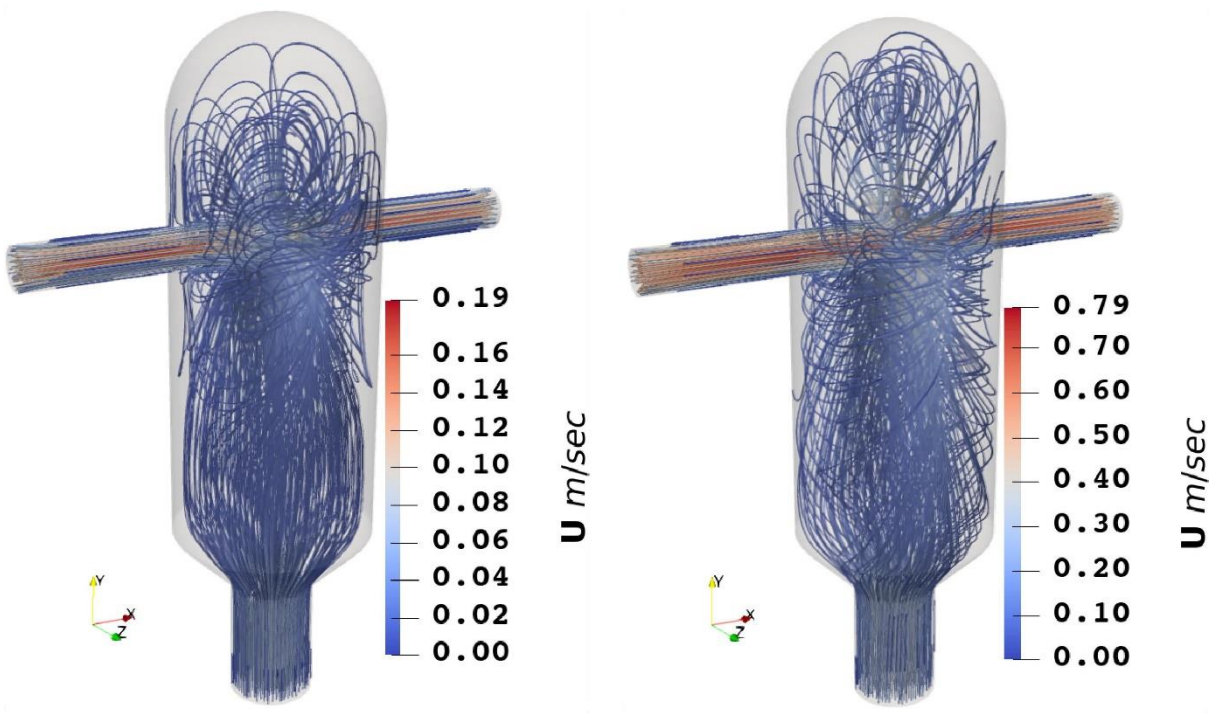

(a) (b)

Fig. 5. Instantaneous contour plot of velocity magnitude for (a) Re=62 and (b) Re=310. The streamlines of the flow for two different Re values. Large and small eddies form inside the mixing chamber and circulate before escaping through the outlet. While both simulations appear to depict turbulent flow, the higher-Re flow is much more chaotic than the lower-Re flow. The higher velocity allows the fluids to reach the top of the mixing chamber and swirl around the boundary. Ultimately, the Launder-Reece–Rodi Turbulence Model (LRR) model yields accurate predictions of the velocity fields, but there appears to be a limit at Re = 310 where the solution produces an error similar in magnitude to those of the Direct Number Simulations (DNS) predictions at Re = 62. [9]

Typically, these systems are notoriously difficult to control the output, furthermore, their use in a GMP context must be nightmarish and costly to change out all that tubing for a cleaning validation. Tying this method to an optimal condition would require a robust analysis method with dramatic feedback loops to effect a change should the output stray from the desired condition.

Gaining insight into the optimal condition is difficult but it is clear moving from a laminar state to a turbulent state computationally 'all hell breaks loose'.

For completeness, the Flash NanoPrecipitation (FNP)[12] method produced by Robert Prud'homme of Princeton University, was primarily a CIJM followed by a MIVM (Multi-Inlet Vortex Mixer) and then scaled down to a μMIVM, essentially a vortex mixing device with multiple inlets (4) to separate the input streams. Intriguing design with a deal of promise, however, the complexity of the system and lack of available data may hinder its adoption for full-scale GMP production. It is worth keeping in mind that it could be a valid technique once the fluid dynamics are shown to be reproducible without great sample loss.

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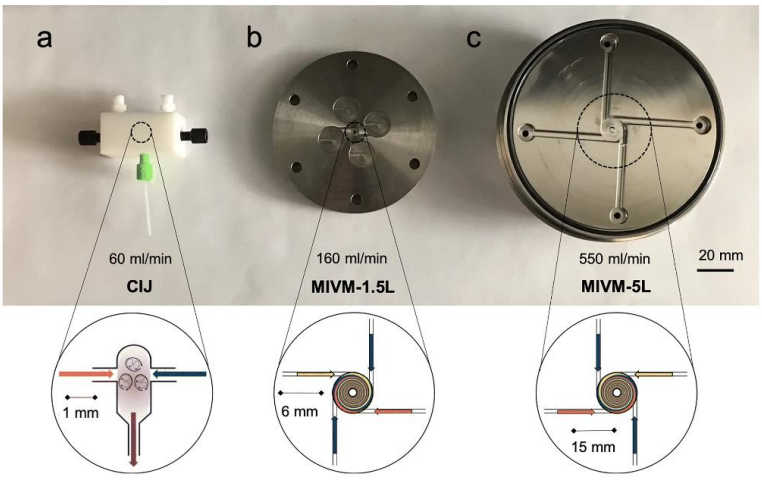

Fig. 6 Images a) CIJM b) MIVM- 1.5 L and c) MIVM 5L [13].

Paradigm shift

Numerous 'me too' systems are attempting to enhance the current popularism and in some instances directly copy existing technology, hoping to ride the wave of good fortune, but lacking novelty as evidenced by the depth of historical research.

Many systems employ a 'one size fits all' approach. Ultimately, they are inflexible operating in the constrains of their technological sweet spot, selling on the small then scale up – actually scale out - as they increase in size the physics falls over relegating them to create duplicates, effectively parallelising.

In science, often it is difficult to be across interdisciplinary technologies, with your goggles on, in your own bubble, but failing to step out and look to alternative solutions. All too frequently we are caught up in the trending technology considering them to be the only options. Unique companies such as ATA Scientific are multidisciplinary spanning a huge array of industries with incredible investment in our people enabling vision to identify solutions, at times to the most perplexing of problems.

Advanced Cross Flow (AXF)

Loughborough University – Micropore Technologies was spun out of the internationally respected Loughborough University Chemical Engineering Department. The patented technology was invented by Professor Richard Holdich, former Head of the Chemical Engineering Department.

The original membrane technology was invented in Japan by Tadao Nakashima and Masataka Shimizu of SPG Research Laboratory, Miyazaki Prefectural Industrial Technology Centre in 1986. The key point to note is this technology was developed for emulsion creation in chemical engineering applications, worlds away from nanoprecipitation and lipid nanoparticles. This highly awarded technology was adapted into the Lipid NanoParticles (LNP) field when COVID -19 was ravaging the planet.

