From Bench to Bedside: The Unsolved Scale-Up Bottlenecks in Lipid Nanoparticle Manufacturing for Personalized RNA Therapeutics

Manasi Vinod Chaudhari[1], Rutuja Pravin Patil[2], Rishabha Motilal Sonawane[3], Om Vinod Wankhede[4], Tejas Nayan Mahajan[5]

[1] Asst prof., Department of pharmaceutical chemistry, Shri Gulabrao Deokar college of pharmacy, Jalgaon.
[2,3,4,5] S.Y. B. Pharm, Shri Gulbarga Deodar College of pharmacy, Jalgaon

Corresponding author:rishabhasonawane44@gmail.com
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Abstract

The advent of RNA-based therapeutics encompassing messenger RNA (mRNA), self-amplifying RNA (saRNA), and CRISPR ribonucleoprotein (RNP) systems—has ushered in a transformative era of precision medicine. Whereas this the transition from laboratory-scale precision to full-scale industrial production presents a fundamental engineering tradeoff. This paper examines how design choices, equipment capabilities focus on biopharmaceutical manufacturing, advanced materials, and Micronanolipidformulation.The bottle necks we add in our review are the formation of nano bio interference, IVIVC failuer, the scalability of process and how the continuous manufacturing is processed Moreover, the rise of personalized medicine introduces N = 1 production scenarios, demanding decentralized Good Manufacturing Practice (GMP) frameworks and sophisticated cold-chain logistics to ensure stability and timely patient access. we are proposing the future plan and road map in the precised RNA therapeutic forming the next generation aisa designed lipid libraries, on Demand self amplifiig RNA factories and regulated autonomous LNP Micro factories.

1. Introduction

The COVID-19 mRNA vaccine is emerged as a best example of RNA therapeutics. New types of RNA like saRNA, circular RNA, and CRISPR systems contribute in various sectors and all requiring efficient nanoscale delivery vehicles.(Kiaie et al., 2022#1) Liposomes and next-generation lipid nanoparticles (LNPs) have become central to RNA therapeutics because they protect naked RNA and enable targeted intracellular delivery. In parallel, personalized "N=1" vaccines and individualized gene therapies are emerging and modular as small-batch GMP manufacturing.(Wu et al., 2024#2) This creates a fundamental bottleneck to counter this and maintaining precise control of nanoscale architecture while scaling and standardizing production for clinical use is emerged as a need of 20th century.(Youssef et al., 2023#3)

2. Engineering the Nano–Bio Interface

RNA therapeutics is a nano-bio interface where mRNA, siRNA, or CRISPR components are precisely delivered by engineered nanoparticles like lipid or polymeric carriers. Targeted gene modulation increases treatment specificity and reduces off-target effects and this is possible by combining molecular biology and nanotechnology which revolutionizing next-generation therapy and personalized medicine.(Zhao et al., 2022#4)

2.1 The Formulation Paradox: Precision vs. Production w.r.t. CQA & IVIV Consideration:

Lipid nanoparticle (LNP) development requires control over particle size, PDI, lamellarity, and RNA-loading sensitivity for consistent therapeutic performance (Roces et al., 2020#5). Microfluidics enable this nano-level precision, producing uniform LNPs with high encapsulation efficiency through controlled mixing at small scales (Li et al., 2022#6). But scaling introduces a trade-off between nano precision and industrial throughput by increasing production rates often disrupts mixing dynamics, resulting in size shifts, broader PDI, altered lamellarity, and reduced batch reproducibility. Large-scale or parallelized mixers improve throughput but control required to maintain strict CQAs

The following equations were used to calculate encapsulation efficiency (EE%) and mRNA recovery as follows:

$$EE\% = 100 \times (CT - CF)/CT$$

CT = total RNA concentration (based on results from the wells prepared with Triton-TE buffer)

CF = free, unentrapped RNA concentration (based on results from the wells prepared with TE buffer) (McMillan et al., 2024#7)

PDI -PDI represents the width of the particle-size distribution.

