RESEARCH PAPER

Design, Optimization And In-Vitro Characterization Of Garcinol-Loaded Liposomal Hydrogel For Topical Management Of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis is a chronic inflammatory disorder that requires long-term therapy and is often associated with systemic adverse effects. The present study aimed to design, optimize, and evaluate a garcinol-loaded liposomal hydrogel for the topical management of rheumatoid arthritis. Garcinol-loaded liposomes were prepared using the thin-film hydration method and optimized through a three-factor, three-level Box–Behnken experimental design. Phospholipon® 90H concentration, cholesterol concentration, and sonication time were selected as independent variables, while particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency were considered as response parameters.

The optimized liposomal formulation exhibited a particle size of 232.8 nm, PDI of 0.285, zeta potential of −32.6 mV, and entrapment efficiency of 85.1%, which were in close agreement with the predicted values, with a prediction error below 2%. The optimized liposomes were incorporated into Carbopol 934 hydrogel to obtain liposomal hydrogel formulations (LH1–LH3). Among the formulations, LH2 (1.5% Carbopol 934) demonstrated suitable physicochemical characteristics, including pH 6.7, viscosity 24,134 cP, spreadability 20.23 g·cm/s, and drug content 97.5%, with no evidence of phase separation.

In-vitro drug release studies revealed sustained release behavior, with cumulative drug release of 92%, 85%, and 75% from LH1, LH2, and LH3 formulations, respectively, over 24 h. Stability studies performed for 30 days under both room temperature (25 ± 2°C) and accelerated conditions (40 ± 2°C/75 ± 5% RH) showed only minor variations in physicochemical parameters, with drug content remaining above 96%, confirming good stability of the optimized formulation.

Overall, the developed liposomal hydrogel exhibited desirable physicochemical properties, sustained drug release, and satisfactory stability, suggesting its potential as an effective topical drug delivery system for the management of rheumatoid arthritis.

Keywords: Garcinol, liposomes, hydrogel, Box–Behnken design, controlled release, rheumatoid arthritis.

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1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disorder characterized by inflammation of synovial joints, leading to cartilage degradation, bone erosion, and joint deformity. The disease affects millions of individuals worldwide and significantly reduces quality of life due to persistent pain, stiffness, swelling, and functional impairment. The pathogenesis of RA involves complex immunological mechanisms, including activation of inflammatory cytokines, infiltration of immune cells, and destruction of joint tissues. If left untreated, the disease may result in severe disability and systemic complications. **[1, 2, 3]**

Conventional therapeutic approaches for the management of rheumatoid arthritis include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs). Although these treatments provide symptomatic relief and slow disease progression, their long-term systemic administration is frequently associated with adverse effects such as gastrointestinal irritation, hepatotoxicity, immunosuppression, and cardiovascular complications. In addition, poor patient compliance and systemic

toxicity remain major limitations of conventional therapy. Therefore, alternative drug delivery strategies capable of reducing systemic exposure while maintaining therapeutic efficacy are highly desirable. **[4]**

Topical drug delivery systems have gained increasing attention as an effective approach for localized treatment of inflammatory joint disorders. Topical formulations offer several advantages, including targeted drug delivery to the affected site, reduced systemic side effects, improved patient compliance, and ease of administration. However, the effectiveness of conventional topical formulations is often limited by poor skin penetration and insufficient drug retention at the site of action. Consequently, the development of advanced topical delivery systems capable of enhancing drug permeation and controlled release is of significant interest. **[5, 6]**

Garcinol is a naturally occurring polyisoprenylated benzophenone isolated from plants of the *Garcinia* species **(Figure 1)**. It has been widely reported to exhibit potent anti-inflammatory, antioxidant, and therapeutic properties. Previous pharmacological studies have demonstrated that garcinol can inhibit inflammatory mediators and cytokine production, which play a critical role in the progression of rheumatoid arthritis. Despite its promising therapeutic potential, the clinical application of garcinol is limited due to its poor aqueous solubility, low bioavailability, and instability in conventional formulations. These limitations necessitate the development of an efficient drug delivery system to enhance its therapeutic effectiveness. **[7, 8]**

Figure 1: *Garcinia indica* fruit

Liposomes are spherical vesicular carriers composed of phospholipid bilayers that can encapsulate both hydrophilic and lipophilic drugs. Due to their biocompatibility, biodegradability, and ability to improve drug solubility and stability, liposomes have been widely explored as drug delivery systems in pharmaceutical research. Liposomal formulations can enhance drug penetration through biological membranes, provide sustained drug release, and improve therapeutic efficacy while minimizing systemic toxicity. These characteristics make liposomes particularly suitable for topical drug delivery applications. **[9, 10]**

Hydrogels are three-dimensional polymeric networks capable of retaining large amounts of water while maintaining structural integrity. They are widely used in topical formulations due to their favorable properties such as biocompatibility, ease of application, non-greasy nature, and controlled drug release capability. Incorporation of liposomes into hydrogel matrices can further enhance the performance of topical formulations by combining the advantages of both delivery systems. Liposomal hydrogels can improve drug stability, prolong drug residence time on the skin, and provide sustained drug release at the site of action. **[11]**

Optimization of pharmaceutical formulations using statistical experimental design has become an essential approach in modern drug delivery research. Response Surface Methodology (RSM), particularly the Box–Behnken design, allows systematic evaluation of multiple formulation variables and their interactions with a reduced number of experimental runs. This approach enables the identification of optimal formulation conditions and ensures reproducibility and quality of the developed formulation.

Therefore, the present study aimed to design, optimize, and evaluate a garcinol-loaded liposomal hydrogel as a controlled topical drug delivery system for the management of rheumatoid arthritis. Liposomes were prepared using the thin-film hydration method and optimized using a Box–Behnken experimental design. The optimized liposomes were subsequently incorporated into a Carbopol-based hydrogel and evaluated for physicochemical properties, in-vitro drug release, and stability characteristics.

2. MATERIALS AND METHODS

2.1 Materials

Garcinol was obtained as a gift sample from Sami-Sabinsa Group Limited, Bengaluru, Karnataka, India, and used as received. Phospholipon® 90H was kindly provided as a gift sample by Lipoid GmbH, Ludwigshafen, Germany. Cholesterol was purchased

from Sigma-Aldrich, St. Louis, MO, USA. Carbopol 934, propylene glycol, glycerin, methyl paraben, propyl paraben, and triethanolamine (TEA) were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Methanol and chloroform (analytical grade) were obtained from Merck Pvt. Ltd., Mumbai, India. Phosphate-buffered saline (PBS, pH 7.4) was prepared in the laboratory according to standard procedures. All other chemicals and reagents used in this study were of analytical grade and were used without further purification. Double-distilled water was used throughout the study.

2.2 Preparation of Garcinol-Loaded Liposomes

Garcinol-loaded liposomes were prepared using the thin-film hydration method. Briefly, accurately weighed quantities of Phospholipon® 90H, cholesterol, and garcinol (25 mg) were dissolved in a mixture of chloroform and methanol (2:1 v/v) to obtain a clear and homogeneous organic solution. The resulting solution was transferred to a round-bottom flask and subjected to solvent evaporation under reduced pressure using a rotary evaporator maintained at 40 °C and 60 rpm. Evaporation of the organic solvents resulted in the formation of a thin and uniform lipid film on the inner wall of the flask. **[13, 14, 15]**.

The dried lipid film was subsequently hydrated with 10 mL of phosphate-buffered saline (PBS, pH 7.4) maintained at 60 °C, which is above the phase transition temperature of Phospholipon® 90H. Hydration was carried out for 30 minutes under continuous rotation, resulting in the formation of multilamellar vesicles (MLVs). **[14, 16]**

The resulting liposomal dispersion was further subjected to probe sonication for 120–240 seconds, according to the experimental design, in order to reduce vesicle size and obtain nanosized liposomes with a relatively uniform size distribution. **[15, 17, 18]**

2.3 Optimization Using Box–Behnken Design

Optimization of the liposomal formulation was carried out using a Box–Behnken experimental design (BBD), which is an efficient statistical approach within response surface methodology (RSM). This design allows the systematic evaluation of the effects of multiple formulation variables and their interactions on selected response parameters while minimizing the number of experimental runs required.

In the present study, a three-factor, three-level Box–Behnken design was employed to optimize the formulation variables affecting the characteristics of garcinol-loaded liposomes. The independent variables selected were phospholipid concentration (X_1), cholesterol concentration (X_2), and sonication time (X_3). These factors were studied at three levels: low (−1), medium (0), and high (+1) based on preliminary experiments. The selected independent variables and their levels used in the design are presented in **Table 1**.

