

**“PREPARATION, DEVELOPMENT AND *IN VITRO* EVALUATION OF RETAPAMULIN
LOADED GLYCEROSOMES FORMULATION”****Kuldeep Kushwaha^{1*}, Pinky Singh², Hemant Agrawal³, Naveen Gupta⁴**¹Student- Madhyanchal Professional University (MPU), Bhopal (M.P)²Professor- Madhyanchal Professional University (MPU), Bhopal (M.P)³Associate Professor- Madhyanchal Professional University (MPU), Bhopal (M.P)⁴Dean- Madhyanchal Professional University (MPU), Bhopal (M.P)***Corresponding Author: Kuldeep Kushwaha**

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ABSTRACT

The present study aimed to develop, optimize, and evaluate Retapamulin-loaded glycosomes using a Quality by Design (QbD) approach. Preformulation studies showed that Retapamulin was a white to off-white, odorless crystalline powder with poor aqueous solubility. The melting point was found to be 128°C and the λ_{max} was observed at 249 nm. A linear calibration curve was obtained over the concentration range of 10–50 µg/mL. FTIR analysis confirmed the characteristic functional groups of Retapamulin. A Box–Behnken design using Response Surface Methodology was employed to optimize the formulation, with phospholipid, cholesterol, and glycerol concentrations as independent variables and particle size and entrapment efficiency as responses. The optimized formulation showed a particle size of 322.7 nm, zeta potential of –30.2 mV, and entrapment efficiency of 97.52%. The predicted values for particle size and entrapment efficiency were 319.313 nm and 95.129%, respectively, showing good agreement with the experimental results. SEM analysis revealed predominantly spherical to oval vesicles with smooth morphology and no significant aggregation. In vitro drug-release studies indicated that the formulation followed Higuchi and Korsmeyer–Peppas models, suggesting predominantly diffusion-controlled release with possible anomalous transport. Overall, QbD-assisted optimization successfully produced Retapamulin-loaded glycosomes with desirable physicochemical characteristics and promising controlled-release properties for topical drug delivery.

KEYWORDS: Retapamulin, Glycosomes, Quality by Design, Box–Behnken Design, Response Surface Methodology, Optimization, Entrapment Efficiency, Topical Drug Delivery.**1. INTRODUCTION**

Glycosomes are novel vesicular structures containing phospholipids, water and glycerol in high amounts. Glycerol is harmless, non-toxic and non-irritating in nature and thus safe to use. It is added to vesicular preparations since it results in an increase in fluidity and stability of vesicles. Increased fluidity of glycosomes aids in better penetration on skin surface than the conventional liposomes (Zaki *et al.*, 2022). Phospholipids are added to glycosomes for the formation of bilayer structures. Both synthetic and natural phospholipids can be added to glycosomes. Cholesterol, stearylamine, diacetyl phosphate are added as stabilizers or for altering the electric charge on

vesicles. These novel vesicular structures can be prepared from the methods used for conventional liposomes (Okafor *et al.*, 2025). Both hydrophilic and lipophilic drugs can be incorporated in glycosomes. Hydrophilic drugs get entrapped in aqueous space whereas hydrophobic drugs get entrapped in phospholipid. Unilamellar or multilamellar vesicles (MLVs) are formed based on the excipients and the method selected for the preparation of glycosomes. These nanostructures are recently gaining interest worldwide due to their ease of preparation, harmless nature and better characteristics than the conventional vesicular structures (Jha *et al.*, 2022).

The performance of glycosomal formulations is strongly influenced by formulation and process variables such as lipid concentration, glycerol concentration, surfactant concentration, drug-to-lipid ratio, hydration conditions and processing parameters. Conventional formulation development generally involves changing one factor at a time, which can require considerable time and may fail to identify interactions among formulation variables (**Salem *et al.*, 2018**). Quality by Design (QbD) provides a systematic and science-based approach to pharmaceutical development in which critical quality attributes (CQAs), critical material attributes (CMAs) and critical process parameters (CPPs) are identified and evaluated to achieve a predefined quality target product profile. The application of QbD and Design of Experiments (DoE) enables the simultaneous investigation of multiple formulation variables and their interactions, thereby facilitating rational optimization and reducing experimental effort (**Yang *et al.*, 2025**).

In the development of vesicular drug delivery systems, important CQAs may include vesicle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, deformability, pH, viscosity and in vitro drug-release characteristics (**Nieves *et al.*, 2026**). These parameters can significantly influence the physical stability, drug retention, release behavior and topical performance of the formulation. A systematic QbD approach can therefore provide a better understanding of the relationship between formulation variables and the resulting quality attributes of retapamulin-loaded glycosomes (**Firmansyah *et al.*, 2026**).

