

FORMULATION AND EVALUATION OF CUBOSOMES FOR ENHANCED DELIVERY OF APIGENIN

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ABSTRACT

The present study focused on the formulation and evaluation of Apigenin-loaded cubosomes for enhanced drug delivery. Pre-formulation studies confirmed the suitability of Apigenin, which showed poor aqueous solubility, a melting point of 346°C, pH 6.4, and λ_{max} at 327 nm. The formulation was optimized using a Box–Behnken Design (BBD) under Quality by Design (QbD) principles, with Glyceryl Monooleate (GMO), Poloxamer 407, and stirring time selected as critical formulation variables. The optimized formulation showed a particle size of 196.32 nm, zeta potential of –23.4 mV, and entrapment efficiency of 95.578%. SEM analysis revealed nearly spherical nanoparticles with a rough surface morphology. The optimized cubosomes exhibited sustained drug release, reaching 92.56% over 12 h. Drug release kinetics showed the best fit with the Zero-order model ($R^2 = 0.951$), indicating controlled drug release. Overall, the findings demonstrate that Apigenin-loaded cubosomes can provide a suitable lipid-based nanocarrier for improving the delivery of poorly water-soluble Apigenin.

KEYWORDS: Apigenin; Cubosomes; Quality by Design; Box–Behnken Design; Glyceryl Monooleate; Poloxamer 407; Nanocarriers; Entrapment Efficiency; Sustained Drug Release; Zero-order Kinetics.

1. INTRODUCTION

Nanotechnology-based drug delivery systems have emerged as promising approaches for overcoming the limitations associated with poorly water-soluble bioactive compounds. Nanocarriers can improve drug solubilization, protect incorporated compounds from premature degradation, enhance drug dispersion, and provide controlled or sustained release. Among various lipid-based nanocarriers, cubosomes have received significant attention because of their unique internal structure and ability to accommodate compounds with different physicochemical characteristics (Sivadasan *et al.*, 2023).

Cubosomes are discrete, sub-micron, nanostructured particles of cubic liquid crystalline phase. These are microstructure particles containing surfactants with proper ratio of water. They are particles with a solid-like rheology that provides unique properties of practical interest (Rao *et al.*, 2018). Cubosomes size ranges from 10-500nm in diameter. They appear like dots, square

shaped, slightly spherical, where each dot corresponds to the presence of pore size of 5-10nm. Cubosomes are typically produced by high-energy dispersion of bulk cubic phase, followed by colloidal stabilization using polymeric surfactants. After formation of the cubosomes, the dispersion is formulated into a suitable dosage form (Sureka *et al.*, 2018).

Cubosomes have great potential in formulating nano sized particulate systems for topical delivery owing to their best advantages such as high drug payload due to high internal surface area, low viscosity and can exist at almost any dilution level. Structurally these are lipid vesicles formed from amphiphilic building blocks, which mimics bio membranes that can be used for carrier potential of hydrophilic, lipophilic and amphiphilic drugs as compared to free drug directly delivered to the particular site of action, thus allowing drug targeting and the sustained or controlled release of conventional medicines (Nath *et al.*, 2024). However the efficiency of cubosomes alone to deliver the drugs in a controlled way

is less when compared with cubosomes incorporated with polymers. The high viscosity of these cubosomes is a challenging task during large scale preparation in industries. Cubosomes can be administrated in many ways such as oral, percutaneous, intravenous route (Sivadasan *et al.*, 2023). Cubic phase is unique and desirable because of its mesoscale structure: a twisted lipid bilayer, separating two continuous but non-intersecting water regions. The tortuous structure of bulk cubic phase provides controlled release of solubilized active ingredients, while cubosomes exhibit burst release because of their sub-micron length scales (Sri *et al.*, 2017).

Apigenin is a naturally occurring flavonoid that shows promise in treating various inflammatory conditions of skin disorders. Apigenin functions by preventing the synthesis and release of chemicals that cause inflammation, such as IL-1 (Interleukin-1), IL-6 (Interleukin-6), and TNF-alpha (Tumor necrosis factor-alpha) (Jamshidi *et al.*, 2024). It also stops mast cells from secreting their contents and inhibits NF-kB (Nuclear factor kappa-light-chain-enhancer) from becoming activated. In doing so, apigenin contributes to a decrease in inflammation, histamine release, and the production of other inflammatory chemicals (Savarese *et al.*, 2021).

Despite its broad biological potential, the pharmaceutical utilization of apigenin is restricted by several physicochemical and biopharmaceutical limitations. Apigenin exhibits poor aqueous solubility and limited dissolution, which can result in inadequate and variable absorption following conventional administration (Tang *et al.*, 2017). Its hydrophobic nature, together with limited bioavailability and susceptibility to metabolic degradation, may reduce the concentration of the active compound reaching the systemic circulation or target site. Therefore, development of an appropriate drug delivery system capable of improving the solubility, stability, and bioavailability of apigenin is important for enhancing its therapeutic performance (Chen *et al.*, 2023).