Table No. 1 Full factorial design: Factors, levels for liposomes formulation

Factors (Independent variables)	**Factor levels used**		
	Low (-1)	Medium (0)	High (+1)
Phospholipon® 90H (mg) X_1	150	225	300
Cholesterol (mg) X_2	15	30	45
Sonication time (seconds) X_3	120	180	240
Responses (Dependent variable)			
Y_1: Particle size (nm)			
Y_2: Polydispersity index (PDI)			
Y_3: Zeta potential (mV)			
Y_4: Entrapment efficiency (%)			

2.4 Experimental Design Matrix

The Box–Behnken design generated 17 experimental runs, including five center points, to evaluate the influence of formulation variables on the selected responses. The dependent responses considered in this study were particle size (Y_1), polydispersity index (Y_2), zeta potential (Y_3), and entrapment efficiency (Y_4). The experimental design matrix along with the corresponding observed responses is presented in **Table 2**.

Table 2: Composition Box Behnken Design with Observed responses.

Batch	**PL90H (mg)**	**Cholesterol (mg)**	**Sonication (s)**	**Particle Size (nm)**	**PDI**	**Zeta Potential (mV)**	**Entrapment Efficiency (%)**
L1	150	30	120	178.2	0.278	–22.1	78.25

L2	225	15	120	191.6	0.289	–28.4	80.81
L3*	225	30	180	162.4	0.246	–27.6	81.55
L4	300	15	180	223.3	0.258	–30.9	83.46
L5*	225	30	180	161.7	0.242	–28.9	80.24
L6	225	15	240	146.9	0.183	–30.5	73.66
L7	300	45	180	246.2	0.261	–29.8	90.25
L8	300	30	120	261.7	0.322	–31.4	87.23
L9*	225	30	180	160.7	0.251	–26.8	82.36
L10	225	45	240	174.2	0.171	–25.4	82.69
L11*	225	30	180	159.9	0.245	–26.2	82.75
L12*	225	30	180	162.8	0.247	–27.5	81.91
L13	150	15	180	140.2	0.211	–27.2	71.26
L14	150	30	240	114.1	0.164	–24.3	73.37
L15	150	45	180	158.2	0.226	–20.6	78.33
L16	300	30	240	186.6	0.206	–34.7	83.87
L17	225	45	120	220.2	0.277	–28.9	87.92

** Indicates center point runs of the Box–Behnken design. Five center points were included in the experimental design to estimate experimental error and evaluate the reproducibility of the model.*

2.5 Mathematical Model

The experimental data obtained from the Box–Behnken design were fitted to a second-order polynomial equation to establish the relationship between the independent variables and the response parameters. The general form of the polynomial equation used to predict the responses is expressed as follows:

$$Y=\beta0+\beta1X1+\beta2X2+\beta3X3+\beta12X1X2+\beta13X1X3+\beta23X2X3+\beta11X12+\beta22X22+\beta33X32Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2Y=\beta0+\beta1X1+\beta2X2+\beta3X3+\beta12X1X2+\beta13X1X3+\beta23X2X3+\beta11X12+\beta22X22+\beta33X32$$

where

Y = predicted response

β_0 = intercept

β_1–β_3 = linear coefficients

β_{12}–β_{23} = interaction coefficients

β_{11}–β_{33} = quadratic coefficients

X_1, X_2, X_3 = coded levels of independent variables.

2.6 Statistical Analysis

The significance and adequacy of the developed polynomial models were evaluated using analysis of variance (ANOVA). ANOVA determines whether the developed model is statistically significant and also evaluates the contribution of each independent variable and their interaction effects on the response parameters.

The F-value and p-value obtained from the ANOVA analysis were used to assess the statistical significance of the model. A p-value less than 0.05 indicates that the model terms are statistically significant. Additionally, the coefficient of determination (R^2) was used to evaluate the goodness of fit between the predicted and experimental responses.

The ANOVA results presented in **Table 3** demonstrate that the developed quadratic model is statistically significant, indicating that the selected formulation variables significantly influence the responses. The statistical parameters obtained from the analysis confirm the adequacy and reliability of the developed model for

predicting the responses within the studied experimental range.

Table 3: Analysis of variance (ANOVA) for the quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	14532.8	9	1614.76	42.18	<0.0001	Significant
X_1 (Phospholipid concentration)	5234.62	1	5234.62	136.71	<0.0001	Significant
X_2 (Cholesterol concentration)	2145.38	1	2145.38	55.99	0.0002	Significant
X_3 (Sonication time)	1894.27	1	1894.27	49.43	0.0003	Significant
X_1X_2	732.61	1	732.61	19.11	0.0032	Significant
X_1X_3	521.74	1	521.74	13.6	0.0075	Significant
X_2X_3	438.29	1	438.29	11.42	0.0114	Significant
X_1^2	1165.24	1	1165.24	30.41	0.001	Significant
X_2^2	852.48	1	852.48	22.25	0.0021	Significant
X_3^2	548.21	1	548.21	14.31	0.0067	Significant
Residual	268.12	7	38.3	—	—	—
Lack of Fit	154.62	3	51.54	1.82	0.2875	Not Significant
Pure Error	113.5	4	28.37	—	—	—
Total	14801	16	—	—	—	—

The significance of the developed quadratic model was evaluated using analysis of variance (ANOVA). The obtained F-value and p-value indicated that the model was statistically significant. A p-value less than 0.05 confirmed the significance of the model terms. The coefficient of determination (R^2) demonstrated good agreement between the predicted and experimental responses. Furthermore, the non-significant lack-of-fit indicated that the developed model was adequate for describing the experimental data.

To further validate the reliability of the developed model, the optimized formulation predicted by the Box–Behnken design was prepared experimentally and evaluated. The predicted responses obtained from the model were compared with the experimentally observed values, and the results are presented in **Table 4.** The low percentage prediction error observed between predicted and experimental responses confirmed the accuracy and validity of the developed optimization model.

Table 4: Statistical parameters of the developed quadratic model

Response	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
Particle Size	0.98	0.97	0.96	22.4
PDI	0.96	0.94	0.92	18.1
Zeta Potential	0.97	0.95	0.93	19.5
Entrapment Efficiency	0.98	0.97	0.95	21.3

2.7 Response Surface Methodology

Response surface methodology (RSM) was applied to evaluate the interaction effects of formulation variables on the selected response parameters and to determine the optimal formulation conditions. Three-dimensional response surface plots and corresponding contour plots

were generated using Design-Expert® software to visually interpret the influence of independent variables on particle size, polydispersity index, and entrapment efficiency of the liposomal formulations.

These graphical representations provide a clear understanding of the relationship between formulation variables and the responses, as well as the interaction effects among the independent variables. The response surface plots also help in identifying the optimal region where the desired formulation characteristics can be achieved.

Effect of Formulation Variables on Particle Size

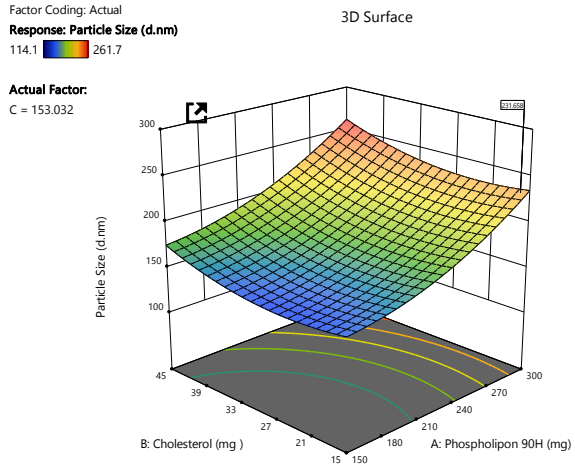


Figure 2. 3D response surface plot showing the effect of phospholipid concentration (X_1) and cholesterol concentration (X_2) on particle size of liposomes.

The three-dimensional response surface plot illustrating the effect of phospholipid concentration (X_1) and cholesterol concentration (X_2) on particle size is presented in **Figure 2**. The response surface plot demonstrates the combined influence of phospholipid and cholesterol concentrations on the particle size of garcinol-loaded liposomes. An increase in phospholipid concentration resulted in an increase in particle size due to the formation of thicker lipid bilayers and greater vesicle aggregation. Similarly, higher cholesterol concentration contributed to increased vesicle rigidity, which also influenced the particle size of the vesicles. The interaction between these two formulation variables indicates that an optimal balance between phospholipid and cholesterol concentrations is required to obtain liposomes with nanoscale particle size suitable for topical drug delivery.

