

DESIGN AND IN-VITRO EVALUATION OF DIOSGENIN-INCORPORATED CUBOSOMES FOR TARGETED THERAPEUTIC DELIVERY

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ABSTRACT

The present study was undertaken to develop and evaluate Diosgenin-loaded cubosomes as a lipid-based nanocarrier for improving the solubility, stability, and controlled release of Diosgenin. Preformulation studies confirmed that Diosgenin was a white to off-white, odorless crystalline powder with poor aqueous solubility. The melting point was 205°C, pH was 6.5, and the λ_{max} was found to be 211 nm. FTIR analysis confirmed the characteristic functional groups and structural integrity of the drug. Five cubosomal formulations (CSF1–CSF5) were prepared and evaluated for particle size, zeta potential, morphology, and entrapment efficiency. Among the formulations, CSF3 demonstrated the most favorable characteristics, with a particle size of 206.7 nm, zeta potential of –34.0 mV, and entrapment efficiency of 94.38%. SEM analysis revealed nearly spherical nanoparticles with smooth surfaces and minimal aggregation. In vitro drug-release studies using a Franz diffusion cell showed sustained release of Diosgenin, with 94.56% cumulative drug release at 12 h. The optimized formulation followed predominantly First-order kinetics ($R^2 = 0.994$), indicating concentration-dependent drug release. Stability studies for 90 days demonstrated satisfactory physical stability under both long-term and accelerated conditions, with only minor changes in particle size and entrapment efficiency. Overall, the optimized CSF3 formulation demonstrated high drug entrapment, nanoscale particle size, good physical stability, and sustained drug release, indicating its potential as a promising lipid-based nanocarrier for improving the therapeutic delivery of Diosgenin.

KEYWORDS: Diosgenin, Cubosomes, Lipid-based Nanocarrier, Nanoparticles, Entrapment Efficiency, Sustained Drug Release, Franz Diffusion Cell, First-order Kinetics, Stability.

1. INTRODUCTION

Cubosomes are bi-continuous lipids containing nanoparticles, and have discrete, sub-micron, cubic liquid crystalline phases. The formulation of these nanocarriers involves a specific ratio of certain surfactants in order to form fine dispersion (**Chaudhary and Sharma, 2021**). Cubosomes are nanoparticles or, more precisely, nanostructure particles, which are generated by the self-assembly of amphiphilic- or surfactant-like molecules in liquid crystalline phases with cubic crystallographic symmetry. Surfactant-controlled cubosome structures are separated by two zones of water, and are also optically isotropic, viscous, and characterized by a solid lipid crystalline cubic symmetry that is comparable to honeycomb (cavernous) structures, and range in sizes from 100 to 500 nm. The continuous repeating and sophisticated 3D structures of cubic liquid crystalline phases provide excellent

opportunities to entrap hydrophobic, hydrophilic, and amphiphilic components (**Umar et al., 2022**).

Cubosomes are isotropic and transparent in nature, and their cubic liquid crystals are physically stable when in excess of water. They are biodegradable, non-toxic, and have the ability to solubilize hydrophobic, hydrophilic, and amphiphilic compounds. Due to their thermodynamic stability, cubosomes last indefinitely (**Chaudhary and Sharma, 2021**). Colloidal dispersions of cubosomes can be stabilized by the addition of polymers. They also possess the potential for the controlled delivery of actives, where diffusion is governed by the tortuous diffusion of the active through the “regular” channel structure of the cubic phase (**Sivadasan et al., 2023**). Due to their narrow pore sizes, cubosomes facilitate the controlled release of drugs and they are vital for maintaining the stability and efficacy of

biologically active compounds, such as proteins and vitamins. Compared with other liquid crystalline drug delivery systems, such as lipo- and hexosomes, cubosomes have lower viscosity and exist at any dilution in water and further cubic phases (Rao *et al.*, 2018).

