RESEARCH PAPER

Rutin Hydrate-Encapsulating Nanohydrogel through Liposome Optimization by QbD

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ABSTRACT

Rutin hydrate is a naturally occurring flavonoid known for its potential antioxidant and anti-inflammatory properties, making it a promising candidate for topical skin protection. However, its pharmaceutical application may be limited by formulation-related challenges, highlighting the need for an appropriate drug delivery system. The present study aimed to develop and optimize rutin hydrate-loaded liposomes using a Quality by Design (QbD) approach and subsequently incorporate the optimized formulation into a topical nanohydrogel. Liposomes were prepared using the thin-film hydration technique, followed by sonication to obtain nanosized vesicles. A three-factor, three-level Box–Behnken design (BBD) was employed using Design-Expert software to evaluate the effects of Phospholipid 90G, cholesterol, and glycerol concentrations on vesicle size, polydispersity index (PDI), and entrapment efficiency. A total of 17 experimental runs, including five centre-point replicates, were conducted, and the optimized formulation was selected using statistical modeling and desirability-based optimization. The optimized liposomal formulation exhibited a mean vesicle size of 149.0 nm, a PDI of 0.255, a zeta potential of −29.82 ± 2.03 mV, and an entrapment efficiency of 82.81 ± 3.85%. The optimized liposomes were subsequently incorporated into a Carbopol 934P-based hydrogel and evaluated for physicochemical characteristics. The developed nanohydrogel demonstrated a pH of 5.7 ± 0.23, drug content of 92.83 ± 1.36%, spreadability of 15.32 ± 0.48 cm, and extrudability of 54.93 ± 2.40 g/cm², with a smooth texture and no visible aggregates. Overall, the findings demonstrate the feasibility of applying a QbD-based strategy to optimize rutin hydrate-loaded liposomes and formulate them into a topical nanohydrogel. Further studies evaluating in vitro drug release, skin permeation, antioxidant efficacy, and stability are required to establish its potential for topical skin protection.

Keywords: Skin, Sunburn, Rutin hydrate, Liposome, Carbopol-gel, DoE

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Introduction

The skin is highly susceptible to photodamage from sun exposure and other factors because it is the body's largest and most exposed organ. Long-term exposure to ultraviolet (UV) radiation carries serious risks, although it is essential for the production of vitamin D in the body and helps in the treatment of diseases like psoriasis, dermatitis, and jaundice. Burning skin, erythema, inflammatory reactions, photoaging, and higher radiation levels are only a few of the negative consequences of prolonged ultraviolet (UVR) exposure. Among the several UV radiation types, UVA (320–400 nm) and UVB (290–320 nm) rays are especially harmful to skin. (1) All living things eventually go through the process of ageing. Its distinguishing characteristic is the progressive deterioration and degradation of cells and organs. The complex process of human ageing includes changes in the body, mind, and society. Ageing is the biological term for all the changes that take place in a living thing over time, including decreased stress tolerance, functional deficits, and eventually death. The global impact of aging has significant implications for healthcare systems and medical practices. Cover the rest of your body from UV rays. The immune system becomes compromised by prolonged exposure to UV light. sunlight, melanoma, erythema, skin pigmentation, early skin aging, suppressed cancer of the skin, a burning sensation, skin injury, precancerous skin proliferation, the appearance of deep and small wrinkles, and the photosensitive reaction. Moisturiser protects the skin against the harmful impacts of sunlight while bolstering the human body's natural defences against harmful radiation. (2) Protective clothing chemicals reflect, disperse, or absorb electromagnetic

radiation through a selection of processes. The most dangerous UVC (100–280 nm) rays are shielded by the ozone layer, although UVB (more than 5%) and UVA (about 90–95%) radiation still reach the Earth's surface and affect ecosystems and human health. As a result, UV radiation exposure can have a major impact on human health.(3)

A naturally occurring flavonoid glycoside, rutin hydrate has been extensively studied for its anti-inflammatory, antioxidant, and perhaps protective properties against oxidative stress. It is present in a variety of plants, fruits, and vegetables and is a member of the flavanol group of polyphenolic chemicals. Consequently, growing attention has been directed toward the incorporation of natural antioxidants into skincare and cosmetic formulations, along with the evaluation of their antioxidant potential. (4) Plant-derived compounds containing flavonoids, phenolic acids, vitamins, alkaloids, and terpenoids have gained considerable interest in pharmaceutical research because of their potential skin-protective properties. Rutin hydrate, a naturally occurring flavonoid, represents a promising candidate for incorporation into skin-care products, including gel nano formulations.(5,6) Ultraviolet (UV) radiation promotes the generation of reactive oxygen species (ROS), which contribute to oxidative stress, cellular damage, and skin injury. Therefore, the development of antioxidant-based formulations containing natural compounds such as rutin hydrate may offer a promising approach to reducing UV-induced oxidative damage and supporting effective skin protection. Rutin has attracted considerable attention in pharmaceutical and pharmacological research due to its potential antioxidant activity, particularly its ability to neutralize free radicals and modulate inflammatory responses.(7)