Effect of Formulation Variables on Zeta potential

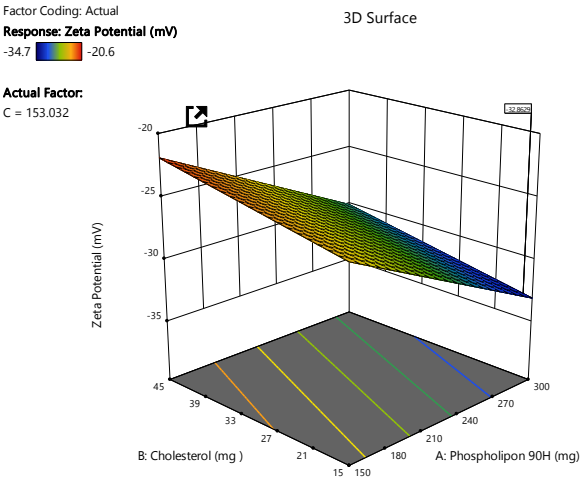


Figure 3. Three-dimensional response surface plot showing the effect of phospholipid concentration (X_1) and sonication time (X_3) on the zeta potential of liposomal formulations.

The three-dimensional response surface plot illustrating the effect of phospholipid concentration (X_1) and sonication time (X_3) on the zeta potential of liposomal formulations is shown in **Figure 3.** Zeta potential is an important indicator of the stability of colloidal systems, as higher absolute values of zeta potential generally indicate stronger electrostatic repulsion between vesicles and improved dispersion stability.

The response surface plot demonstrates that phospholipid concentration significantly influences the surface charge of liposomal vesicles due to changes in membrane composition and structural organization of the lipid bilayer. Additionally, sonication time affects the zeta potential by reducing vesicle size and promoting uniform distribution of liposomal particles within the dispersion.

The optimized formulation exhibited a zeta potential of approximately −32.6 mV, indicating good electrostatic stability of the liposomal system and suggesting reduced aggregation of vesicles during storage.

Effect of Formulation Variables on Entrapment Efficiency

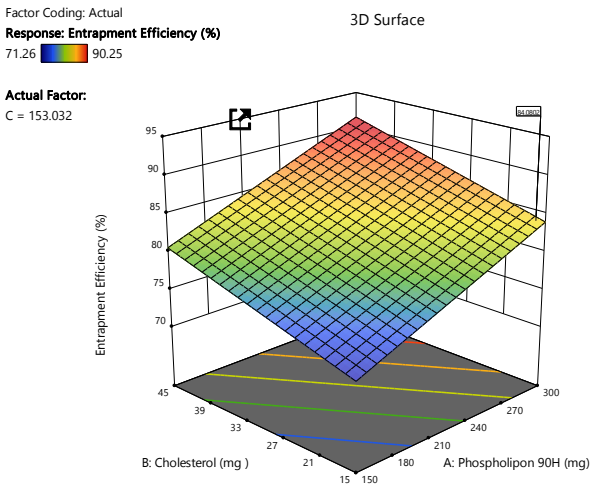


Figure 4. Three-dimensional response surface plot showing the effect of cholesterol concentration (X_2) and sonication time (X_3) on the entrapment efficiency of liposomal formulations.

The three-dimensional response surface plot illustrating the effect of cholesterol concentration (X_2) and sonication time (X_3) on the entrapment efficiency of liposomal formulations is presented in **Figure 4.** The response surface plot demonstrates the combined influence of cholesterol concentration and sonication time on the entrapment efficiency of garcinol within the liposomal vesicles.

An increase in cholesterol concentration resulted in improved entrapment efficiency due to enhanced stabilization of the lipid bilayer and reduced permeability of the vesicle membrane, thereby minimizing drug leakage from the liposomes. However, excessive sonication time slightly decreased the entrapment efficiency, which may be attributed to disruption of the liposomal vesicles and possible leakage of the encapsulated drug during the sonication process.

These results indicate that an optimal combination of cholesterol concentration and sonication time is required to achieve maximum drug encapsulation and maintain structural stability of the liposomal vesicles.

2.8 Numerical Optimization

Numerical optimization of the liposomal formulation was performed using the desirability function approach provided in Design-Expert® software. This approach allows simultaneous optimization of multiple response variables by assigning specific goals to each response parameter. In the present study, the optimization criteria were defined to obtain minimum particle size and polydispersity index (PDI), while maximizing zeta potential and entrapment efficiency in order to achieve a stable and efficient liposomal drug delivery system.

Each response was assigned a desirability function ranging from 0 to 1, where a value closer to 1 indicates a more desirable formulation condition. The software generated several possible solutions within the experimental design space, and the formulation with the highest overall desirability value was selected as the optimized formulation. The desirability plot illustrating the optimized formulation conditions is presented in **Figure 5.**

Based on the numerical optimization results, the optimal formulation was predicted to contain Phospholipon® 90H (approximately 297.43 mg), cholesterol (approximately 15.95 mg), and a sonication time of about 153 seconds. The predicted responses for the optimized formulation included a particle size of approximately 231.66 nm, a polydispersity index of 0.287, a zeta potential of −32.86 mV, and an entrapment efficiency of approximately 84.08%. These predicted values indicate the formation of nanosized liposomes with desirable physicochemical characteristics suitable for topical drug delivery.

The optimized formulation predicted by the statistical model was subsequently prepared experimentally and evaluated to verify the reliability and predictive ability of the optimization process.

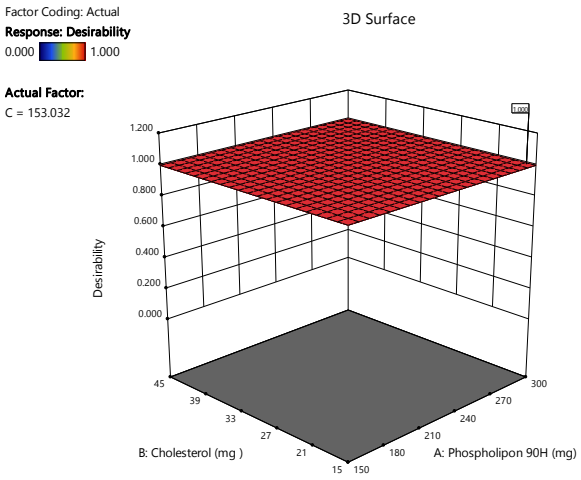


Figure 5. Desirability plot showing the optimized formulation conditions obtained using the Box–Behnken design.

2.9 Validation of Optimized Liposomes

To verify the reliability and predictive ability of the developed Box–Behnken design model, the optimized liposomal formulation suggested by the numerical optimization process was prepared experimentally under the predicted optimal conditions. The optimized formulation consisted of Phospholipon® 90H (approximately 297.43 mg), cholesterol (approximately 15.95 mg), and a sonication time of about 153 seconds.

The prepared optimized formulation was evaluated for the selected response parameters, including particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The experimentally obtained values were compared with the predicted responses generated by the statistical model to assess the accuracy and validity of the optimization process.

The results demonstrated that the experimentally observed responses were in close agreement with the predicted values, with prediction errors below 2% for all response parameters. The optimized liposomal formulation exhibited a particle size of 232.8 nm, a PDI of 0.285, a zeta potential of −32.6 mV, and an entrapment efficiency of 85.1%. These results confirm that the developed quadratic model is reliable for predicting formulation responses within the studied experimental range.

The comparison between the predicted and experimental values is presented in **Table 5**, which indicates good agreement between the model predictions and the experimentally obtained results. The minimal deviation between predicted and observed responses confirms the adequacy and validity of the Box–Behnken design for optimization of the liposomal formulation.