- Low PDI → narrow distribution → homogeneous nanoparticles
- High PDI → broad distribution → instability, aggregation
- $PDI=\left(\frac{d}{\sigma}\right)^2$
- σ = standard deviation of particle size and is
- d = mean particle diameter[8#]

Table:2.1. CQAs Considerations:

No.	CQAs	Possible Impact	Reference

1	Size	Too small → rapid clearance; Too large → spleen trapping; Optimal → best PK	(Haghighi et al., 2024#9)
2	PDI	High → unstable & inconsistent dosing; Low → reproducible delivery	(Mehta et al., 2023#10)
3	ζ-potential	High +ve → toxicity; Too –ve → low uptake; Near-neutral → ideal in vivo	(Shivaswamy et al., 2024#11)
4	Morphology / Lamellarity	Wrong structure → poor release & escape	(Nele et al., 2024#12)
5	Internal Structure	Dense → slow release; Loose → leakage	(John et al., 2024#13)
6	Lipid Ratio	Wrong ratio → low stability & EE%; PEG↑ → uptake↓	(B. Li et al., 2023#14)
7	Lipid Purity	Impurities → toxicity & immunogenicity	(Xu et al., 2025#15)
8	N/P Ratio	Low → poor EE; High → toxicity/aggregation	(Catenacci et al., 2024#16)
9	Residual Solvents (e.g. Ethanol)	Ethanol↑ → toxicity & instability	(Dikpati et al., 2020#17)
10	EE% (Encapsulation Efficiency)	Low EE → dose ↑, potency ↓	(Liu et al., 2025#18)
11	RNA Loading %	Under-loading → low potency; Over-loading → unstable LNP	(Liao et al., 2025#19)
12	RNA Integrity	Breakdown → reduced protein expression	(Zhai et al., 2024#20)
13	Free RNA (Unencapsulated)	Triggers innate immunity & toxicity	(Hou et al., 2021#21)
14	Complexation (RNA–lipid interaction)	Poor complexation → leakage + low transfection	(Gaspar et al., 2020#22)
15	pKa of Ionizable Lipid	Wrong pKa → failed endosomal escape	(Habrant et al., 2016#23)
16	Density / Internal Packing	Alters organ targeting (liver vs spleen)	(Heredero et al., 2025#24)

Table:2.2. IVIVC Consideration:

No	IVIVC Consideration	Observations	Reason	Reference
1	Protein Corona Dynamics & Tissue-Specific Clearance	In vitro cannot replicate dynamic, competitive protein corona formed in vivo.	Corona controls ApoE binding, PEG shedding, complement opsonization → governs Kupffer/MPS uptake.	(Akinc et al., 2010#25; Voke et al., 2025#26)
2	Species-Dependent Biodistribution	Rodent → NHP → human biodistribution patterns do not match.	Differences in ApoE isoforms, complement pathways & liver fenestration size shift organ targeting.	(Lemdani et al., 2024#27)
3	Failure of Predictive Models in Large Animals / Humans	In vitro & advanced organ-models fail to recapitulate whole-body clearance & immune–LNP interactions.	NHP–human differences in lipid transport, immune amplification, endosomal escape → dose–response mismatch.	(Hou et al., 2021#21b; Lemdani et al., 2024#27; W. Liu et al., 2025#28) (Gilleron et al., 2013#29)
4	Nano–Bio Interactions Are Engineering-Driven Problems	Scale-up changes nanoscale structure → changes biological identity.	Size, PDI, PEG density, N/P ratio, flow rate, ethanol dilution alter morphology → collapse IVIVC.	(Fell et al., 2025#30; Schoenmaker et al., 2021#31)

3. LNP Architecture, Materials, and Physicochemical Considerations

Why LNPs Are Needed in RNA Therapy?

