White Paper | leon-nanodrugs GmbH & Glatt Pharmaceutical Services GmbH & Co. KG | March 2025

Feasibility of preparing naproxen-loaded liposomes using the FR-JET® technology

Dr. Blerina Shkodra[1], Dr. Shuai Shi[2], Dr. Annette Grave[3]

1 – Senior Lead Scientist, Process Development & Lab Manager, leon-nanodrugs GmbH
2 – Scientist Formulation & Process Development, Glatt Pharmaceutical Services GmbH & Co. KG
3 – Senior Scientific Expert Pharmaceutical Technologies, Glatt Pharmaceutical Services GmbH & Co. KG

Over the past 60 years, research on liposomal formulations has been significant, aiming to develop and optimize these nanocarriers for next-generation therapeutics.[1,2] Recently, there has been increasing interest, particularly in oncology, to repurpose existing cancer therapies by formulating them into liposomal formulations to enhance their targeting and efficacy.[3] The improvements in drug efficacy enabled by liposomal delivery systems are particularly beneficial for cancer patients. Additionally, they offer drug developers a business incentive by creating opportunities to market their repurposed drug products for new indications.[4]

Liposomes are versatile nanocarriers composed of phospholipids and cholesterol that form bilayered spherical vesicles, capable of encapsulating hydrophilic drugs in their aqueous core and lipophilic drugs within their lipid bilayer.[5] As such, liposomes have been used as a delivery platform to encapsulate small molecules as well as larger biomolecules (e.g., nucleic acids) and are the first nano-sized drug delivery model approved for clinical use.[1,2] As self-assembly systems, their size and structural morphology are mainly controlled by the formulation composition (i.e., lipid composition and aqueous medium). Conventional technologies used for the preparation of liposomes include methods such as thin-film hydration, ethanol injection, and membrane extrusion.[5,6] The thin-film hydration method involves dissolving lipids in an organic solvent, followed by evaporation to form a thin lipid film, which is then hydrated with an aqueous solution.[7] The ethanol injection method involves injecting a lipid-ethanol solution into an aqueous phase, which results in the formation of liposomes.[7] Whereas the membrane extrusion method involves forcing a lipid suspension through polycarbonate membranes with defined pore sizes under high pressure, resulting in liposomes with a size distribution typically reflecting the membrane pore sizes.[7]

Despite their widespread application, these methods have several limitations, including the need for high temperatures (unsuitable for biomolecules), the use of large volumes of organic solvents (posing explosion risks and environmental challenges), broad particle size distributions, low encapsulation efficiencies, multiple passes required during membrane extrusion to achieve the desired size, potential degradation of sensitive compounds, and challenges with scalability.[7,8] To circumvent the limitations, bottom-up approaches are being explored and developed to improve the reproducibility of the manufacturing process and enable scale-up of liposomal products that meet the desired critical quality attributes (CQAs) of the product.

The FR-JET® is a jet-impinging reactor in which liposomes are formulated by mixing two starting solutions at high velocity. The impinging of the liquid jets of two phases, i.e., lipids (and drugs) in ethanol and aqueous buffer, triggers a rapid nucleation process that results in the formation of bilayered particles with uniform sizes within a confined mixing chamber. This white paper explores the feasibility of preparing conventional liposomes with the FR-JET® technology, using naproxen as a model payload. Naproxen, a non-steroidal anti-inflammatory drug (NSAID) widely used for pain relief, has previously been encapsulated in liposomes using the thin-film hydration and ethanol injection methods.[9–11]

The target size and polydispersity index (PDI) of the naproxen containing liposomes were ≤200 nm and ≤0.2, respectively. Three liposomal formulations with differing lipid compositions, total lipid concentration as well as naproxen feed concentrations were prepared. The lipid components—soy phosphatidylcholine (SoyPC), cholesterol (Chol) and poly(ethylene glycol) lipid (PEG-lipid)—each play distinct and complementary roles in liposome formation and function.[12,13] SoyPC forms the basic structural matrix of the liposome, cholesterol enhances its structural stability and reduces membrane permeability, whereas the PEG-lipid improves the circulation time, while also allowing for further modifications (e.g., particle size) and preventing particle aggregation both during formulation and storage.[12]

This study highlights the robustness of FR-JET® technology in producing stable liposomal formulations with particle sizes ≤150 nm and PDI ≤0.14 during a screening phase.

Materials & Methods

Liposomes composed of SoyPC were prepared to encapsulate naproxen as a model drug (Table 1, Figure 1). Based on preliminary solubility screening results, total lipid concentrations of 15 mg/mL, 30 mg/mL and/or 100 mg/mL were used to prepare the lipids (Table 1). Liposomes were formulated using the FR-JET® technology at a total flow rate (TFR) of 80

mL/min and a flow rate ratio (FRR) of 2:1 (aqueous:organic). The FR-JET® reactor with a 2 mm mixing chamber diameter was used, with a pinhole combination of 200 µm on the aqueous phase inlet and 100 µm on the organic phase inlet. After formulation, liposomes were dialyzed overnight against 10 mM phosphate buffer under stirring at 50 to 150 rpm, using dialysis cassettes with a 100 kDa MWCO of cellulose membrane. Dialyzed samples were filtered using polyethersulfone (PES) filters with a pore size of 0.22 µm. Particle size and PDI of liposomes was measured using dynamic light scattering (DLS) (Zetasizer NANO ZS, Malvern Panalytical).

