Recipharm
Advanced Bio

NUCLEIC ACID-LNP ULTRAFILTRATION AND DIAFILTRATION: TFF FLAT SHEET CASSETTES VS HOLLOW FIBER CARTRIDGES

Lipid nanoparticles (LNPs) are the leading non-viral gene delivery platform in gene therapy, owing to their lower manufacturing costs compared with adeno-associated viruses (AAVs), reduced immunogenicity relative to viral vectors, and high nucleic acid cargo capacity.

The flexibility of LNP and its tested proof of concept during the COVID pandemic have made the LNP delivery system the golden platform for both vaccine application and gene editing.

Moving forward with the knowledge accumulated during the pandemic and the resources that were invested into non-viral gene delivery, we still have a lot to learn and uncover with how the drug product (DP) components can interfere with the API biological activity.

These effects are usually not observed at the discovery level but the complexity surfaces during DP scaled up. Scale-up or scale out presents distinct challenges all together. In scale-out, there may not be a problem if the same mixing technology is used. However, in scale-up with different equipment, process performance can change especially since parameters such as linear velocity and Reynolds numbers do not necessarily ensure scalable process transfer. Consequently, increasing batch size introduces additional complexity, particularly when there are different mixing technologies.

Ultrafiltration and diafiltration steps (UF/DF) are used to increase RNA concentration to a more desired concentration, remove ethanol content to below detection, and perform buffer exchange to the final buffer the DP will be frozen in.

During discovery phase, companies typically produce small batches that can be processed using dialysis and centrifugal filtration devices. Despite easy and rapid form of concentration and buffer exchange, they are not readily translatable to large-scale manufacturing.

For scale-up, Tangential Flow Filtration (TFF) is the primary method used for processing of LNP formulations, typically using hollow fibers filters or by flat sheet cassettes. As scale-up increases, both membrane selection and the molecular weight cutoff become critical process parameters to retain the nanoparticles.

The buffers used during small scale processing might not be compatible with the selected TFF technology of choice, often requiring going back to the drawing table to test several buffer systems. Numerous challenges can arise during downstream processing as processes are scaled up. Even after optimizing scale-up parameters, biological performance may still be affected, particularly with the drug substance (DS) activity.

Therefore, navigating downstream processing of LNP formulation while preserving biological activity is a critical challenge.

Yes, during process optimization and troubleshooting, certain modifications can compromise DS biological activity. Such losses represent a major risk in product development.

Hollow fiber membranes have traditionally been the preferred choice in downstream LNP processing. These membranes consist of hollow tubular filaments with one or multiple axial empty cores, through which filtration occurs through radial transmembrane permeation (Figure 1). Due to the fabrication process of these fibers, hollow fiber systems typically generate relatively low shear forces, which is the advantage for processing shear-sensitive nanoparticles such as LNPs. Hollow fiber membranes are manufactured from materials such as polyether sulfone (mPES), polyether sulfone (PES), and Poly sulfone (PS).

Because LNPs are structurally sensitive nanoparticles, hollow fiber filtration has historically been considered the gold standard for LNP downstream processing. However, as more chemistries are introduced to the ionizable lipid libraries, and more robust processes are being developed, additional challenges have emerged during process scale-up using hollow fibers.