Retapamulin is a pleuromutilin-class topical antibacterial agent used primarily for the treatment of superficial bacterial skin infections. It exerts its antibacterial activity by selectively binding to the bacterial 50S ribosomal subunit and inhibiting bacterial protein synthesis (**Dallo *et al.*, 2023**). Although retapamulin is suitable for topical application, conventional semisolid formulations may not provide optimal drug deposition and penetration into the relevant layers of the skin. The development of an appropriate nanocarrier can therefore improve the interaction of retapamulin with the skin, enhance drug deposition and provide a more controlled release profile (**Jain *et al.*, 2022**).

Therefore, the present investigation was designed to develop a retapamulin-loaded glycosomal formulation using a systematic Quality by Design approach. Formulation and process variables were studied using an appropriate Design of Experiments strategy to establish their influence on selected critical quality attributes and to identify an optimized formulation.

2. MATERIALS AND METHODS

2.1 Chemicals

Retapamulin, were obtained from Kavya Pharma. Loba Chemie Pvt. Ltd provided the Phosphatidylcholine, Propylene glycol and Potassium dihydrogen phosphate.

Merck Life Science Pvt. Ltd provided the Glycerol, Ethanol, Triethanolamine, Methanol, Sodium hydroxide and Hydrochloric acid while Sigma-Aldrich, India supplied Cholesterol. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Ams Fine Chemical, Pandora Industries, Suvchem, Manas Petro Chem and Vizag chemical.

2.2 Pre-formulation Studies

Pre-formulation studies were performed to evaluate the fundamental physicochemical properties of Retapamulin prior to formulation development. These investigations provided essential information regarding the drug's behavior in different environments, including its solubility, stability (**Chaurasia, 2016**).

2.2.1 Organoleptic Properties

The organoleptic characteristics of *Retapamulin* including color, odor, and physical appearance, were examined using standard evaluation methods. These parameters were assessed to ensure the identity, purity, and overall quality of the drug before proceeding with the development of the Retapamulin-loaded glycosomal formulation (**Elgammal, 2025**).

2.2.2 Solubility Study

The solubility profile of *Retapamulin* was evaluated in various solvents using a simple qualitative method. A measured quantity of the drug was introduced into different solvents such as water, chloroform, ethanol, methanol, phosphate buffer (pH 7.4), and DMSO, followed by continuous stirring. The solutions were then visually examined for clarity to assess the extent of solubility. The results indicated that *Retapamulin* exhibited high solubility in ethanol, methanol, and phosphate buffer (pH 7.4), while limited solubility was observed in certain other solvents. These findings were useful in selecting suitable solvents and excipients for effective formulation development (**Breda *et al.*, 2009**).

2.2.3 Melting Point Determination

The melting point of *Retapamulin* was determined using the capillary method. This determination helped in assessing the purity and identity of the drug sample prior to formulation development (**Fedosejevs *et al.*, 2025**).

2.2.4 pH Determination of the Retapamulin

The pH of Retapamulin was determined by preparing a suitable solution of the drug in distilled water or an appropriate solvent system. The solution was stirred to ensure uniform dispersion, and the pH was measured using a calibrated digital pH meter at room temperature (**Nalwade and Reddy, 2014**).

2.2.5 Standard λ Max Determination

Retapamulin stock solution (1 mg/mL) was prepared in ethanol and suitably diluted to obtain a working solution of 100 μ g/mL. The solution was scanned from 200–400 nm using a Shimadzu 1700 double-beam UV–Visible spectrophotometer with ethanol as blank. The absorption

spectrum was recorded, and the wavelength of maximum absorbance (λ_{max}) was identified for further quantitative analysis and formulation studies (Paramelle *et al.*, 2014).

2.2.6 Calibration Curve Analysis

To construct the calibration curves for Retapamulin, stock solutions of drug were prepared and appropriately diluted to obtain a series of working solutions with concentrations of 10, 20, 30, 40, and 50 $\mu\text{g/mL}$ for Retapamulin. The absorbance of each solution was measured using a UV-Visible spectrophotometer, with ethanol used as a blank to calibrate the instrument. The absorbance values obtained were plotted against their corresponding concentrations, with concentration on the X-axis and absorbance on the Y-axis. The resulting calibration curves for both drugs displayed a linear relationship, indicating compliance with Beer-Lambert's law over the tested concentration range, thereby validating the method for accurate and reliable quantitative analysis of Retapamulin in formulation studies.

2.3 Fourier Transform Infrared (FT-IR) Spectroscopy of selected drug

FT-IR spectroscopy of Retapamulin was performed to identify its characteristic functional groups and to confirm the chemical integrity of the drug prior to formulation development. In this method, a small amount of the finely powdered drug sample was accurately weighed and mixed thoroughly with dry potassium bromide (KBr) in an appropriate ratio. The mixture was

then compressed under high pressure using a hydraulic press to form a transparent KBr pellet.

The prepared pellet was placed in the sample holder of the FT-IR spectrophotometer, and the spectrum was recorded over a wavelength range of 4000–400 cm^{-1} . The obtained spectrum displayed distinct absorption peaks corresponding to various functional groups present in the drug molecule. These peaks were analyzed and compared with reported reference values to confirm the identity and purity of Retapamulin (Paramelle *et al.*, 2014).

