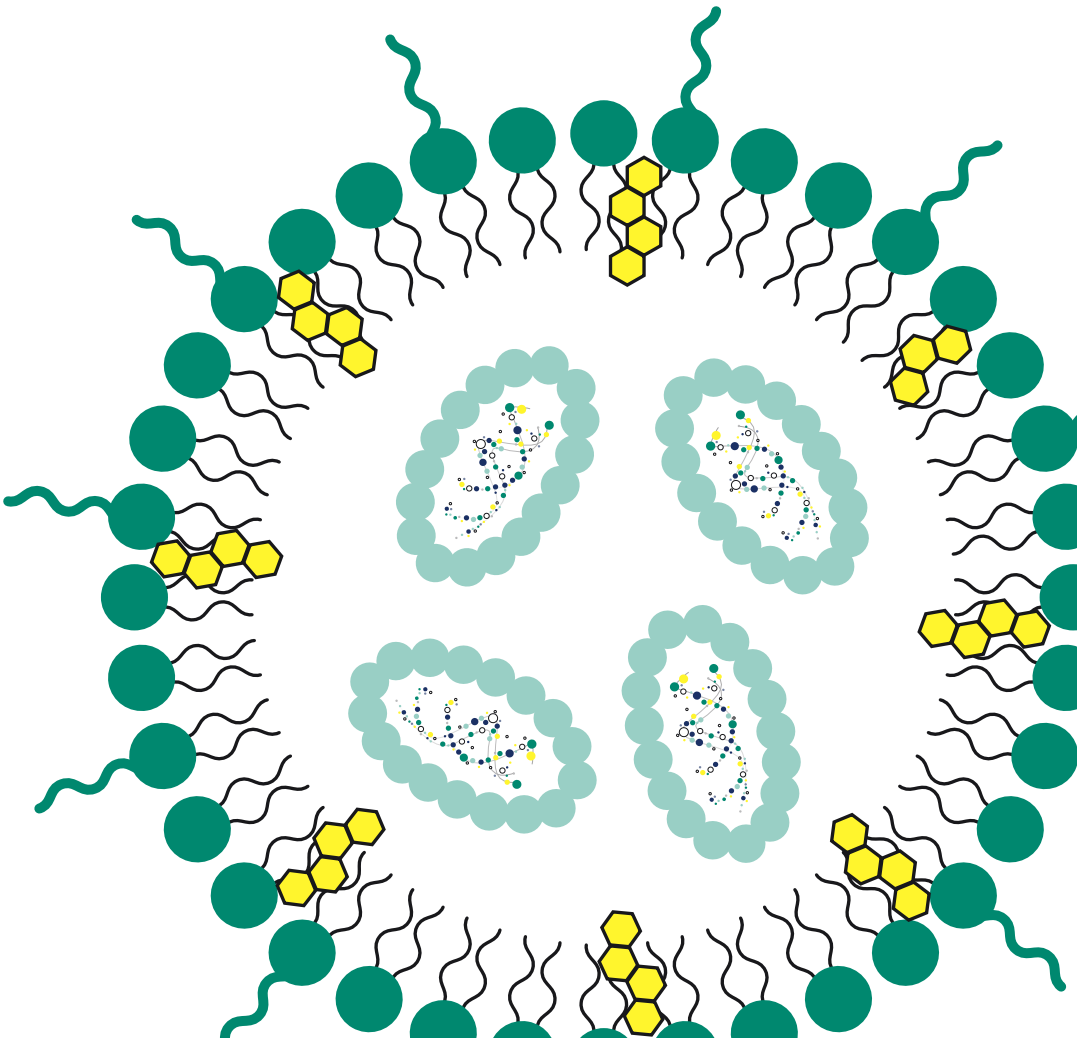
cytiva

The crucial role of downstream processing in optimizing RNA-LNP drug development

In today's dynamic pharmaceutical landscape, RNA lipid nanoparticle (LNP) drugs stand out for the innovation, scientific ingenuity, and precision engineering driving them forward. But while the clinical promise of this class of therapeutics is truly exciting, the key to their success will come in refining formulation and manufacturing processes — an undertaking that requires insights into complex parameter interactions.



What does successful RNA-LNP drug development entail? For one, it requires optimizing LNP formulations where excipients can be mixed, matched, and modified to target different organs in the body. To ensure a drug will work as intended, it's important to fine tune pharmacokinetic and pharmacodynamic profiles to maximize cellular uptake and efficacy. Downstream, tangential flow filtration (TFF) plays a key role in purification, with appropriate cryoprotectant selection helping to maintain LNP stability during storage and transport. In summary, each step must carefully balance and consider drug efficacy, safety, stability, and scalability to enhance efficiency and help de-risk processes, all while complying with complex regulatory standards.