Liposomes have risen to prominence owing to their exceptional biocompatibility, biodegradability, and versatility in delivering diverse therapeutic payloads. Consisting of concentric phospholipid bilayers that enclose an aqueous core, these spherical vesicles can simultaneously house hydrophilic compounds in their aqueous center and lipophilic molecules within their lipid membranes. (8) When applied topically, liposomal carriers enhance the uniform spread of active compounds across the skin, shield delicate ingredients against environmental breakdown, and tailor the rate of drug release. (9) Furthermore, their lipid composition enables favorable interactions with the stratum corneum—the skin's outermost defensive barrier—which can boost percutaneous penetration and improve targeted drug accumulation within the tissue. Additionally, liposomes can buffer the skin's direct contact with potent active compounds, which helps minimize irritation and boost overall skin tolerability. (10) However, their therapeutic effectiveness relies heavily on key physicochemical properties, such as vesicle dimensions, lipid makeup, surface charge, entrapment efficiency, and long-term shelf stability. Embedding liposomes within topical vehicles such as hydrogels, creams, and gels significantly enhances their spreadability, retention time on the skin, and effectiveness for site-specific treatments. Consequently, liposomes provide a flexible framework for designing sophisticated dermal formulations that boost therapeutic efficiency while minimizing unnecessary systemic absorption. (11)

These flexible vesicles are formulated from a combination of phospholipids and 20–30% (v/v) biocompatible glycerol. (12) They can also integrate components like cholesterol to reinforce lipid bilayer stability, as well as charged lipid molecules (basic or acidic) to adjust surface electrical potential and prevent aggregation. Glycerol plays a crucial role by elevating the vesicular compactness index, which in turn boosts percutaneous drug permeability and skin delivery. Meanwhile, cholesterol acts as a protective barrier to improve overall structural resilience and maintain membrane integrity. Upon hydration in an aqueous medium, these components naturally self-assemble into bilayer vesicles. (13)

In this investigation, a rutin hydrate-loaded liposome delivery system was successfully engineered and systematically optimized using a Box-Behnken design (BBD) to maximize dermal photoprotection and antioxidant performance.

Materials and methods

Materials

Rutin hydrate (RTN) was obtained from Sigma-Aldrich, China, while Phospholipid 90G was supplied by Lipoid, Ludwigshafen, Germany. Cholesterol was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Other chemicals, including glycerol, HPLC-grade water, disodium hydrogen phosphate, potassium dihydrogen phosphate, and hydrochloric acid (HCl), were procured from SD Fine-Chem Ltd., Mumbai, India. Methanol, chloroform, ethanol, and octanol were obtained from Merck, Mumbai, India, and were of laboratory grade.

Methods

Identification of components under Quality by Design (QbD)

Quality by Design (QbD) is a structured, science-driven method for pharmaceutical development that guarantees product quality by setting clear goals and conducting risk assessments. It aids in pinpointing key formulation variables and process parameters to enhance product performance, consistency, and quality.(14) A Quality Target Product Profile (QTPP) was created to secure that the final expression satisfies the necessary criteria for safety and effectiveness, laying the groundwork for quality by design and ensuring the product delivers its intended performance. The QTPP also encompasses Critical Quality Attributes (CQAs), which are derived from it and implemented to conduct the exploitation of products and procedures. Moreover, CQAs define the range or boundaries for validating high-quality products,

maintaining the desirable level of quality for the product. Variations in Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs), related to formulation composition and manufacturing conditions, respectively, can significantly affect Critical Quality Attributes (CQAs).(15) To systematically identify and evaluate these potential risks, the International Council for Harmonisation (ICH) guideline Q9 recommends the use of various risk assessment tools to support quality risk management throughout pharmaceutical development. Commonly used risk assessment techniques include Fault Tree Analysis (FTA), Failure Mode and Effects Analysis (FMEA), and the Ishikawa fishbone diagram. In the present study, the Ishikawa fishbone diagram was utilized to identify potential factors affecting formulation quality and to systematically illustrate the cause-and-effect relationships between formulation variables, process parameters, and critical quality attributes.(16,17)

