

FORMULATION AND OPTIMIZATION OF RUTIN-LOADED NANOSTRUCTURED LIPID CARRIERS: A PHARMACEUTICS-FOCUSED REVIEW

Yogesh R^{1*}, Sobana S², Kiruthigaa Sri K³, Dharshini Priya P⁴, Ritthish G⁴, Venkateshwari
K⁴, Gayathri Abiraami K⁵

¹Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Namakkal, Tamil Nadu, India.

²Assistant Professor, Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Namakkal, Tamil Nadu, India.

³Department of Pharmaceutics, Vellalar College of Pharmacy, Erode, Tamil Nadu, India.

⁴Department of Pharmaceutics, Paavai College of Pharmacy and Research, Namakkal, Tamil Nadu, India.

⁵Department of Pharmaceutics, Jaya College of Pharmacy, Chennai, Tamil Nadu, India.

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***Corresponding Author: Yogesh R.**

Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Namakkal, Tamil Nadu, India.

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ABSTRACT

Rutin is a naturally occurring flavonoid glycoside with considerable pharmaceutical and nutraceutical interest; however, its poor aqueous solubility, limited dissolution, instability, and restricted systemic availability present important formulation challenges. Nanostructured lipid carriers (NLCs), composed of solid and liquid lipids stabilized by surfactants, offer a promising lipid-based platform for improving the incorporation, protection, and controlled release of poorly water-soluble compounds such as rutin. This review focuses on the formulation development and optimization of rutin-loaded NLCs, with particular emphasis on lipid and surfactant selection, drug-to-lipid ratio, preparation methods, formulation and process variables, and critical quality attributes. The influence of formulation composition and processing conditions on particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and in vitro drug release is discussed. The application of Quality by Design (QbD) and Design of Experiments (DoE) approaches for systematic optimization and understanding formulation-process-performance relationships is also examined. Reported studies demonstrate that variations in lipid composition, drug concentration, surfactant system, and processing conditions can substantially influence the physicochemical characteristics and release performance of rutin-loaded NLCs. In addition, drug-excipient compatibility, solid-state characterization, stability, reproducibility, and scale-up challenges are considered. Overall, QbD- and DoE-guided development provides a systematic approach to obtaining robust rutin-loaded NLC formulations with desirable physicochemical characteristics and controlled drug-release profiles, but further work is needed to establish long-term stability, standardised characterisation, scalable manufacturing, safety, and pharmaceutical translation.

KEYWORDS: Rutin, Nanostructured Lipid Carriers, Formulation Optimization, Quality by Design, Drug Release.

INTRODUCTION

Rutin (quercetin-3-O-rutinoside) is a naturally occurring flavonoid glycoside with considerable pharmaceutical and nutraceutical interest because of its diverse biological activities. However, its formulation and therapeutic utilization are limited by poor aqueous solubility, restricted dissolution, instability under certain conditions, and limited systemic availability, which can compromise its reproducible performance following administration. From a pharmaceutical perspective, these limitations highlight the need for delivery systems capable of improving the incorporation, dispersion, protection, and release of rutin while maintaining formulation stability. Various approaches have been investigated to overcome these limitations, but the performance of such systems depends strongly on drug–excipient interactions and formulation composition.^[1,2]

Nanostructured lipid carriers (NLCs) are lipid-based nanocarriers consisting of a mixture of solid and liquid lipids stabilized by surfactants. The incorporation of a liquid lipid into the solid lipid matrix produces a less ordered structure that can provide greater opportunities for drug incorporation and controlled drug release compared with conventional solid lipid nanoparticles.^[3,4] These characteristics make NLCs particularly attractive for the formulation of poorly water-soluble bioactive compounds such as rutin. Experimental studies have demonstrated the successful incorporation of rutin into NLCs, with formulation composition and processing conditions influencing important characteristics such as particle size, polydispersity index, encapsulation efficiency, drug loading, release behaviour, and physical stability.^[5,6] Consequently, the development of a robust rutin-loaded NLC requires systematic evaluation of critical formulation variables, including lipid composition, solid-to-liquid lipid ratio, surfactant concentration, drug-to-lipid ratio, and processing conditions.