Accordingly, the present research focuses on the formulation, characterization, and evaluation of cubosomes for enhanced delivery of apigenin. The study aims to develop a stable nanosized cubosomal formulation capable of improving the delivery characteristics of apigenin.

2. MATERIAL AND METHODS

2.1 Chemicals

Apigenin were obtained from ZES Bioscience, a reputable supplier of analytical reagents. HiMedia provided the Dialysis Membrane, and BASF provided the Poloxamer 407 (Pluronic® F127). Sigma-Aldrich provided the Glyceryl Monooleate (Monoolein) and Potassium Bromide (KBr). Merck provided the Hydrochloric Acid, Sodium Hydroxide, Potassium

Dihydrogen Phosphate, Acetone, Chloroform, Methanol and Ethanol.

2.2 Pre-formulation studies of selected Drug

Pre-formulation studies were conducted to investigate the physicochemical properties of Apigenin prior to formulation development. These studies provide critical information regarding solubility, stability, compatibility, and analytical characteristics that influence formulation design and performance.

2.2.1 Organoleptic Properties

The organoleptic characteristics of Apigenin were evaluated by visual and sensory examination. Parameters such as color, odor, appearance, and physical state were assessed (Singh *et al.*, 2025).

2.2.2 Solubility Study

The solubility of Apigenin was evaluated in distilled water, ethanol, methanol, chloroform, DMSO, and PBS using the equilibrium solubility method. Apigenin (1 mg) was added to 1 mL of each solvent, stirred at room temperature until equilibrium, filtered, and analyzed by UV-visible spectrophotometry at λ max. Solubility was expressed as mg/mL, and the solvent showing the highest solubilizing capacity was selected for further analytical studies and cubosomal formulation development (Shakeel *et al.*, 2017).

2.2.3 pH Determination

The pH of the Apigenin-loaded cubosomal formulations was determined using a calibrated digital pH meter to evaluate the suitability of the formulation for pharmaceutical applications and to ensure formulation stability (Pápay *et al.*, 2016).

2.2.4 Melting Point Determination

The melting point of Apigenin was determined to assess its purity and to confirm its identity before formulation development using digital melting point apparatus (Wang *et al.*, 2022).

2.2.5 Spectroscopic Analysis and Calibration of Apigenin

2.2.5.1 Determination of λ max

A stock solution of Apigenin (1 mg/mL) was prepared using methanol. Appropriate dilution was made to obtain a working solution of 100 μ g/mL. The solution was scanned in the wavelength range of λ max, 200–400nm using a UV-visible spectrophotometer. The wavelength showing maximum absorbance was recorded as the λ max of Apigenin (Mevada *et al.*, 2024).

2.2.5.2 Standard Calibration Curve Analysis

A standard calibration curve of Apigenin was prepared using UV-visible spectrophotometry. A 1000 μ g/mL stock solution was prepared in methanol and diluted to obtain concentrations of 2–12 μ g/mL. The absorbance of each solution was measured at λ max, and a calibration curve was plotted against concentration. The linear

response confirmed compliance with Beer–Lambert’s law (Arun *et al.*, 2015).

2.2.6 Fourier Transform Infrared (FT-IR) Spectroscopy of Apigenin

FT-IR spectroscopy was used to identify the characteristic functional groups of Apigenin and confirm its chemical identity before formulation. About 1–2 mg of Apigenin was mixed with 100–200 mg of dry KBr, compressed into a transparent pellet, and analyzed over 4000–400 cm^{-1} at room temperature using a blank KBr pellet as the background. The spectrum was evaluated by comparing the observed absorption bands with reported values. Characteristic peaks for –OH, C=O, aromatic C=C, and C–O groups confirmed the chemical structure of Apigenin. The spectrum was also used as a reference for subsequent drug–excipient compatibility studies (Saberian *et al.*, 2025).

2.3 Quality by Design (QbD)-Based Preparation of Apigenin-Loaded Cubosomal Nanocarriers

Apigenin-loaded cubosomal nanocarriers were prepared by the emulsification–homogenization method based on

Quality by Design (QbD) principles. The concentrations of Glyceryl Monooleate (GMO) and Poloxamer 407, along with homogenization conditions, were optimized using Design of Experiments (DoE) to obtain cubosomes with suitable physicochemical properties. GMO was melted at 60–70°C to form a uniform lipid phase, and 50mg of Apigenin dissolved in a small amount of methanol was incorporated under continuous stirring. Separately, Poloxamer 407 was dissolved in distilled water and heated to the same temperature before being gradually added to the lipid phase to form a coarse emulsion. The emulsion was then homogenized at the optimized speed and duration to reduce particle size and promote cubosome formation. The dispersion was cooled to room temperature with continuous stirring, allowing the lipid components to self-assemble into cubic liquid crystalline structures. The resulting homogeneous formulation was stored in a tightly closed container at 4°C until further physicochemical characterization and *in vitro* evaluation (Abdo *et al.*, 2025).