It was during this time Micropore Technologies contacted Prof Yvonne Perrie, Head of Institute, Strathclyde Institute of Pharmacy and Biomedical Sciences - University of Strathclyde – Glasgow, to determine if the Membrane technology would be useful in encapsulating RNA into an LNP for large scale manufacture. During a 2021 webinar, Perrie noted that not only was it reproducible across a range of flow rates, the AXF system was showing very good Encapsulation Efficiency and volume14. There is ongoing collaboration with Strathclyde University.

Figure 7 is a basic schematic of the AXF-1 as noted in a paper by Holdich R., Dragosavac M., Williams B., Trotter S.[15] which largely discussed the technology as a single pass annular cross-flow membrane for the emulsions and dispersions industry elucidates some key fundamentals.

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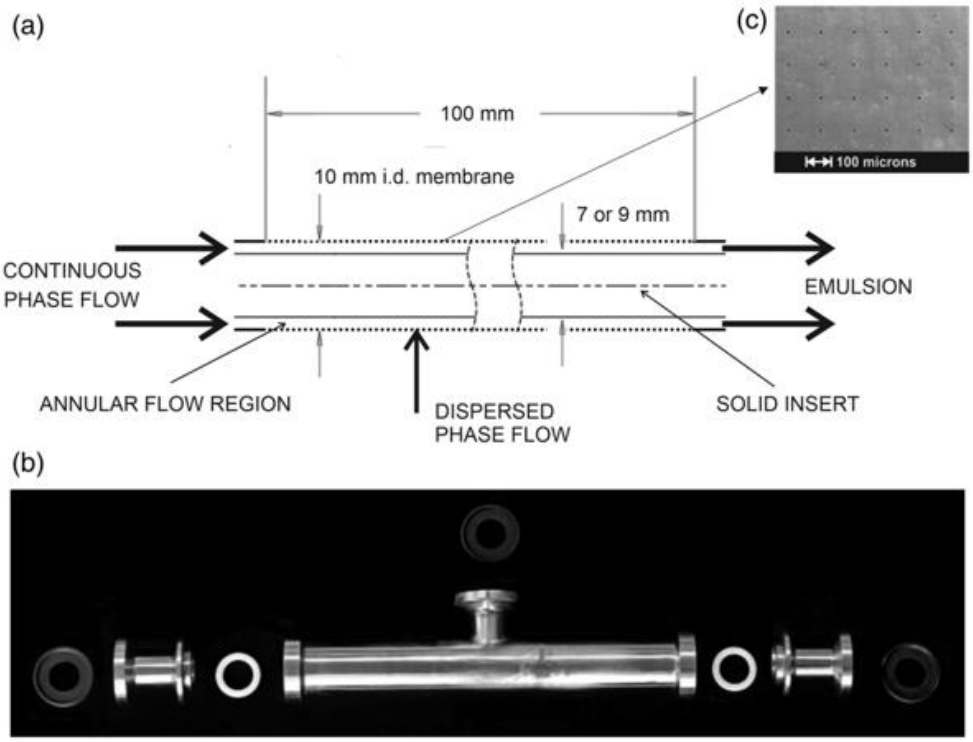

Fig. 7 Single-pass crossflow membrane emulsification[15]: (a) Schematic illustration of the annular flow system with insert and tubular membrane in place (note that outer shroud is not shown); (b) external image of the shroud and fittings for sealing the internal components; and (c) SEM of laser drilled stainless steel membrane.

Deceptively simple, the complex flow interactions afford a diverse applicability across a range of emulsions and dispersions plus, as we will explore LNPs. Holdich et al identified predictive equations defining drop size as a function of shear stress.

$$x = \frac{\sqrt{18\tau^2 r_p^2 + 2\sqrt{81\tau^4 r_p^4 + 4r_p^2\tau^2\gamma^2}}}{3\tau}$$

Equation (1)

'For interpretation of the results, a previously published equation for drop diameter (x) as a function of membrane pore radius (rp), shear stress at the surface of the membrane (τ), and interfacial tension (γ) was used,[3] Equation (1). The equation results from considering a force balance at the surface of a pore as a drop emerges, where the only forces considered relevant are the capillary pressure retaining the drop to the surface and the drag force induced by the wall shear stress. This equation has generally been found to predict the drop size at very low injection rates, the drop size increasing with injection rate, while maintaining the same surface shear stress.'[15]

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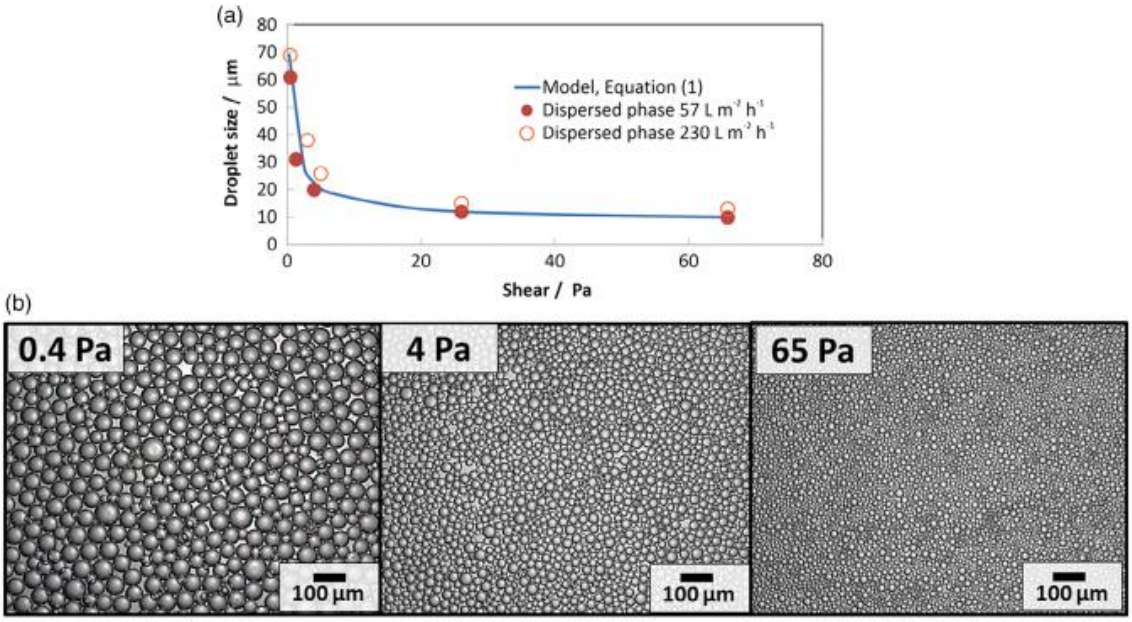

Fig. 8 (a) Drop size of silica precursor as a function of shear stress for the hydrophobic membrane system and comparison with Equation (1). (b) Images of the silica precursor beads produced at various shear stresses.

Whilst the above is a drastically abridged version of the findings, there is merit in noting the predictability of this platform. Confidence that simple equations can be built on to describe phenomena noted in practice, in distinct contrast to turbulent flow models.

Assessing this system initially for pertinence with LNPs was encouragingly simple. A basic lipid mix of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) 52%, Cholesterol (ovine) 45%, DSPE-PEG 3% was formulated against a aqueous phase of Phosphate Buffer Solution (PBS) using a syringe pump and a smaller version of the AXF-1 called the Mini (Fig. 9).

The pore sizes had been changed to that used for emulsions given the method required little shear as it operates with nanoprecipitation. Uniform particles were formed in seconds. In a request for further control, the formulation was presented to the high throughput AXF™ Pathfinder (Fig. 10). This equipment enables formulations to be ramped through a range of flow rates to aid in defining the optimum state. It took seconds to assess the ideal condition to produce particles of 55 nm with a polydispersity Index (PDI) of 0.06.