Preparation of Rutin Hydrate-Liposomes

Rutin hydrate-encapsulated liposomes (RTN-LM) were synthesized using a standard thin-film hydration method. Initially, precise quantities of rutin hydrate, Phospholipid 90G, and cholesterol were dissolved within a chloroform-methanol (2:1 v/v) solvent system inside a round-bottom flask. This mixture was subjected to magnetic stirring at 40°C for 60 minutes to achieve complete dissolution and homogeneity. Organic solvents were eliminated under reduced pressure using a rotary evaporator (model HS-2005V-N, Hahnshin Scientific Co.), which deposited a thin, uniform lipid film along the inner walls of the flask. Complete elimination of trace organic solvents was achieved by maintaining the flask under vacuum overnight. The dried lipid layer was subsequently hydrated via a two-stage process using an aqueous glycerol solution, accompanied by continuous stirring at 40°C for 1 hour to promote spontaneous vesicle assembly. To reduce vesicle dimensions, the resulting crude liposomal dispersion underwent probe sonication for 60 seconds (interrupted by 3-second pauses after every 5 seconds of active sonication). Finally, unencapsulated drug molecules were separated from the vesicular suspension via cooling centrifugation at 7,500 rpm for 10 minutes at 4°C. The purified liposomal formulation was subsequently lyophilized and stored for future characterization.(18)

Optimization of Rutin Hydrate-Loaded Liposomes Using Box–Behnken Design

Design of Experiments (DoE) methodologies were effectively employed to guide the engineering and optimization of the vesicular carriers. Specifically, the formulation of rutin hydrate-loaded liposomes was refined using a Box-Behnken design (BBD)—a robust statistical experimentation framework comprising three distinct factors evaluated at three operational levels. Data generation and statistical modeling were executed using Design-Expert software (Version 12, Stat-Ease, Minneapolis, MN, USA). The primary objective of this experimental design was to investigate and quantify the interactive effects of key formulation parameters—namely, Phospholipid 90G concentration (X_1), cholesterol level (X_2), and glycerol content (X_3)—on critical dependent responses, including vesicle size (Y_1), polydispersity index (Y_2), and entrapment efficiency (Y_3).The independent variables were investigated at three levels—low (−1), intermediate (0), and high (+1)—to identify the optimal formulation composition, as presented in Table 1. The Box–Behnken design comprised 17 experimental runs, including five centre-point replicates, to evaluate the effects of the selected formulation variables on the responses. The influence of these variables was analyzed using polynomial regression equations and response surface plots. A quadratic model was selected as the most appropriate model for describing the relationship between the independent variables and dependent responses, as it accounted for linear, interaction, and quadratic effects on the formulation characteristics.(19)

Table 1: To develop and optimize the rutin hydrate-loaded liposomes, independent and dependent variables were systematically evaluated across three distinct operational levels (low, medium, and high).

Independent variable	**Minimum (-1)**	**Maximum (+1)**
Phospholipid 90G (mg) X_1	40mg	60mg
Cholesterol (mg) X_2	2.5mg	7.5mg
Glycerol (%) X_3	1%	3%
Dependent variable	**Constraints**	
Vesicle Size (nm) Y_1	Minimize	
Polydispersity Index (PDI) Y_2	Maximize	
Entrapment efficiency (%) Y_3	Maximize	

Characterization of Optimized Rutin Hydrate-Loaded Liposomes

Vesicle diameter, polydispersity index (PDI), and surface charge (zeta potential).

Vesicle size, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Before analysis, the liposomal dispersion was diluted approximately 50-fold with distilled water. Measurements were performed at a scattering angle of 90° and a controlled temperature of 25 ± 2°C. Distilled water was used as the dispersing medium, and each parameter was

measured in triplicate to ensure the reliability and reproducibility of the results.(20)

Entrapment efficiency

The encapsulation efficiency (% EE) of the rutin-loaded liposomes was quantified using an ultracentrifugation method. Aliquots of the liposomal dispersion were placed in centrifugal filter units and centrifuged at 25,000 x g for 45 minutes at 4°C using an ultracentrifuge (model LE 80, Beckman Coulter). Following separation, the concentration of unentrapped (free) drug remaining in the clear aqueous supernatant was determined by diluting the sample with methanol and analyzing it via UV-visible spectrophotometry (model UV-1600, Shimadzu Corporation) at an absorption wavelength of 259 nm. The percentage of entrapped drug was subsequently calculated using the standard mathematical formula relating total drug versus free drug in the supernatant.(21)

$$\text{Entrapment efficinecy \%} = \frac{\text{total drug} - \text{free drug}}{\text{total drug}} \times 100$$