Table 1: Composition of Cubosomes Formulation.

Formulation Code	Apigenin (mg)	GMO %	Poloxamer 407	Methanol (mL)	Distilled Water (mL)
CF1	50	2	0.5	10	10.0
CF 2	50	6	1	10	10.0
CF 3	50	6	1.5	10	10.0
CF 4	50	2	1.5	10	10.0
CF 5	50	4	1.5	10	10.0
CF 6	50	2	1	10	10.0
CF 7	50	4	0.5	10	10.0
CF 8	50	4	1	10	10.0
CF 9	50	2	1	10	10.0
CF 10	50	4	1.5	10	10.0
CF 11	50	6	0.5	10	10.0
CF 12	50	6	1	10	10.0
CF 13	50	4	0.5	10	10.0

2.4 Evaluation Parameters of Apigenin-Loaded Cubosomal Nanocarriers

2.4.1 Physical Appearance

The prepared Apigenin-loaded cubosomal formulations were visually inspected for their physical appearance, color, homogeneity, and presence of any visible aggregates or phase separation. (Shetti, 2022).

2.4.2 Particle Size and Polydispersity Index (PDI) Measurement

The mean particle size and polydispersity index (PDI) of the Apigenin-loaded cubosomal formulations were determined using Dynamic Light Scattering (DLS) with a Malvern Zetasizer (Gilani *et al.*, 2025).

2.4.3 Zeta Potential Measurement

The zeta potential of Apigenin-loaded cubosomes was measured using a Malvern Zetasizer based on electrophoretic mobility analysis (Imam *et al.*, 2022).

2.4.4 Morphological Analysis by Scanning Electron Microscopy (SEM)

The surface morphology and structural characteristics of the optimized Apigenin-loaded cubosomal formulation were examined using Scanning Electron Microscopy (SEM). A small quantity of the lyophilized cubosomal sample was mounted on an aluminum stub using double-sided adhesive tape and coated with a thin layer of gold by sputter coating. The coated samples were analyzed under SEM at appropriate accelerating voltages. High-resolution micrographs were obtained to evaluate particle shape, surface texture, and aggregation behavior. SEM analysis provided valuable information regarding the structural integrity and morphology of the cubosomal nanocarriers (Zafar *et al.*, 2021).

2.4.5 Drug Entrapment Efficiency

The entrapment efficiency of Apigenin within the cubosomal system was determined using the centrifugation method. An accurately measured quantity

of cubosomal dispersion was centrifuged at high speed to separate free drug from the entrapped drug. The supernatant containing untrapped Apigenin was collected and analyzed spectrophotometrically at the predetermined λ max using a UV-visible spectrophotometer. The amount of entrapped drug was calculated by subtracting the amount of free drug from the total amount of drug initially added (Jafari *et al.*, 2023).

The percentage entrapment efficiency was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = \left(\frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \right) \times 100$$

Determination of entrapment efficiency is essential for evaluating the drug-loading capacity and effectiveness of the cubosomal delivery system.

2.5 In Vitro Drug Release Study of Apigenin-Loaded Cubosomal Nanocarriers

The *in vitro* release of Apigenin from the optimized cubosomal formulation was evaluated using the dialysis membrane diffusion method. The dialysis membrane was hydrated overnight in distilled water, after which a known quantity of the formulation equivalent to a fixed amount of Apigenin was placed inside and both ends were securely sealed. The bag was immersed in phosphate buffer (pH 6.8 or 7.4) maintained at $37 \pm 0.5^\circ\text{C}$ with continuous stirring. Samples were withdrawn at predetermined intervals (0, 1, 2, 3, 5, 7, 9, 10, and 12 h) and replaced with an equal volume of fresh medium. The samples were diluted and analyzed spectrophotometrically at the λ max of Apigenin. Cumulative drug release was calculated and plotted against time to assess the release profile.

Kinetic Analysis of Drug Release

The release data obtained from the *in vitro* drug release study were fitted into various kinetic models to

determine the mechanism governing drug release from cubosomes.

Zero-Order Kinetics

Cumulative % Drug Release vs. Time

$$Q = K_0 t$$

A linear plot indicates a constant drug release rate independent of drug concentration.

First-Order Kinetics

Log % Drug Remaining vs. Time

$$\log Q = \log Q_0 - K_1 t / 2.303$$

Indicates concentration-dependent release.

Higuchi Model

Cumulative % Drug Release vs. Square Root of Time

$$Q = K \sqrt{t}$$

A linear relationship indicates diffusion-controlled drug release.

Korsmeyer–Peppas Model

Log Cumulative % Drug Release vs. Log Time

$$M_t/M_\infty = K t^n$$

2.6 Stability Studies

The optimized Apigenin-loaded cubosomal formulation was evaluated for accelerated stability according to ICH guidelines. The formulation was stored in airtight glass containers at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for three months. Samples were examined at 0, 30, 45, 60, and 90 days for physical appearance, particle size, and entrapment efficiency. The results were compared with initial values to assess changes during storage and determine the stability and quality of the formulation (Danaei *et al.*, 2018).