Naked RNA rapidly degrades in biological fluids due to abundant RNases, making direct delivery impossible and LNPs protect mRNA from RNases.(Jung et al., 2022#32)Ionizable lipid–based LNPs encapsulate negatively charged RNA and enable cellular entry.(Sui et al., 2025#33) LNPs protect RNA, improve stability, and greatly increase in vivo and in vitro delivery efficiency.(Kong et al., 2024#34)Composition w.r.t to siRNA is listed and also various RNA constraints table(Kulkarni et al., 2018#35)

No	**Lipid Component**	**Typical % mol**	**Findings wrt si RNA**
1	Ionizable Cationic Lipid	~50 mol%	KC2 induces pH-dependent structural transition (bilayer vesicles at pH 4 → amorphous solid-core at pH 7.4). • Increasing KC2 mol% decreases LNP size at pH 4. • At neutral pH, neutral KC2 forms an oil-like phase leading to vesicle fusion. • Higher KC2 content increases core size (40–90 mol%)
2	PEG-Lipid	1.5mol%	PEG-lipid is positioned on the nanoparticle surface. • PEG-content influences and controls LNP size.
3	Phospholipid (DSPC)	~10 mol%	• DSPC localizes to the LNP surface monolayer due to amphipathic structure. • DSPC is not soluble in the KC2 oil phase.
4	Sterol Lipid (Cholesterol)	38.5 mol%	Cholesterol has limited solubility in neutral KC2 (≈8 mol%). • Minor presence in the amorphous KC2 core structure.

MOA of RNA therapeutics

Starts with Injection (IV or IM),Cellular Uptake,Endosomal Escape,Protein Translation (mRNA/saRNA),Gene Silencing (siRNA),Genome Editing (CRISPR)(Hou et al., 2021b)

4. Manufacturing Technologies for Nano-Scale Control

Nanoparticle fabrication involves top-down (breaking bulk materials) extraction, HPH, Sonication and bottom-up (molecular self-assembly) approaches such as thin layer formation and various nano precipitation techniques. Thin-layer formation enhances uniformity and encapsulation efficiency. Despite these methods, microfluidics surpasses traditional techniques and batch and high energy methods by offering precise control over mixing, particle size, reproducibility, and scalability, making it the preferred approach for RNA therapeutics.(Bi et al., 2025#36)

Impact of Microfluidic Mixing Parameters 1. Total Flow Rate (TFR): o Sum of aqueous + organic phase flow rates. o Higher TFR → faster mixing → smaller nanoparticles. o Primary control parameter for tuning LNP size. 2. Flow Rate Ratio (FRR): o Ratio of aqueous to organic phase flow rates. o Moderate effect on size; strong effect on encapsulation efficiency. o Typical: FRR ≈ 3:1 → EE% > 95% for RNA–LNP systems. 3. Temperature: o Influences lipid solubility, ethanol–water miscibility, and mixing kinetics. o Affects: particle size, encapsulation efficiency, internal LNP structure(O'Brien Laramy et al., 2023#37; Vogelaar et al., 2023#38)

4.1 Microfluidic & Nanofluidic Assembly

T-junction and staggered herringbone mixer (SHM) microfluidic designs are widely used to enhance mixing at low Reynolds numbers where diffusion is slow, especially for biomolecules with very low diffusivity (~10^{-11} m^2/s). Many lamination-based micromixers exist—interdigital, split-and-recombine, geometric focusing, secondary-flow and chaotic mixers—to overcome poor diffusive mixing and clogging. Confining polymers from micrometre to nanometre scales alters nucleation and crystallization, producing fractionated crystallization or requiring large supercoolings. Heterogeneous nucleation dominates in impurity-containing domains, whereas homogeneous nucleation occurs only in clean, isolated microdomains. Microfluidic strategies such as SHM, serpentine channels and SAR mixers improve mixing and controlled assembly.(Kee & Gavriilidis, 2008#39)

4.2 CFD Modeling of Mixing & Particle Formation

. CFD simulates mixing and particle formation by solving mass, momentum, and energy conservation equations Chaotic advection—modeled using geometries such as twisted pipes or micromixers—improves mixing in laminar flows by stretching and folding fluid elements; CFD tools like Poincaré maps help identify chaotic regions. Mixing efficiency improves over diffusion-limited systems but may be hindered by coherent flow structures. LES. High energy-dissipation rates accelerate formation of sub-100 nm particles;