Importantly, formulation development should extend beyond the preparation of nanosized particles and should establish clear relationships between formulation variables, process parameters, and critical quality attributes. Quality-by-Design (QbD) and Design-of-Experiments (DoE) approaches provide a systematic framework for identifying influential variables and optimizing multiple formulation responses simultaneously, thereby reducing dependence on empirical trial-and-error development.^[7,8] Although recent literature has reviewed the broader application of nanocarriers for rutin delivery, encompassing several different nanosystems,^[2,9] a focused assessment of the formulation and optimization of rutin-loaded NLCs remains valuable. Therefore, this review critically examines the formulation development of rutin-loaded NLCs, with emphasis on lipid and surfactant selection, formulation and process variables, preparation techniques, critical quality attributes, physicochemical characterization, drug-release behaviour, optimization strategies, stability, and scale-up considerations. The review further highlights the relationship between formulation variables and NLC performance and identifies opportunities for QbD- and DoE-guided development of robust rutin-loaded NLC formulations.

Formulation Development of Rutin-Loaded NLCs

The development of rutin-loaded nanostructured lipid carriers (R-NLCs) requires careful selection of formulation components and processing conditions because these factors collectively determine particle formation, drug incorporation, physical stability, and release behavior. NLCs are composed of a mixture of solid and liquid lipids stabilized by one or more surfactants, and the characteristics of the resulting system are strongly influenced by the type and proportion of each component.^[4,10]

Lipid Selection

Selection of suitable solid and liquid lipids is a critical step in R-NLC development. Solid lipids provide the structural matrix, whereas incorporation of a liquid lipid disrupts the highly ordered crystalline arrangement of the solid lipid and creates imperfections that can facilitate drug accommodation within the lipid matrix.^[4,10] Lipid selection should consider the solubility of rutin in the lipid phase, miscibility between solid and liquid lipids, melting characteristics, physicochemical compatibility, and the intended stability of the formulation. In a rutin-specific study, rutin was successfully incorporated into food-grade NLCs using different rutin-to-lipid ratios, demonstrating the feasibility of lipid-based encapsulation for this flavonoid.^[5] More recent experimental work has also used stearic acid as the solid lipid and oleic acid as the liquid lipid for preparation of rutin-loaded NLCs, highlighting the importance of lipid composition in formulation design.^[11]

Surfactant Selection

Surfactants stabilize the newly formed lipid-water interface and influence particle size, polydispersity, surface characteristics, and physical stability. The concentration and type of surfactant should therefore be optimized rather than selected solely to obtain nanosized particles.^[4,10] Excessive surfactant concentrations may alter interfacial properties and can affect drug incorporation and formulation stability. In reported rutin-NLC formulations, surfactants such as Span 60 have been employed to stabilize the lipid dispersion, while earlier rutin-NLC work demonstrated that formulation composition significantly influenced the resulting colloidal characteristics.^[5,11] Thus, surfactant selection should be considered together with lipid composition and processing conditions.

Drug-to-Lipid Ratio

The drug-to-lipid ratio is an important formulation variable because it directly influences rutin incorporation, encapsulation efficiency, drug loading, and the physical stability of the carrier. Increasing the amount of rutin relative to the lipid matrix may increase drug loading up to the incorporation capacity of the system; however, excessive drug concentration can result in incomplete incorporation or drug precipitation. In a reported rutin-NLC study, different rutin-to-lipid ratios were evaluated, and a 10% rutin-to-lipid ratio was selected as the optimum formulation based on the investigated physicochemical characteristics and encapsulation performance.^[5] Therefore, optimization of the drug-to-lipid ratio should balance maximum rutin incorporation with acceptable particle characteristics and formulation stability.