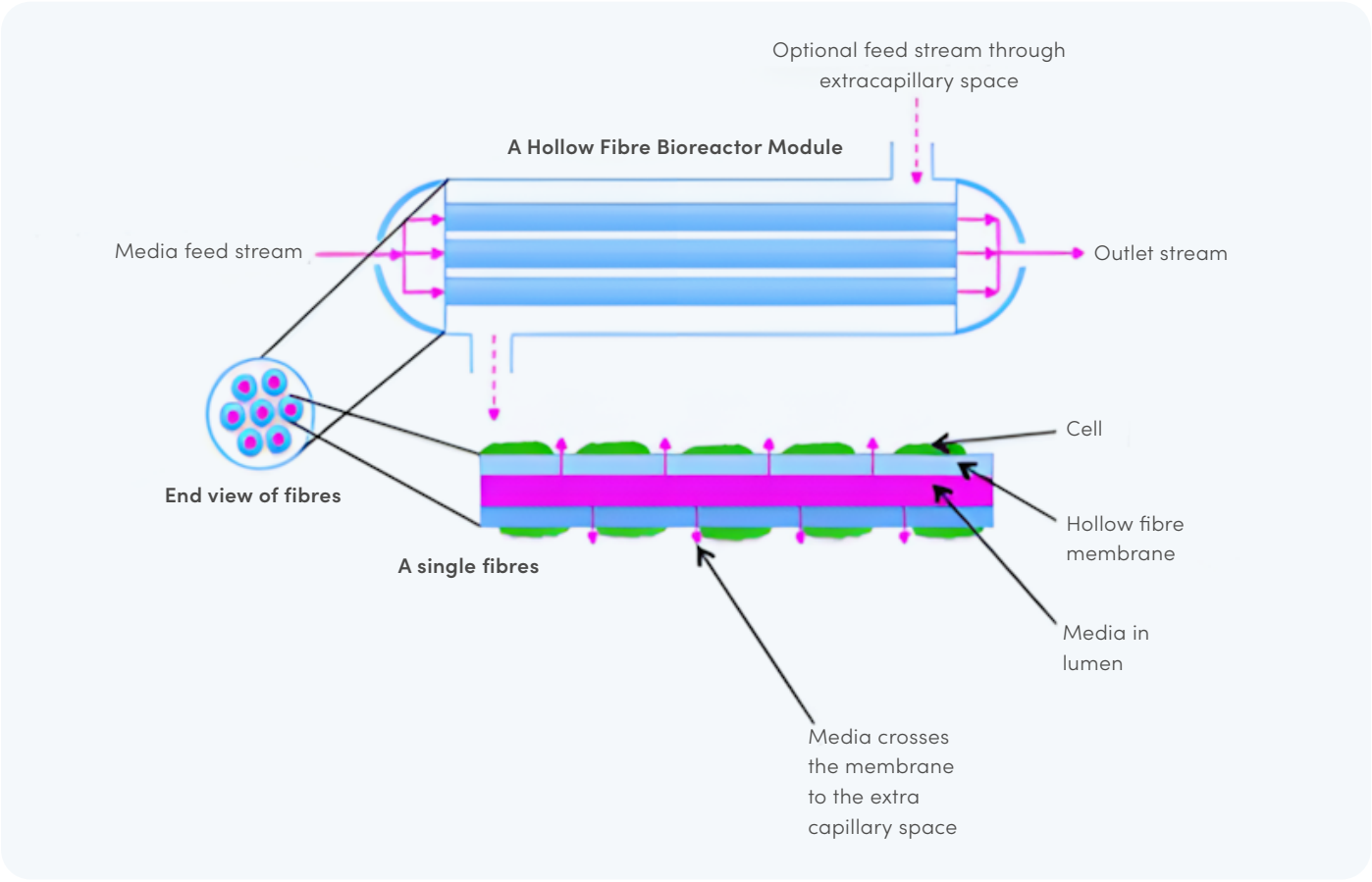


Figure 1. Hollow fiber inner components[6].

Increasing yield requirements, driven by gene editing applications and *in vivo* CAR-T therapies, have motivated the adoption of flat sheet cassette system for TFF. Flat sheet cassettes consist of multiple stacked membrane layers, creating a multilayer filtration system (Figure 2). The feed channel contains screens that promote turbulence, delaying gel layer formation and reducing membrane fouling. The screen also enables higher permeate flux compared with hollow fiber systems. Furthermore, the effective filtration area can be readily increased by stacking additional membrane layers, facilitating process scale-up.