For this Kolmogorov scale is widely used

$$\text{Kolmogorov scale}(\eta) = \left(\frac{v^3}{\varepsilon}\right)^{\frac{1}{4}}$$

Where,

$$\varepsilon(epsilon) = \ The\ energy\ sissipation\ rate$$

v (mu)=Kinetic Viscosity of fluid(m^2/sec)

CFD challenges include data quality, turbulence-model uncertain CFD modelling and simulations of atomization-based processes for production of drug particles(Baassiri et al., 2025#40)

4.3 From Batch to Continuous Nanomanufacturing Engineering

Continuous nano-manufacturing replaces stop-and-go batch processing with steady, higher-quality production using microreactors, microfluidic chips,(Pattanayak et al., 2021#41) 3-D-printed channels and continuous dense-gas systems(Kang et al., 2018#42). Coiled-channel designs generate toroidal (Dean) vortices that enhance mixing, prevent clogging and narrow size distributions. In GMP genetic-drug manufacturing, success depends on high-quality materials, defined unit operations, validated cleaning, strong QC and compliant

infrastructure(Bretaudeau et al., 2020#43). The CTAT method, tested at BCRT and UC-Davis, maps core and support processes to quantify operational and variable manufacturing costs.(Abou-El-Enein et al., 2013#44)

4.4 Continuous Freeze-Drying and Integrated Fill-Finish

Continuous fill–freeze–dry systems integrate vial filling, controlled freezing and lyophilisation into an automated line, improving throughput and batch uniformity. (Lamoot et al., 2023#45)Vial-based continuous freezing uses controlled nucleation to ensure consistent ice crystal formation, while spray freeze-drying (SFD) rapidly freezes atomised droplets into fine, free-flowing powders. For thermostable mRNA-LNPs, lyophilisation is enabled by cryoprotectant blends, bio-inspired additives such as antifreeze proteins (AFPs), and NADES to prevent degradation. (Y. Liu et al., 2018#46). Protein therapeutics avoid aggregation through supercharging and targeted charge clustering to enhance structural stability and reduce immunogenic interactions.(Ebo et al., 2020)

4.5. Regulatory Problems:

Regulatory pathways for RNA-LNP therapeutics remain complex because ICH Q13 demands real-time control of continuous manufacturing, yet nano-specific PAT tools for RNA integrity, encapsulation, and particle-formation dynamics are still limited. FDA and EMA highlight characterization gaps for LNPs, especially for personalized small-batch GMP production. Integrating Quality-by-Design (QbD) with advanced PAT is essential to ensure reproducibility, safety, and regulatory compliance.(Alshaer et al., 2022#50)

5.The Scale-Up Bottleneck: Where Bench Meets the Factory

• Traditional pharma works for only ~60% of people because it follows a reactive, “one-size-fits-all” model that ignores genetic and environmental variability

• Advances in PK/PD show drug response is highly individualized, driving growth of Personalized Medicine (> $250B)](Harvey et al., 2012#51).

• Conventional manufacturing (milling, blending, compression) lacks flexibility for individualized dosing.

**• Industry is shifting to 3D printing, microfluidics, flow chemistry, pharmacy-on-demand, continuous/smart manufacturing, and single-tablet systems to enable customization(Goetz & Schork, 2018#52)

5.1 Raw Material Shortage

• GMP/cGMP are essential for large-scale production of complex biologics like IVT mRNA(Feddema et al., 2023#53)
• Manufacturing includes:
A) DNA template design
B) DNA template production
C) Bacterial fermentation
D) Synthetic DNA routes
E) mRNA–LNP formulation
• Regulatory ambiguity slows commercialization of new mRNA products; streamlined pathways are required((PDF) Decentralized Manufacturing Systems Review: Challenges and Outlook, 2012#54)

5.2 Hydrodynamic Barrier

• Microfluidics manipulates μL–pL volumes using microscale channels; highly powerful for precision formulations(Chan et al., 2025#55)
• Personalized microfluidic systems face challenges:

– Large datasets needed for patient stratification
– Slow characterization workflows
– Hydrodynamic constraints during personalized processing(C. Liu et al., 2025#56).