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Fig. 9 Micropore AXF-Mini

Testing this in real world conditions was the next challenge. A simple syringe pump and Micropore Mini rig was run independently at the University of NSW RNA Institute, Sydney, Australia where a SM-102 formulation was created encapsulating RNA produced on site. Dr Febrina Sandra noted the following with a flow rate of 12 ml / min: Particle size (z-average) 110 ±1.54 nm, PDI 0.178 ±0.012, Encapsulation Efficiency (EE) 96.14 %. This was tremendous given it was a very simple, uncontrolled rig. The University of Strathclyde are typically enjoying EE beyond 98%, in some formulations they are approaching 100%. It is worth noting, there is a significant cost attributed to every 1% drop in EE in the context of GMP production.

From a research point of view, there is a lot to like about the Pathfinder.

Fig. 10 Micropore Pathfinder

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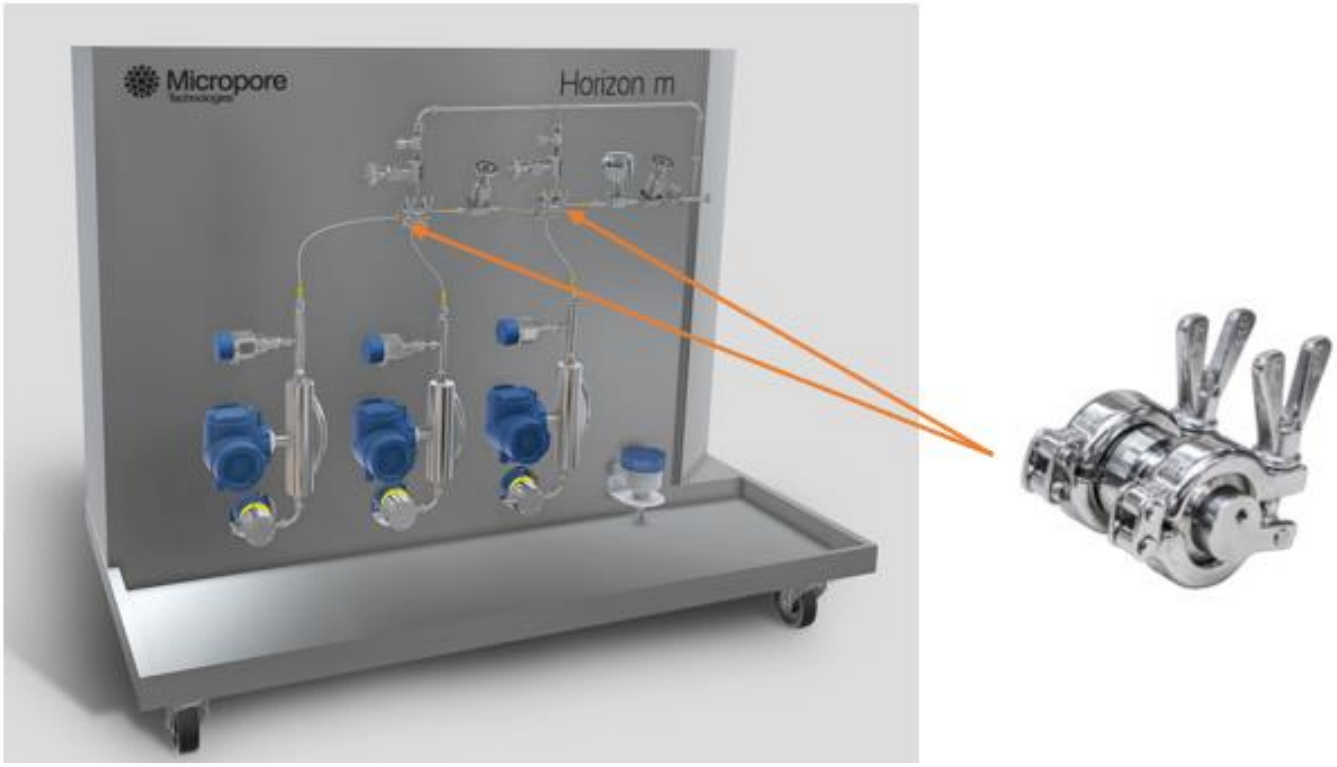

Fig. 11 Horizon m

Micropore Pathfinder

The key is the enormous flexibility it offers. Breaking the 'one size fits all' limitation decreed by alternate methods, the Micropore AXF system constructed with 316 stainless steel, with seemingly limitless variants of membrane design enables fundamental research to move forward beyond the current paradigm of formulation constraints and consumable costs into unchartered, innovative discoveries utilising formulation constituents currently avoided due to equipment incapability. Accepting a novel method requires rigor in testing and comparison to known entities, this has been easily established for the Pathfinder. As with most R&D techniques, the real issue is how to scale up, after all, if this research is to be translated to treatments, it needs to build to sufficient volumes.

How does the Micropore AXF handle scale?

The flexibility of the Micropore AXF system becomes clear when moving to scale. The identical mixer can move from a single device to deliver a 200 ul sample to 5 -10 litres on the Pathfinder series. Specifically, what you formulate is absolutely scalable without tweaks and changes, which for those who need to meet the requirements of the regulators, is spectacular.

If a closed system is required for GMP production, the very same mixer on the Pathfinder (AXF-Mini) can be used in the Horizon™ m (Fig. 11). This is an open skid design with custom control architecture, SS 316L throughout – no consumables. Integrated LNP manufacture and dilution in a system that is fully CFR 21 Part 11 compliant capable of production volumes of 0.5 – 2000 Liters / hr.

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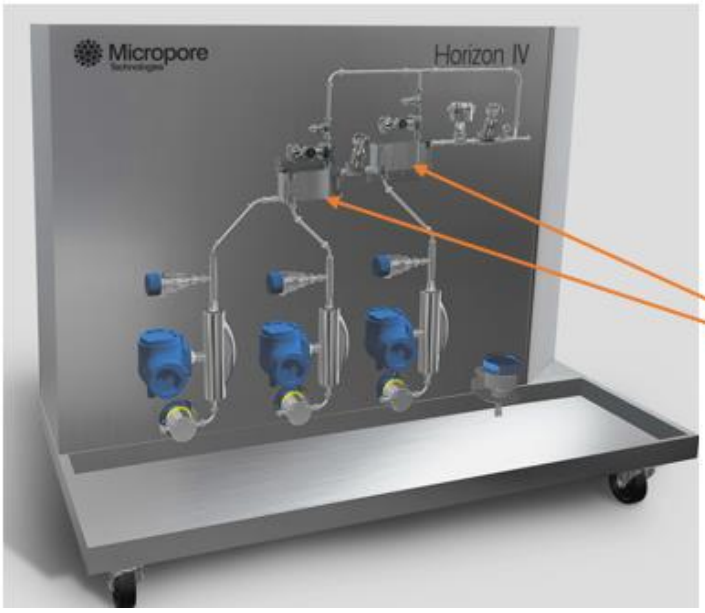


Fig. 12 Horizon IV

Custom built to client specification for capacity, feed system (pumped or pressure), full DQ / IQ / OQ / PQ support and PLC Integration.
Whilst this is impressive, consider it is the smallest version of the AXF. The range is astonishing. The AXF-1 as shown in Fig. 7 above, has a total flow rate of 2000 ml / min. Parallelise the AXF-1 to an AXF-4 and we begin to approach pandemic readiness (Fig. 12). Empirically, it is likely to produce the following under the stated conditions.