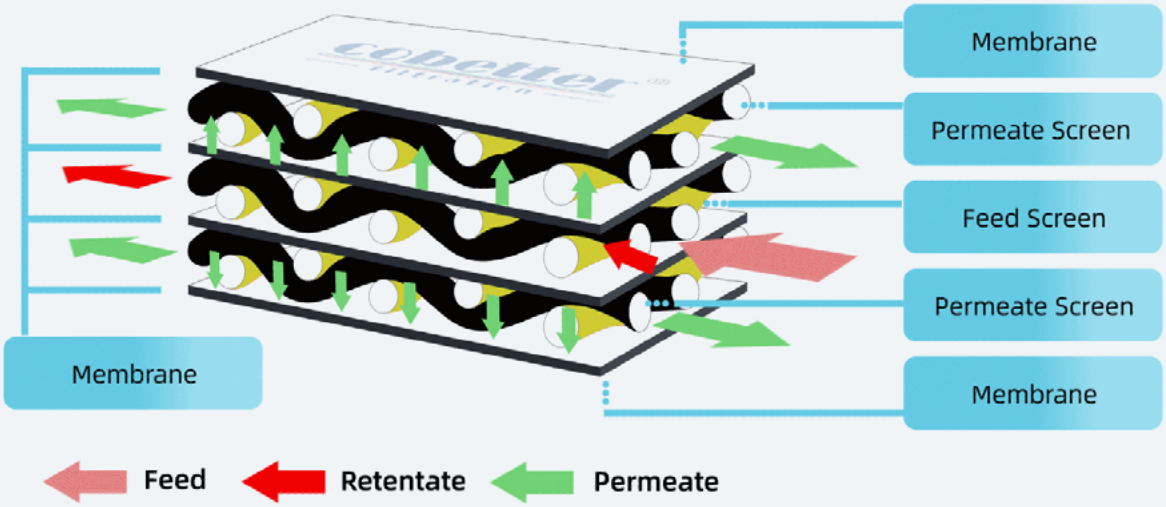


Figure 2. Flatsheet layers and inner structures[2].

CASE STUDY

Using a commercially available formulation, a decline in permeate flux during TFF along with lipid precipitation on the membrane surface under both conservative and high shear conditions were observed. Initial investigations focused on the buffer system, which was systematically evaluated and modified. However, the issues persisted and the investigation focused on whether the lipid composition contributed to the observed behavior. Increasing the concentration of DMG-PEG-2000 was explored as a potential strategy to prevent shedding which may contribute to the increase in particle size and polydispersity index (PDI) during TFF. DMG-PEG-2000 is a C14 functionalized PEG lipid with an ester linker. Because ester linkers are more prone to hydrolysis, it is hypothesized that even the slightest shear conditions may promote PEG detachment from the LNP surface. Loss of surface PEG could subsequently lead to particle growth and increased PDI as seen in Figure (3).

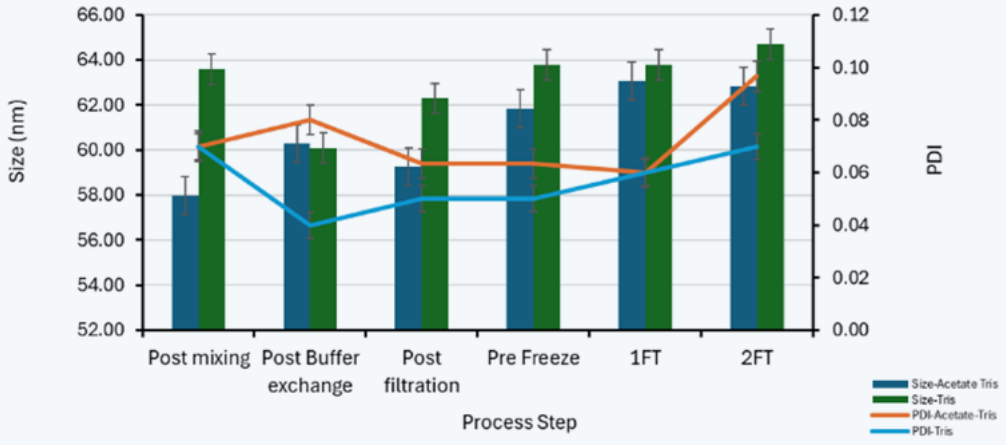
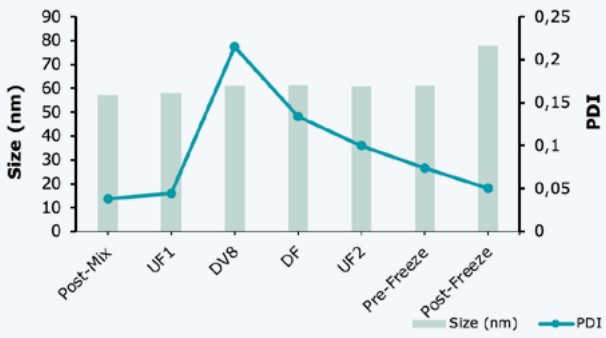


Figure 3. Testing two different buffer systems: acetate as acidic buffer and ILD with Tris as exchange buffer; and testing Acetate as acidic buffer with Tris as ILD and exchange buffer. As can be seen when transitioning from Acetate as ILD to Tris as ILD, there is a significant improvement in the PDI, but the size is higher when using Tris as ILD with better stability throughout the process. However, we saw lipids crashing out during the TFF UF2 step.