Preparation of Optimized Rutin Hydrate-Loaded Liposomes Carbopol gel

Carbopol 934P NF was used as the gelling agent to prepare the gel formulation. Briefly, the required quantity of Carbopol powder was dispersed in distilled water to obtain a 1% (w/w) polymer concentration and left overnight to ensure complete hydration. On the following day, 2–4 drops of triethanolamine (TEA) were gradually added to the polymer dispersion under continuous stirring to adjust the pH and facilitate gel formation. The optimized rutin hydrate-loaded liposomes were then incorporated into the prepared gel base with gentle, continuous stirring to ensure uniform distribution while minimizing air bubble formation. The resulting mixture was further stirred using a magnetic stirrer for 60 minutes to obtain a homogeneous Carbopol gel containing optimized rutin hydrate-loaded liposomes.(12)

Characterization of Optimized Rutin Hydrate-Loaded Liposomes Gel

pH evaluation

To determine the pH of the optimized rutin-loaded liposomal gel, a 1.0 g sample of the formulation was accurately weighed and transferred into a 50 mL volumetric flask, diluted with double-distilled water, and allowed to equilibrate for 2 hours. The pH values were recorded using a calibrated glass microelectrode (Mettler Instruments, Giessen, Germany) immersed directly into the diluted hydrogel dispersion and maintained in contact for 1 minute to ensure a stable reading. All measurements were performed across three independent batches to guarantee reproducibility.(12)

Homogeneity

All developed hydrogel formulations were transferred into clear, transparent glass vessels and subjected to rigorous visual evaluation to assess overall physical appearance and macroscopic homogeneity. batch was carefully examined against a light background to detect the presence of any particulate matter, air entrapment, or vesicular aggregation within the gel matrix.(12)

Spreadability

The spreadability of the optimized rutin-loaded liposomal hydrogel was evaluated using a parallel plate technique. In this procedure, a 1 cm diameter circle was marked on a clean glass plate, onto which exactly 500 mg of the hydrogel formulation was placed. A second glass plate was carefully positioned directly over the sample. A standardized 500-g weight was then placed on top of the upper glass plate for a duration of five minutes, allowing the semi-solid matrix to spread outward. The resulting increase in the diameter of the spread circle was measured. All experiments were performed in triplicate ($n = 3$), and average values were utilized to compute the final spreadability coefficient according to the standard mathematical equation.(22)

Spreadability was calculated using the following equation:

$$S = \frac{\mathrm{M} \times \mathrm{L}}{T}$$

Where,

L represents the diameter of the spread gel (cm)

S denotes spreadability (g/sec)

M is the weight applied to the upper glass slide (g)

T represents the time required for spreading (sec)

Results and discussion

Development of a Robust Formulation Using the Quality by Design (QbD) Approach

Quality by Design (QbD) is a systematic approach to developing a suitable, reliable, and robust pharmaceutical formulation. The Quality Target Product Profile (QTPP) in Tables 2, 3, and 4 was established, and the corresponding Critical Quality Attributes (CQAs) were identified to ensure the desired product quality.

Table 2: Quality Target Product Profile (QTPP) for the Development of Optimized Rutin Hydrate-Loaded Liposomes

QTPP Parameter	Target	Justification
Drug delivery system	Liposome-based formulation	Liposomes may improve skin interaction and facilitate topical delivery of the encapsulated drug.
Drug release profile	Sustained drug release.	Prolonged drug release may help maintain drug availability at the application site and support the intended therapeutic effect.

Route of administration	Topical	Topical administration enables localized drug delivery to the affected skin while potentially minimizing systemic exposure.
Dosage form	Hydrogel	A hydrogel offers convenient application, a suitable consistency, and prolonged contact with the skin.

Table 3: Critical Formulation Variables and Their Influence on the Quality of Optimized Rutin Hydrate-Loaded Liposomes

Formulation Component	Target	Justification
Phospholipid 90G	Optimal vesicle formation and size	Phospholipid 90G acts as the primary vesicle-forming material, contributing to bilayer formation and influencing vesicle size and structural integrity.(23)
Cholesterol	Appropriate membrane rigidity and fluidity	Cholesterol modulates phospholipid bilayer packing, membrane fluidity, and vesicular integrity, thereby influencing vesicle size and stability.(24)
Glycerol	Improved vesicle flexibility and deformability	Glycerol may enhance vesicle flexibility and deformability, potentially facilitating interactions with the skin barrier and improving topical drug delivery.(25,26)
Hydration medium	Uniform hydration and stable vesicle dispersion	The hydration medium facilitates lipid-film hydration and vesicle formation. Its composition can influence vesicle characteristics, dispersion uniformity, and physical stability.(27,28)
Rutin hydrate (API)	Adequate drug incorporation and high entrapment efficiency	The drug's physicochemical properties and its interactions with the lipid bilayer and aqueous compartment influence drug incorporation, vesicle characteristics, and entrapment efficienc.(28)