Preparation Methods and Process Variables

The preparation method determines the energy input, particle disruption, lipid crystallization, and ultimately the characteristics of the NLC dispersion. Common approaches include high-pressure homogenization, high-shear homogenization, ultrasonication, microemulsion methods, and solvent-based techniques.^[10,12] High-shear and high-pressure homogenization are particularly useful for producing nanosized lipid dispersions, while the choice of process depends on drug stability, lipid properties, desired particle characteristics, and scalability. In the reported rutin-NLC study, high-shear homogenization was used for preparation, whereas a recent rutin-NLC formulation employed hot high-shear homogenization at controlled temperature and processing speed.^[5,11] Important process variables include temperature, homogenization speed, processing time, number of homogenization cycles, and cooling conditions. Consequently, formulation composition and process parameters should be evaluated together to achieve reproducible R-NLCs with desirable critical quality attributes.

Formulation Optimization and Characterization

The optimization of rutin-loaded NLCs requires systematic evaluation of formulation composition and processing conditions to obtain reproducible physicochemical characteristics. The relationship between critical formulation variables and critical quality attributes is particularly important because changes in lipid concentration, surfactant level, drug loading, and processing conditions can directly influence particle characteristics, drug entrapment, and release behavior.^[13,14]

Critical Formulation and Process Variables

Important formulation variables include the type and concentration of solid and liquid lipids, solid-to-liquid lipid ratio, surfactant concentration, and drug-to-lipid ratio. Process variables such as homogenization speed, homogenization time, sonication intensity, processing temperature, and cooling conditions can also influence particle formation and lipid organization. These variables affect the size, polydispersity, surface charge, entrapment efficiency, and drug-release characteristics of NLCs.^[4,13] In rutin-loaded lipid nanoparticles, increasing the theoretical drug concentration has been reported to influence particle size and homogeneity, demonstrating the importance of controlling drug loading during formulation development.^[15] Recent rutin-NLC research has further demonstrated that high-speed homogenization followed by ultrasonication can be systematically optimized using experimental design to obtain desirable formulation characteristics.^[16]

Critical Quality Attributes

The major critical quality attributes of rutin-loaded NLCs include particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, drug content, physical appearance, and in vitro drug release. Particle size and PDI provide information regarding the nanodispersion and size uniformity, while zeta potential provides an indication of surface charge and colloidal stability. Entrapment efficiency and drug loading are important indicators of the ability of the lipid matrix to incorporate rutin effectively.^[13,14] In rutin-loaded lipid nanoparticles, variations in drug concentration have been shown to alter particle size and aggregation behavior, emphasizing the need to define suitable quality targets during optimization.^[15] Therefore, these attributes should be evaluated collectively rather than as isolated responses.

DoE and QbD-Based Optimization

A Quality by Design approach provides a systematic framework for identifying formulation variables that significantly influence the performance of NLCs. Initial risk assessment can be used to identify critical material attributes and critical process parameters, followed by experimental design to establish their effects on selected CQAs. Factorial, Box-Behnken, and central composite designs have been successfully applied to optimize lipid-based nanosystems by evaluating the individual and interactive effects of lipid concentration, surfactant concentration, lipid ratio, and processing conditions on particle size, PDI, entrapment efficiency, and drug release.^[14,17] Importantly, a 2025 study specifically developed rutin-loaded NLCs using a three-factor, three-level Box-Behnken design, demonstrating the applicability of DoE for systematic optimization of rutin-NLC formulations.^[16] Such an approach can reduce experimental burden while providing a better understanding of formulation-response relationships.