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of drug

Table 2: Organoleptic properties of Apigenin.

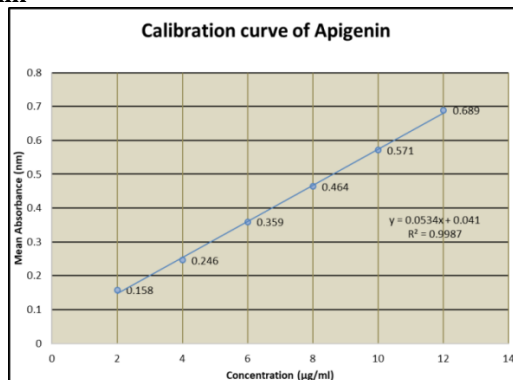
Drug	Organoleptic properties	Observation
Apigenin	Color	Pale yellow to yellow crystalline powder
	Odor	Odorless or faint characteristic odor
	Appearance	Fine crystalline powder
	State	Solid

3.1.2 pH and Melting point determination

Table 3: pH and Melting point of Apigenin.

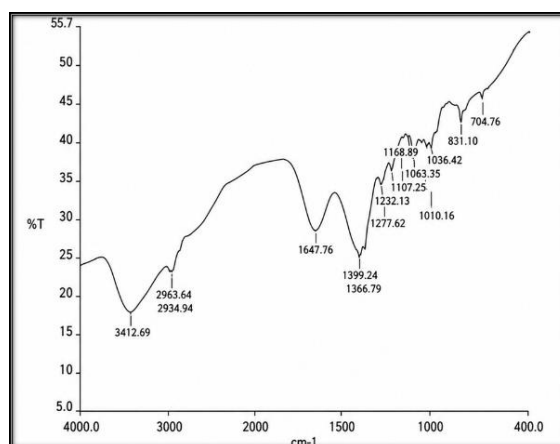
Drugs	Observed (pH)	Reference (pH)	Observed (Melting point)	Reference (Melting point)
Apigenin	6.4	6.2 to 6.8	346 °C	345 to 350 °C

3.1.3 Calibration curve of Apigenin



Graph 1: Calibration curve of Apigenin.

3.1.4 FTIR study of Apigenin



Graph 2: IR study of Apigenin.

Table 4: Interpretation of FTIR of Apigenin.

Peak Obtained (cm ⁻¹)	Reference Peak (cm ⁻¹)	Functional Group	Name of Functional Group
3412.69	3550–3200	O–H stretching	Phenolic alcohol
2963.64	3000–2850	C–H stretching	Alkane
1647.76	1680–1620	C=O stretching	Conjugated ketone (Flavone)
1399.24	1450–1350	O–H bending	Phenol
1277.62	1300–1200	C–O stretching	Phenolic ether

3.2 Optimization of Cubosomes formulations by the DoE approach

3.2.1 Build Information

Table 5: Build information of DOE software.

File Version	12.0.1.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	13
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	190.00		

3.2.2 Formulation trials as per Box–Behnken design.

Table 6: Formulation trials as per Box–Behnken design.

Formulation Code	Apigenin (mg)	GMO %	Poloxamer 407 %	Stirring time (hrs)	Particle size (nm)	Entrapment efficiency (%)
CF 1	50	2	0.5	2.5	493.26	76.51
CF 2	50	6	1	4	258.35	88.26
CF 3	50	6	1.5	2.5	577.2	76.33
CF 4	50	2	1.5	2.5	498.01	79.63
CF 5	50	4	1.5	1	855.4	64.1
CF 6	50	2	1	4	214.82	91.58

CF 7	50	4	0.5	1	883.62	66.13
CF 8	50	4	1	2.5	603.11	81.79
CF 9	50	2	1	1	793.08	65.42
CF 10	50	4	1.5	4	243.45	89.92
CF 11	50	6	0.5	2.5	552.71	74.2
CF 12	50	6	1	1	953.13	65.22
CF 13	50	4	0.5	4	233.62	92.86

3.2.3 Limits of Variables (Constraints)

Table 7: Variables operating range for Cubosome formulation.