Table 4. Critical Quality Attributes (CQAs) of Optimized Rutin Hydrate-Loaded Liposomes

CQA Parameter	Target Specification	Justification
Vesicle size	Less than 300 nm	A nanoscale vesicle size may support favorable skin interactions and drug distribution. The optimal size should be established through experimental evaluation.
Polydispersity index (PDI)	Less than 0.5	A lower PDI indicates a narrower vesicle-size distribution and greater dispersion uniformity.
Entrapment efficiency (EE)	More than 80%	High entrapment efficiency indicates effective incorporation of rutin hydrate into the glycerosomal formulation and reduces the proportion of unencapsulated drug.
Zeta potential	A value consistent with adequate dispersion stability	Zeta potential provides information about the surface charge of vesicles and helps assess their tendency to

		aggregate. The acceptance criterion should be based on the formulation's composition and observed stability.
Physical stability	No significant aggregation or undesirable changes in vesicle characteristics	Maintaining vesicle size, PDI, and drug entrapment during storage supports formulation consistency and quality.
Drug content	Within predefined acceptance limits	Consistent drug content supports batch-to-batch reproducibility and uniform drug incorporation.

Model fitting and optimization using Box-Behnken design

The optimization of rutin hydrate-loaded liposomes was performed using Box–Behnken Design (BBD) in Design-Expert software (Version 13; Stat-Ease, Inc., Minneapolis, MN, USA). A three-factor, three-level experimental design was employed to generate 17 experimental runs, comprising 12 distinct formulation combinations and five replicates at the center point. The selected independent variables were Phospholipid 90G concentration (X_1), cholesterol concentration (X_2), and glycerol concentration (X_3), while the dependent responses included vesicle size (Y_1), polydispersity index (PDI; Y_2), and entrapment efficiency (Y_3). The effects of the independent variables on the responses were evaluated using polynomial regression equations and response surface plots, as presented in Figure 2. Various statistical models were assessed to establish the relationships between formulation variables and the measured responses. The quadratic model was selected as the most suitable model because it accounted for the individual effects of the variables, their interactions, and quadratic effects on the dependent responses, thereby supporting systematic formulation optimization.(29)

Optimization of Rutin Hydrate-Loaded Liposomes by Statistical design

The optimization of nano-formulation. Among the different statistical experimental designs, the Box-Behnken design is an effective optimization strategy that is widely used for comparison with traditional optimization techniques among the many statistical experimental designs. Due to fewer experiments and evaluations done in a sufficient amount of time, it has been extensively examined. Based on the important observations, the independent variables and their effects on the response were used here for the optimization and evaluation process. Glycerol concentrations of 10% or less produced vesicles with lower flexibility, fast deformation, and perhaps limited skin penetration. The flexibility of the vesicles grew as the glycerol percentage in the formulation increased; at 30% glycerol content, the liposome was significantly more flexible and resisted the force applied. Glycerol may function as a phospholipid bilayer active component. When applied topically, the presence of glycerol in the liposomes formulation reduces skin irritation caused by the presence of alcohol in liposome lipid formulations. The addition of cholesterol to the liposome composition enhances the long-term stability of the phospholipids, enhances the positive charges, and prevents vesicle agglomeration**.** The table summarizes the results of the experimental runs as obtained by Box-Behnken design. Box–Behnken design (BBD) was employed to optimize the Rutin hydrate-loaded liposomal formulation using Design-Expert software. The effects of glycerol, phospholipid, and cholesterol concentrations on the selected formulation responses were systematically evaluated. Higher glycerol concentrations improved vesicle flexibility, whereas lower concentrations resulted in less flexible vesicles. Glycerol may also help reduce skin irritation in topical formulations, while cholesterol contributes to vesicular membrane stability and reduces aggregation. The observed response values obtained from the experimental runs are summarized in the table.

Observed values of response in Box-Behnken Design (BBD) for optimization of Rutin hydrate-loaded liposome formulation created by Design-Expert software.