Total flow rate (mL/min): 8000
RNA concentration (µg/mL): 59
RNA dose (µg): 30
Annual production (doses): 3.5 billion.

This is not a pipe dream, the first GMP Pathfinder has been installed and commissioned, the first Horizon system has been ordered and is likely to be commissioned Q1 2024, this is for a 1 billion dose facility.

Clean in place (CIP)/ steam in place (SIP) makes economic sense, considering single use fluid paths can be very expensive, often well beyond the CIP / SIP option. Micropore Technologies is working on resolving a major bottleneck in the production of nanomedicines. The Tangential Flow Filtration step can take many hours to complete and is detrimental to the particles reducing EEs considerably – costing millions of dollars in payload and time. Not yet developed fully, but seductively intriguing, imagine using the AXF system as a single pass TFF.
To wrap up, Micropore offers opportunities to work with novel carriers not only due to the construction material but also the scope to make changes. Not all lipids react in the same way and with an increasing number of lipids in development, profit-driven awash with IP constraints, it's limiting access to 1000's of researchers and perhaps the discovery of a lifesaving treatment. Current offerings for LNP manufacture have very strict boundaries of optimal operation evidenced by their inability to effectively scale up. **There is a need to hyperdrive the use of novel delivery modalities and the Micropore Pathfinder is nicely poised to accommodate.**

CHAPTER THREE

Overcoming Roadblocks to Achieve Efficient Production of Nanoparticles

In the next section, we provide a quick comparison guide showing the benefits of using the Micropore Pathfinder AXF across all scales.

Overcoming Roadblocks

During lipid nanoparticle (LNP) manufacturing

One of the most common roadblocks faced in lipid nanoparticle (LNP) manufacturing processes is achieving consistent and reproducible product quality. Several factors contribute to this challenge.

Scale-up Challenges

Transitioning from small-scale laboratory production to large-scale manufacturing introduces challenges in maintaining the same particle characteristics. Scale-up can affect mixing dynamics, heat transfer, and other parameters, leading to variations in particle size, distribution, and stability.

Batch-to-Batch Variability

LNP production often involves multiple complex steps, and subtle variations in conditions or raw materials can lead to batch-to-batch variability. Small changes in parameters during these steps, such as temperature, pressure, or shear forces can influence the final product. Achieving consistent quality across different batches is critical, especially for applications like drug delivery where efficacy and safety depend on the characteristics of the nanoparticles.

Addressing these challenges requires a combination of implementing robust process development and quality control measures, and adherence to regulatory guidelines. Continuous research and innovation in the field aim to overcome these roadblocks and enhance the reliability and scalability of lipid nanoparticle manufacturing processes. Micropore Technologies overcomes scale-up and batch variability challenges with ease using advanced cross-flow mixing technology. The result is a faster, cheaper and more efficient LNP manufacturing process.

Quick Comparison Table

Micropore Technologies versus the rest

Here we summarise how the Micropore Pathfinder differs compared to other technologies in use.

	Micropore Technologies AXF Pathfinder 	Other mixing technologies – e.g Microfluidics, IJMs 
Consumables	**$** **Simple PTFE O-rings, low-cost consumable. Stainless steel, 316L precision-engineered mixing device.**	**$$$$$$$** High-cost consumables and reagents from suppliers increases operational costs.
Cost of Acquisition	**LOW** **Single device designed to cover entire process from lab to GMP.**	**VERY HIGH** Barrier for small research groups or organisations with budget constraints.
Scale-up Challenges	**NONE** **Fully scalable from development to manufacturing quantities.**	**HIGH** Transitioning to commercial-scale production may require additional considerations.
Material Compatibility	**EXCELLENT** **All stainless-steel materials allow a wide range of solvents.**	**LIMITED** Leachable/extractable material from plastic cartridges in some solvents.
Complexity/ Maintenance	**MINIMAL** **Simple to clean and can disassemble completely.**	**HIGH** Regular training to operate effectively, high maintenance can limit accessibility
Throughput	**CUTOMISABLE** **Membrane can be changed from nanoparticles to microemulsions building potential use for an array of fields not just LNPs.**	**LIMITED** Inflexible and fine tuned to specific flow rates limiting scope of use.

Formulating made simple

Our top 6 considerations that are key to success

If you had a wish list for the ideal platform that can be used to engineer nanoparticles with optimised formulations for drug delivery from the design stage through to commercial scale manufacturing, what would this be? Here are our top six.

1. Robust Control of Parameters

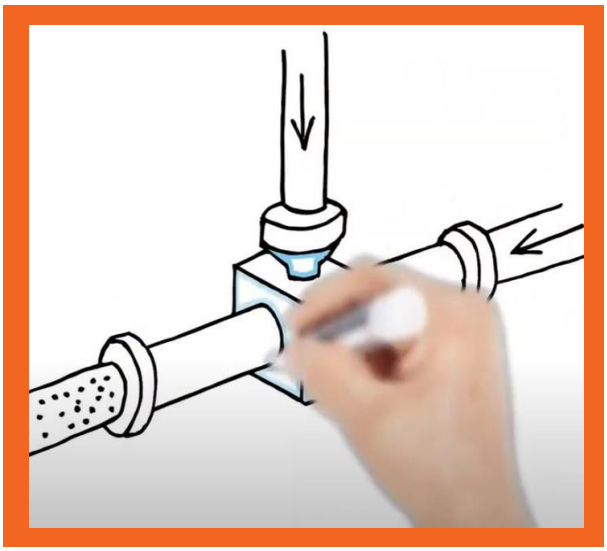
Accurate control over particle size, particle charge, polydispersity/particle size distribution, composition and encapsulation efficiency is crucial. The ideal platform would offer the ability to control parameters for optimising these factors during production, ensuring reproducibility across all scales

Micropore technologies allows for seamless scaleup with consistent physics, mechanisms conditions and geometry across its equipment range. Advanced cross-flow (AXF) mixing technology allows precision controlled, low shear, continuous flow mixing from nano to micro formulations to avoid roadblocks during your product development journey. It uses the same shear conditions, same physics and same technology from lab bench to manufacturing scale to enable scale-up with confidence, from 200µL up to 20 L per hour from a device that would still fit inside a briefcase.

2. Versatile/ Customisable

The platform should accommodate a wide range of materials and nanoparticle types, including organic, inorganic, and hybrid nanoparticles. It should support iterative testing and optimisation of formulations from the early design stages to commercial scale, allowing for fine-tuning. This versatility enables the production of nanoparticles tailored for diverse applications.

Micropore AXF technology uses a stainless-steel, 316L precision-engineered membrane, enabling a faster, cheaper and more efficient LNP manufacturing process. Start-up waste is very small - approximately 400 µL for the entire Pathfinder family. The system operates at very low pressure – approximately 2 Bar compared to T-mixer devices operate at much higher pressures – about 30 Bar. This enables the size of the particles created to be very predictable with any change in flow rate.



Formulating made simple

Our top 6 considerations that are key to success

3. Scalable/ Continuous Manufacturing Capability

The platform should seamlessly transition from small-scale research and development to large-scale commercial manufacturing. It should be flexible enough to handle varying production volumes without compromising quality and efficiency. The ideal platform would incorporate continuous production processes, enabling a more streamlined and consistent workflow.