2.4 Formulation of Retapamulin- Loaded Glycosomes

Glycosomes were prepared by the thin-film hydration method according to the DoE formulation compositions (GS1–GS12). Specified amounts of phospholipid and cholesterol were dissolved in methanol and chloroform (10 mL each) and evaporated under reduced pressure using a rotary evaporator to form a thin lipid film. The film was vacuum-dried and hydrated with glycerol-containing phosphate buffer (pH 6.8) at 40°C with continuous stirring for 1 h. The resulting dispersion was ultrasonicated to obtain a homogeneous nanosized glycosomal formulation and stored under suitable conditions for further evaluation. Formulations were varied according to the phospholipid, glycerol, and cholesterol concentrations specified in the experimental design (Rani *et al.*, 2021).

Table 1: Composition of Glycosomes Formulation.

Formulation table as per Design of experiment software (DOE)					
Formulation Code	Phospholipid (mg)	Glycerol (%)	Cholesterol %	Methanol (mL)	Chloroform (mL)
GS1	15.0	10.0	3.5	10.0	10.0
GS2	30.0	20.0	5.0	10.0	10.0
GS3	22.5	10.0	2.0	10.0	10.0
GS4	22.5	30.0	2.0	10.0	10.0
GS5	30.0	20.0	2.0	10.0	10.0
GS6	15.0	20.0	5.0	10.0	10.0
GS7	22.5	30.0	5.0	10.0	10.0
GS8	30.0	10.0	3.5	10.0	10.0
GS9	22.5	10.0	5.0	10.0	10.0
GS10	15.0	20.0	2.0	10.0	10.0
GS11	15.0	30.0	3.5	10.0	10.0
GS12	30.0	30.0	3.5	10.0	10.0

2.5 Characterization parameter of Drug loaded Glycosome

2.5.1 Physical Appearance

The physical appearance of the Retapamulin -loaded glycosomal formulation was evaluated by visual inspection. The formulation was observed for parameters such as color, clarity, homogeneity, and presence of any particulate matter or phase separation (Dhaka *et al.*, 2026).

2.5.2 Particle size analysis

The vesicle size and size distribution of Retapamulin-loaded glycosomes were determined using the dynamic light scattering (DLS) technique (Salem *et al.*, 2018).

2.5.3 Zeta Potential

The zeta potential of Retapamulin-loaded glycosomal formulations was determined using a Malvern Zetasizer

(Malvern Instruments, UK) based on the principle of electrophoretic light scattering (Md *et al.*, 2021).

2.5.4 Morphological analysis of the optimized Glycosome formulation by SEM

The surface morphology of the optimized Retapamulin-loaded glycosomes was evaluated using Scanning Electron Microscopy (SEM). A small amount of lyophilized sample was mounted on a metal stub, gold-coated under vacuum, and examined at suitable magnifications. The obtained micrographs were analyzed for vesicle shape, surface texture, size distribution, and morphological integrity, confirming the formation and uniformity of the glycosomal vesicles (Aubin, 2012).

2.5.5 Entrapment Efficiency Determination

The entrapment efficiency of Retapamulin-loaded glycosomes was determined to quantify the amount of drug successfully encapsulated within the vesicular system. The formulation was subjected to centrifugation at a suitable speed and time to separate the untrapped (free) drug from the vesicle-entrapped drug. After centrifugation, the supernatant containing the free drug was carefully collected and analyzed using a UV-Visible spectrophotometer at the predetermined λ max. The concentration of untrapped drug was calculated using a standard calibration curve.

The entrapment efficiency was then determined using the following formula:

$$\text{Entrapment Efficiency (\%)} = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100$$

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 2: Organoleptic Properties of Retapamulin.

Drug	Organoleptic properties	Observation
Retapamulin	Color	White to off-white
	Odor	Odorless
	Appearance	Crystalline solid / powder.
	State	Solid

3.1.2 pH and melting point determination

Table 3: pH and melting point of Retapamulin

Drugs	Observed (pH)	Reference (pH)	Observed (melting point)	Reference (melting point)
Retapamulin	6.4pH	6.0 -8.0 pH	128 °C	127°C to 129°C

All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. This parameter was important for evaluating the drug loading capacity and effectiveness of the glycosomal delivery system (Ghadiri *et al.*, 2012).