5.3 Quality Drift During Scale-Up

• Vaccine/biologic supply chains lack robustness, remain siloed, and react to disruptions instead of anticipating them(Kumar et al., 2024#57)
• Lipid nanocarrier quality depends on particle size and PDI, which drift easily during scale-up.(Obinna Ogbuagu et al., n.d.#58)
• mRNA stability is biologically limited: deadenylation (Pan2/Pan3, Ccr4/Pop2/Not) → exosome-mediated degradation• Biological instability amplifies variability during manufacturing scale-up. 9(Pajić et al., 2024#58)

5.4 Analytical Limitations

• IoT-enabled systems produce massive process data, but historian-based analytics are too slow.
• RT-DAP systems are required for fast, real-time process understanding and control(Fatorachian et al., 2025#60)
• Impurity profiling (residual solvents, degradants, byproducts) is critical for safety but technically challenging(Danaei et al., 2018#61)• Nanomedicine progress needs multifunctional, biologically contextual nanocarriers and improved analytical tools(Randhawa & Sigalov, 2025#62)

Stability — Residual Ethanol Disrupts Final Nanoparticle Structure

(a) Residual solvent alters particle size, zeta potential, assembly)
• *Residual ethanol significantly changes liposome stability, size distribution, and charge; ethanol removal is required for final structural organization.(Bnyan et al., 2020#63)*
(b) Microfluidic LNP synthesis requires solvent removal because high ethanol fraction keeps particles "partially assembled."
• *Microfluidic LNPs require downstream buffer exchange/dialysis to remove ethanol and allow proper self-assembly*(Mehraji & DeVoe, 2024#64).
[14]🔗

2. Toxicity — Ethanol & Low pH Make Crude LNP Suspensions Biologically Unsafe

(a) Ethanol in LNP formulations is cytotoxic; must be removed for in-vitro/in-vivo use.
• *FDA-oriented review notes ethanol is toxic above low percentages and must be removed during LNP purification.(Pittiu et al., 2024#65)*

(b) Microfluidic LNP formation uses 20–40% ethanol; crude output unsuitable for biological use until purified.
• *Solvent removal is mandatory before biological testing to avoid cytotoxicity and instability.*(Mehraji & DeVoe, 2024#64)

3. Purity — Removal of Free Drug, Unassembled Lipids, and Polymer Precursors

(a) Post-formulation purification removes free drug and excess lipids to improve performance & reproducibility.
• *Critical review of purification strategies in nanomedicine showing removal of free cargo, polymers, and surfactants is essential.*
(Tehrani et al., 2025#66)

(b) In microfluidic LNP synthesis, unassembled lipids remain in crude suspension and require purification (TFF, dialysis).
• *Impurities like free nucleic acid, lipids, and solvent must be removed to ensure uniformity and therapeutic performance.*(Mehraji & DeVoe, 2024#64)

Endosomal escape remains a major barrier in RNA therapeutics because most internalized nanoparticles become trapped in endosomes, where cargo faces degradation before reaching the cytosol. Only a small fraction (<2%) typically escapes, limiting therapeutic efficacy. Improving escape efficiency is critical for successful mRNA, siRNA, and CRISPR delivery(Dowdy, 2023#67)

6. Personalized Medicine Manufacturing Mode

- Aims to predict, prevent, and treat illnesses tailored to individual needs.
- Driven by technologies enabling detailed molecular-level biological profiling.
- Future development requires novel technologies for data collection and analysis that are:
  - o Integrated: understanding system-level functioning.
  - o Dynamic: capturing biological systems in flux.
- Key factors: standardisation, integration, and harmonisation across research sites(Harvey et al., 2012#51)

Applications / Key Areas:

1. Patient guidance services for personalised health management.
2. Personalised web-based assistive and social computing solutions.
3. Patient-specific data and organ models.
4. Personalised health systems.
5. Personalised digital media in personalised products and services.