Micropore technologies delivers reproducible, scalable LNPs using laminar flow mixing across a permanent stainless steel membrane. It differs from conventional mixing LNP techniques by considering the process not as discrete or separate unit operations but as one single whole process. The AXF Mini (and larger versions) sits at the heart of every Pathfinder AXF system. This award-winning device is the smallest cross-flow system in the AXF series. The AXF-1 and AXF-n extend throughput volumes from 0.06 to >2000 L per hour as a continuous manufacturing process.

4. Cost-Effective/Eco friendly

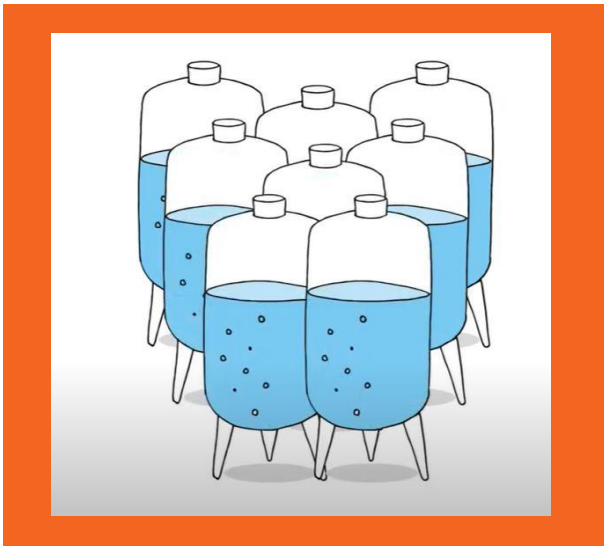
The platform should minimise raw material wastage and offer efficient processes to ensure cost-effectiveness at scale. It should incorporate environmentally friendly principles, removing the use of single use plastics and waste generation.

Micropore Technologies offer a stainless-steel mixing device that is simple to clean and requires PTFE O-rings - a simple, low-cost consumable. The AXF™ mini's extremely low internal volume means that start-up waste for the Pathfinder unit is only around 400 µL and sample sizes can be as small as 200 µL. High throughput screening of up to 96 samples can be achieved in less than a minute for rapid DOE and process optimisation.

> *"The sheer flexibility of the system to morph to a bespoke product to solve a particular need is outstanding."*
>
> **Pete Davis**
> Applications Scientist, ATA scientific

Formulating made simple

Our top 6 considerations that are key to success



5. Regulatory Compliance/ Process Monitoring

Adherence to regulatory standards is non-negotiable, especially in industries like pharmaceuticals. The platform should facilitate compliance with regulatory requirements, streamlining the path from research to GMP manufacturing. It should feature consistent nanoparticle production with real-time monitoring and feedback mechanisms, crucial for quality control.

The Pathfinder AXF is a fully integrated Design of Experiment (DoE) system with intuitive software that avoids single-use components that can be used for Discovery, Preclinical and Clinical work on wide range of particles sizes and different applications. Samples sizes can be upgraded using the same device as requirements dictate without compromising on process quality, efficiency or safety. Constructed of 316L stainless steel Micropore disrupts the current drug development paradigm, by allowing rapid formulation screening and manufacturing without the need for a disposable cartridge and technology transfer, helping to save thousands of dollars.

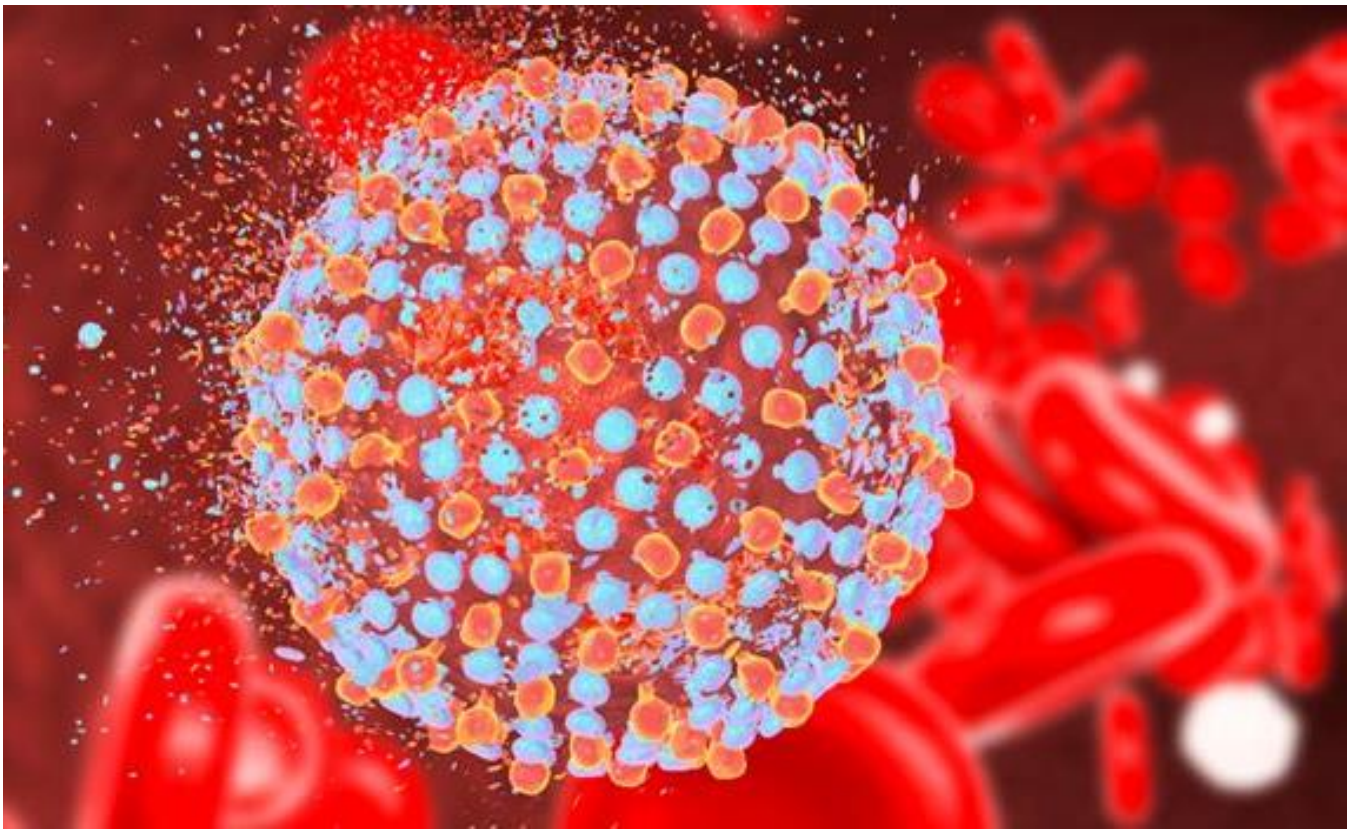
6. Global Support

Access to global technical support ensures that users can maximise the platform's potential. Ongoing support is crucial for troubleshooting and addressing challenges throughout the nanoparticle production lifecycle.

Micropore is a technology provider with global experience in manufacturing all different types of vaccine modalities can further ensure a cost-effective, high-quality process. Partnering with Micropore will enable a stronger benchmark with in-depth expertise and the ability to leverage novel technologies will also help reduce risk and shorten timelines.

CHAPTER FOUR

Essential analytical technologies for developing and manufacturing Nanoparticles



In the next section, we discuss a suite of robust orthogonal analytical tools that can help you accurately measure and control a range of key attributes to determine stability and help guide product design and production.