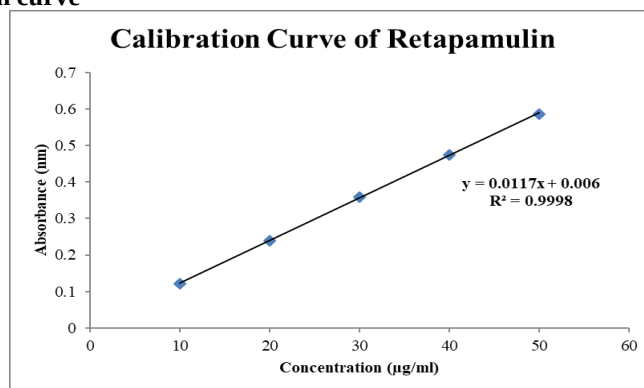
2.6 In vitro drug release study of Drug loaded Glycosome formulation

The in-vitro drug release of the optimized drug-loaded glycosomal formulation was evaluated using the dialysis membrane diffusion technique. A known quantity of formulation was placed in a pre-soaked dialysis membrane and immersed in 100 mL PBS (pH 7.4) maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm. At predetermined intervals, aliquots were withdrawn and replaced with equal volumes of fresh pre-warmed PBS. The samples were analyzed by UV-Visible spectrophotometry at the drug's λ_{max} . Cumulative percentage drug release was calculated using the calibration curve and plotted against time (Mast *et al.*, 2021).

2.6.1 Kinetic Modelling of Drug Release

The obtained *in-vitro* drug release data of the optimized drug-loaded glycosome formulation were fitted to different mathematical kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, to determine the release pattern and possible mechanism of drug release. The model showing the highest correlation coefficient (R^2) was considered the best-fit model for describing the drug-release behavior of the optimized glycosomal formulation.

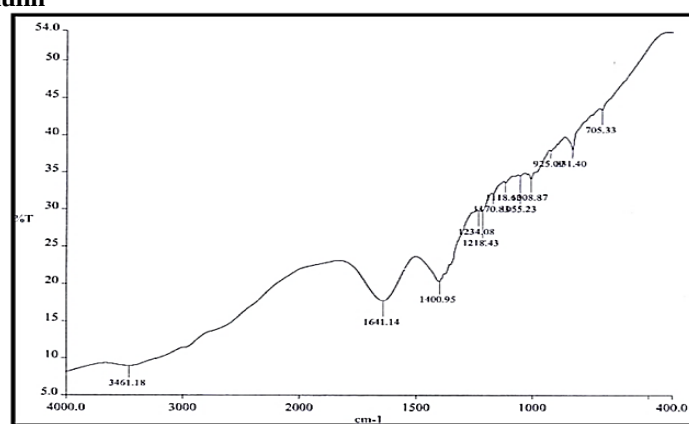
3.1.2.1 Standard calibration curve



Graph 1: Calibration Curve of Retapamulin.

3.1.3 Functional group identified by Infra-Red spectroscopy

3.1.3.1 FTIR of Retapamulin



Graph 2: FTIR of Retapamulin.

3.2 Optimization of glycosomes formulations by the DoE approach

3.2.1 Formulation trials as per Box–Behnken design

Table 4: Formulation trials as per Box–Behnken design.

Formulation Code	Phospholipid (mg)	Glycerol (%)	Cholesterol %	Particle size (nm)	Entrapment Efficiency
GS1	15	10	3.5	408.11	93.63
GS2	30	20	5	436.42	94.55
GS3	22.5	10	2	463.17	92.46
GS4	22.5	30	2	756.81	77.07
GS5	30	20	2	230.44	95.21
GS6	15	20	5	753.19	85.26
GS7	22.5	30	5	533.78	80.11
GS8	30	10	3.5	280.46	96.28
GS9	22.5	10	5	401.21	93.18
GS10	15	20	2	561.23	84.44
GS11	15	30	3.5	647.75	85.23
GS12	30	30	3.5	588.35	87.06

3.2.2 Limits of Variables (Constraints)

Table 5: Variables Operating Range for Glycosome Formulation.

Name	Goal	Lower Limit	Upper Limit	Importance
A:Phospholipid	is in range	15	30	3
B:Glycerol	is in range	10	30	3
C:Cholesterol	is in range	2	5	3
Particle size	none	230.44	756.81	3
Entrapment efficiency	none	77.07	96.28	3

3.2.3 Fit Summary

Response 1: Particle size

Table 6: Response 1: Particle size (Fit Summary).

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.0247	0.5477	0.2599	Suggested
2FI	0.9339	0.3313	-0.7509	
Quadratic	0.8871	-0.0291	-3.4905	Aliased

3.3 Effect of formulation variables on particle size (ANOVA for Linear model)

3.3.1 Response 1: Particle size

Table 7: Response 1: Particle size (ANOVA for Linear model).