Figure 4. DMG-PEG percentage against ALC-0159 at different process unit operations with four buffer systems. In the above experiment, we noticed improvement in stability with DMG-PEG content at 2-2.5% on centrifugal tubes (amicon, Sigma). When these two concentrations were transferred to TFF using hollow fiber cartridges (mPES, Repligen), lipids still crashed out of solution.



% LIPID RECOVERY

DMG-PEG2000	Cholesterol	DSPC	ALC-0315	Total
50%	43%	44%	45%	45%

None of the applied mitigation strategies changed the outcome. Even when the membrane was operated at low loading densities (0.10 and 0.15 mg/cm^2), lipid precipitation persisted, indicating that lipids were consistently coming out of solution during the process (Figure 5).

The consistent results suggested that the observations were not an anomaly. Attention was then directed towards the hollow fibers as a cause of the problem. Several factors were considered. First, mPES may contribute to the observed behavior since its hydrophobic nature with modifications that renders it more hydrophilic that could trigger nanoparticle aggregation. Second, the length of the hollow fiber can induce high pressure surge during the process, impacting the integrity of the LNP and leading to particle aggregation.

Figure 5. Surge in particle size and PDI during diafiltration using hollow fiber cartridge. This increase was accompanied by surge in feed pressure with lipids precipitating out of solution as seen in the table with less than 50% lipid recovery.

INVESTIGATION OF FLATSHEETS

Considering the compounding issues with the LNP formulation during hollow fiber TFF studies, flat sheet cassette systems were evaluated as an alternative solution. The objective was to observe the performance of regenerated cellulose (RC) and polyether sulfone (PES) membranes to understand the impact of a hydrophilic membrane (RC) and a less hydrophilic membrane (PES).

An excursion style study was designed using RC membranes using molecular weight cut off (MWCO) of 100KdA, 50cm² surface area (Hydrosart®, Sartorius). Different loading capacitites were tested at 0.15, 0.25, 0.35 and 0.45 mg/cm². At each loading, both permeate flux and transmembrane pressure (TMP) were systemically varied throughout the TFF process, including the initial ultrafiltration step (UF1), diafiltration (DF) and the final ultrafiltration step (UF2)

At low loading of 0.15 mg/cm², experiments were conducted at various TMP ranging from passive TMP up to 18psi. The optimal performance was observed at a TMP of approximately 10 psi across all TFF steps, including UF1, DF, and UF2. At this condition, the flux maxed out at 50.4 LMH during UF1. Then, the flux dropped from 50LMH to 30-40LMH during DF and UF2. Particle size PDI stayed constant with minimal change. There was a noticeable drop in encapsulation efficiency (%EE) during freeze/thaw cycle. This may be due to high shear on the particles during the excursion study and possibly due to low loading.

To test the theory of low loading, a new experiment at 0.25 mg/cm² loading was tested on RC while targeting similar TMP and flux rate. Under those conditions, particle size and PDI were maintained with minimal change during UF1, DF and UF2 with high encapsulation maintained as seen in Figure 6.

Figure 5. Particle size and PDI values at various shear rates and TMP using RC cassette at 0.25 mg/cm² loading. The values of shear rate and TMP reported in the graphs were in UF1, DF and UF2 steps.

0.25 MG/CM² LOADING

Component	Theoretical (mg/mL)	Actual (mg/mL)	% Recovery
Ionizable Lipid	11.62	9.22	79%
DSPC	2.4	1.86	78%
Cholesterol	4.52	3.45	76%
DMG PEG	1.14	0.86	75%
Total	19.68	15.39	78%

Table 1. Values of the lipids at 0.25 mg/cm² loading. The results account for a total recovery of 77%.

0.35 MG/CM² LOADING

Component	Theoretical (mg/mL)	Actual (mg/mL)	% Recovery
Ionizable Lipid	11.38	8.04	71%
DSPC	2.35	1.68	72%
Chol.	4.42	3.05	69%
DMG PEG	1.12	0.76	68%
Total	19.26	13.53	70%

Table 2. Values of the lipids at 0.35 mg/cm² loading. The results show losses of lipids at higher loading.

Cutoff loading by pushing the RC loading to 0.45 mg/cm² was tested. Particle size and PDI data showed clear indications that this loading density is too high for this formulation for RC cassettes (Figure 7).