Outsourcing to help navigate complex LNP development challenges

Companies often choose to navigate the complex LNP journey in-house, drawing on internal expertise and inside resources to precisely engineer nanoparticles and final drug products.

However, this approach can come with some risk. From the initial lipid synthesis and encapsulating RNA within stable nanoparticles to then optimizing characteristics and setting critical quality attributes (CQAs), the LNP development process demands specialized skills and experience — as well as time and investment.

Designing dedicated labs, validating manufacturing processes, and developing in-house capabilities can take years. And hiring and training the scientists, engineers, and technicians who understand the complexity of RNA-LNP drugs and can continuously upgrade their skills to stay ahead in such a dynamic field is costly.

Outsourcing offers an alternate route, potentially speeding up access to the expertise, facilities, and end-to-end workflows needed for efficient formulation, process development, and scale-up to GMP batches. With the potential for quick progress and customization, this strategic move can position biopharmaceutical companies at the forefront of innovation.

Optimizing tangential flow filtration for RNA-LNP drugs

In looking at the essentials of LNP development, tangential flow filtration proves vital to ensuring RNA-LNP formulations' purity, potency, safety, and stability.

Post-formulation, downstream LNP processing removes excess materials used in formulation, introduces the correct storage components, and adjusts product concentration. These key steps are commonly performed using TFF or cross flow filtration (CFF).

How do these filtration processes work? As LNP-containing solutions are passed tangentially across a filter membrane, pressure applied to the membrane forces components generally smaller than the pore size of the filter across the membrane and into the permeate stream. The rate of material entering the permeate stream is known as the flux. The LNPs are retained and recirculated back to a feed reservoir.

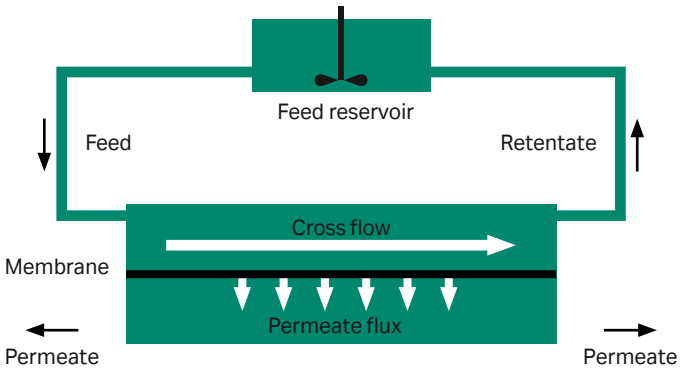


Fig 1. Schematic showing an overview of TFF process fundamentals.

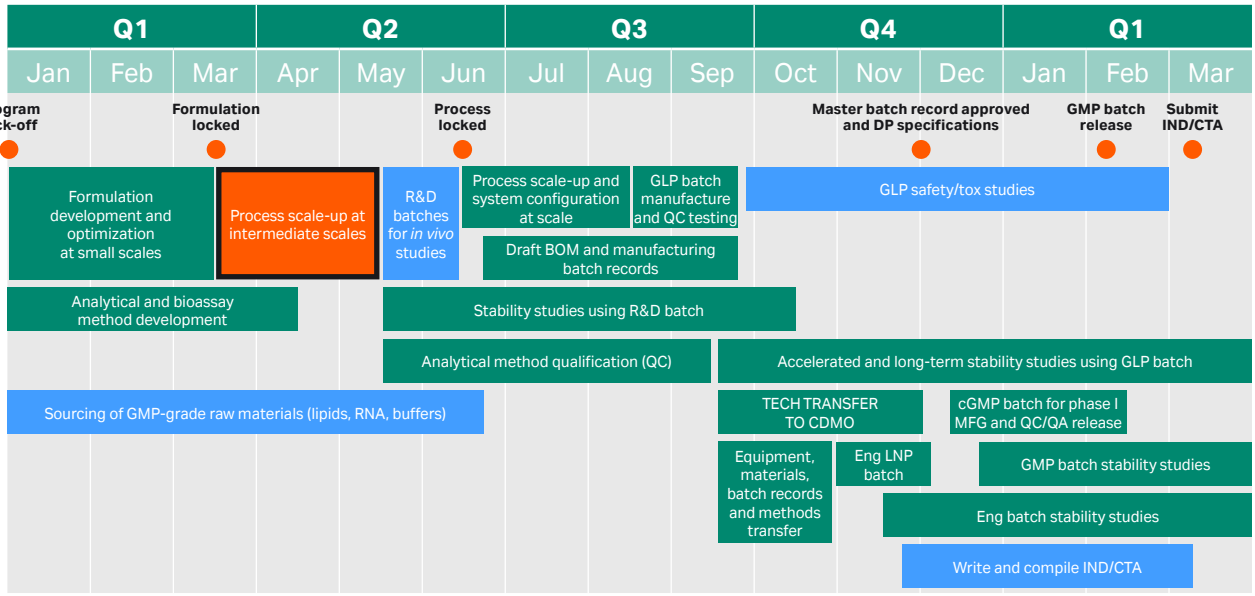
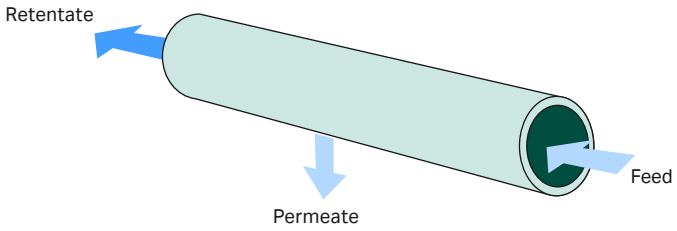