Name	Goal	Lower Limit	Upper Limit	Importance
A: GMO	is in range	2	6	3
B: Poloxamer 407	is in range	0.5	1.5	3
C: Stirring Time	is in range	1	4	3
Particle size	none	214.82	953.13	3
Entrapment efficiency	none	64.1	92.86	3

3.2.4 Fit Summary

Response 1: Particle size

Table 8: Response 1: Particle size (Fit Summary)

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.9852	0.9789	Suggested
2FI	0.3213	0.9871	0.9785	
Quadratic	0.0981	0.9960		
Cubic				Aliased

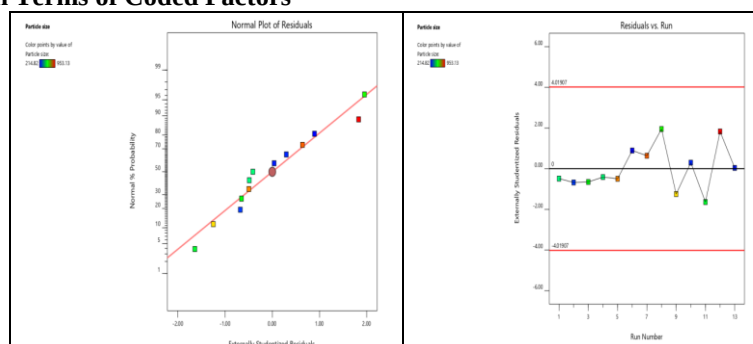
3.3 Effect of formulation variables on particle size (ANOVA for Linear model)

3.3.1 Response 1: Particle size

Table 9: Response 1: Particle size (ANOVA for Linear model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	8.179E+05	2.726E+05	266.58	< 0.0001	significant
A-GMO	14639.32	14639.32	14.31	0.0043	
B-Poloxamer 407	14.72	14.72	0.0144	0.9072	
C-Stirring Time	8.033E+05	8.033E+05	785.40	< 0.0001	
Residual	9204.81	1022.76			
Cor Total	8.271E+05				

3.3.2 Final Equation in Terms of Coded Factors



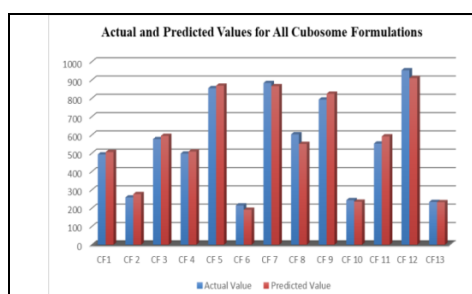
Graph 3: Diagnostic Residual Plots (Normal Plot and Residuals vs. Run) for Particle Size Response.

3.3.3 Predicted value and actual value of all Formulations

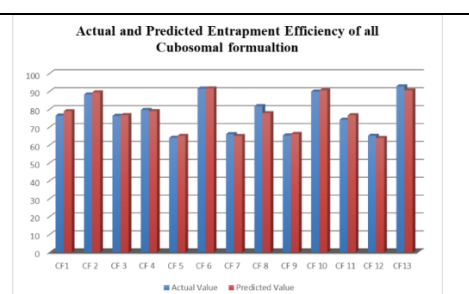
Table 10: Predicted value and actual value of Particle size and Entrapment efficiency of all Cubosome Formulations.

Formulations	Actual Value (Particle size)	Predicted Value (Particle size)	Actual Value (Entrapment efficiency)	Predicted Value (Entrapment efficiency)
CF1	493.26	506.62	76.51	78.95
CF 2	258.35	276.65	88.26	89.42
CF 3	577.20	594.88	76.33	76.74

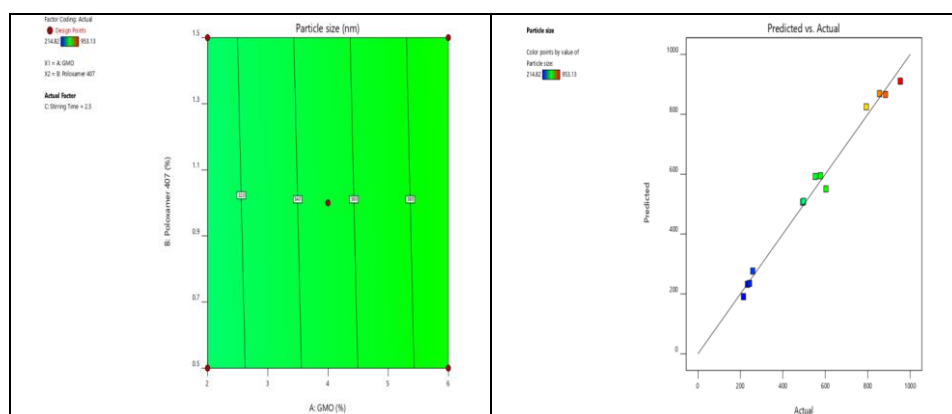
CF 4	498.01	509.33	79.63	79.02
CF 5	855.40	868.98	64.10	65.16
CF 6	214.82	191.10	91.58	91.70
CF 7	883.62	866.27	66.13	65.09
CF 8	603.11	550.75	81.79	77.84
CF 9	793.08	824.85	65.42	66.26
CF 10	243.45	235.23	89.92	90.60
CF 11	552.71	592.17	74.20	76.67
CF 12	953.13	910.40	65.22	63.98
CF13	233.62	232.52	92.86	90.53



Graph 4: Comparison of Actual and Predicted Values for All Cubosome Formulations



Graph 5: Graphical Correlation between Actual and Predicted Entrapment Efficiency



Graph 6: Response Surface Plot Illustrating the Combined Effect of Formulation Variables on Cubosome Characteristics.