Personalised Medical Product Design & Development

- Particularly prominent over the last decades.
- Enabled by Rapid Prototyping (RP) and Rapid Manufacturing (RM).((PDF) Personalised Medical Product Development: Methods, Challenges and Opportunities, n.d.)#52

6.1 manufacturing

Tablet customization is challenging due to novelty. Data mining extracts insights from large datasets for market-driven design.(S. Li et al., 2015#53) Modularization enables small, decentralized, customer-focused units.((PDF) Decentralized Manufacturing Systems Review: Challenges and Outlook, n.d.)54 RNA medicine relies on LNP lipid composition; COMET predicts efficacy, adapting to non-canonical LNPs. (Algorri et al., 2025#55)CSFV E2 mRNA-LNP vaccines enhance immunogenicity and reduce costs.(C. Liu et al., 2025b56) AI-driven cancer vaccines optimize epitopes, mRNA/DNA instructions, and enable personalized therapeutic strategies (Kumar et al., 2024#57).

6.2 Decentralize manufacturing hubs

Decentralized Supply Chain & Cold Chain Logistics
Optimizing supply chains is vital for personalized medicine, ensuring faster drug discovery and reliable patient delivery. JIT inventory, real-time tracking, blockchain, and cold chain logistics maintain product quality. (Avramescu et al., 2021#68)Inefficient handling or delays can lead to product damage, spoilage, and costly re-delivery, emphasizing the need for robust systems.(Ouranidis et al., 2021#69)

6.3AI- driven supply chain and cold chain engineering

AI and ML optimize cold chain logistics, improving demand forecasting, inventory management, and product quality. Predictive analytics (ARIMA, MLR) enables dynamic stock adjustments(Fatorachian et al., 2025b60)Agile, decentralized manufacturing enhances flexibility and resilience. Blockchain ensures traceability and fraud prevention(Harrison et al., 2017#70) DApps track operations, providing automation, transparency, and data integrity throughout the supply chain.(Panda & Satapathy, 2021)

7.Case Studies: Translational Lessons and renaissance

No.	**RNA Type**	**Primary Role in RNA Therapeutics**	References

1	**mRNA** Messenger RNA	Delivers genetic instructions to produce therapeutic proteins (e.g., vaccines, antibodies, enzymes).	(Jung et al., 2022b)
2	**[saRNA** Self-amplifying RNA	Encodes both antigen + replicase, leading to **in-cell RNA amplification** → high protein expression with low dose.	(Schmidt & Schnierle, 2023#71)
3	**siRNA** Small interfering RNA	Silences disease-causing genes via **RNA interference** by degrading target mRNA.	(Hu et al., 2020#72)
4	**CRISPR RNA (gRNA/RNP)** Guide RNA for CRISPR systems	Directs CRISPR-Cas nucleases for **genome editing**, gene correction, knockout, or base editing.	(Uddin et al., 2020#73)

7.1 mRNA COVID-19 Vaccines: A Scale-Up Blueprint

COVID-19 vaccines (Pfizer/BioNTech and Moderna) that use lipid nanoparticles (LNPs) to deliver spike protein mRNA. The lipid nanoparticle protect the mRNA from degradation also enables entry into cells through endocytosis . (Demongeot & Fougère, 2022#74).mRNA vaccines (Pfizer-BioNTech, Moderna) are rapid ,scalable and it stimulate both humoral (antibody) and cellular immune responses. It do not integrate in the human genome unlike the DNA vaccines. High efficacy was demonstrated in clinical trials (Pfizer ~90% in Phase III). The mRNA vaccines include Coding sequence for antigen (spike protein or RBD) and Regulatory regions (5′ cap, UTRs, poly-A tail). It mimics viral infection in this case SARS-CoV-2 infection, producing spike protein inside host cells. Moderna (100 µg dose) tends to produce stronger immune responses than Pfizer (30 µg dose). (Mirtaleb et al., 2023#75)so their precise work in COVID-19 has opened pathways for infectious diseases, cancer, and genetic disorders, with lipid nanoparticles as the key in delivering technology (Gote et al., 2023#76)