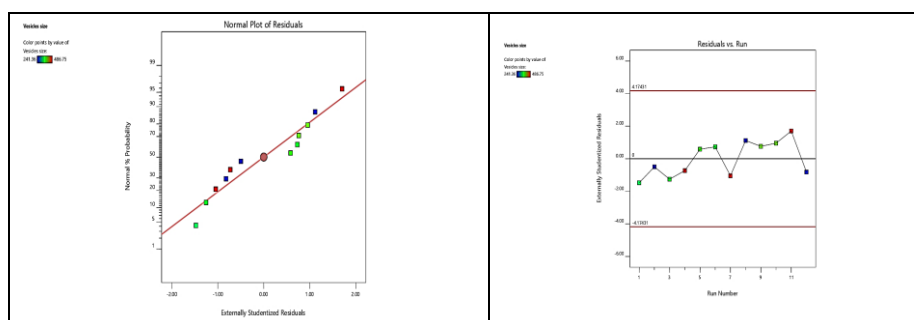
Source	Sum of Squares	Mean Square	F-value	p-value	
Model	2.072E+05	69062.55	5.44	0.0247	significant
A-Phospholipid	87071.73	87071.73	6.86	0.0307	
B-Glycerol	1.185E+05	1.185E+05	9.34	0.0157	
C-Cholesterol	1594.71	1594.71	0.1256	0.7322	
Residual	1.015E+05	12693.52			
Cor Total	3.087E+05				

3.3.2 Final Equation in Terms of Coded Factors

The relationship between the selected formulation variables and particle size was established using a polynomial equation generated through the Box–Behnken experimental design. The developed regression model provides a mathematical representation of the

influence of formulation factors on the particle size of Retapamulin-loaded glycosomes and can be used to predict the response within the experimental domain.

Particle size (A) = 505.08 (Intercept) -104.33A + 121.72 B + 14.12C.

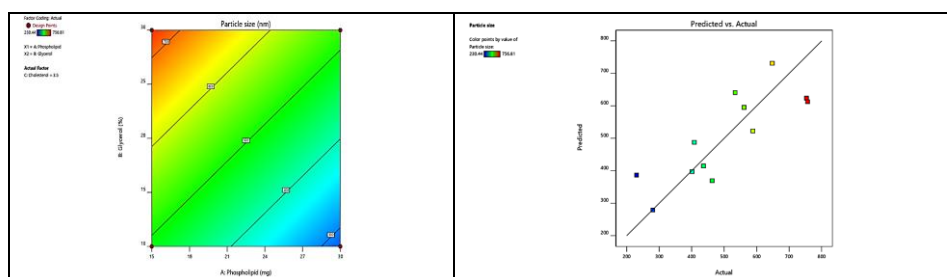
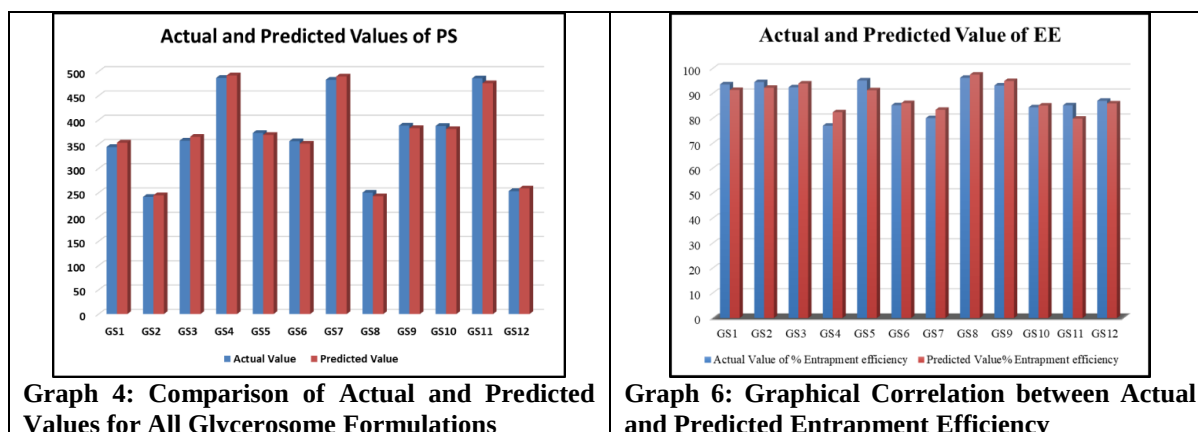


Graph 3: Diagnostic Residual Plots (Normal Plot and Residuals vs. Run) for Particle Size Response.

3.3.3 Predicted value and actual value of all Formulations

Table 8: Predicted value and actual value of Particle size and Entrapment efficiency all Glycosome Formulations.

Formulations	Actual Result of particle size	Predicted Result of particle size	Actual Value of % Entrapment efficiency	Predicted Value% Entrapment efficiency
GS1	344.25	353.28	93.63	91.40
GS2	241.36	244.80	94.55	92.26
GS3	357.33	365.29	92.46	93.98
GS4	486.75	491.68	77.07	82.46
GS5	373.22	369.22	95.21	91.28
GS6	356.26	351.32	85.26	86.13
GS7	482.90	489.71	80.11	83.44
GS8	250.06	242.83	96.28	97.53
GS9	388.33	383.19	93.18	94.96
GS10	387.53	381.23	84.44	85.15
GS11	485.79	475.74	85.23	79.88
GS12	253.28	258.77	87.06	86.01



Graph 5: Response Surface Plot Illustrating the Combined Effect of Formulation Variables on Glycosome Characteristics.