3.3.4 Effect of formulation variables on Entrapment efficiency.

Table 11: Response 2: Entrapment efficiency (Fit Summary).

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.9605	0.9465	Suggested
2FI	0.9220	0.9450	0.8975	
Quadratic	0.3807	0.9554		
Cubic				Aliased

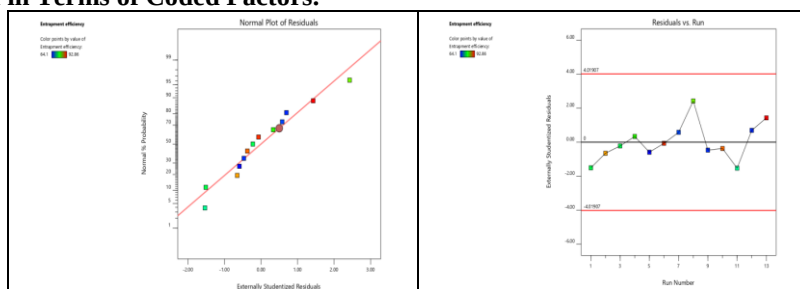
3.4 ANOVA for linear model

3.4.1 Response 2: EE (ANOVA Linear)

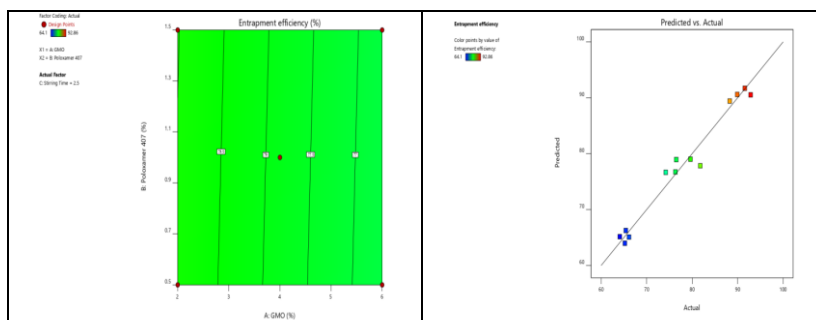
Table 12: Response 2: Entrapment efficiency.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1304.56	434.85	98.17	< 0.0001	significant
A-GMO	10.42	10.42	2.35	0.1595	
B-Poloxamer 407	0.0098	0.0098	0.0022	0.9635	
C-Stirring Time	1294.13	1294.13	292.16	< 0.0001	
Residual	39.87	4.43			
Cor Total	1344.43				

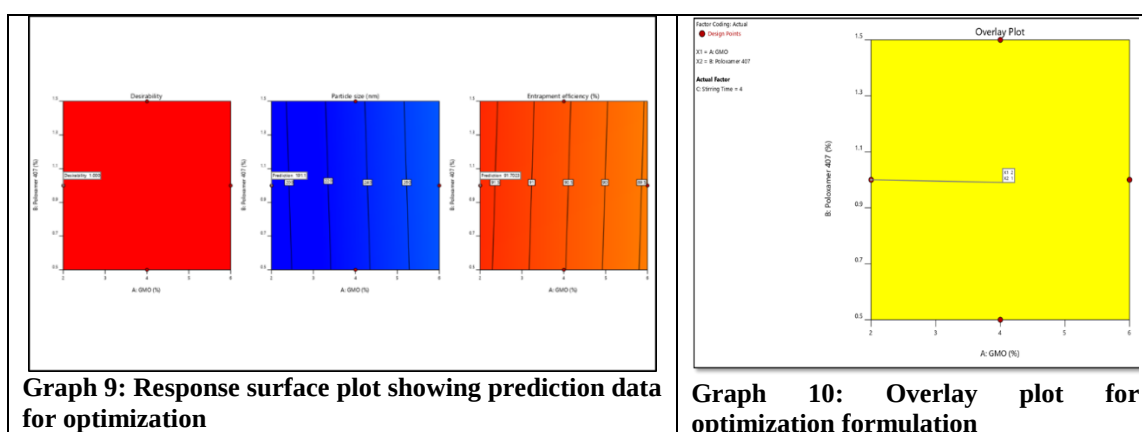
3.4.2 Final Equation in Terms of Coded Factors.



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



Graph 8: Two-Dimensional Contour Plots for the Effect of GMO and Poloxamer 407 Concentration on % Entrapment Efficiency and Predicted vs. Actual Values.



Graph 9: Response surface plot showing prediction data for optimization

Graph 10: Overlay plot for optimization formulation

3.4.4 Optimized formula of Cubosome formulation.

Table 13: Optimized formula of Cubosome formulation.