Diosgenin is biosynthesized from cholesterol via the isoprenoid pathway. This pathway produces squalene, which then undergoes cyclization to form lanosterol and is further converted into cholesterol through multiple enzymatic steps. Cholesterol is sequentially converted into glycosides, and subsequently spirostanols through the enzymatic removal of glucose units at the C-26 position. In addition to this primary pathway, diosgenin might be formed from squalene-2,3-oxide in two alternative ways; (i) cycloartenol via the formation of sitosterol, (ii) from lanosterol via the formation of cholesterol (Jyotishi *et al.*, 2026). In the pharmaceutical industry, diosgenin serves as a main precursor in the synthesis of synthetic steroids. Although it is structurally similar to steroids, diosgenin itself is neither synthesized nor metabolically converted into steroid by-products in the mammalian body and hence, considered a safe phytochemical. For example, it does not exhibit any systemic toxicity, genotoxicity, or estrogenic activity at very high concentration of 3.5% (wt/wt). Although there is a claim that diosgenin might act like estrogen in humans, there is no scientific evidence to support this claim (Wu and Jiang, 2019).

In vitro evaluation of optimized cubosomes is essential for establishing their physicochemical characteristics and drug-delivery performance (Eldeeb *et al.*, 2019). Parameters such as particle size, polydispersity index, zeta potential, entrapment efficiency, surface morphology, drug content, and in vitro drug-release behavior provide important information regarding the quality and potential functionality of the developed nanocarrier. The release kinetics can also provide insight into the mechanism by which diosgenin is released from the cubosomal matrix.

Therefore, the present study was undertaken to design, optimize, and evaluate diosgenin-incorporated cubosomes using a systematic formulation-development approach. The study aimed to investigate the influence of selected formulation variables on important quality attributes, identify an optimized cubosomal formulation, and evaluate its physicochemical properties and in vitro drug-release behaviour (Aslam *et al.*, 2025).

2. MATERIALS AND METHOD

2.1 Chemicals

Diosgenin were obtained from ZES Bioscience Ltd., India. Sigma-Aldrich, USA provided the Glyceryl Monooleate (GMO) and Tween 80. BASF Corporation, Germany provided the Poloxamer 407, while Merck Specialities Pvt. Ltd., India supplied Propylene Glycol. Loba Chemie Pvt. Ltd., India obtained from Chloroform and Sodium Hydroxide. SD Fine Chemicals Ltd., India

provided the Methanol. Ethanol were procured from Changshu Yangyuan Chemicals Co. Ltd., China, a well-known provider of high-quality laboratory chemicals.

2.2 Pre-formulation studies of selected Drug

Pre-formulation studies of Diosgenin were carried out to evaluate its physicochemical characteristics and compatibility with the excipients selected for the development of cubosomal formulations. The results of the pre-formulation studies provided valuable insights that facilitated the successful development and optimization of Diosgenin - loaded cubosomes with enhanced skin penetration and controlled drug release properties (Mahapatra *et al.*, 2023).

2.2.1 Organoleptic Properties

The organoleptic properties of Diosgenin were evaluated through visual and sensory examination. The drug sample was carefully inspected for its color, appearance, texture, and physical state under normal daylight conditions (Saada *et al.*, 2024).

2.2.2 Solubility Study

The solubility of Diosgenin was determined in different solvents including water, ethanol, methanol, chloroform, acetone, dimethyl sulfoxide (DMSO), and phosphate buffer saline (PBS pH 7.4). An excess amount of drug was added separately into test tubes containing 1 mL of each solvent. The mixtures were shaken continuously using a mechanical shaker at room temperature for 24 hours to attain equilibrium. After equilibration, the solutions were filtered through Whatman filter paper to remove undissolved drug particles. The filtrates were visually observed for solubility behavior, and the solubility of Diosgenin in each solvent was recorded as freely soluble, soluble, slightly soluble, or insoluble (Knopp *et al.*, 2015).