Table 5: Predicted and experimental responses of the optimized liposomal formulation

Response	Predicted Mean	Experimental Mean	% Error
Particle Size (nm)	231.66	232.8	0.49
PDI	0.287	0.285	0.7
Zeta Potential (mV)	−32.86	−32.6	0.79
Entrapment Efficiency (%)	84.08	85.1	1.2

2.10 Preparation of Hydrogel

Hydrogel formulations were prepared using Carbopol 934 as the gelling agent. Accurately weighed quantities of Carbopol 934 (1%, 1.5%, and 2% w/w) were gradually dispersed in an appropriate volume of distilled water under continuous stirring to avoid the formation of lumps. The dispersion was allowed to hydrate and swell for 24 hours at room temperature to obtain a uniform gel base. **[19, 20, 21]**

Subsequently, propylene glycol and glycerin were added to the hydrated Carbopol dispersion as humectants and co-solvents, followed by the incorporation of methyl paraben and propyl paraben as preservatives. The mixture was stirred continuously to ensure uniform distribution of all excipients within the gel matrix. **[20, 23]**

The pH of the formulation was then adjusted to approximately 6.5–7.0 by the dropwise addition of triethanolamine (TEA) under gentle stirring. Neutralization of Carbopol resulted in the formation of a clear and homogeneous gel. The prepared hydrogel formulations were evaluated for their physicochemical properties and were used as the base for the preparation of liposomal hydrogel formulations. **[21, 22, 24]**

Table 6: Composition of Hydrogel Formulations (H1–H3)

S. NO	Formulation	Carbopol 934	Propylene glycol	Glycerin	Propyl paraben	Methyl paraben	Triethanolamine	Distilled water
1	H1	1 gm	5 ml	2 ml	0.02 %	0.18 %	0.5 ml	100 q.s.
2	H2	1.5 gm	5 ml	2 ml	0.02 %	0.18 %	0.5 ml	100

								q.s.
3	H3	2 gm	5 ml	2 ml	0.02 %	0.18 %	0.5 ml	100 q.s.

2.11 Preparation of Liposomal Hydrogel

The optimized garcinol-loaded liposomal formulation obtained from the Box–Behnken design optimization was incorporated into the prepared Carbopol hydrogel base to develop the liposomal hydrogel formulation. A required quantity of the optimized liposomal dispersion was slowly added to the previously prepared hydrogel base under gentle stirring to ensure uniform distribution of liposomes within the gel matrix.

The mixing process was carried out at room temperature using continuous mechanical stirring to avoid disruption of the liposomal vesicles. The liposomal dispersion was gradually incorporated into the hydrogel base in order to maintain the structural integrity of the liposomes and achieve homogeneous dispersion throughout the gel. **[25, 26]**

The final liposomal hydrogel formulations were adjusted to an appropriate pH (approximately 6.5–6.8) to ensure compatibility with skin and improve patient acceptability. The prepared formulations were stored in airtight containers and allowed to equilibrate for 24 hours before further physicochemical evaluation. **[27, 28, 29] [26, 30]**

The developed liposomal hydrogel formulations were subsequently subjected to various evaluation parameters including physical appearance, pH determination, viscosity measurement, spreadability, extrudability, drug content analysis, and in-vitro drug release studies.

Table No. 7. Composition of liposomal hydrogel formulation

Compositions	LH1	LH2	LH3
Liposomal dispersion	10 ml	10 ml	10 ml
Carbopol 934 (% w/w)	1%	1.5%	2%
Glycerin	2 ml	2 ml	2 ml
Propyl paraben	0.02%	0.02%	0.02%
Methyl paraben	0.18%	0.18%	0.18%
Triethanolamine	0.5 ml	0.5 ml	0.5 ml
Distilled water (q. s.)	100 q.s.	100 q.s	100 q.s

2.12 Characterization of Liposomes

The prepared garcinol-loaded liposomal formulations were characterized to evaluate their physicochemical properties and to determine their suitability for topical drug delivery. Various characterization parameters including drug–excipient compatibility, particle size, polydispersity index, zeta potential, entrapment efficiency, surface morphology, and in-vitro drug release were investigated using standard analytical techniques.

2.12.1 FTIR Compatibility Study

Fourier Transform Infrared (FTIR) spectroscopy was used to evaluate the compatibility between garcinol and the excipients used in the liposomal formulation. FTIR spectra of pure garcinol, phospholipid, cholesterol, and the optimized liposomal formulation were recorded using an FTIR spectrophotometer.

The samples were prepared by the potassium bromide (KBr) pellet method, and spectra were recorded over a scanning range of 4000–400 cm^{-1}. The obtained spectra were analyzed to identify characteristic functional group peaks and to detect any possible chemical interactions between the drug and formulation components**. [31]**

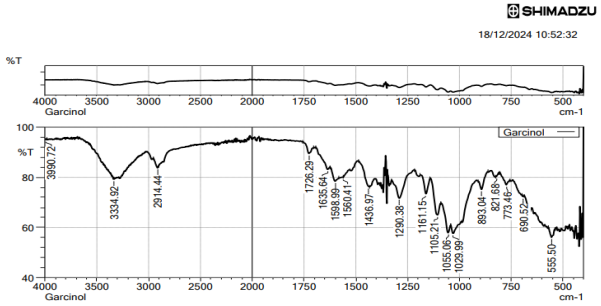


Figure 6: FTIR spectra of Garcinol

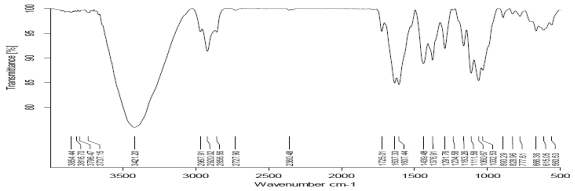


Figure 7: FTIR spectra of Garcinol with excipients

2.12.2 Particle Size and Polydispersity Index

The particle size and polydispersity index (PDI) of the prepared liposomal formulations were determined using dynamic light scattering (DLS) technique. The measurements were performed using a particle size analyzer at room temperature.

Before analysis, the liposomal dispersion was diluted with distilled water to prevent multiple scattering effects. The particle size distribution and PDI values were recorded, where PDI values less than 0.3 indicate a narrow size distribution and good homogeneity of the vesicles**. [32, 33]**

2.12.3 Zeta Potential

The zeta potential of the liposomal formulations was determined to evaluate the surface charge and stability of the vesicles. Measurements were carried out using a zeta potential analyzer based on electrophoretic mobility principles.

The liposomal dispersion was diluted appropriately with distilled water and analyzed at room temperature. Zeta potential values greater than ±30 mV generally indicate good stability of colloidal dispersions due to electrostatic repulsion between particles**. [34]**

2.12.4 Entrapment Efficiency

Entrapment efficiency was determined to evaluate the percentage of garcinol encapsulated within the liposomal vesicles. The liposomal dispersion was centrifuged at high speed (approximately 15,000 rpm for 30 minutes) to separate the free drug from the liposome-entrapped drug. The supernatant containing the unentrapped drug was collected and analyzed spectrophotometrically at the maximum absorption wavelength of garcinol using a UV-visible spectrophotometer **[35, 36].** The entrapment efficiency was calculated using the following equation:

% Entrapment efficiency= Entrapped drug /Total drug added ×100

2.12.5 FESEM Analysis

The surface morphology and structural characteristics of the optimized liposomal formulation were examined using Field Emission Scanning Electron Microscopy (FESEM). A small quantity of the liposomal sample was placed on a sample holder and dried under vacuum.

The dried samples were coated with a thin layer of gold to enhance conductivity before imaging. The samples were then examined under FESEM at appropriate magnification to observe the shape, surface morphology, and structural characteristics of the liposomes**. [37, 38]**

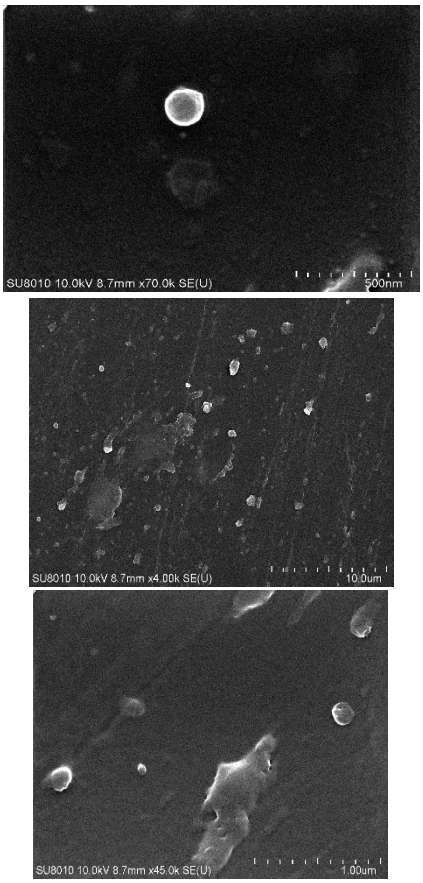


Figure 8: SEM of optimized Liposomes formulation

2.12.6 In-Vitro Drug Release of Liposomes

The in-vitro drug release study of garcinol from liposomal formulations was carried out using the dialysis bag diffusion method. The dialysis membrane was soaked in distilled water prior to use to ensure proper hydration.