GMO	Poloxamer 407	Stirring Time	Particle size	Entrapment efficiency	Desirability	
2.000	1.500	2.500	509.330	79.019	1.000	
2.000	1.000	4.000	191.100	91.702	1.000	Selected

3.5 Characterization of optimized Cubosome formulation

3.5.1 Physical Appearance

Table 14: Physical Appearance of Apigenin loaded Cubosome.

Parameter	Observation
Color	Pale yellow
Odor	Characteristic
Appearance	Homogeneous, smooth and uniform dispersion
State	Liquid dispersion

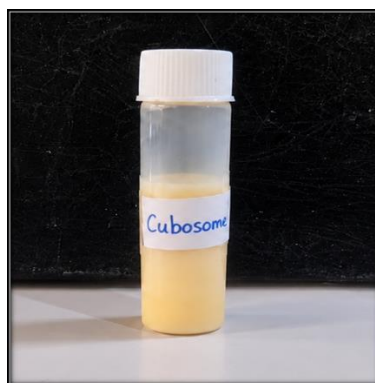
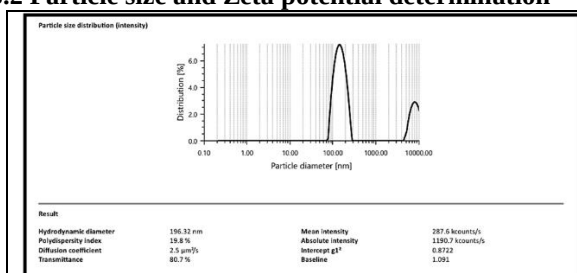
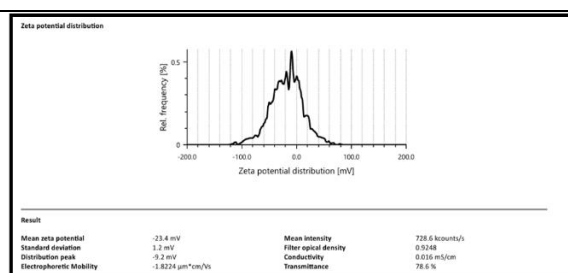


Figure 1: Cubosome Formulation.

3.5.2 Particle size and Zeta potential determination



Graph 11: Particle size of optimized formulation.



Graph 12: Zeta potential of optimized formulation.

Table 15: Particle size, Entrapment efficacy and Zeta potential of optimized formulation.

Formulation	Particle size (Predicted value)	Particle size (Actual value)	Zeta potential	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)
Cubosome	191.10 nm	196.32 nm	-23.4 mV	91.702%	95.578%

3.5.3 SEM analysis of Optimized formulation Cubosome

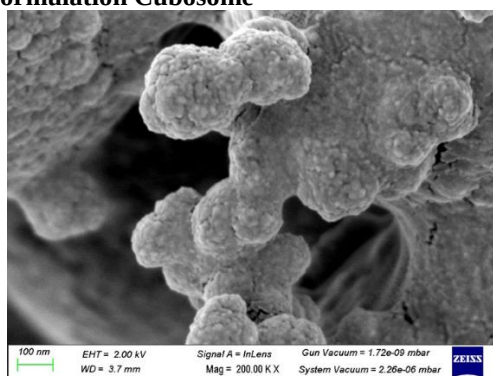
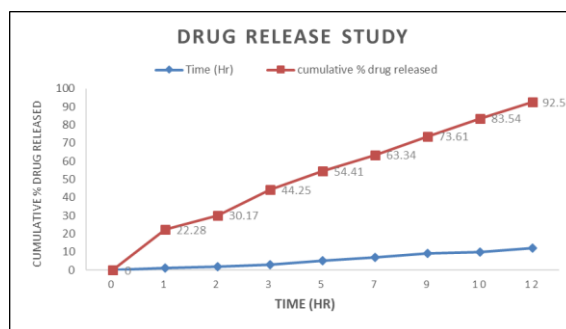


Figure 2: Scanning electron microscope (SEM).

3.6 In Vitro drug release study of drug loaded Cubosome formulation

Table 16: In-vitro drug release studies.

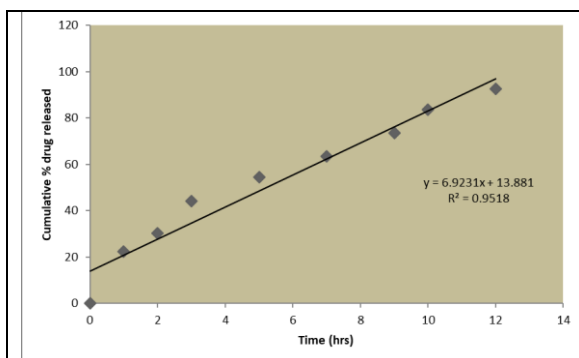
Time (Hr)	Cumulative % drug released
0	0
1	22.28
2	30.17
3	44.25
5	54.41
7	63.34
9	73.61
10	83.54



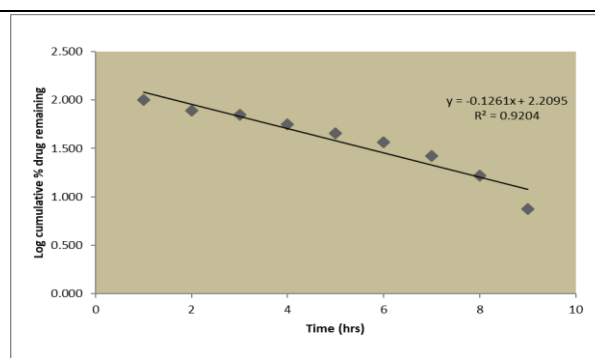
Graph 13: Drug release study.