7.2 Personalized Cancer Vaccines (BioNTech, Moderna)

Personalized therapeutic cancer vaccines represent a successful but complex immunotherapy approach. They target a patient-specific tumor mutations, but their success depends totally on overcoming challenges in antigen selection, dosing, immune monitoring, and regulatory pathways. They present antigens to dendritic cells, which prime T cells and generate long-lasting immunological memory. Personalized cancer vaccines are engaged in innate immunity and adaptive immunity . They often target neoantigens (this are the mutations unique to each patients which are more immunogenic than shared tumor-associated antigens(Shemesh et al., 2021#77)

7.3 A CRISPR-Cas9 is a gene-editing tool that cuts DNA at precise locations to correct mutations. Together with a lipid nanoparticles form a non viral delivery system that offer great biocompatibility and scalability.(Lin et al., 2023#78)

8. Future Roadmap and some problem solution

8.1 Digital Twins & Predictive Scale-Up Simulators

A brand‑new digital‑twin platform was built for continuous mRNA manufacturing, stitching together IVT, TFF, CCTC, LNP formulation and freeze‑drying in a modular, plug‑and‑play framework. It runs full‑scale simulations that produce time‑series and 3D visuals, while Morris‑based sensitivity analysis pinpoints the most influential variables for each unit, guiding optimization and validation.

(Shahab et al., 2025#79)

8.2 Next-Generation Lipid Libraries (AI-designed)

The team leveraged machine-learning predictors, GANs for inventive lipid structures, and neural-network models to forecast biodistribution. These AI-tuned LNPs showed better tumor homing, and the GAN-crafted lipids kept high encapsulation while offering novel chemistries; graph-neural nets accurately predicted RNA-LNP binding.(Wang et al., 2024#80)

8.3 On-Demand Self-Amplifying RNA (saRNA) Factories and screening optimization

Self-amplifying RNA (saRNA) lets cells crank out antigen at lower doses, boosting immune response and cutting costs. Because the RNA replicates inside cells, only a tiny synthetic transcript is needed for strong immunity—something the first approved saRNA vaccine proves.(Casmil et al., 2025#81). Along with this CQAs,CMAs,CPPs and QTPP contribute most for mRNA therapeutics. Also PLGA is plying role in non viral RNA therapeutics and can be analysed by Ai and DOE software w.r.t. QBD approaches. (Toma et al., 2022#82).Along with this various analytical techniques are used for screening and optimization some are listed in table below (Nogueira et al., 2024#83)

Table 1: analytical techniques are used for screening and optimization

No.	Technique	Principle	References
1	**DLS**	Light scattering fluctuation → hydrodynamic size & PDI	(Rodriguez-Loya et al., 2023#84)
2	**NTA**	Tracks Brownian motion of individual particles	(Nogueira et al., 2024#83)
3	**Cryo-TEM**	Direct imaging at cryogenic temp	(Ma et al., 2022#84)

9. Conclusion

RNA therapeutics hold transformative potential across vaccines, protein replacement, and gene modulation, but their full realization is hindered by key challenges. Chief among these are delivery limitations — including inefficient tissue targeting, narrow biodistribution beyond liver, and endosomal escape inefficiency — which hinder clinical translation. (MDPI)(Pozdniakova et al., 2025#85)
Manufacturing and scalability remain bottlenecks: current production requires specialized facilities, stringent quality controls, and cold-chain logistics, limiting global access and increasing costs. (MDPI)(Youssef, Hitti, Fulber, et al., 2023#86)
Finally, issues of immunogenicity, stability, and precise dosing complicate long-term and repeated-dose applications, particularly for chronic diseases requiring controlled protein expression. (ScienceDirect)(Shahid, 2025#87)

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