Table. 5: X_1: Phospholipid 90G (mg), X_2: Cholesterol (mg), X_3 Glycerol (%w/v), Y_1: Vesicle Size (nm), Y_2: Polydispersity Index, Y_3: Entrapment Efficiency (%).

Independent variables				Dependent variable					
				Observed variables			Predicted variables		
S.NO.	X1	X2	X3	Y1	Y2	Y3	Y1	Y2	Y3
F1	40	7.5	2	171.64 ± 3.59	0.1947±0.0030	79.13±0.53	171.31	0.1986	79.44
F2	50	5	2	149.20 ± 6.93	0.2907±0.0061	83.56±1.53	149.16	0.2977	82.84
F3	60	7.5	2	186.16 ± 9.04	0.2592±0.0036	95.34±0.98	185.91	0.2578	95.09
F4	50	7.5	3	176.26 ± 3.03	0.2378±0.0013	88.16±0.59	176.08	0.2392	88.23
F5	60	5	1	157.31 ± 5.94	0.3132±0.0023	91.40±3.17	157.49	0.3108	91.61
F6	**50**	**5**	**2**	**149.57 ± 6.45**	**0.2971±0.0011**	**82.56±1.79**	**149.00**	**0.2555**	**82.84**
F7	50	5	2	149.46 ± 6.47	0.2963±0.0016	82.61±1.05	149.16	0.2977	82.84
F8	50	5	2	149.95 ± 5.01	0.2971±0.0011	82.74±1.89	149.16	0.2977	82.84
F9	50	2.5	1	118.07 ± 5.98	0.3568±0.0003	80.04±0.96	117.92	0.3567	80.28
F10	40	5	1	141.34 ± 8.95	0.2445±0.0010	74.50±3.26	141.29	0.2554	74.76
F11	50	2.5	3	123.26 ± 3.40	0.3759±0.0093	81.01±2.61	123.2	0.3775	81.55
F12	50	7.5	1	181.76 ± 6.08	0.2207±0.0050	85.13±3.94	181.1	0.2185	84.85
F13	50	5	2	149.16 ± 4.95	0.2966±0.0008	82.64±0.20	149.16	0.2977	82.84
F14	40	5	3	142.1 ± 3.95	0.2749±0.0012	76.52±2.12	141.91	0.2734	76.74

The effects of the independent variables on the measured responses were evaluated using suitable statistical models, with the quadratic model identified as the most appropriate for formulation optimization. The optimal values for the three responses—vesicle size, polydispersity index (PDI), and entrapment efficiency—were determined using the desirability function to identify the most favorable formulation conditions.

Response surface analysis

Influence of independent factors on vesicle size (Y_1)

A quadratic polynomial equation could explain the effect of phospholipid concentration, cholesterol concentration, and glycerol concentration on vesicle size, as shown in Table 2.

The size of the liposome vesicles was significantly influenced favorably by the phospholipid concentrations (P values less than 0.05). The optimized formulations' vesicle size analyses indicated a size range of 112.15 ± 4.03 nm to 186.16 ± 9.04 nm. The particle size at low concentrations was found to be 112.15 ± 4.03 nm, and when the phospholipid concentration was increased to 50 mg, a significant increase in particle size was found to be 149.00 ± 5.01 nm. (F6). The increased phospholipid concentration to 60 mg resulted in the greatest increase in particle size to 186.16 ± 9.04 nm. (F3), as shown in the table. 5. The final result was comparable to the previously reported result.

The concentration of cholesterol had a positive effect on the size of liposomes vesicles. The increase in vesicle size was caused by increasing the cholesterol concentration from 2.5 to 7.5 % w/v. It should be noted that the same concentration of cholesterol was used, and a significant decrease in vesicle size was observed in (F17), which could be attributed to the combined effect of phospholipid and glycerol concentrations.

The particle size was less positively affected by the glycerol concentration. The vesicle size for the (F12) formulation was 181.76 ± 6.80 nm at 10% glycerol (w/v). The vesicle size of formulations (F5) and (F7), which both included the same amount of glycerol (10% w/v), was 157.31 ± 5.94 nm and 149.46 ± 6.47 nm, respectively. Besides the above results, formulation (F9) showed a low vesicle size of 118.07 ± 5.98 nm at 10% w/v glycerol concentration, which may be caused by the combined effect of phospholipid and cholesterol concentrations. At a concentration of 20% w/v glycerol, the vesicle sizes of (F1), (F2), (F3), (F8), and (F17) were 171.64 ± 3.59 nm, 149.20 ± 6.93 nm, 186.16 ± 9.04 nm, 149.57 ± 6.47nm, and 112.15 ± 4.03 nm, respectively. Similar outcomes were seen with formulation (F16), which had vesicles measuring 129.47 ± 9.61 nm. More, (F1)7 showed a significant reduction in vesicle size at the same glycerol concentration, which may be the result of the combined action of phospholipid and cholesterol concentrations As the content of aqueous glycerol increased, the viscous fluid of glycerol induced an increase in vesicle formation. .