Fig 2. Sample timeline of drug development at Cytiva. (BOM: bill of materials; CDMO: contract development and manufacturing organization; CTA: clinical trial application; GLP: good laboratory practice; IND: investigational new drug; MFG: manufacturing; QA: quality assurance; QC: quality control; R&D: research and development).

Downstream TFF for LNP formulations is complex, and a one-size-fits-all approach won't work. Membrane and key process parameter selections are formulation-specific and critical to ensuring an efficient workflow that delivers the intended final drug product.

During early development, it's therefore critical to rapidly implement an optimal TFF processing strategy to shorten overall development timelines, utilize less materials, and reduce costs. If the incorrect downstream parameters are chosen, LNP characteristic changes, filter clogging, and processing challenges can lead to costly project delays and wasted materials.

Cartridge (hollow fiber)



Cassette

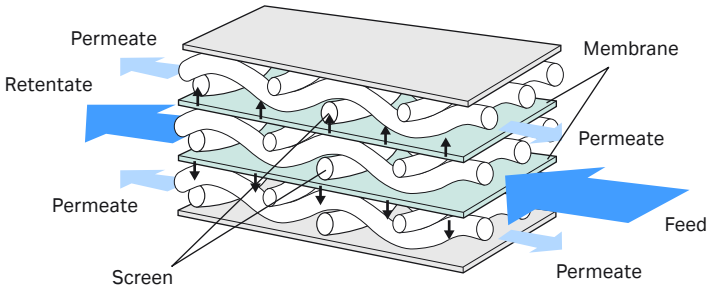


Fig 3. Configuration of TFF hollow fiber cartridges and flat-sheet cassettes.

TFF filters are available in different membrane configurations, the most common being hollow fiber and flat-sheet cassettes. For hollow fiber filters, product flows directly through many individual fibers. With flat sheets, product passes through channels that may be supported by screens to promote fluid turbulence at lower flowrates.

Different LNP formulations may interact with different membrane materials in different ways. Whereas one formulation may be processed smoothly with a certain membrane, another may readily aggregate or clog the filter using the same operating conditions. As a result, the selection of membrane configuration, membrane material, and pore size can have a dramatic impact on processing efficiency. LNP performance must also be considered carefully in early development.

TFF optimization through flux excursion

Important process parameters for TFF include the crossflow rate, transmembrane pressure (TMP), and resultant permeate flux. Flux excursion, also known as TMP scouting, is an established method for TFF optimization that can be used to simultaneously optimize both crossflow rate and TMP. In principle, as the transmembrane pressure is increased, the driving force across the membrane is also increased, leading to higher flux across the membrane. However, this increased pressure also pushes more LNP material into the concentration polarization layer that forms by the membrane, leading to greater flow resistance.

To obtain a higher flux and shorter processing time during TFF processing, the effects of concentration polarization may be mitigated by choosing a higher flowrate. An additional advantage of a short processing time is less exposure of the formulation to the shear forces of pumping. However, this strategy can amplify the risk of product degradation as the shear stress particles experience in the TFF recirculation loop increases directly with higher crossflow rates. It's also critical that flux excursions using different TFF filter types be performed on novel formulations to obtain robust, optimized process performance. Performing flux excursions allows the formulation-specific balance between flux and TMP to be determined, leading to data-driven selection of optimized process parameters and filter candidates.

Incorporating an LNP sensitivity study into a traditional flux excursion is an effective way to understand the impact of shear stress on formulations at increasing TMPs and crossflow rates. At each processing condition tested, material samples can be analyzed for signs of degradation. Analytical techniques, such as dynamic light scattering (DLS) sizing and the Thermo Fisher Scientific RiboGreen assay, among others, are effective at detecting changes in the physicochemical properties of LNPs after exposure to the shear stresses of TFF.