A predetermined volume of liposomal dispersion containing a known amount of drug was placed inside the dialysis bag, which was then immersed in phosphate buffer saline (PBS, pH 7.4) maintained at 37 ± 0.5°C under constant stirring using a magnetic stirrer.

At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The withdrawn samples were analyzed using a UV-visible spectrophotometer at the appropriate wavelength to determine the cumulative percentage drug release**. [40, 41, 42]**

Table 8: Drug release of from Liposomes

Time (h)	% Drug Release (Liposomes)
0	0
0.5	8.6± 0.3
1	14.2± 0.5
2	21.8± 0.3
4	33.5± 0.9
6	42.7± 1.0
8	50.3± 0.8
10	57.1± 0.4
12	63.4± 0.6
16	72.6± 0.5
20	79.8± 0.3
24	**85.2**± 0.7

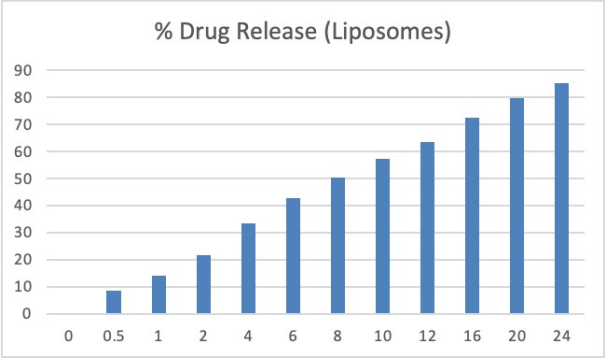


Figure 9. Cumulative percentage drug release profile of garcinol from liposomal formulation.

2.13 Evaluation of Hydrogel Formulations

The prepared hydrogel formulations were evaluated for various physicochemical parameters to determine their suitability for topical application. The evaluation parameters included physical appearance, pH, viscosity, spreadability, extrudability, and drug content.

2.13.1 Physical Appearance

The prepared hydrogel formulations were visually inspected to evaluate their color, transparency, homogeneity, and presence of any particulate matter or phase separation. A small quantity of each formulation was examined against a white background to assess clarity and uniformity. The formulations were also checked for smooth texture and absence of lumps, which are important characteristics for topical gel preparations.

2.13.2 pH Determination

The pH of the hydrogel formulations was measured using a digital pH meter. Approximately 1 g of gel was dispersed in 10 mL of distilled water, and the electrode of the calibrated pH meter was immersed in the dispersion. The pH value was recorded once the reading stabilized. Maintaining the pH within the range of 5.5–7.0 is important to ensure compatibility with skin and to avoid irritation upon topical application.

2.13.3 Viscosity Measurement

The viscosity of the hydrogel formulations was determined using a Brookfield viscometer equipped with a suitable spindle at room temperature. A predetermined quantity of gel was placed in the sample container, and viscosity was measured at a fixed rotational speed. The viscosity values were recorded in centipoise (cP), which indicates the flow behavior and consistency of the hydrogel formulations.

2.13.4 Spreadability

Spreadability of the hydrogel formulations was determined to evaluate the ease of application on the skin. A fixed amount of gel was placed between two glass slides, and a known weight was applied on the upper slide to allow uniform spreading of the gel. **[43]**

The time required for the upper slide to move a specified distance was recorded, and spreadability was calculated using the following equation:

$$S = M \times L / T$$

Where:

S = Spreadability (g·cm/s)

M = Weight applied to the upper slide (g)

L = Length of the glass slide (cm)

T = Time taken for the slides to separate (s)

Higher spreadability values indicate better ease of application of the gel on the skin.

2.13.5 Extrudability

Extrudability of the hydrogel formulations was evaluated to determine the ease with which the gel could be extruded from collapsible tubes. The gel formulations were filled into aluminum tubes, and the force required to extrude the gel from the tube was measured by applying constant pressure. The extruded gel was visually evaluated and categorized as excellent, good, or poor based on the ease of extrusion**.[44, 45]**

Table 9: Physicochemical Evaluation of Hydrogel Formulations (H1–H3)

S. No.	Formulation	Physical Appearance	Ph	Viscosity (cP)	Spreadability (g·cm/sec)	Extrudability

1	H1	Transparent, smooth, homogeneous gel	6.3 ± 0.2	21,120± 27	10±0.13	Excellent
2	H2	Slightly opaque, smooth gel	6.5 ± 0.5	25,326± 34	9.25±0.2	Good
3	H3	Opaque, thick gel	6.8 ± 0.3	38,230± 23	8.33±0.15	Poor

2.14 Evaluation of Liposomal Hydrogel Formulations

The prepared liposomal hydrogel formulations were evaluated to determine their physicochemical properties and suitability for topical drug delivery. Various parameters including physical appearance, phase separation, pH, viscosity, spreadability, extrudability, and drug content were assessed using standard procedures.

2.14.1 Physical Appearance

The liposomal hydrogel formulations were visually inspected to evaluate their color, transparency, homogeneity, and consistency. A small quantity of each formulation was examined against a white background to detect any visible particulate matter, lump formation, or irregularities in the gel matrix. The formulations were also checked for smooth texture and uniform distribution of liposomes within the hydrogel base**. [46, 47, 48, 49]**

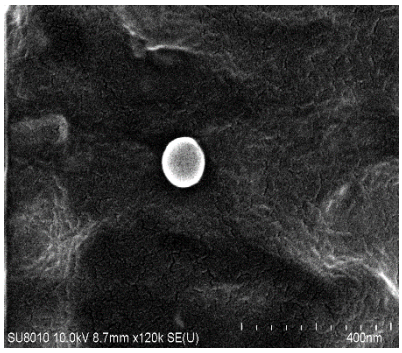
2.14.2 Phase Separation

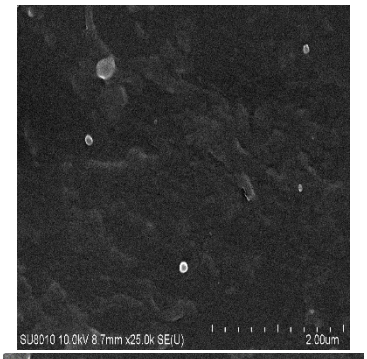
The prepared liposomal hydrogel formulations were observed visually for any signs of phase separation during storage. The formulations were kept at room temperature and periodically examined for changes in consistency, separation of liquid components, or instability of the gel matrix. Absence of phase separation indicates good stability of the liposomal hydrogel system.

2.14.3 Field Emission Scanning Electron Microscopy (FE-SEM)

The surface morphology of the optimized liposomal hydrogel was examined using field emission scanning electron microscopy. A small quantity of the hydrogel was freeze-dried to remove water and preserve the internal microstructure. The dried sample was mounted on an aluminum stub and coated with a thin layer of gold using a sputter coater to enhance electrical conductivity. The prepared sample was observed under a field emission scanning electron microscope (SU8010 SERIES, HITACHI, JAPAN.) operated at an suitable accelerating voltage. Micrographs were recorded at different magnifications to examine the surface morphology and structural characteristics of the hydrogel matrix.

The FE-SEM images revealed a porous three-dimensional network structure of the hydrogel with uniformly distributed liposomal vesicles embedded within the polymer matrix. The observed morphology indicated effective incorporation of liposomes into the hydrogel system and confirmed the formation of a homogeneous liposomal hydrogel structure**. [47]**





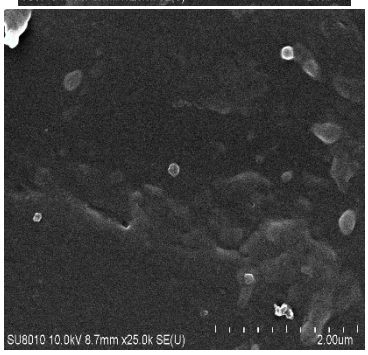


Figure 10: SEM of optimized Liposomal hydrogel formulation.