Influence of independent variables on PdI

The polydispersity index quadratic equation that was observed is shown in Table 5. The Model F value of 29560.93 the model has been determined to be significant According to the “Lack of Fit F-value” 6.41 indicates it was non - significant. The "Pred R2" of 0.9996 and the "Adj R2" of 0.9999 had a respectable degree of correlation. PdI values for Rutin hydrate-loaded liposomes prepared according to the experimental design ranged between 0.194 ± 0.003 and 0.395 ± 0.0006. These indications point to a linear model for liposome polydispersity index analysis. All of the formulations had polydispersity index values that were less than 0.3, except for F16, which had a PDI value of 0.395 and demonstrated issues with a wider range of size distribution and agglomeration. The effects of individual parameters on PdI were observed using the response Y2 coded quadratic equation. PdI was observed to (F6) rise significantly as the concentration of phospholipid 90G continued to increase.

Influence of independent factor on EE%

The determined quadratic equation for % entrapment efficiency (%EE) has a Model F value of 1512.66 the model has been determined to be significant. According to the "Lack of Fit F-value," 4.0 indicates it was non - significant. The "Pred R^2" of 0.9988 and the "Adj R^2" of 0.9936 had a respectable degree of correlation. The range of the improved Rutin hydrate-loaded liposome formulation's entrapment efficiency was found to be F6 82.81 ± 2.47% to 28.4. ± 1.30% The significant favorable impact P value less than 0.05 the higher the phospholipid concentration had on it, as seen in, the percentage of Rutin hydrate-loaded liposome increased. The level of cholesterol has a favorable effect on the %EE. It was observed that formulation entrapment was enhanced when cholesterol concentration increased from 2% to 7.5% w/v, as seen in the table. Furthermore, formulations (F2), (F6), (F7), (F8), and (F13) showed mixed effects at moderate cholesterol concentrations (5% w/v). The Formulations (F2) and (F13) showed an improved %EE of 76.52 ± 2.12% and 83.56 ± 1.53%, respectively, whereas (F5) and (F15) showed an increased %EE of 91.40 ± 3.17% and 94.71 ± 0.82%, respectively. This is most likely caused by the combined effect of various phospholipid, glycerol, and cholesterol concentrations. The concentration of glycerol significantly improved EE ($p < 0.05$). The %EE increased from 76.52 ± 2.12% to 94.71 ± 0.82% when the glycerol concentration was increased from 10% to 30% w/v. All of the observations were consistent with previous research.

Validation of experimental design

The consistency between predicted and experimental values is utilized to evaluate the validity of the optimization strategy. Predicted values of the individual runs are presented in the table. 5. Based on the observed correct responses, the composition of the optimum formulation was determined. The software also produced the optimal formulation's expected responses, which were compared to the optimized experimental formulation. In the BBD-optimized recipe, 50 mg of phospholipid, 5 mg of cholesterol, and 2% glycerol were included. The observed responses were 149.0 nm, 0.255, and 82.04 %, while the expected responses for particle size, PdI, and %EE were 148.4 nm, 0.292, and 82.81 %, respectively. The findings showed a strong correlation between the predicted and actual values. As an outcome, the Rutin hydrate-loaded liposome developed with the optimal composition was used for further research.

Optimized level of formulation by point prediction

Using the Design-Expert software's point prediction approach, the appropriate formulation of RUT-loaded liposome was selected based on the parameters of optimum entrapment efficiency, desirable particle size, and PDI. The optimized formulation (F6) is shown in the table. 6 predicted values of vesicle size, entrapment efficiency, and PDI are 149 nm, 82.84%, and 0.255, respectively.

Table 6: The corresponding experimental values are shown below.

Response	Experimental value	Predicted value
Vesicle size (nm)	149.4	149.0
PDI	0.292	0.255
Entrapment Efficiency (%)	82.81	82.4

Characterization of optimized RUT-loaded-Liposome

Determination of vesicle size, PDI, Zeta potential, and EE%

The optimized liposomal formulation was observed to have a mean vesicle size of 149.0 nm, uniform size distribution, and a PDI value of 0.255 ± 0.04 (Fig. A. 1). The zeta potential value, which was determined to be -29.82 ± 2.03 mV, indicated that the vesicle surface was negative, as shown in (fig B. 2); hence, the formulation was observed to be stable. the optimized Rutin hydrate-loaded liposomal, the observed entrapment efficiency and drug content were 82.04, respectively. This may contribute to improved colloidal stability by reducing vesicle aggregation. Furthermore, the optimized Rutin hydrate-loaded liposomal formulation (RTN-loaded-LM) demonstrated an entrapment efficiency of 82.81 ± 3.85%. The drug content value should be added separately if available.