Drug-Excipient Compatibility and Solid-State Characterization

Drug-excipient compatibility studies are essential for confirming the physical and chemical suitability of rutin with the selected lipid and surfactant system. Fourier-transform infrared spectroscopy (FTIR) can be used to identify major

changes in characteristic functional-group bands and assess potential chemical interactions, whereas differential scanning calorimetry (DSC) provides information regarding thermal transitions and changes in the physical state of rutin within the lipid matrix. X-ray diffraction (XRD) can further assess changes in crystallinity and the degree of molecular organization of the incorporated drug and lipid matrix. In rutin-loaded lipid nanoparticle systems, spectroscopic and thermal analyses have demonstrated changes in drug-lipid interactions and physical organization following encapsulation.^[6,15] These studies are useful for determining whether rutin remains crystalline or undergoes changes in its solid-state characteristics after incorporation into the lipid matrix.

In Vitro Drug Release

In vitro drug release studies are important for evaluating the ability of NLCs to control and sustain rutin release. Release behavior is influenced by particle size, lipid composition, drug loading, matrix structure, and the distribution of rutin within the lipid phase. Rutin-loaded lipid nanoparticle systems have demonstrated sustained release compared with the corresponding free drug, with the release profile generally reflecting diffusion from the lipid matrix and gradual drug partitioning into the aqueous release medium.^[6,15] Mathematical kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas models can be applied to investigate the release mechanism. However, model selection should be based on the experimental release profile and supported by appropriate statistical evaluation rather than relying solely on the highest correlation coefficient. Overall, in-vitro release should be considered together with particle characteristics, entrapment efficiency, and solid-state properties when assessing the optimized rutin-NLC formulation.^[13,17]

Comparative Analysis of Reported Rutin-NLC Formulations

Reported rutin-loaded NLC formulations demonstrate considerable variation in lipid composition, drug concentration, surfactant system, preparation method, and processing conditions. Consequently, differences in particle size, PDI, entrapment efficiency, surface charge, and release behavior are observed between studies. Rather than identifying a single universally optimal formulation, comparison of these studies indicates that the performance of rutin-NLCs is strongly dependent on the interaction between formulation composition and processing conditions.^[5,16,18,19]

Effect of Formulation Composition

Lipid composition is one of the major determinants of rutin-NLC performance. In a 2016 study, rutin was incorporated into food-grade NLCs using different rutin-to-lipid ratios, and a 10% rutin-to-lipid ratio was selected as the optimum formulation based on the evaluated physicochemical characteristics and encapsulation performance.^[5] Similarly, a 2023 study using oleic acid and stearic acid as liquid and solid lipids, respectively, demonstrated that changing the lipid ratio affected particle size and entrapment efficiency; the 1:1 lipid ratio produced the smallest particles and highest entrapment efficiency among the investigated formulations.^[18] These findings indicate that the balance between solid and liquid lipids can influence the internal structure of the lipid matrix and consequently the incorporation of rutin.

The concentration of rutin itself can also influence formulation performance. In a 2024 ocular NLC study, increasing rutin concentration from 0.05 to 0.5% w/v resulted in differences in particle characteristics and entrapment, with 0.1% w/v selected as the optimized drug concentration.^[19] More recently, a three-factor, three-level Box-Behnken study further demonstrated that the combined effects of formulation variables should be considered rather than optimizing individual components independently.^[16]

Effect of Process Conditions

The preparation method and processing intensity also contribute substantially to the final characteristics of rutin-NLCs. High-shear homogenization was used for the preparation of food-grade rutin-NLCs in the 2016 study, whereas other investigations have employed emulsification-solvent evaporation followed by probe ultrasonication or melt-emulsification and ultrasonication.^[5,18,19] The 2025 DoE-based study used high-speed homogenization followed by ultrasonication and systematically optimized the formulation using a Box-Behnken design.^[16] A more recent study prepared rutin-NLCs by hot high-shear homogenization, demonstrating another approach to produce lipid nanoparticles with defined particle characteristics.^[11]

Differences in temperature, homogenization intensity, sonication conditions, and cooling rate can alter droplet disruption, lipid crystallization, and particle growth. Therefore, direct comparison of formulations prepared by different methods should be interpreted cautiously because apparently similar compositions may produce different CQAs when processed under different conditions.^[5,11,16]