2.14.4 pH Determination

The pH of the liposomal hydrogel formulations was measured using a digital pH meter. Approximately 1 g of the gel was dispersed in 10 mL of distilled water, and the electrode of the calibrated pH meter was immersed in the dispersion. The pH value was recorded once the reading stabilized. Maintaining the pH within the range of 5.5–7.0 is important to ensure compatibility with skin and minimize the risk of irritation during topical application**. [48]**

2.14.5 Viscosity Measurement

The viscosity of the liposomal hydrogel formulations was determined using a Brookfield viscometer equipped with an appropriate spindle at room temperature. A known quantity of gel was placed in the sample container and allowed to equilibrate before measurement. The viscosity was recorded at a constant rotational speed, and the values were expressed in centipoise (cP). Viscosity measurement helps determine the consistency and flow behavior of the hydrogel formulations**. [49]**

2.14.6 Spreadability

Spreadability of the liposomal hydrogel formulations was evaluated to assess the ease of application of the gel on the skin surface. A fixed quantity of gel was placed between two glass slides, and a specified weight was applied to the upper slide to allow uniform spreading of the gel**. [50]**

The time taken for the upper slide to move a certain distance was recorded, and spreadability was calculated using the following equation:

$$S=M\times L/T$$

Where:

S = Spreadability (g·cm/s)

M = Weight applied to the upper slide (g)

L = Length of the glass slide (cm)

T = Time taken for the slides to separate (s)

Higher spreadability values indicate better applicability of the gel formulation.

2.14.7 Extrudability

Extrudability of the liposomal hydrogel formulations was evaluated to determine the ease with which the gel can be extruded from collapsible tubes. The formulations were filled into aluminum tubes and subjected to uniform pressure. The amount of gel extruded from the tube under constant pressure was observed and categorized as excellent, good, or poor based on the ease of extrusion and consistency of the formulation**. [50]**

2.14.8 Drug Content

Drug content analysis was performed to determine the uniform distribution of garcinol within the liposomal hydrogel formulations. Approximately 1 g of liposomal hydrogel was accurately weighed and dissolved in phosphate buffer saline (PBS, pH 7.4) with continuous stirring to ensure complete extraction of the drug.

The solution was filtered to remove any undissolved components, and the absorbance of the filtrate was measured using a UV-visible spectrophotometer at the maximum absorption wavelength of garcinol. The drug content was calculated as the percentage of drug present in the formulation relative to the theoretical drug content. **[51, 52]**

Table 10: Physicochemical Evaluation of Liposomal Hydrogel Formulations (LH1–LH3)

S. No.	Formulation	Physical Appearance	Phase separation	Drug content	pH	Viscosity (cP)	Spreadability (g·cm/sec)	Extrudability
1	LH1	Transparent, , Smooth gel	No	95.3±0.8 %	6.6 ± 0.5	20154± 12	28.57±0.9	Excellent
2	LH2	smooth Slightly opaque gel	No	97.5±0.15 %	6.7 ± 0.2	24134± 26	20.23±0.4	Good
3	LH3	Opaque, thick gel	No	98.4±0.12 %	6.9 ± 0.3	36467± 34	16.34±0.7	Poor

2.15 In-Vitro Drug Release Study of Liposomal Hydrogel

The in-vitro drug release study of garcinol from liposomal hydrogel formulations was carried out using the Franz diffusion cell method. A dialysis membrane previously soaked in distilled water for 12 hours was used as the diffusion membrane. The hydrated membrane was mounted between the donor and receptor compartments of the Franz diffusion cell.

A predetermined quantity of the liposomal hydrogel formulation equivalent to a known amount of drug was placed in the donor compartment, while the receptor compartment was filled with phosphate-buffered saline (PBS, pH 7.4) to simulate physiological conditions. The receptor medium was continuously stirred using a magnetic stirrer to maintain uniform drug distribution.

The entire system was maintained at a temperature of 37 ± 0.5 °C to mimic physiological conditions. At predetermined time intervals, aliquots of the receptor medium were withdrawn, and an equal volume of fresh buffer solution was added to maintain constant volume and sink conditions**. [53]**

The collected samples were filtered and analyzed using a UV-visible spectrophotometer at the maximum absorption wavelength of garcinol. The cumulative percentage of drug released was calculated and plotted against time to obtain the drug release profile of the liposomal hydrogel formulations**.[54]**

Table 11: In-vitro Drug Release Profile of Liposomal Hydrogel Formulations (LH1–LH3)

Time (hrs)	LH1 (% Drug Release)	LH2 (% Drug Release)	LH3 (% Drug Release)
0	0	0	0
1	18 ± 0.8	12 ± 0.9	8 ± 0.8
2	32 ± 0.5	25 ± 0.5	18 ± 1.0
4	50 ± 0.9	40 ± 0.8	30 ± 0.4
6	65 ± 1.0	55 ± 0.5	42 ± 0.7
8	75 ± 0.6	65 ± 0.4	52 ± 1.0
10	82 ± 0.4	72 ± 0.6	60 ± 0.5
12	88 ± 0.8	78 ± 1.0	68 ± 0.3
24	92 ± 0.5	85 ± 0.7	75 ± 0.4

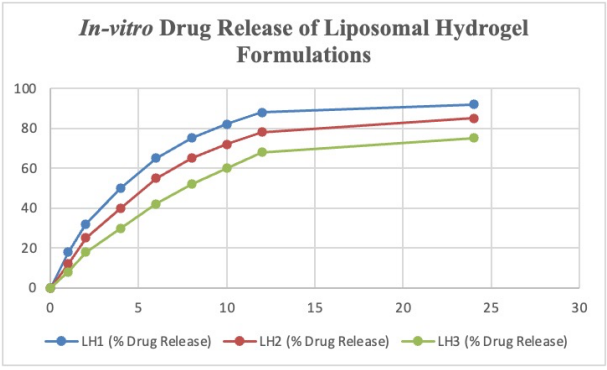


Figure 11: *In-vitro* Drug Release Profile of Liposomal Hydrogel Formulations (LH1–LH3)

2.16 Stability Studies of Optimized Liposomal Hydrogel

Stability studies were conducted to evaluate the physical and chemical stability of the optimized liposomal hydrogel formulation during storage. The stability studies were performed in accordance with International Council for Harmonisation (ICH) guidelines under both room temperature and accelerated storage conditions. The optimized formulation was stored in airtight containers and evaluated periodically for changes in physicochemical properties.

The parameters evaluated during the stability study included pH, drug content, viscosity, spreadability, and physical appearance. The samples were analyzed at predetermined time intervals of 0, 15, and 30 days.

2.16.1 Stability Study at Room Temperature (25 ± 2 °C)

For the room temperature stability study, the optimized liposomal hydrogel formulation was stored at 25 ± 2 °C under normal laboratory conditions. Samples were withdrawn at specified time intervals (0, 15, and 30 days) and evaluated for changes in pH, drug content, viscosity, spreadability, and physical appearance to determine the stability of the formulation during storage**. [43]**

Table 12: Stability Study of Optimized Liposomal Hydrogel Formulation

Time (Days)	pH	Drug Content (%)	Viscosity (cP)	Spreadability (g·cm/s)	Physical Appearance
0	6.7± 0.2	97.5± 0.6	24134 ±22	20.23±0.6	Smooth, slightly opaque

15	6.7± 0.5	97.2± 0.3	24000 ±34	19.71±0. 4	No signific ant change
30	6.6± 0.4	97±0. 5	23874 ±28	19.33±0. 7	Stable

2.16.2 Accelerated Stability Study (40 ± 2 °C / 75 ± 5% RH)

Accelerated stability studies were carried out to evaluate the stability of the optimized formulation under stress storage conditions. The liposomal hydrogel formulation was stored at 40 ± 2 °C and 75 ± 5% relative humidity in a stability chamber.

Samples were withdrawn at 0, 15, and 30 days and analyzed for physicochemical parameters including pH, drug content, viscosity, spreadability, and physical appearance**. [43]**

Table 13: Accelerated Stability Study of Optimized Liposomal Hydrogel Formulation

Tim e (Da ys)	pH	Drug Cont ent (%)	Visco sity (cP)	Spreada bility (g·cm/s)	Physica l Appear ance
0	6.7± 0.5	97.5± 0.1	24134 ±21	20.23±0. 4	Smooth, slightly opaque
15	6.7± 0.1	97.1± 0.5	23264 ±19	18.76±0. 2	Slight change
30	6.5± 0.3	96.8± 0.4	22954 ±33	17.64±0. 2	Accepta ble

3. Results and Discussion

The developed garcinol-loaded liposomal hydrogel was systematically evaluated to investigate the influence of formulation variables on liposomal characteristics and to assess the performance of the final topical formulation. The results obtained from optimization, physicochemical characterization, hydrogel evaluation, drug release, and stability studies are discussed below.

3.1 Optimization of Liposomal Formulation Using Box–Behnken Design

The Box–Behnken design was employed to optimize the formulation variables affecting the physicochemical characteristics of garcinol-loaded liposomes. The independent variables selected for the study were phospholipid concentration (X_1), cholesterol concentration (X_2), and sonication time (X_3), while the responses evaluated were particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency.