At Cytiva, we can use our extensive analytical capabilities to effectively optimize TFF conditions in as little as one batch, saving our customers months of development work and materials.

Flux excursion testing in action

To assess performance across TFF cassettes, we performed flux excursion testing using a proprietary lipid mix and self-amplifying RNA (saRNA) payload. The formulations were generated using a NanoAssemblr™ Blaze instrument and added to the TFF system immediately after formulation. A peristaltic pump was utilized as the feed pump for the TFF system, a 250 mL centrifuge tube was the feed reservoir for both systems, and 3.1 mm inner diameter (ID) peristaltic tubing was used for all system tubing. The system was configured for full recycle during flux excursion activities, and the filters used were made of regenerated cellulose and polyethersulfone.

Crossflow rates (L per m^2 per min, LMM) were selected at 2.5, 5, and 7.5 LMM for the flat-sheet cassettes based on recommended operating range.

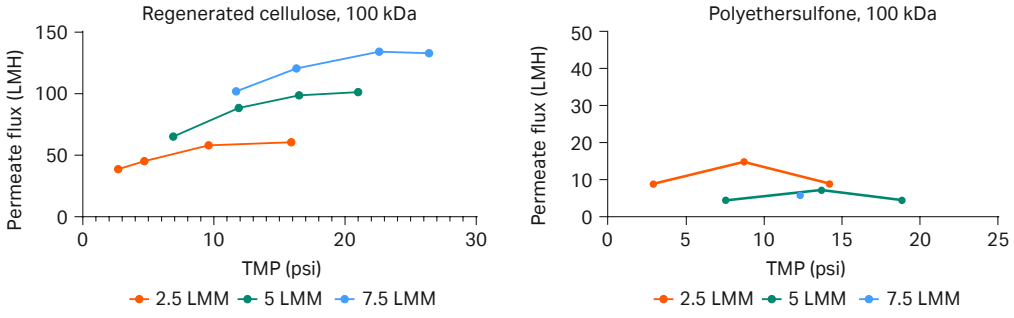


Fig 4. Graphs showing flux excursion performance results for regenerated cellose, 100 kDa (A) and polyethersulfone, 100 kDa (B) TFF cassettes at different crossflow rates.

As demonstrated, flux excursion can highlight performance differences and guide filter selection, as not all filters may be compatible with a specific formulation. Once performance is evaluated for fouling at each crossflow rate, further investigation is needed to ensure that key attributes of the nanoparticles are maintained.

Our TFF runs indicate that while the permeate flux increased with crossflow rate and TMP, the particle size and polydispersity index (PDI) also increased substantially at the highest crossflow rates and TMP values. This finding suggests that the LNP formulation under study is likely sensitive to shear at the harshest conditions tested. Therefore, to avoid risk to product CQAs during at-scale batch production, moderate crossflow conditions should be selected.

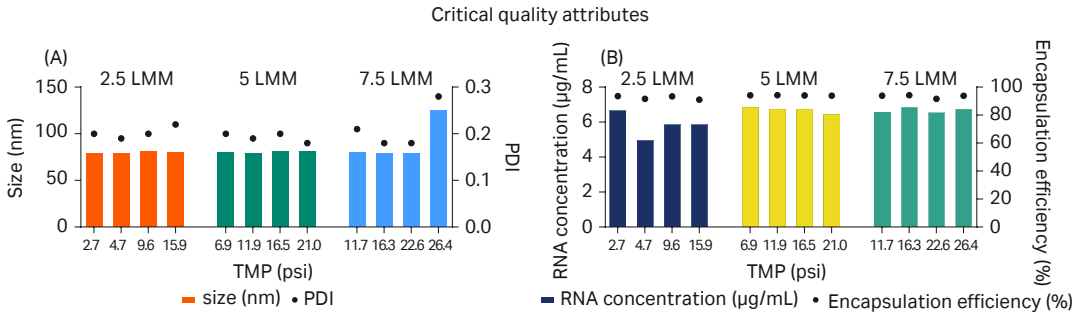


Fig 5. Graphs showing critical quality attributes size and polydispersity index (PDI) (A) and RNA concentration and encapsulation efficiency (EE) (B) at different crossflow rates.

Performance testing paired with LNP characterization and analysis

Focusing on improved process time doesn't always give the full picture of a successful downstream process — it's critical to pair this performance testing with LNP characterization and analysis (Fig 5).

It's important to consider that formulations may behave differently depending on the filters and membrane materials used, calling for optimization. Flux excursion is a tool that can be used to quickly assess the most favorable filter configuration and process conditions. Our experience and expertise enable us to optimize filtering conditions based on a formulation's specific characteristics, saving time in process development and batch runs.

The next step for downstream processing is to assess if the optimized conditions obtained during flux excursion screening translate to overall improvement in batch processing time while maintaining a product's CQAs.