Influence on Particle Characteristics and Entrapment

Particle size and PDI are important indicators of the physical characteristics and uniformity of rutin-NLC dispersions, whereas entrapment efficiency reflects the ability of the lipid matrix to incorporate the drug. Reported rutin-NLC studies have generally produced nanosized systems, although substantial differences exist between formulations. The 2023 oleic acid-stearic acid system produced a particle size of approximately 240 nm with an entrapment efficiency of 97.34%, while the 2024 ocular formulation showed a particle size of approximately 230 nm and an entrapment efficiency of 69.36%.^[18,19] The 2025 DoE-optimized formulation showed a particle size of 172.8 ± 3.10 nm, PDI of 0.32 ± 0.20 , zeta potential of -27.2 ± 2.10 mV, and entrapment efficiency of $79 \pm 4.31\%$.^[16] In contrast, the 2026 formulation prepared by hot high-shear homogenization exhibited a larger mean particle size of 566.9 ± 73.9 nm, with a PDI of 0.294 and zeta potential of -43.7 ± 7.07 mV.^[11]

These differences demonstrate that particle size alone should not be considered as the sole indicator of formulation quality. The lipid composition, drug concentration, surfactant system, and processing conditions must be considered together when interpreting particle size, PDI, surface charge, and entrapment efficiency.

Influence on Drug Release and Overall Performance

The release behavior of rutin from NLCs is influenced by the location of the drug within the lipid matrix, lipid composition, particle size, and matrix crystallinity. Reported formulations generally demonstrate an initial release phase followed by a more sustained release phase, indicating the presence of both surface-associated and matrix-incorporated drug.^[11,16,18] The 2023 rutin-NLC formulation containing an oleic acid-stearic acid lipid matrix showed 94.5% drug release within 12 h, whereas the 2025 optimized formulation demonstrated 82.12% release over 30 h, indicating a more prolonged release profile.^[16,18] The 2018 microencapsulated NLC system also demonstrated that additional encapsulation with hydroxypropyl- β -cyclodextrin could modify particle characteristics, entrapment, and rutin-release behavior.^[6]

Overall, the available evidence suggests that an optimized rutin-NLC should not simply maximize entrapment efficiency or minimize particle size. A balanced formulation should provide acceptable particle size and PDI, adequate drug incorporation, sufficient colloidal stability, reproducible release, and stability during storage. The comparison of

reported studies therefore supports the use of a multidimensional optimization strategy in which formulation composition and process parameters are optimized simultaneously.^[5,11,16,18,19]

Table 1: Simplified comparison of reported rutin-loaded NLC formulations.^[5,6,11,16,18,19]

S. No.	Formulation / Lipid system	Preparation method	Major observation
1	Solid + liquid lipid NLC	High-shear homogenization	Lipid composition and rutin-to-lipid ratio influenced encapsulation and particle characteristics
2	Rutin-loaded NLC with additional microencapsulation	NLC preparation followed by microencapsulation	Microencapsulation modified particle characteristics and release behavior
3	Rutin-loaded NLC for ocular delivery	Melt-emulsification and ultrasonication	Formulation optimization improved particle characteristics and rutin entrapment
4	Rutin-loaded NLC	High-speed homogenization + ultrasonication; DoE	DoE successfully optimized formulation variables and CQAs
5	Stearic acid + oleic acid NLC	Hot high-shear homogenization	Demonstrated successful rutin incorporation with controlled nanosystem characteristics

Stability and Translational Challenges

Although rutin-loaded NLCs have demonstrated promising formulation characteristics, their successful development requires adequate control of physical stability, chemical stability, reproducibility, and manufacturing-related variables. These factors become particularly important when moving from laboratory-scale formulation development toward reproducible and scalable production.^[4,10]