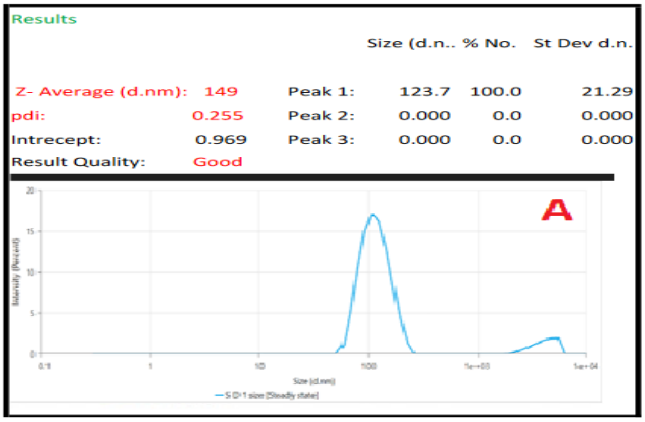


Figure 1: Particle size and PDI of optimized formulation

Determination of Zeta potential

The optimized Rutin hydrate-loaded liposomal zeta potential was found to be -29.82 ± 2.03 mV. The graph in Fig. 2 B shows that the optimized nano formulation will resist flocculation and aggregation for a longer period. This means that the formulation will remain physically stable

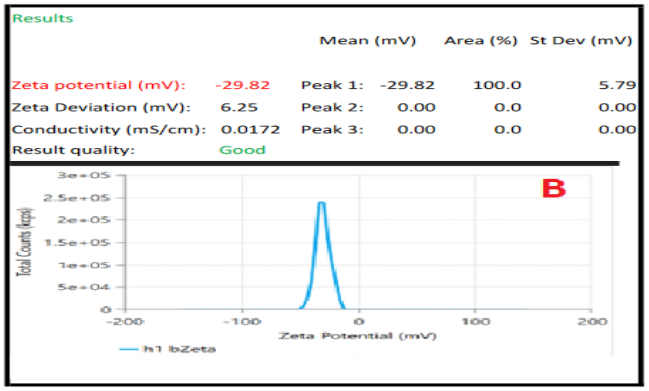


Figure 2: Zeta potential of optimized formulation

Characterization and evaluation of RUT-loaded liposomal nano hydrogel

pH and homogeneity

The pH of the gel formulation F6, including Rutin hydrate-loaded liposomal, was determined using a pH meter and found to be 5.7±0.23 (Table 7), within the normal skin pH range. The gel texture was consistent and smooth, with no grittiness.

Spreadability and Extrudability

The spreadability records indicated that the gels may be easily spread with minimal shear force. The spreadability of Rutin hydrate-loaded liposomal gel F6 formulation was found to be 15.32±0.48 cm. Gel characterization was performed to evaluate the gel based on several parameters, and the results are summarized in Table 7 below. The optimized Rutin hydrate-loaded liposomal extrudability was found to be 54.93±2.40 gm/cm^2.

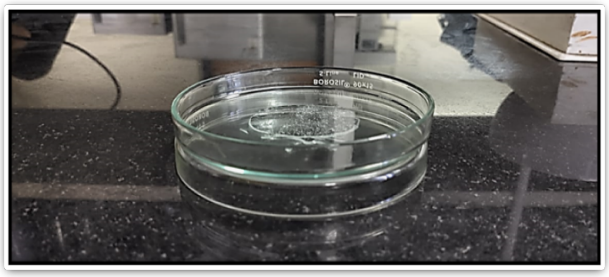


Figure. 7.10: Spreadability Evaluation of RUT-Loaded liposomal nanohydrogel

Table. 7: Characterization parameters of Rutin hydrate-loaded liposomal nanohydrogel

Rutin hydrate liposomal Nanohydrogel		
S. No	**Parameters (Mean±SD) (n=3)**	**Value**
1	Homogeneity	Good Texture no aggregates were found
2	Drug content (%)	92.83±1.36
3	pH	5.7±0.23
4	Extrudability	54.93±2.40
5	Spreadability	15.32±0.48

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