Essential Analytical technologies

Developing and manufacturing safe and effective LNP therapies

Accurately measuring and carefully controlling a range of properties can help inform LNP design, and stability while also optimising production processes. Here we discuss some of the key analytical technologies that characterise critical quality attributes (CQAs) of LNPs.

The importance of LNP size, polydispersity, and concentration

Size is a critical attribute for the function of LNP-based therapies. It can determine tissue penetration and efficacy and identify potential instability typically displayed as aggregation due to external stresses.

Sizing up your LNPs: available tools & techniques

LNP-based therapy developers have a selection of size-measuring analytical techniques at their disposal when it comes to efficiently and reliably measuring LNP vector size — namely single-angle Dynamic Light Scattering (DLS), Multi-Angle Dynamic Light Scattering (MADLS), and nanoparticle tracking analysis (NTA). These techniques cover a wide particle size range.

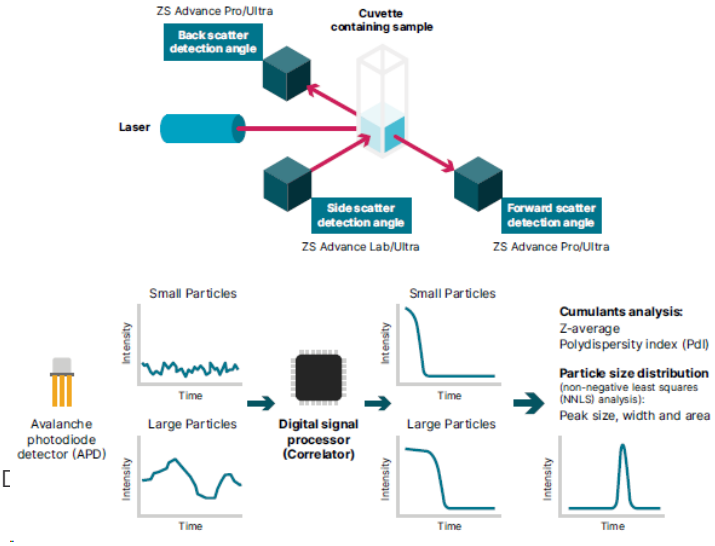
Dynamic Light Scattering (DLS)

In DLS, a light source illuminates a dispersion of particles that scatter light in all directions, with some of this scattering detected at a single, specified angle. Analysing the scattering intensity fluctuations gives the velocity of the Brownian motion, which is then used to calculate the particle size using the Stokes-Einstein relationship.

Malvern Panalytical Zetasizer Ultra

The Malvern Panalytical Zetasizer DLS system can detect particles ranging from 10s of µm to 1 nm and below, including mRNA-LNPs (which typically range from ~50–150 nm) and at high concentrations. DLS is ideal as a rapid screen for sample degradation or aggregation, indicating whether deeper investigation of size is needed. DLS also uses minimal sample volumes (~20 µl) non-destructively, meaning precious samples can be reused in other assays.

However, when it comes to single-angle (backscatter) DLS, larger aggregates tend to scatter more light in the forward angle, meaning it can be difficult to detect the presence of LNP aggregates. For this reason, complementary techniques like MADLS and NTA can be used to verify results.

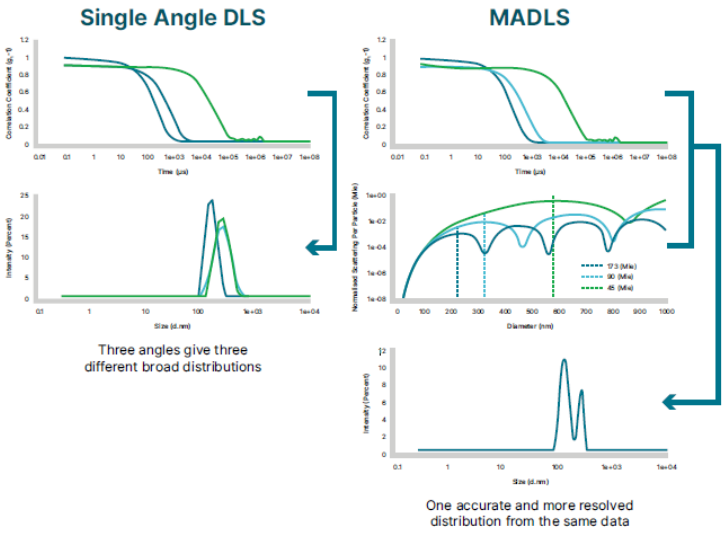


Essential Analytical technologies

Developing and manufacturing safe and effective LNP therapies

Multi-angle dynamic light scattering (MADLS)

Malvern Panalytical Zetasizer Ultra includes MADLS which measures samples at multiple angles, offering improved resolution as well as angle independent particle size distribution. It provides a more accurate representation of the different populations present in the sample, and a higher resolution size determination of multi-modal samples. It can also consistently detect low numbers of larger aggregates (which are inherently harder to detect with single angle DLS).



Like DLS, MADLS can detect even the smallest LNPs, with a detectable size range of 10 µm to 1nm and below. Using a known refractive index is a key consideration for using MADLS as is the absorption of the sample material and dispersant. Since RNA can change the refractive index of a sample, users need to know if samples contain RNA or not. To do this, a RiboGreen assay can be used to calculate the refractive index or it can calculated from compositional analysis data using Size Exclusion Chromatography (SEC).

Nanoparticle Tracking Analysis (NTA)

Using the properties of both light scattering and Brownian motion Nanoparticle tracking analysis (NTA) can determine nanoparticle size distribution of samples in liquid suspension.

For this technique, a laser beam illuminates particles in liquid suspension which are loaded into a sample chamber. Particles in the path of the beam scatter the light, which is then collected by a microscope and viewed with a digital camera. The camera captures a video of the individual particles moving under Brownian motion, with software analysing many particles individually and simultaneously, particle-by-particle.

By using the Stokes Einstein equation, NTA software then calculates the hydrodynamic diameters of the particles. NTA does not require any knowledge about the material such as RI or absorbance. This orthogonal technique described by the ISO standard 19430 tracks particles in real time to provide size, concentration and fluorescence data.

NTA is a higher resolution technique when compared to DLS and MADLS and can be particularly useful when analysing polydisperse LNP samples and to detect subtle changes in the characteristics of LNP populations. NTA uses very low volumes (1 µl, before dilution) which is fully recoverable and requires little sample preparation, however is not suitable for particles below 50nm.

Malvern Panalytical NanoSight Pro

Essential Analytical technologies

Developing and manufacturing safe and effective LNP therapies

Nanoparticle Tracking Analysis (NTA)

In addition to size and concentration, NTA also provides scatter intensity, which resolves adjacent populations of particles and differentiates materials of sufficiently-differing refractive indices. This unique ability potentially allows the user to probe whether nanoscale drug delivery structures such as LNPs vary in their contents, i.e. empty LNPs may have a lower refractive index (light scattering power) than those loaded with a higher refractive index material. This would allow them to be differentiated even though they may be of very similar sizes.
In addition, fluorescence detection capability allows differentiation of suitably labeled particles from complex backgrounds.