Physical and Chemical Stability

The physical stability of NLC dispersions is influenced by particle aggregation, lipid crystallization, polymorphic transitions, phase separation, and changes in particle size or PDI during storage. The incorporation of a liquid lipid into the solid lipid matrix can reduce the highly ordered structure of the lipid phase and improve drug incorporation; however, changes in lipid organization during storage may still influence drug retention and release.^[4] Chemical stability is also important because degradation of rutin or other formulation components can affect drug content and overall product quality. Therefore, stability evaluation should monitor particle size, PDI, zeta potential, drug content, entrapment efficiency, and drug-release characteristics under appropriate storage conditions.^[10,20]

Reproducibility and Scale-Up

Reproducible production of NLCs requires consistent control of critical material attributes and process parameters. Changes in homogenization conditions, temperature, mixing intensity, cooling rate, or processing equipment can influence particle size, size distribution, lipid crystallization, and drug entrapment. Although high-pressure homogenization and related techniques provide potential for scale-up, process conditions established at laboratory scale cannot always be transferred directly to larger equipment without appropriate optimization and process control.^[10,21] A QbD-based approach can support scale-up by defining critical process parameters and acceptable ranges for the associated critical quality attributes.

Manufacturing and Translation Challenges

The translation of rutin-loaded NLCs from laboratory research to pharmaceutical manufacturing remains challenging because formulation composition, process reproducibility, sterilization, storage, quality control, and regulatory

requirements must be addressed simultaneously. Large-scale production should maintain consistent particle characteristics and drug content while minimizing batch-to-batch variability. Manufacturing strategies such as high-pressure homogenization, controlled continuous processing, and systematic process monitoring may facilitate scale-up.^[10,21] In addition, standardized characterization methods and well-defined quality specifications are required before rutin-NLC formulations can progress toward broader pharmaceutical development. Thus, future studies should emphasize reproducible manufacturing, long-term stability, safety evaluation, and regulatory considerations alongside formulation optimization.

Future Perspectives

Future development of rutin-loaded NLCs should move beyond laboratory-scale formulation optimization toward robust, reproducible, and clinically relevant delivery systems. Greater emphasis should be placed on establishing clear relationships between lipid composition, processing parameters, critical quality attributes, and rutin release behavior. The integration of Quality by Design (QbD), Design of Experiments (DoE), and process analytical technologies can improve formulation understanding and support reproducible manufacturing.^[13,22]

Further research should focus on systematic evaluation of lipid–rutin interactions, long-term physical and chemical stability, scalable manufacturing processes, and standardized characterization methods. Although several rutin-NLC studies have demonstrated promising physicochemical and release characteristics, differences in formulation composition and experimental conditions make direct comparison between studies difficult. Development of standardized testing approaches and clearly defined quality specifications would therefore strengthen the translational potential of these systems.^[14,22]

The application of continuous manufacturing, process monitoring, mechanistic modelling, and data-driven approaches may further facilitate scale-up and batch-to-batch consistency. Recent advances in QbD-based scale-up of lipid nanocarriers also suggest that integration of process analytical technology with emerging computational and machine-learning approaches could support more efficient development of robust manufacturing processes.^[13,23] For rutin specifically, future studies should prioritize optimized formulations with reproducible performance, adequate stability, appropriate safety evaluation, and a clear pathway toward pharmaceutical translation.

CONCLUSION

Rutin-loaded nanostructured lipid carriers offer a promising formulation platform for improving the pharmaceutical performance of rutin. Their successful development depends on rational selection of lipid composition, surfactant, drug-to-lipid ratio, and processing conditions, which collectively determine critical quality attributes and drug-release behavior. QbD and DoE-based optimization can provide a systematic approach to achieving robust and reproducible formulations. However, further emphasis on long-term stability, scale-up, manufacturing reproducibility, and standardized characterization is required to translate rutin-NLCs from laboratory formulations into practical pharmaceutical products.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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