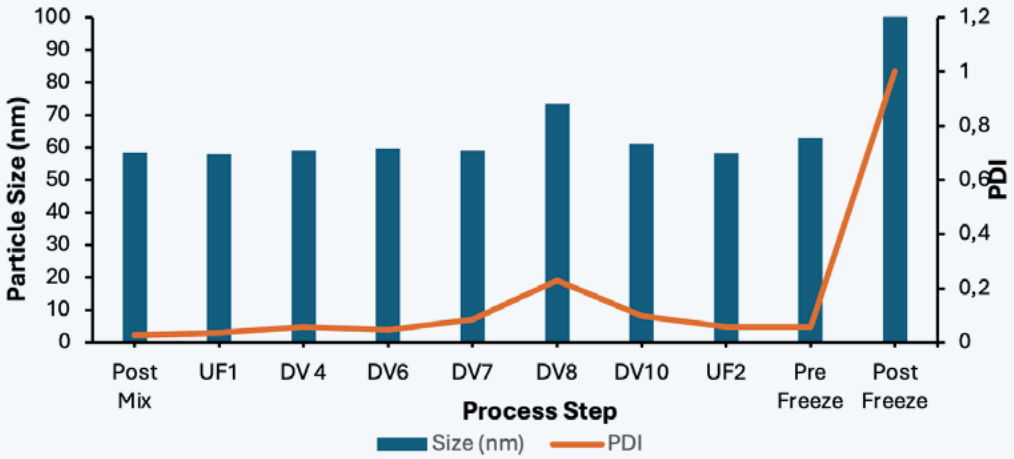


Figure 7. Result of 0.45 mg/cm² density. Critical process parameters: particle size and PDI were stable across the TFF process with particles size roughly at 65 nm And PDI of less than 0.1. However, post freeze size and PDI shot up showing instability of the formed nanoparticles indicating that this loading is past the threshold of the cassette.

This study with RC cassettes demonstrated that higher membrane loading could be achieved while maintaining elevated flux compared with hollow-fiber systems, with product recovery reaching about 77% at low batch size.

PES cassettes (Sartorius) at100kda with 50cm² surface area cassettes were evaluated subsequently. Studies were performed using the same loading conditions that were evaluated for RC membranes (0.15, 0.25 and 0.35 mg/cm²), while maintaining the best shear and TMP conditioned derived from RC cassettes studies.

The TFF process was operated at a crossflow rate of 3LMM during UF1 and DVs from 1-8. For DVs 8-10 and UF2 the crossflow rates were reduced to 2LMM.

Across the three TFF runs, particle size and PDI showed minimal growth and lipid content was consistent with values observed from RC membranes. However, the ionized lipid exhibited lower recovery when processed with PES membranes compared with RC Tables 3 and 4.

0.25 MG/CM² LOADING

Component	Theoretical (mg/mL)	Actual (mg/mL)	% Recovery
Ionizable Lipid	11.68	8.47	73%
DSPC	2.41	2.05	85%
Cholesterol	4.54	3.74	82%
DMG PEG	1.35	1.07	79%
Total	19.96	15.33	77%

Table 3. Values of the lipids at 0.25 mg/cm² loading using PES membrane. The results account for a total recovery of 77%.

0.35 MG/CM² LOADING

Component	Theoretical (mg/mL)	Actual	% Recovery
Ionizable Lipid	11.48	8.64	75%
DSPC	2.37	1.97	83%
Chol.	4.46	3.56	80%
DMG PEG	1.32	1.05	77%
Total	19.63	15.21	77%

Table 4. Values of the lipids at 0.35 mg/cm² loading using PES membrane. The results account for a total recovery of 77%.

The PES study results are shown to be consistent with the results obtained from the RC study. Both membrane systems demonstrated improved product recovery compared with the hollow-fiber system.

SUMMARY

Studies were conducted using both RC and PES membranes to understand their effects to key process parameters, including membrane loading, TMP, permeate flux, and shear rate. Hollow fiber cartridges have historically been the preferred platform in LNP process development. These systems are well established and commonly implemented as single-use techniques. However, applications requiring larger batch volumes or higher dosing programs such as gene editing, may benefit from flat sheet cassettes, which offer more capacity due to its higher allowable membrane loading and scalable configuration. Also, certain LNP formulations require hydrophilic membranes such as RC which are not available in hollow fiber formats.

The results of this study demonstrated that flat sheet cassettes are easy to use and provide product recoveries comparable to those achieved with hollow-fiber systems. Importantly, critical quality attributes (CQAs), including particle size, PDI, percent encapsulation and lipid content were maintained throughout the flat sheet TFF process, indicating that cassette based filtration can support robust processing of LNP formulation.

References

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