An LNP formulation consisting of a proprietary lipid mix and a GFP mRNA payload formulated on the NanoAssemblr commercial formulation system was utilized to evaluate the CQAs for both filter types: the regenerated cellulose filter (Filter 1) and polyethersulfone filter (Filter 2).

The amount of LNP material loaded onto each filter was normalized to a loading of ~500 mg mRNA payload/m^2 with crossflow rates selected from the flux excursion runs. Peristaltic pumps were utilized as the feed pump, a 1 L bioprocess container was used as the feed reservoir, and 3.1 mm ID peristaltic tubing was utilized for all system tubing. During the diafiltration process, the LNP formulations were diafiltered into a 10% sucrose storage buffer. Post-filtration, the collected LNPs were adjusted to the target concentration, sterile filtered using 0.2 µm syringe filters, and then stored at -80°C.

Table 1. Process condition summary for TFF testing

TFF filter	**Filter 1: regenerated cellulose cassette**	**Filter 2: polyethersulfone hollow fiber**
Amount of payload added	10 mg	6 mg
Loading (mg/m^2)	538	522
Filter surface area (cm^2)	186	115
Feed flow rate	93 mL/min (cross flow filtrations: 5 LMM)	106 mL/min (shear: 4000 s^{-1}, cross flow filtration: 9.2 LMM)
TMP (psi)	15	3
Initial concentration factor	13×	1×
Diavolumes	4×	4×
Final concentration factor	3×	3×

Particle samples were taken at the end of each major processing step for both filter set-ups. The samples were analyzed for size, PDI, zeta potential, total RNA concentration and encapsulation efficiency (%EE), and potency (final samples only). Analytical results from both batches show similar size, PDI, and encapsulation efficiency values between both filters across the TFF process. Bioassays are also recommended to correlate CQAs with *in vitro* activity, with an additional recommendation to calculate the half maximal effective concentration (EC50) values to gauge activity.

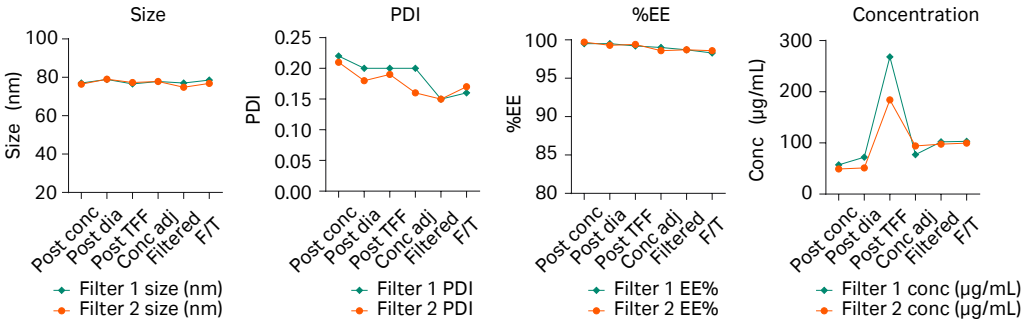


Fig 6. Graphs showing measurement of critical quality attributes for Filter 1 and Filter 2 at each major processing step.

Table 2. Description of process stages where in-process samples were collected

Sample point	Process stage
Post conc	Post initial concentration
Post dia	Post diafiltration
Post TFF	Post product collection after TFF
Conc adj	Post adjustment to target concentration
Filtered	Post sterile filtration
Freeze/thaw (F/T)	Post one F/T cycle at -80°C

Beyond impacting key quality attributes and activity, filters can affect overall processing time as well. Depending on LNP properties, some formulations may have more sensitivity to shear stress which must be taken into account while evaluating filters and TFF conditions.

In one such formulation, while maintaining all the same particle physicochemical properties and *in vitro* potency, the regenerated cellulose cassette filter was able to process the LNP batch in roughly half the time. This finding highlights the importance of testing to guide filter selection, as the optimal material may be formulation-specific and can affect both processing time and product exposure to shear stresses, such as pumping.