2.2.3 pH determination

The pH of the Diosgenin solution was determined using a calibrated digital pH meter. A specified quantity of the drug was dissolved in distilled water to prepare an appropriate solution (Frankaer *et al.*, 2018).

2.2.4 Melting Point determination

The melting point of Diosgenin was determined using a melting point apparatus. A small quantity of the finely powdered drug was accurately filled into a capillary tube sealed at one end (Liu *et al.*, 2022).

2.2.5 Spectroscopic Analysis and Calibration of Diosgenin

2.2.5.1 Lambda (λ) max analysis

The λ_{max} of Diosgenin was determined using a Shimadzu UV-1700 spectrophotometer with methanol as solvent and blank. A 1 mg/mL stock solution was prepared and diluted to 100 $\mu\text{g/mL}$. The solution was scanned from 200–400 nm, and the wavelength of maximum absorbance was recorded as the λ_{max} of Diosgenin for further calibration and analysis of

Diosgenin-loaded cubosomal formulations (Qiao *et al.*, 2023).

2.2.5.2 Standard calibration curve analysis

A standard stock solution of Diosgenin (1 mg/100 mL) was prepared in methanol and suitably diluted to obtain different concentrations. The absorbance of each solution was measured at the selected λ_{max} using methanol as blank. A calibration curve was plotted by taking concentration ($\mu\text{g/mL}$) on the X-axis and absorbance on the Y-axis. The regression equation obtained from the curve was used for quantitative estimation of Diosgenin in the formulation studies (Azadeh *et al.*, 2017).

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

FT-IR analysis of Diosgenin was performed to identify its characteristic functional groups and confirm drug identity. A small quantity of Diosgenin was mixed with dry KBr, compressed into a pellet, and scanned from 4000–400 cm^{-1} using an FT-IR spectrophotometer. The obtained spectrum was analyzed for characteristic absorption peaks to confirm the structural integrity and

purity of Diosgenin before formulation development (Rosi *et al.*, 2017).

2.3 Preparation of Cubosomes containing Diosgenin

The Diosgenin -loaded cubosomes were prepared using the emulsification homogenization technique. In this method, Glyceryl monooleate (GMO) was first melted at 60–70°C to form a clear lipid phase. Diosgenin (5 mg) was dissolved in a 10 mL of Methanol and incorporated into the molten lipid with continuous stirring to ensure uniform drug distribution. In a separate vessel, Poloxamer 407 was dissolved in distilled water to prepare the aqueous phase. The aqueous phase was then gradually added to the lipid phase under continuous stirring to form a coarse emulsion. The resulting dispersion was further subjected to high-speed homogenization to reduce particle size and facilitate the formation of cubosomal nanostructures. Finally, the prepared formulation was allowed to cool and equilibrate at room temperature, resulting in stable and uniform Diosgenin -loaded cubosomal dispersion (Gaballa *et al.*, 2020).

Table 1: Composition of Cubosomes Formulation.

Formulation Code	Diosgenin (mg)	Glyceryl Monooleate (GMO) (g)	Poloxamer 407 (% w/v)	Methanol (mL)	Distilled Water (mL)
CSF1	5	0.75	0.25	10	q.s. to 10 mL
CSF2	5	1.00	0.50	10	q.s. to 10 mL
CSF3	5	1.25	0.75	10	q.s. to 10 mL
CSF4	5	1.50	1.00	10	q.s. to 10 mL
CSF5	5	1.75	1.25	10	q.s. to 10 mL

2.4 Evaluation parameter of Diosgenin loaded cubosomes formulation

2.4.1 Physical appearance

The physical appearance of Diosgenin -loaded cubosomes was evaluated through visual observation under normal daylight conditions. The prepared cubosomal dispersions were examined for their color, clarity, homogeneity, and presence of any visible particulate matter or phase separation (Chaiken *et al.*, 2022).