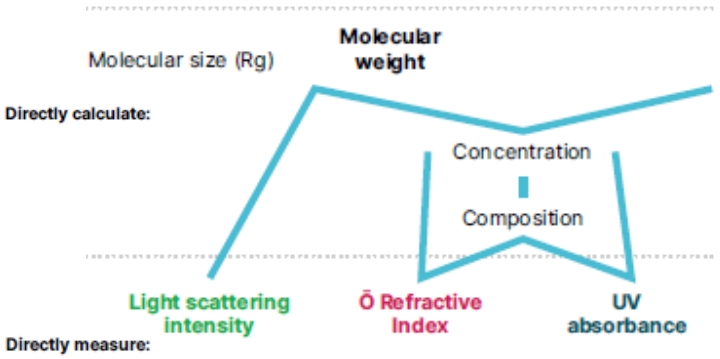
Size Exclusion Chromatography (SEC)

To achieve more in-depth characterisation of the different size populations in a polydisperse LNP sample, it is essential to use a separation technique like size exclusion chromatography (SEC) coupled with multiple in-line detectors. Using SEC ahead of size measurement improves the resolution of the identified populations. Multi-detection SEC works by separating molecules based on their hydrodynamic radius as they pass through a chromatography column, with larger components being eluted first followed by smaller ones. After the separation step, one or more advanced detectors (such as refractive index, UV/Vis-PDA, and right-angle light scattering and multi-angle light scattering, RALS and MALS) can be used to gather further information about the sample including size, molecular weight, and aggregation profile.

Malvern Panalytical OMNISEC Multi-detector SEC/GPC

Multi-detector SEC for LNP payload quantification

Understanding the therapeutic payload of LNP vectors particularly how much of the payload has been incorporated into the LNP is critical to ensuring patients receive the correct therapeutic dose. Traditional analytical methods for quantifying LNP vector payload can be labour intensive, require complex method development, where protocols are not easily transferable between different LNP formulations. SEC with multiple detectors has emerged as a key approach for LNP quantification.
By observing the sample's concentration using both the RI and UV/Vis-PDA detectors, equations can determine the concentration of two components within a single sample (in this case, LNPs and the genetic payload). By comparing the concentrations of the two components, you can then obtain the weight fraction (%) of the LNP payload. SEC-LS has several benefits and does not require the dedicated reagents needed in traditional methods.



Overview of the multi-detection pyramid, displaying what each SEC-coupled detector can directly measure and calculate.

Essential Analytical technologies

Developing and manufacturing safe and effective LNP therapies

The importance of LNP surface charge

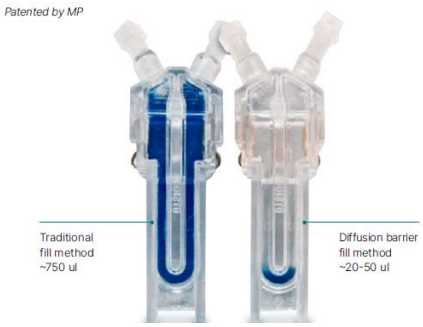
Optimal surface charge (or zeta potential) is a key attribute in the development of LNP therapies that will influence solubility and interaction with cellular membranes. Knowledge of the surface charge can therefore help predict the in vivo fate and activity of an LNP therapy. Several factors can influence the measured zeta-potential including pH, ionic strength and the concentration of other components in the solution (such as additives, coagulants, and surfactants).

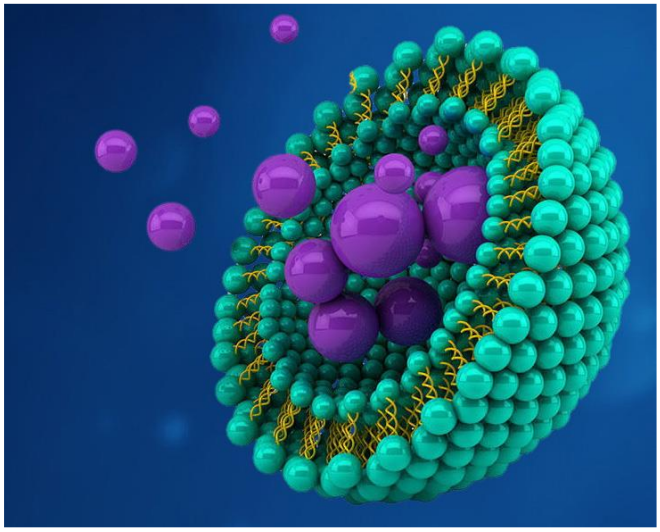
Electrophoretic Light Scattering (ELS)

ELS is most often used to validate the apparent surface charge. A dispersion is introduced into a cell containing two electrodes, and an electrical field is applied across them. Particles with a net charge (or zeta potential) migrate towards the oppositely charged electrode with a velocity (known as the mobility) related to their zeta-potential. A laser is passed through the bottom of the cell, with the charged particles producing scattered light that is frequency shifted in proportion to their velocity. By detecting the frequency shifts, we can calculate the zeta potential to evaluate formulations for stability and predicted uptake efficiency in target tissues.

However, LNP therapies which are prepared in physiological buffers, are high conductivity, and can suffer from heating effects, degrade or form aggregates during measurement.

The **diffusion barrier method** prevents degradation by separating the sample from the electrodes. A small 'plug' or aliquot (~20–100 ul) of sample is inserted into a folded capillary cell containing the same buffer. The amount of sample required for zeta potential measurements is also reduced.





Measuring LNP Stability and Structure

Beyond size and size distribution, structure and structural stability are the key attributes of a biotherapeutic drug determining its ability to consistently deliver and maintain the desired function throughout the manufacturing process, administration, and longer-term storage.

Thermal stability using Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a valuable and well-established tool for monitoring the thermal stability and thermally induced transitions of biomolecules like LNPs and nucleic acids. DSC measures the heat change associated with a sample's structural transitions when heated at a constant rate.

A reference cell with buffer, and a sample cell with the sample solution, are maintained at the same temperature as they are heated. The absorption of heat that occurs when a molecule undergoes a structural changes causes a temperature difference (ΔT) between the cells, resulting in a thermal gradient. The voltage, converted into power is used to return ΔT to 0°C. The output of a DSC measurement is a thermogram which provides multiple parameters for describing the thermally induced transitions of samples.

Essential Analytical technologies

Developing and manufacturing safe and effective LNP therapies

– What DSC thermograms tell us about a sample?

- **T_m (thermal transition midpoint).** The higher the Tm, the more stable the sample. Changes in Tm can indicate structural heterogeneity of the sample, or degradation.
- **T_{onset} (thermal transition onset)** is the onset of a thermal transition event and is used to determine the temperature ranges to avoid to maximise sample stability.
- **$T_{1/2}$ (the width of thermal transition at half-height)** reflects the extent of cooperativity of the thermal transition. The narrower the transition, the more cooperative it is.
- **Enthalpy change (ΔH)** is the total energy spent in a thermal transition and reflects the amount of native biomolecule in your sample.
- **Higher order structure (HOS)**: The thermogram shape can give us a fingerprint of the molecule's HOS and provide a stability profile.
- **Reversibility** reveals the ability of biomolecules to re-adopt their native structure upon cooling. Low reversibility is characteristic of unfolding events accompanied by aggregation and/or chemical degradation.