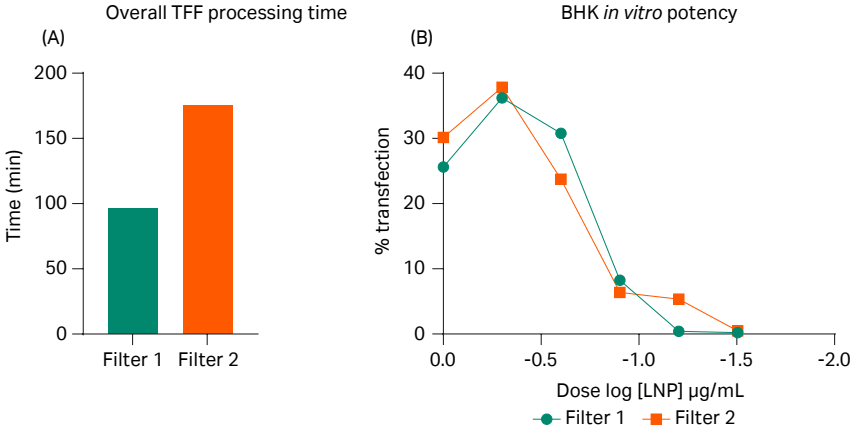


Fig 7. (A) Overall TFF batch processing time. (B) *In vitro* potency results – Filter 1 EC50: 140 ng/mL, Filter 2 EC50: 211 ng/mL in baby hamster kidney (BHK) cells.

Our results indicate that combining flux excursion and CQA evaluation can help optimize downstream processing for a shear-sensitive LNP formulation. We found that an optimization methodology incorporating flux excursions and analytical insight can aid users in selecting both a filter and operating conditions, driving success with LNP formulations.

TFF optimization is a critical component of drug development scale up for LNP drug products. Flux excursion methodology, to optimize TFF conditions in a single batch, helps in optimizing overall process conditions to preserve desired formulation properties and *in vitro* activity. Selecting the appropriate filter type can provide robust performance during flux excursion screening and offer quick processing flux rates for LNPs.

LNP formulations are highly variable and there is no one-size-fits-all approach to achieving downstream processing success. Our experience and expertise can help clients select the most effective process conditions while shortening process timelines and reducing material demands.

"Leveraging LNP experience during TFF optimization accelerates process development and can significantly reduce batch processing time."

Kelsey Schwartz,
Associate Sr. Engineer, Process Development

Batch splitting for normal flow filtration optimization

Normal flow filtration (NFF) is necessary to sterilize bulk drug product prior to fill finish, but LNPs pose unique challenges due to their size relative to filter pores. Filter fouling can drastically reduce filtration throughput and, while increasing filter size can alleviate this problem, it can also result in substantial product losses due to increased holdup volumes.

Post-filtration buffer flushes can help reduce this loss but, in turn, may lead to undesirable product dilution. Additionally, any uncertainty in the filtration capacity can create risks during at-scale production, since unexpected filter fouling can cause a batch failure. It's therefore critical to be confident in the capacity of your filter for each formulation, selecting a membrane and methodology to both minimize losses and avoid exceeding filtration capacity while scaling up.

Batch splitting can make normal flow filtration optimization more efficient, enabling you to evaluate different membranes and process conditions with a single development batch. During scale-up process development, each batch run can be split into three or more aliquots to test the impact of different filtration conditions — particularly flux rate — on filter throughput. Applying this method allows for the screening and selection of filtration conditions and membranes, with testing of three or more normal flow filtration parameters per filter processing step.

Application of this filtration screening methodology allows for optimization of final batch yields. Selecting the optimal filter size based on filtration capacity evaluated through batch splitting minimizes holdup volume while ensuring capacity is sufficient to filter the entire volume of product. Conversely, overestimation of filter capacity can lead to multiple filters being required for a run, further reducing yields or, in the worst case, resulting in costly batch failures.

During normal flow filtration, LNP formulations are often exposed to high pressures. Our methodology incorporates sample testing at each stage of the filtration screening to evaluate the impact of the selected conditions on the LNP formulation. Our team has the analytical expertise and broad LNP formulation and characterization experience needed to robustly screen for impacts associated with screened filtration, allowing you to select conditions that simultaneously optimize throughput and ensure preservation of CQAs at scale.

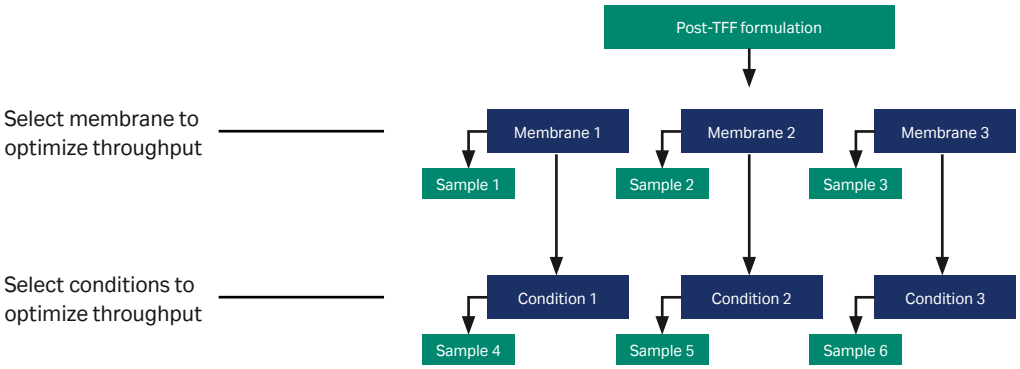


Fig 8. Split-batch filtration optimization workflow.