The experimental design matrix and observed responses are presented in **Table 2.** The results indicated that the selected formulation variables significantly influenced the characteristics of the liposomal formulations. Among the studied variables, phospholipid concentration showed a pronounced effect on particle size and entrapment efficiency, whereas sonication time had a significant influence on particle size reduction and size distribution of the vesicles.

The developed quadratic model was statistically significant as confirmed by ANOVA analysis **(Table 3).** The model demonstrated high R^2 values, indicating good agreement between predicted and experimental responses. Additionally, the non-significant lack-of-fit confirmed that the developed model was adequate for predicting formulation responses within the studied experimental range.

Response surface plots further illustrated the interaction effects of formulation variables on the selected responses (Figures 1–3). Numerical optimization using the desirability function approach suggested an optimized formulation with suitable physicochemical characteristics. The optimized formulation predicted by the model showed particle size of 231.66 nm, PDI of 0.287, zeta potential of −32.86 mV, and entrapment efficiency of 84.08%.

3.2 Characterization of Garcinol-Loaded Liposomes

3.2.1 FTIR Compatibility Study

FTIR analysis was performed to evaluate possible interactions between garcinol and the excipients used in the liposomal formulation.

The characteristic peaks corresponding to functional groups of garcinol were retained in the spectra of the liposomal formulation without significant shifts or disappearance of peaks. This indicates the absence of chemical interaction between the drug and formulation components, confirming the compatibility of garcinol with the excipients used in the liposomal formulation.

3.2.2 Particle Size and Polydispersity Index

Particle size plays a crucial role in determining the stability, drug release behavior, and skin penetration ability of liposomal formulations. The optimized

liposomal formulation exhibited a particle size of 232.8 nm with a PDI of 0.285, indicating a narrow size distribution and uniform vesicle population.

The nanosized particle size observed in the optimized formulation is advantageous for topical drug delivery as it facilitates improved skin penetration and enhances drug retention at the application site. **[55]**

3.2.3 Zeta Potential

Zeta potential is an important parameter used to evaluate the stability of colloidal dispersions. The optimized liposomal formulation showed a zeta potential of −32.6 mV, indicating good electrostatic stability of the vesicles. Generally, zeta potential values greater than ±30 mV indicate sufficient repulsive forces between particles to prevent aggregation. The observed zeta potential therefore suggests good stability of the liposomal system. **[56]**

3.2.4 Entrapment Efficiency

Entrapment efficiency is an important parameter that determines the ability of liposomes to encapsulate the drug within the lipid bilayer. The optimized liposomal formulation exhibited an entrapment efficiency of 85.1%, indicating efficient incorporation of garcinol within the liposomal vesicles.

The high entrapment efficiency may be attributed to the lipophilic nature of garcinol, which favors its incorporation within the phospholipid bilayer of the liposomes**. [57]**

3.2.5 FESEM Analysis

The surface morphology of the optimized liposomal formulation was examined using FESEM. The FESEM micrograph **(Figure 8)** revealed that the liposomes were spherical in shape with smooth surface morphology. The vesicles appeared well-dispersed without significant aggregation, confirming successful formation of liposomal nanovesicles.

3.3 Evaluation of Hydrogel Formulations

The prepared hydrogel formulations were evaluated for various physicochemical properties including physical appearance, pH, viscosity, spreadability, and extrudability. The results are summarized in **Table 9.**

All hydrogel formulations appeared smooth, homogeneous, and free from visible particulate matter. The pH values ranged between 6.3 and 6.8, which falls within the acceptable range for topical applications and ensures compatibility with skin.

Viscosity measurements indicated that increasing Carbopol concentration resulted in increased viscosity of the gel formulations. Spreadability values decreased slightly with increasing polymer concentration due to higher viscosity. These results indicate that the gel base with 1.5% Carbopol concentration provided a balanced combination of viscosity and spreadability suitable for topical application. **[59]**

3.4 Evaluation of Liposomal Hydrogel Formulations

The liposomal hydrogel formulations (LH1–LH3) were evaluated for physicochemical parameters including physical appearance, phase separation, drug content, pH, viscosity, spreadability, and extrudability. The results are presented in **Table 10.**

All liposomal hydrogel formulations appeared smooth and homogeneous with no evidence of phase separation. Among the formulations, LH2 demonstrated desirable physicochemical properties including pH 6.7, viscosity 24134 cP, spreadability 20.23 g·cm/s, and drug content of 97.5%. These results indicate uniform distribution of liposomes within the hydrogel matrix and good formulation stability.

3.5 In-Vitro Drug Release Study

The in-vitro drug release profiles of liposomal hydrogel formulations are shown in Table 10 and Figure 7. The results demonstrated sustained drug release behavior over a period of 24 hours.

Among the formulations, LH1 exhibited the highest cumulative drug release (92%), followed by LH2 (85%) and LH3 (75%) after 24 hours. The observed release pattern indicates that increasing Carbopol concentration leads to higher viscosity of the gel matrix, which subsequently slows down drug diffusion.

The sustained drug release behavior observed in liposomal hydrogel formulations may be attributed to the combined effect of liposomal encapsulation and the gel matrix, which together control the diffusion of drug molecules**. [58]**

3.6 Stability Studies

Stability studies were performed to evaluate the stability of the optimized liposomal hydrogel formulation under room temperature (25 ± 2 °C) and accelerated conditions (40 ± 2 °C / 75 ± 5% RH). The results are presented in **Tables 12 and 13.**

The results indicated only minor variations in pH, viscosity, spreadability, and drug content over the study period. The drug content remained above 96%, indicating minimal degradation of garcinol during storage.

4. Conclusion

In the present study, a garcinol-loaded liposomal hydrogel was successfully developed and optimized for topical delivery using a Box–Behnken experimental design. The optimization process demonstrated that formulation variables, including phospholipid concentration, cholesterol concentration, and sonication time, significantly influenced the physicochemical characteristics of the liposomal formulations. The optimized liposomes exhibited a particle size of 232.8 nm, PDI of 0.285, zeta potential of −32.6 mV, and entrapment efficiency of 85.1%, indicating good stability and efficient drug encapsulation.

The optimized liposomes were successfully incorporated into a Carbopol-based hydrogel, resulting in a stable liposomal hydrogel formulation with suitable physicochemical properties such as acceptable pH, appropriate viscosity, good spreadability, and uniform drug distribution. In-vitro drug release studies demonstrated sustained drug release over 24 hours, confirming the controlled release capability of the developed formulation. Stability studies further indicated that the optimized liposomal hydrogel remained physically and chemically stable under both room temperature and accelerated conditions.

Overall, the developed liposomal hydrogel system shows promising potential as an effective topical drug delivery system for the management of rheumatoid arthritis, offering improved drug stability, controlled release, and enhanced therapeutic efficacy.

No significant changes in physical appearance or phase separation were observed during the study period. These results confirm that the optimized liposomal hydrogel formulation possesses good physical and chemical stability under both storage conditions.

5. REFERENCES

1. Firestein GS, McInnes IB. Immunopathogenesis of rheumatoid arthritis. *Immunity*. 2017;46(2):183-196. DOI: 10.1016/j.immuni.2017.02.006
2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *The Lancet*. 2016;388(10055):2023-2038. DOI: 10.1016/S0140-6736(16)30173-8
3. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *The Lancet*. 2010;376(9746):1094-1108. DOI: 10.1016/S0140-6736(10)60826-4
4. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. *The Lancet*. 2017;389(10086):2338-2348. DOI: 10.1016/S0140-6736(17)31491-5
5. Prausnitz MR, Langer R. Transdermal drug delivery. *Nature Biotechnology*. 2008;26(11):1261-1268. DOI: 10.1038/nbt.1504
6. Benson HAE. Transdermal drug delivery: penetration enhancement techniques. *Current Drug Delivery*. 2005;2(1):23-33. DOI: 10.2174/1567201052772915
7. Padhye S, Ahmad A, Oswal N, Sarkar FH. Emerging role of garcinol in cancer therapy. *Journal of Hematology & Oncology*. 2009;2:38. DOI: 10.1186/1756-8722-2-38
8. Aggarwal BB, Yuan W, Li S, Gupta SC. Garcinol: a potential anti-inflammatory agent. *Biochemical Pharmacology*. 2013;85(6):847-855. DOI: 10.1016/j.bcp.2013.01.003
9. Bozzuto G, Molinari A. Liposomes as nanomedical devices. *International Journal of Nanomedicine*. 2015;10:975-999. DOI: 10.2147/IJN.S68861
10. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: classification, preparation and applications. *Nanoscale Research Letters*. 2013;8:102. DOI: 10.1186/1556-276X-8-102
11. Ahmed EM. Hydrogel: preparation, characterization, and applications. *Journal of Advanced Research*. 2015;6(2):105-121. DOI: 10.1016/j.jare.2013.07.006
12. Ferreira SLC, Bruns RE, Ferreira HS, et al. Box–Behnken design: an alternative for the optimization of analytical methods. *Analytica Chimica Acta*. 2007;597(2):179-186. DOI: 10.1016/j.aca.2007.07.011
13. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology*. 1965;13:238-252. DOI: 10.1016/S0022-2836(65)80093-6
14. Mozafari MR, Johnson C, Hatziantoniou S, Demetzos C. Nanoliposomes and their applications. *Journal of Liposome Research*. 2008;18(4):309-327. DOI: 10.1080/08982100802443541
15. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery.