Table 1. *Formulation parameters of the liposomes.*

Lipid composition	Total lipid conc.	Payload	Lipid ratio % (w/w)
SoyPC/Chol/PEG	30 mg/mL	-	62/26.7/11.5
SoyPC/Chol/PEG	100 mg/mL	-	62/26.7/11.5
SoyPC/Chol/PEG	30 mg/mL	3%	62/26.7/11.5
SoyPC/Chol/PEG	30 mg/mL	6%	62/26.7/11.5
SoyPC/Chol/PEG	100 mg/mL	3%	62/26.7/11.5
SoyPC/Chol/PEG	100 mg/mL	6%	62/26.7/11.5
SoyPC/Chol	15 mg/mL	-	55/45
SoyPC/Chol	15 mg/mL	3%	55/45
SoyPC/Chol	15 mg/mL	6%	55/45
SoyPC	30 mg/mL	-	100
SoyPC	100 mg/mL	-	100
SoyPC	30 mg/mL	3%	100
SoyPC	30 mg/mL	6%	100
SoyPC	100 mg/mL	3%	100
SoyPC	100 mg/mL	6%	100

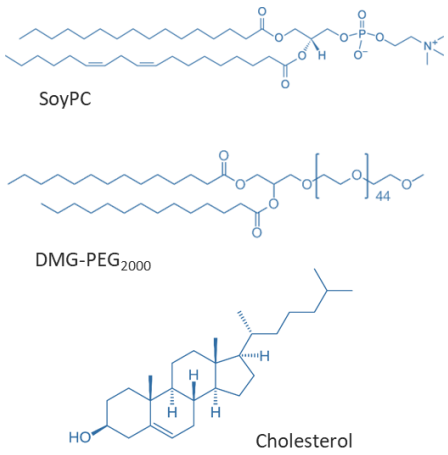

Figure 1. *Chemical structure of the lipids.*

Results & Discussion

The particle size for all liposomal formulations prepared with the FR-JET® technology was below 200 nm and varied depending on the formulation composition and total lipid concentrations (Figure 2). As expected, formulations prepared with a higher lipid concentration resulted in larger liposomes. Furthermore, it was observed that feeding naproxen in the formulation increased the particle size by up to 30 nm for liposomes consisting of SoyPC and SoyPC/Chol/PEG, and by approximately 100 nm for liposomes consisting of SoyPC/Chol. Meanwhile, the PDI for all liposomes remained ≤ 0.2, except for the empty SoyPC/Chol liposomes, which displayed a PDI of 0.26. SoyPC/Chol liposomes were prepared at a lower total lipid concentration of 15 mg/mL due to the solubility limit of cholesterol in the absence of additional lipid components, such as the PEG-lipid. The same liposomes resulted in the formation of larger liposomes, due to the absence of a stabilizing agent. In general, the PEG-lipid stabilized the liposomal formulation by maintaining both particle size and PDI values more consistent for SoyPC/Chol/PEG liposomes, regardless of the total lipid concentration and the amount of naproxen fed in the formulation. However, it should be noted that since the PEG-lipid affects the size and structure of the particles,[12] thus influencing intracellular pathway of particles, the optimal molar content should be investigated *in vitro* to optimize formulation parameters.

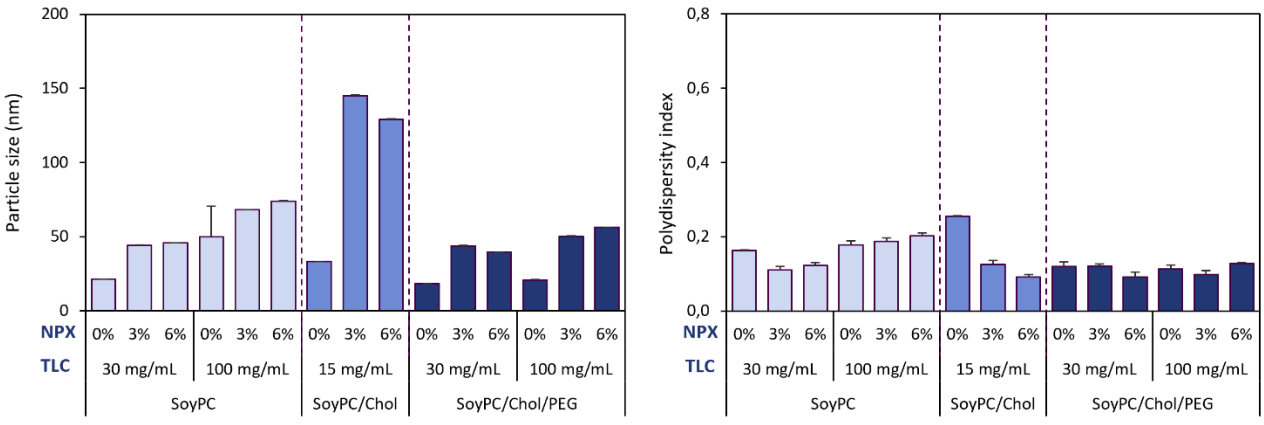

Figure 2. *Particle size and PDI of naproxen-loaded liposomes after filtration prepared at a TFR of 80 mL/min using the FR-JET® technology. Error bars represent standard deviations from three DLS measurement replicates. NPX refers to naproxen, whereas TLC refers to the total lipid concentration.*