Table 17: Correlation value (R^2 value).

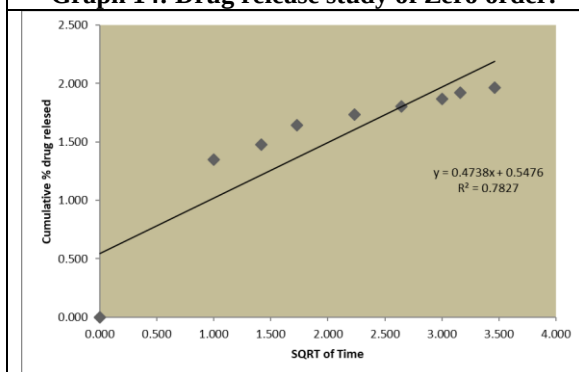
Formulation	Model	Kinetic parameter values
Cubosome Formulation	Zero Order	$R^2 = 0.951$
	First Order	$R^2 = 0.920$
	Higuchi	$R^2 = 0.782$
	Korsmeyerpeppas	$R^2 = 0.569$



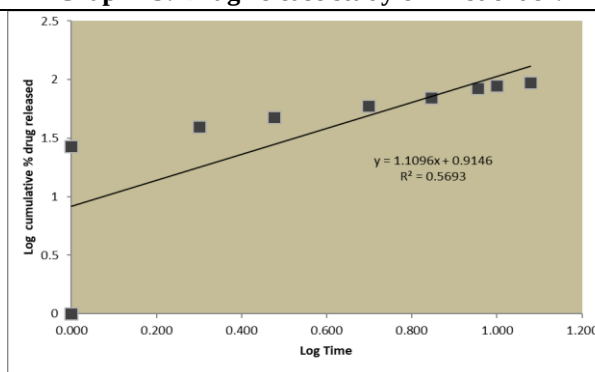
Graph 14: Drug release study of Zero order.



Graph 15: Drug release study of First order.



Graph 16: Drug release study of Higuchi order.



Graph 17: Drug release study of Korsmeyer-peppas.

DISCUSSION

The study demonstrated successful development and optimization of Apigenin-loaded cubosomes using a QbD-based Box–Behnken design. Pre-formulation studies confirmed the physicochemical characteristics and poor aqueous solubility of Apigenin, supporting the need for a suitable nanocarrier system. Among the formulation variables, stirring time showed the strongest influence on particle size and entrapment efficiency. The optimized formulation exhibited a particle size of 196.32 nm, zeta potential of -23.4 mV, and high entrapment efficiency of 95.578%, confirming effective drug incorporation and acceptable dispersion stability. SEM analysis showed nearly spherical particles with a rough

surface. The formulation provided sustained Apigenin release of 92.56% over 12 h, with the release profile best fitted to the Zero-order model ($R^2 = 0.951$). These findings indicate that cubosomes may serve as a promising lipid-based system for the controlled delivery of poorly water-soluble Apigenin.

4. CONCLUSION

The present study successfully developed and optimized Apigenin-loaded cubosomal nanocarriers using a Quality by Design (QbD) approach with Box–Behnken design and Response Surface Methodology (RSM). GMO concentration, Poloxamer 407 concentration, and stirring time were optimized to achieve the desired formulation

characteristics. Pre-formulation studies confirmed the suitability of Apigenin, while UV and FTIR methods provided reliable drug identification and estimation. Stirring time showed the greatest influence on particle size and entrapment efficiency. The optimized formulation showed a particle size of 196.32 nm, entrapment efficiency of 95.58%, and zeta potential of -23.4 mV, indicating good nanoscale characteristics and colloidal stability.

The formulation provided sustained drug release, with 92.56% cumulative release over 12 hours. The release profile followed the Zero-order model ($R^2 = 0.951$), suggesting a relatively constant release rate. The close agreement between experimental and predicted responses further supported the reliability of the optimization model. Overall, the optimized cubosomal system demonstrated desirable physicochemical properties, high drug entrapment, and sustained release, indicating its potential as a lipid-based delivery system for poorly water-soluble Apigenin. However, further long-term stability, *in vivo* pharmacokinetic and pharmacodynamic, biodistribution, and clinical studies are required to establish its safety, efficacy, and therapeutic potential.

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