3.3.4 Effect of formulation variables on Entrapment efficiency

Table 9: Response 2: Entrapment efficiency (Fit Summary).

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.0065	0.6805	0.4772	Suggested
2FI	0.9907	0.4990	-0.3118	
Quadratic	0.3109	0.6168	-0.6723	Aliased

3.4 ANOVA for linear model

3.4.1 Response 2: EE (ANOVA Linear)

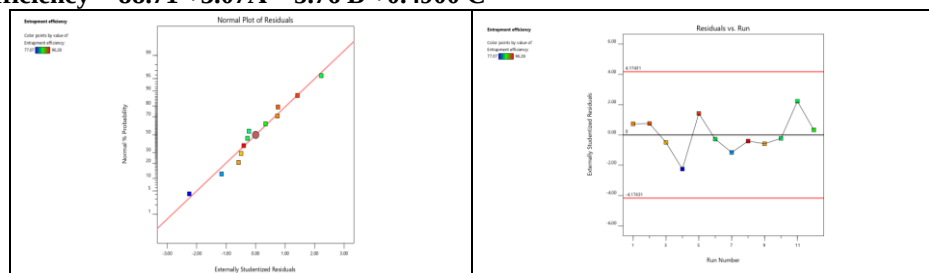
Table 10: Response 2: Entrapment Efficiency.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	342.62	114.21	8.81	0.0065	significant
A-Phospholipid	75.28	75.28	5.81	0.0425	
B-Glycerol	265.42	265.42	20.47	0.0019	
C-Cholesterol	1.92	1.92	0.1482	0.7103	
Residual	103.71	12.96			
Cor Total	446.33				

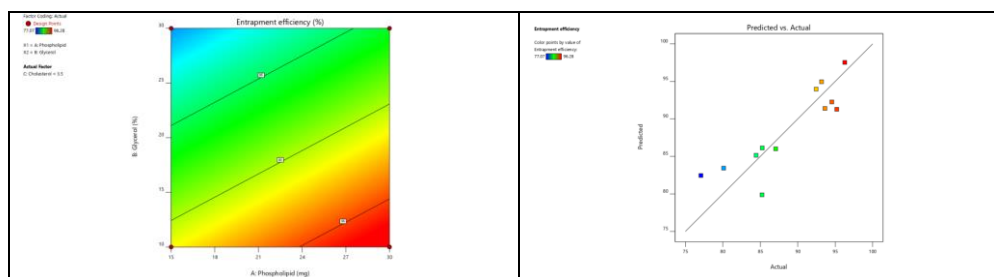
3.4.2 Final Equation in Terms of Coded Factors

The regression model developed for entrapment efficiency, expressed in terms of coded variables, is presented as follows:

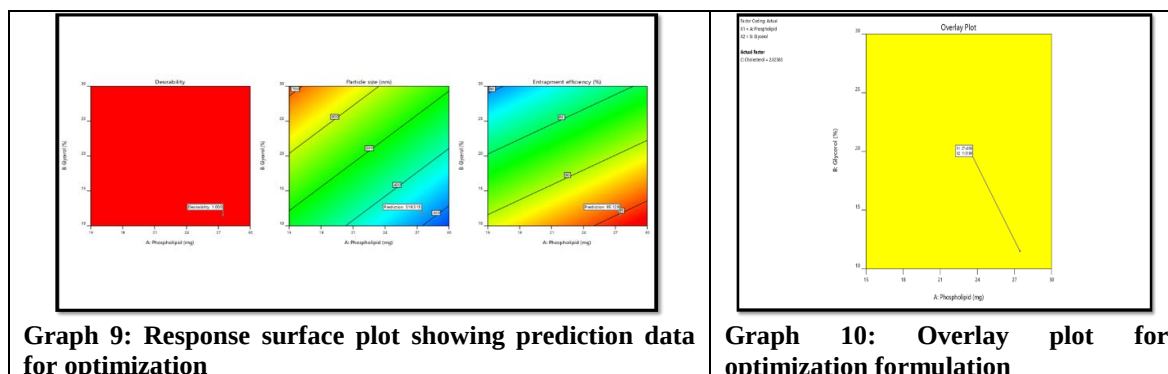
$$\text{Entrapment efficiency} = 88.71 + 3.07A - 5.76B + 0.4900C$$



Graph 7: Graphical representation of Residuals vs. run, Normal plot of Glycosome formulation on Entrapment efficiency.



Graph 8: Two-dimensional contour plots for the effect of Phospholipid and cholesterol concentration on % entrapment efficiency and predicted vs. actual.



Graph 9: Response surface plot showing prediction data for optimization

Graph 10: Overlay plot for optimization formulation

3.4.4 Optimized formula of Glycosome formulation

Table 11: Optimized formula of Glycosome formulation.