Conclusion

This study demonstrated the successful preparation of liposomes with particle sizes of ≤150 nm and an average PDI of ≤0.14 using the FR-JET® technology. Liposomes comprising three distinct lipid compositions across varying total lipid concentrations (15, 30, and/or 100 mg/mL) were investigated. Formulations containing cholesterol but without a stabilizing agents exhibited lower stability, however, addition of the PEG-lipid improved particle size distribution. This initial study showcases the robustness of FR-JET® technology in efficiently producing liposomal formulations to screen for optimal formulation compositions as well as drug feed concentration ranges. It should be noted that further investigations are necessary to optimize the properties of these liposomes.

References

[1] J. Chen, S. Hu, M. Sun, J. Shi, H. Zhang, H. Yu, Z. Yang, *European Journal of Pharmaceutical Sciences* **2024**, *193*, 106688.

[2] P. R. Cullis, P. L. Felgner, *Nat Rev Drug Discov* **2024**.

[3] S. T. Galatage, A. S. Manjappa, R. R. Waghmode, S. S. Harale, R. B. Katkar, S. A. Desai, S. S. Chopade, K. S. Bille, R. U. Watangi, S. N. Kalebere, A. S. Hebalkar, S. V. Dhobale, H. N. Gunjate, P. R. Dhenge, P. S. Ikke, S. A. Shaikh, R. J. Patil, S. B. Shinde, R. V. Khatavakar, A. B. Patil, P. N. Khatavakar, S. S. Hegaje, S. G. Killedar, S. T. Galatage, A. S. Manjappa, R. R. Waghmode, S. S. Harale, R. B. Katkar, S. A. Desai, S. S. Chopade, K. S. Bille, R. U. Watangi, S. N. Kalebere, A. S. Hebalkar, S. V. Dhobale, H. N. Gunjate, P. R. Dhenge, P. S. Ikke, S. A. Shaikh, R. J. Patil, S. B. Shinde, R. V. Khatavakar, A. B. Patil, P. N. Khatavakar, S. S. Hegaje, S. G. Killedar, *Drug Repurposing - Advances, Scopes and Opportunities in Drug Discovery* **2023**.

[4] K. H. van der Pol, M. Aljofan, O. Blin, J. H. Cornel, G. A. Rongen, A. G. Woestelandt, M. Spedding, *Appl Health Econ Health Policy* **2023**, *21*, 831–840.

[5] H. Nsairat, D. Khater, U. Sayed, F. Odeh, A. Al Bawab, W. Alshaer, *Heliyon* **2022**, *8*, e09394.

[6] P. Liu, G. Chen, J. Zhang, *Molecules* **2022**, *27*.

[7] V. V. S. N. L. Andra, S. V. N. Pammi, L. V. K. P. Bhatraju, L. K. Ruddaraju, *Bionanoscience* **2022**, *12*, 274.

[8] H. Elsana, T. O. B. Olusanya, J. Carr-wilkinson, S. Darby, A. Faheem, A. A. Elkordy, *Sci Rep* **2019**, *9*.

[9] C. Puglia, F. Bonina, L. Rizza, R. Cortesi, E. Merlotti, M. Drechsler, P. Mariani, C. Contado, L. Ravani, E. Esposito, *J Pharm Sci* **2010**, *99*, 2819–2829.

[10] S. Ghanbarzadeh, A. Khorrami, S. Arami, *J Pharm Investig* **2014**, *44*, 33–39.

[11] F. Oliverio, E. Nuin, I. Andreu, G. Ragno, M. A. Miranda, *European Journal of Pharmaceutics and Biopharmaceutics* **2014**, *88*, 551–555.

[12] C. Hald Albertsen, J. A. Kulkarni, D. Witzigmann, M. Lind, K. Petersson, J. B. Simonsen, *Adv Drug Deliv Rev* **2022**, *188*, 114416.

[13] N. T. T. Le, V. Du Cao, T. N. Q. Nguyen, T. T. H. Le, T. T. Tran, T. T. H. Thi, *International Journal of Molecular Sciences 2019, Vol. 20, Page 4706* **2019**, *20*, 4706.

Abbreviations

Chol - Cholesterol
DLS – Dynamic light scattering
DMG-PEG_{2000} – 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
kDa (unit) – kilo Dalton
MWCO – Molecular weight cut-off
NPX - Naproxen
NSAID – Non-steroidal anti-inflammatory drugs
PDI – Polydispersity index
PEG – Polyethylene glycol
PES – Polyethersulfone
SoyPC – Soy phosphatidylcholine
TFR – Total flow rate
TLC – Total lipid concentration