Chemical Reviews. 2015;115(19):10938-10966. DOI: 10.1021/acs.chemrev.5b00046

16. Laouini A, Jaafar-Maalej C, Limayem-Blouza I, et al. Preparation and characterization of liposomes. *Journal of Colloid Science and Biotechnology*. 2012;1(2):147-168. DOI: 10.1166/jcsb.2012.1020
17. Torchilin VP. Liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*. 2005;4(2):145-160. DOI: 10.1038/nrd1632
18. Mozafari MR. Liposome manufacturing techniques. *Cellular and Molecular Biology Letters*. 2005;10:711-719. DOI: 10.2478/s11658-005-0039-2
19. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. *Pharmaceutical Press*. 2009. DOI: 10.1016/B978-0-85711-027-5.00001-2
20. Aulton ME, Taylor K. Aulton's Pharmaceutics: The Design and Manufacture of Medicines. *Elsevier*. 2018. DOI: 10.1016/C2016-0-04691-9
21. Bonacucina G, Cespi M, Palmieri GF. Characterization and stability of Carbopol hydrogels. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009;72(2):411-418. DOI: 10.1016/j.ejpb.2009.01.017
22. Patel RP, Patel MM. Formulation and evaluation of topical gel. *International Journal of Drug Delivery*. 2009;1(1):41-45. DOI: 10.5138/ijdd.2009.0975.0215.01007
23. Khurana S, Jain NK, Bedi PMS. Development of topical gels. *Indian Journal of Pharmaceutical Sciences*. 2013;75(2):188-193. DOI: 10.4103/0250-474X.117409
24. Ahmed EM. Hydrogel preparation and applications. *Journal of Advanced Research*. 2015;6(2):105-121. DOI: 10.1016/j.jare.2013.07.006
25. Verma DD, Verma S, Blume G, Fahr A. Liposomes increase skin penetration. *European Journal of Pharmaceutics and Biopharmaceutics*. 2003;55(3):271-277. DOI: 10.1016/S0939-6411(02)00175-6
26. Sinico C, Fadda AM. Vesicular carriers for dermal drug delivery. *Expert Opinion on Drug Delivery*. 2009;6(8):813-825. DOI: 10.1517/17425240903004464
27. El-Maghraby GM, Williams AC, Barry BW. Skin delivery of drugs from liposomes. *International Journal of Pharmaceutics*. 2000;204:159-169. DOI: 10.1016/S0378-5173(00)00477-1
28. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal drug delivery. *Drug Discovery Today: Technologies*. 2005;2:67-74. DOI: 10.1016/j.ddtec.2005.05.004
29. Benson HAE. Elastic liposomes for transdermal drug delivery. *Current Drug Delivery*. 2009;6:217-226. DOI: 10.2174/156720109788680707
30. Touitou E, Dayan N, Bergelson L, et al. Ethosomes as vesicular carriers. *Journal of Controlled Release*. 2000;65:403-418. DOI: 10.1016/S0168-3659(99)00222-9
31. Stuart B. Infrared Spectroscopy: Fundamentals and Applications. *John Wiley & Sons*. 2004. DOI: 10.1002/0470011149
32. Malvern Instruments. Dynamic Light Scattering Guide. *Malvern Instruments Ltd*. 2011.
33. Hunter RJ. Zeta Potential in Colloid Science: Principles and Applications. *Academic Press*. 1981. DOI: 10.1016/C2013-0-10868-2
34. Tadros T. Colloids in Cosmetics and Personal Care. *Wiley-VCH*. 2008. DOI: 10.1002/9783527612000
35. Li X, Anton N, Zuber G, et al. Nanocarriers for drug delivery. *Journal of Controlled Release*. 2011;152:49-57. DOI: 10.1016/j.jconrel.2010.11.033
36. Costa P, Sousa Lobo JM. Modeling dissolution profiles. *European Journal of Pharmaceutical Sciences*. 2001;13:123-133. DOI: 10.1016/S0928-0987(01)00095-1
37. Higuchi T. Mechanism of sustained action medication. *Journal of Pharmaceutical Sciences*. 1963;52:1145-1149. DOI: 10.1002/jps.2600521210
38. Singhvi G, Singh M. In-vitro drug release characterization models. *International Journal of Pharmaceutical Studies and Research*. 2011;2:77-84.
39. United States Pharmacopeia. USP 43–NF 38. *USP Convention*. 2020.
40. Shah VP, Elkins JS, Williams RL. In vitro release testing for topical products.

Pharmaceutical Development and Technology. 1999;4:377-385. DOI: 10.1081/PDT-100101363

41. Franz TJ. Percutaneous absorption studies. *Journal of Investigative Dermatology*. 1975;64:190-195. DOI: 10.1111/1523-1747.ep12533356
42. Hadgraft J, Lane ME. Skin permeation principles. *International Journal of Pharmaceutics*. 2005;305:2-12. DOI: 10.1016/j.ijpharm.2005.08.034
43. ICH. Stability Testing of New Drug Substances and Products Q1A(R2). *International Conference on Harmonisation*. 2003.
44. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. *Lippincott Williams & Wilkins*. 2011.
45. Sinko PJ. Martin's Physical Pharmacy and Pharmaceutical Sciences. *Lippincott Williams & Wilkins*. 2011.
46. Bhowmik D, Chiranjib B, Chandira RM. Advances in topical drug delivery systems. *The Pharma Innovation*. 2012;1(9):12-31.
47. Tadros T. Rheology of Dispersions. *Wiley-VCH*. 2010. DOI: 10.1002/9783527632145
48. Bhardwaj V, Bansal M. Evaluation of topical gels. *International Journal of Pharmaceutical Sciences Review and Research*. 2013;20:66-70.
49. Bonacucina G, Cespi M, Palmieri GF. Rheological properties of hydrogels. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009;72:411-418. DOI: 10.1016/j.ejpb.2009.01.017
50. Costa P, Sousa Lobo JM. Dissolution profile comparison. *European Journal of Pharmaceutical Sciences*. 2001;13:123-133. DOI: 10.1016/S0928-0987(01)00095-1
51. Verma DD, Verma S, Blume G, Fahr A. Liposomes enhance skin penetration. *European Journal of Pharmaceutics and Biopharmaceutics*. 2003;55:271-277. DOI: 10.1016/S0939-6411(02)00175-6
52. Hunter RJ. Zeta potential in colloidal stability. *Academic Press*. 1981.
53. Akbarzadeh A, Rezaei-Sadabady R, Davaran S. Liposome drug delivery systems. *Nanoscale Research Letters*. 2013;8:102. DOI: 10.1186/1556-276X-8-102
54. Honeywell-Nguyen PL, Bouwstra JA. Vesicular dermal drug delivery. *Drug Discovery Today: Technologies*. 2005;2:67-74. DOI: 10.1016/j.ddtec.2005.05.004
55. Bonacucina G, Cespi M, Palmieri GF. Hydrogels in topical formulations. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009;72:411-418. DOI: 10.1016/j.ejpb.2009.01.017
56. Singhvi G, Singh M. Drug release mechanisms from gels. *International Journal of Pharmaceutical Studies and Research*. 2011;2:77-84.
57. Shah VP, Elkins JS. Topical drug release testing. *Pharmaceutical Development and Technology*. 1999;4:377-385. DOI: 10.1081/PDT-100101363
58. Hadgraft J, Lane ME. Skin drug delivery mechanisms. *International Journal of Pharmaceutics*. 2005;305:2-12. DOI: 10.1016/j.ijpharm.2005.08.034
59. Touitou E, Dayan N. Vesicular carriers in dermal delivery. *Journal of Controlled Release*. 2000;65:403-418. DOI: 10.1016/S0168-3659(99)00222-9