Phosphatidylcholine (mg)	Cholesterol (mg)	Glycerol (%)	Vesicle size (nm)	% EE	Desirability	
23.155	17.175	3.595	462.473	90.633	1.000	
27.436	11.519	2.026	319.313	95.129	1.000	Selected

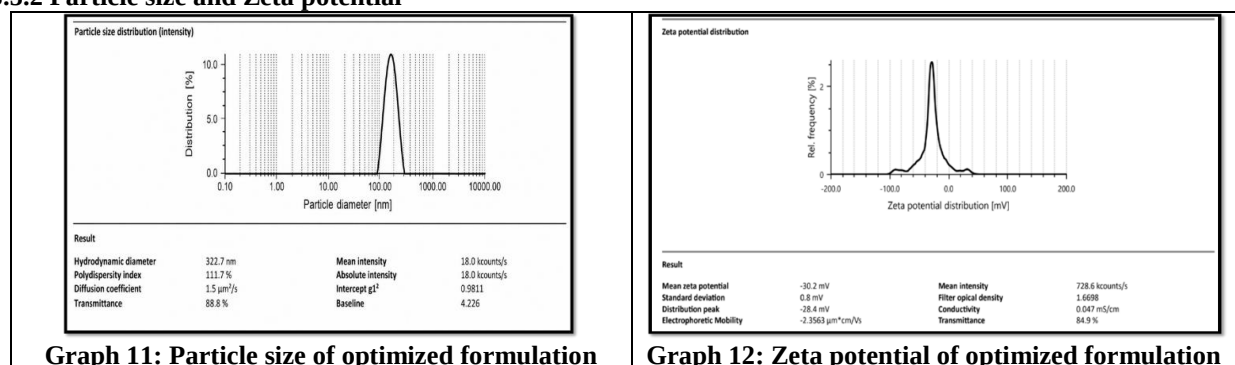
3.5 Characterization of optimized Glycosome formulation

3.5.1 Physical Appearance

Table 12: Physical Appearance of Retapamulin loaded Glycosome.

Parameter	Observation
Color	White to off-white
Odor	Odorless
Appearance	Smooth, uniform
State	Liquid

3.5.2 Particle size and Zeta potential



Graph 11: Particle size of optimized formulation

Graph 12: Zeta potential of optimized formulation

Table 13: Particle size, Entrapment efficiency and Zeta potential of Glycosome formulation

Formulation	Zeta potential	Particle size (Predicted value)	Particle size (Actual value)	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)
Glycosome	-30.2 mV	319.313 nm	322.7 nm	95.129 %	97.52 %

3.5.3 SEM analysis of Glycosome formulation

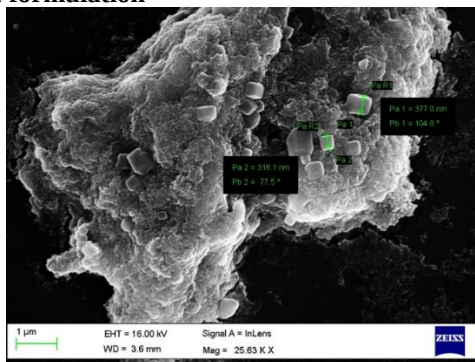
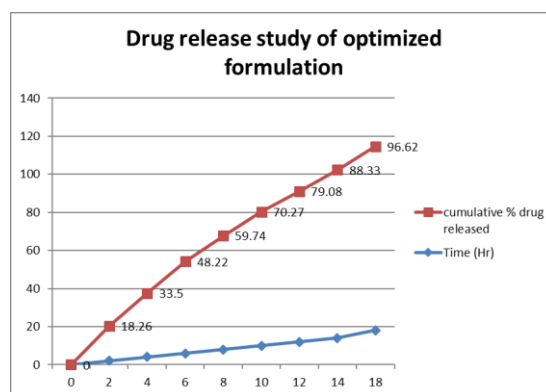


Figure 1: SEM.

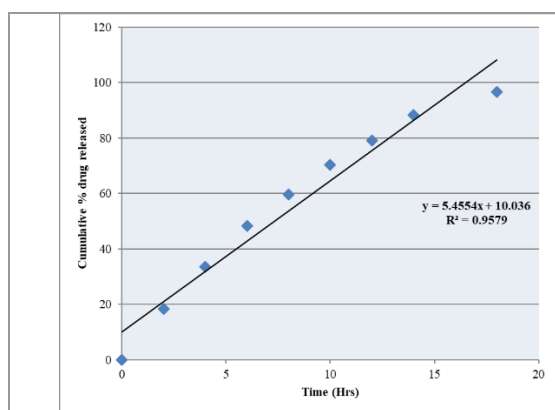
3.6 In Vitro drug release study



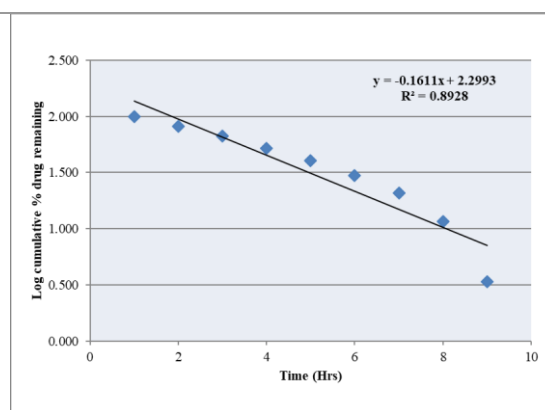
Graph 13: Kinetics Models of optimized formulation.