Filtration testing was completed using Cytiva LNPs formulated on the NanoAssemblr commercial formulation system. The filtration was run at constant flowrate using a peristaltic pump and capsule polyethersulfone filters with 0.2 µm pore sizes.

The optimal filter flux rate was evaluated across several filters differing in modifications to polyethersulfone (mPES) to determine the impact of the membrane material on filtration capacity. As shown in Figure 9, different filter materials can have an impact on capacity, while having no impact on LNP CQAs post-filter for any of the conditions tested (Fig 10). It's therefore important to evaluate multiple conditions to ensure there is sufficient information to optimize your filtration process.

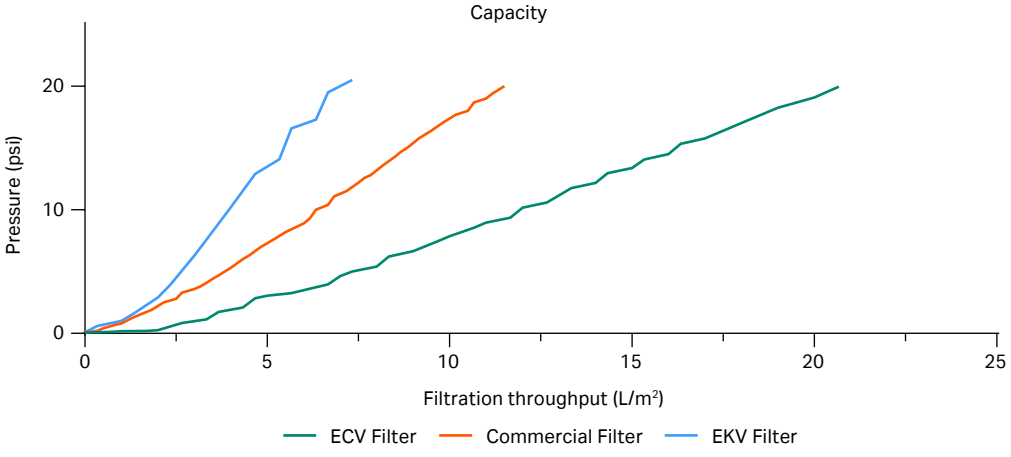


Fig 9. Membrane material and impact on capacity at constant flow rate.

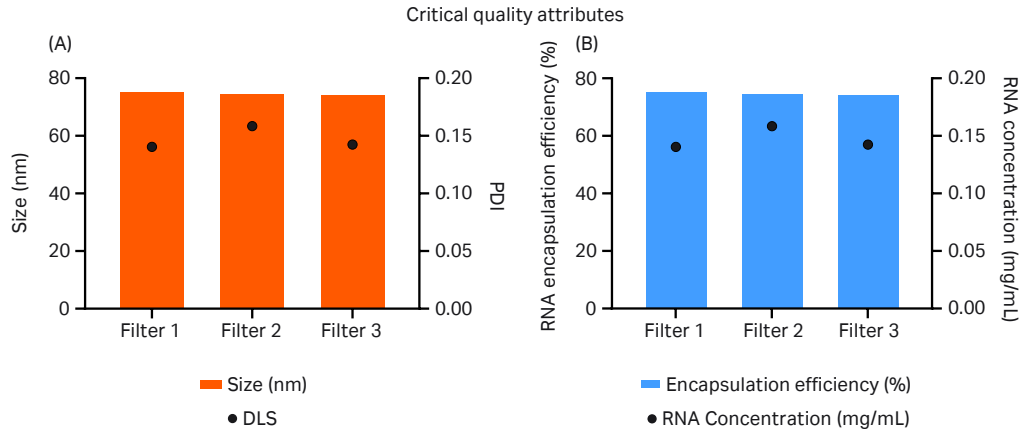


Fig 10. Critical quality attributes (A) size and polydispersity Index (PDI) and (B) RNA concentration and encapsulation efficiency (EE) post-filter with various membrane materials.

In parallel, individual filters were tested under various flux rates ranging from 10 to 20 LMM to determine impact of flux rate on pressure. For all filters, the flux rate was observed to substantially impact the filtration capacity, with the highest filter capacity at 20 LMM. No impact on the LNP CQAs was observed at any of the filtration conditions tested.