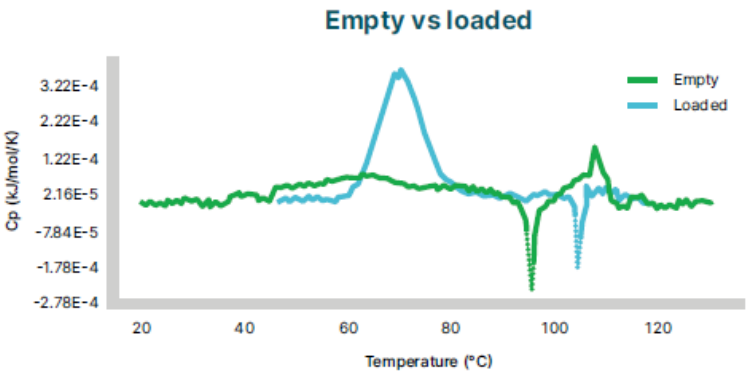
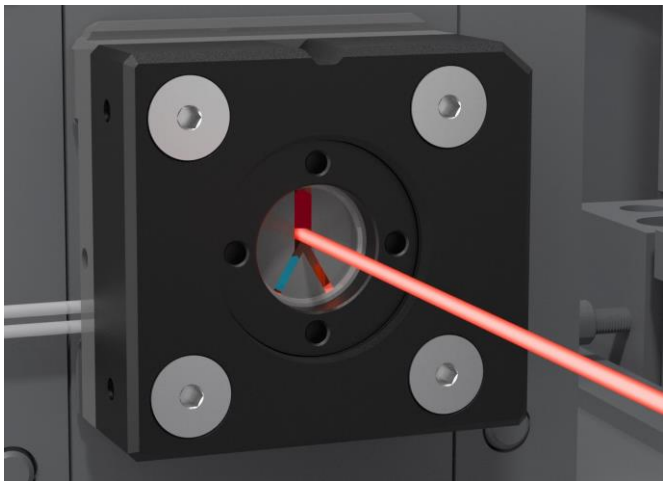


Illustration of mRNA-LNP thermogram



MMS is A Game Changer in RNA-Ligand Analysis

Microfluidic Modulation Spectroscopy (MMS) and provides ultra-sensitive, ultra-precise structural analysis of a wide range of biomolecules like proteins, peptides, antibodies, mRNA, ADCs, and AAVs. It measures structural changes due to buffer/pH/formulation, stress, point mutations, binding partners, and storage conditions. When compared to CD or FTIR, MMS can detect structural change 20x faster and with 30x greater sensitivity. MMS combines a high-power Quantum Cascade Laser with real-time buffer referencing. This provides the power to analyse both low and high-concentration samples, in formulation buffer without excipient interference, to detect small but critical structural changes.

Essential Analytical technologies

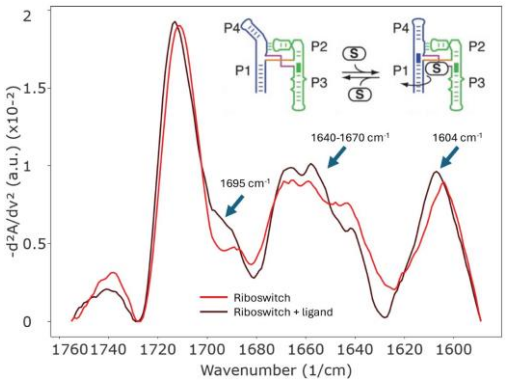
Developing and manufacturing safe and effective LNP therapies

RedSiftBio Aurora TX

Benefits of Aurora MMS

What sets this system apart is its ability to perform these analyses with minimal sample requirements (50µL of sample), high sensitivity and exceptional accuracy - all within an automated space-saving unit that is simple to operate.

The Aurora MMS system provides a wealth of information about the protein's secondary structure, allowing users to gain insights into its folding, stability, and conformation. Proteins can be analysed at concentrations as low as 0.2 mg/mL to >200 mg/ml, a capability that was once considered impossible with traditional methods.



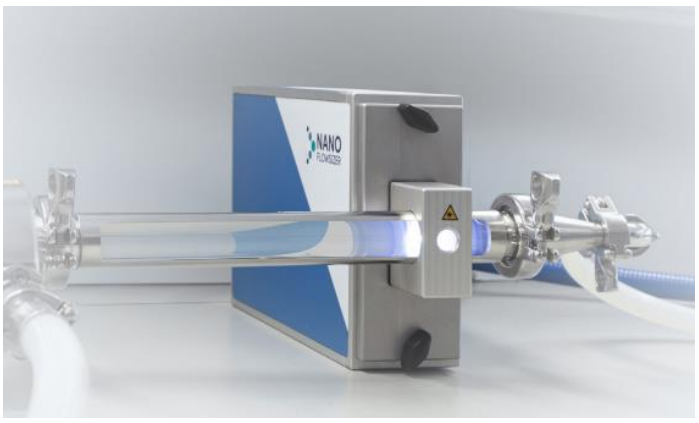
MMS similarity plot used to detect RNA structural changes in the presence of small molecule ligands.

Process Analytical Technology (PAT) - Inline Sizing of Nanoparticles

PAT aims to ensure constant final product quality, with real time in/on-line measurement of product characteristics, ideally to provide feedback for process control and to reduce waste, minimise batch rejects, and reduce production cycling time.

Spatially Resolved Dynamic Light Scattering (SR-DLS)

SR-DLS offers a non-invasive, online approach that can measure particle size distributions (PSDs) of undiluted, highly turbid nanosuspensions, in process and in flow. The spatial resolution allows automated selection of information-rich single scattered light from strongly scattering turbid nanosuspensions. NanoFlowSizer can continuously monitor particle formation and determine the size and polydispersity index (PDI) at any point during particle synthesis. Importantly, using the NanoFlowSizer, the quality, purity and particle size characteristics can be quickly monitored (every 10 seconds) allowing for in-depth interpretation, understanding and continuous improvement of processes.



NanoFlowSizer uses novel SR-DLS technology for inline, real-time sizing of flowing and turbid suspensions.

Understanding future needs for Nanoparticle manufacturing

Join Us In Transforming The Nanoparticle World

Scaleable Technology from Lab to Production

What sets Micropore Technologies apart from other systems is its true scalability on an unprecedented scale, ease of use and robust GMP-ready stainless-steel construction. Adaptable to a broad spectrum of applications, Micropore removes barriers and bottlenecks in manufacturing to reduce risk, stabilise costs maximise capacity and speed up time to market. Micropore is equipped to handle the entire process from lab to production delivering future life-saving therapeutics by democrating medicine.

Analytical Technologies Enable Success

We offer a suite of robust, accurate, and highly reproducible biophysical techniques to help overcome current challenges and better characterise the critical quality attributes of LNPs — from size and polydispersity to surface charge and composition. These tools offer powerful, complementary approaches to tracking the development and manufacture of therapies, delivering deeper insights while also offering opportunities to minimise sample use, save time, and reduce costs.

Full scale-up to GMP manufacturing

	Pathfinder 20	Pathfinder 50	Pathfinder Pro 250	Pathfinder Pro 1000	Horizon System
Minimum sample volume 200µL	✓	✓	✓	✓	
Up to 20 mL batches	✓	✓	✓	✓	
Up to 50 mL batches		✓	✓	✓	
Up to 250 mL batches			✓	✓	
Up to 5 L				✓	
Fully continuous					✓
In-line dilution			✓	✓	✓
GMP Electropolished 316L			✓	✓	✓
USP Class VI PTFE o-rings			✓	✓	✓
Equipment Qualification			✓	✓	✓
21 CFR Part 11 & EU Annex 11 compliant			✓	✓	✓
Components cleanable with common acid, alkali & solvent cleaning agents	✓	✓	✓	✓	✓
Components sterilisable by moist heat or dry heat	✓	✓	✓	✓	✓
Compact for operation in laminar flow cabinet, isolators etc	✓	✓	✓	✓	
Closed system					✓
Process instrumentation					✓
Integrated PAT					OPTIONAL

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