Table 14: Correlation value (R^2 value).

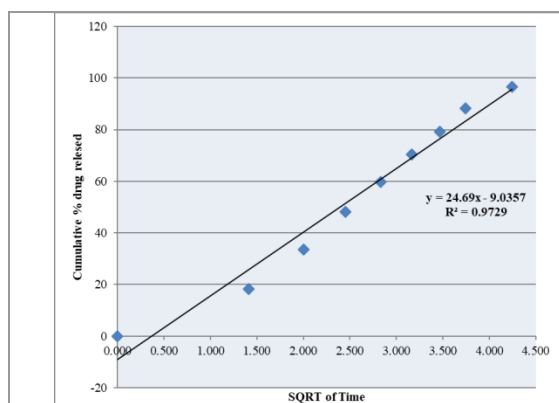
Formulation	Model	Kinetic parameter values
Glycosomes formulation	Zero Order	$R^2 = 0.9579$
	First Order	$R^2 = 0.8928$
	Higuchi	$R^2 = 0.9729$
	Korsmeyer peppas	$R^2 = 0.8401$



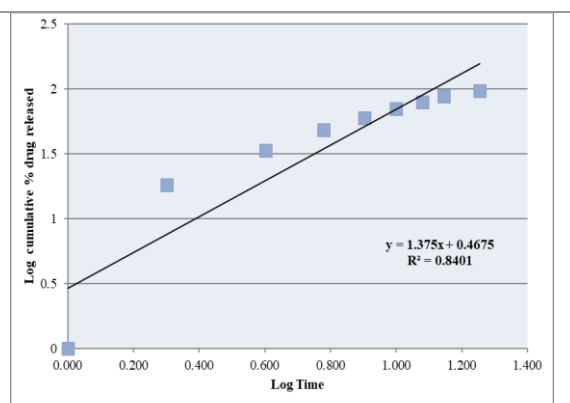
Graph 14: Zero order



Graph 15: First Order



Graph 16: Higuchi model



Graph 17: Korsmeyer peppas

DISCUSSION

The present study successfully demonstrated the development and optimization of Retapamulin-loaded glycosomes using a Quality by Design approach. Preformulation studies confirmed the acceptable physical characteristics of Retapamulin, while its poor aqueous solubility supported the use of a lipid-based vesicular system. The Box–Behnken design effectively evaluated the influence of phospholipid, cholesterol, and glycerol concentrations on particle size and entrapment efficiency. The optimized formulation exhibited a particle size of 322.7 nm, zeta potential of -30.2 mV, and high entrapment efficiency of 97.52%, indicating satisfactory vesicle formation, physical stability, and drug-loading capacity. The close agreement between predicted and experimental values confirmed the reliability of the developed optimization model. SEM analysis showed predominantly spherical to oval vesicles with smooth surfaces and no prominent aggregation. *In vitro* release studies showed better fitting with the Higuchi and Korsmeyer–Peppas models, indicating predominantly diffusion-controlled release with possible anomalous transport. Overall, the findings suggest that QbD-assisted Retapamulin-loaded glycosomes possess favorable characteristics and may serve as a promising vesicular system for topical drug delivery.

4. CONCLUSION

The present investigation successfully established a QbD-assisted approach for the development and optimization of Retapamulin-loaded glycosomes. Preformulation investigations confirmed the physicochemical suitability of Retapamulin for incorporation into a lipid-based vesicular delivery system, particularly considering its poor aqueous solubility. The application of the Box–Behnken design provided an efficient and systematic means of understanding the effects of phospholipid, glycerol, and cholesterol on particle size and entrapment efficiency. The optimized formulation exhibited a particle size of 322.7 nm, high entrapment efficiency of 97.52%, and zeta potential of -30.2 mV, indicating satisfactory vesicle formation, drug incorporation, and physical stability. The close correlation between predicted and

experimental values further validated the statistical optimization procedure.

The optimized glycosomes demonstrated progressive drug release, with 96.62% Retapamulin released within 18 h, and the release behavior was better explained by the Higuchi and Korsmeyer–Peppas models, suggesting predominantly diffusion-controlled and potentially anomalous release. The formulation retained satisfactory entrapment efficiency and exhibited only a limited increase in particle size during 90 days of storage, particularly under room-temperature conditions. Therefore, the developed Retapamulin-loaded glycosomal system represents a promising vesicular platform for topical delivery, with the potential to improve drug localization, prolong drug release, and enhance the efficiency of Retapamulin administration compared with conventional delivery approaches. However, the present findings are based primarily on physicochemical characterization and *in vitro* evaluation, and further studies are necessary before definitive therapeutic claims can be made.

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