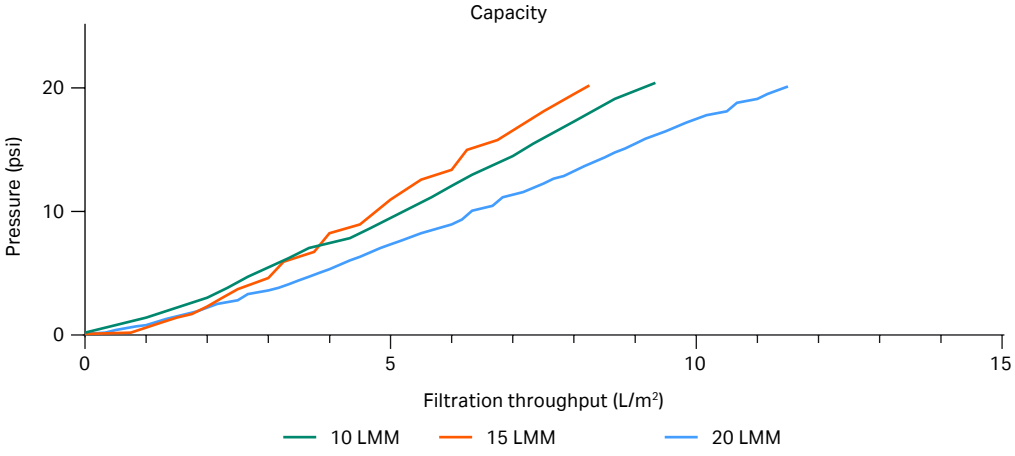


Fig 11. Representative graph showing the impact of filtration flowrate on capacity.

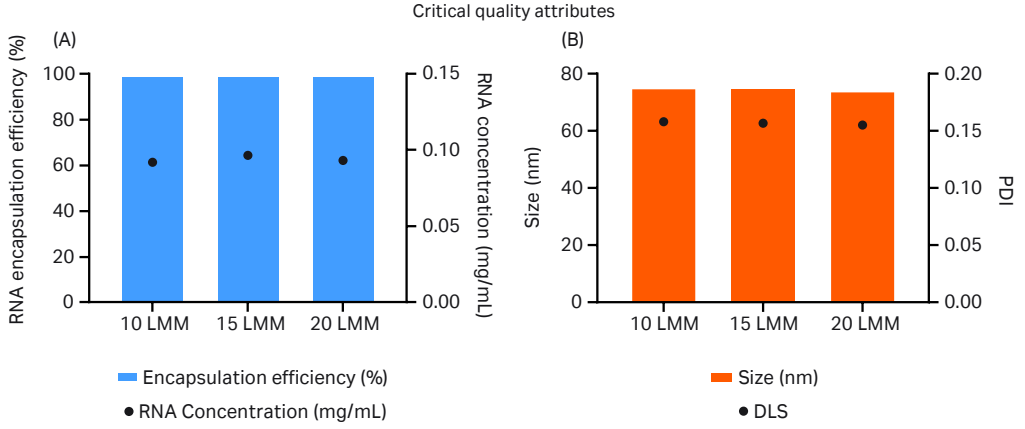


Fig 12. Critical quality attributes (A) size and polydispersity Index (PDI) and (B) RNA concentration and encapsulation efficiency (EE) post-filtration at different flux rates.

In summary, filtration capacity was found to vary significantly with different filtration flux rates and membranes, with changes in filter membrane having the most pronounced impact. This study demonstrates the value of testing filtration conditions to optimize filtration capacity.

This methodology can also be applied to LNP development scale up to evaluate the optimal filter flux rate and determine the filtration capacity at this flux rate. When this information is known, the optimal filter size can be chosen for each development batch of increasing scales to maximize final yields and de-risk at-scale filtrations.

"When our LNP customers aren't sure where to start with optimizing normal flow filtration, we can provide the experience needed to maximize yields and de-risk critical batches."

Logan Ingalls,
Associate Engineer II, Process Development

Optimizing downstream processing to maximize LNP success

Optimization of downstream processing during scale-up development of LNP formulations is critical to maximizing efficiency and yields while de-risking at-scale batches. TFF optimization can be accelerated using a flux excursion methodology to rapidly screen TFF processing conditions. Normal flow filtration can be optimized using a split-batch screening method to evaluate filter capacity and ensure appropriate filter sizes are selected at scale. Incorporation of both workflows can save months of project time and raw material expenses.

At Cytiva, we can support you in LNP formulation and more, with broad expertise extending across the nanomedicine journey from concept to clinic. Our end-to-end nanomedicine development capabilities and technologies for both downstream process optimization and LNP manufacture can help ensure critical process parameters are established at each step to maintain consistent formulation quality and potency from preclinical programs to cGMP manufacturing.

When you collaborate with Cytiva and our highly experienced BioPharma Services team, we'll work with you to implement downstream processing strategies and methods that accelerate and de-risk the development of scalable formulations to maximize nanomedicine success.

Contact our specialists to discuss your project.

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