2.4.2 Particle Size Measurement

The particle size of Diosgenin -loaded cubosomes was determined using a dynamic light scattering (DLS) technique. The instrument provided the average particle size (Z-average) along with the polydispersity index (PDI), which indicates the size distribution uniformity of the formulation (Deepak *et al.*, 2025, Fennelly *et al.*, 2020).

2.4.3 Zeta Potential Measurement

The zeta potential of Diosgenin -loaded cubosomes was determined to evaluate the surface charge and physical stability of the colloidal system. The measurement was carried out using a zeta potential analyzer based on electrophoretic light scattering technique (Lunardi *et al.*, 2021).

2.4.4 Scanning Electron Microscopy (SEM) Analysis

The surface morphology and structural characteristics of Diosgenin -loaded cubosomes were examined using Scanning Electron Microscopy (SEM) (Mohammed *et al.*, 2018).

2.4.5 Drug Entrapment Efficiency

The entrapment efficiency (EE%) of Diosgenin -loaded cubosomes was determined to evaluate the amount of drug successfully incorporated within the cubosomal lipid matrix. The method was carried out by separating the free (untrapped) drug from the cubosomal dispersion using a suitable centrifugal filtration technique. A known quantity of cubosomal formulation was placed in Amicon Ultra centrifugal filter tubes (molecular weight cut-off 3,000 Da) and centrifuged at controlled speed and time. The filtrate containing free drug was collected and analyzed using a UV spectrophotometer at the predetermined wavelength of Diosgenin (Lv *et al.*, 2018).

The entrapment efficiency was calculated using the following formula:

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

2.5 In Vitro Drug Release Study

The *in vitro* drug release study of Diosgenin -loaded cubosomes was performed using a Franz diffusion cell apparatus to evaluate the release behavior of the drug from the cubosomal system. A dialysis membrane (previously soaked overnight in phosphate buffer saline, pH 7.4) was mounted between the donor and receptor compartments of the diffusion cell. A known quantity of Diosgenin -loaded cubosomal formulation was placed in the donor compartment. The receptor compartment was filled with phosphate buffer saline (PBS, pH 7.4) and maintained at $37 \pm 0.5^\circ\text{C}$ with continuous magnetic stirring to simulate physiological conditions. At predetermined time intervals, aliquots were withdrawn from the receptor medium and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were analyzed spectrophotometrically at the λ_{max} of Diosgenin, and the cumulative percentage drug release was calculated and plotted against time (Deepak et al., 2025, Paswan and Saini 2021).

Kinetic Model Analysis

To understand the drug release mechanism, the *in vitro* release data were fitted into various kinetic models, including

- Zero-order kinetics (cumulative drug release vs. time)
- First-order kinetics (log cumulative % drug

remaining vs. time)

- Higuchi model (cumulative % drug release vs. square root of time)
- Korsmeyer–Peppas model (log cumulative % release vs. log time)

2.6 Stability Testing

The optimized Diosgenin -loaded cubosomal formulation was evaluated for its stability by subjecting it to accelerated stability testing as per ICH guidelines. The samples were stored under two different controlled conditions: $25 \pm 2^\circ\text{C}$ with $60 \pm 5\%$ RH and $40 \pm 2^\circ\text{C}$ with $75 \pm 5\%$ RH, for a period of three months. The formulations were packed in suitable airtight containers and protected from light to prevent any drug degradation. At predetermined time intervals (0, 20, 50, 70, and 90 days), the formulations were withdrawn and analyzed for critical quality parameters such as particle size and entrapment efficiency. These studies were performed to assess any possible changes in the physicochemical properties of the cubosomal system during storage. The results obtained from stability studies were used to determine the physical and chemical stability of the formulation and to ensure its consistency, performance, and suitability for further pharmaceutical application (Mieloszyk et al., 2020).

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 2: Organoleptic properties of Diosgenin.

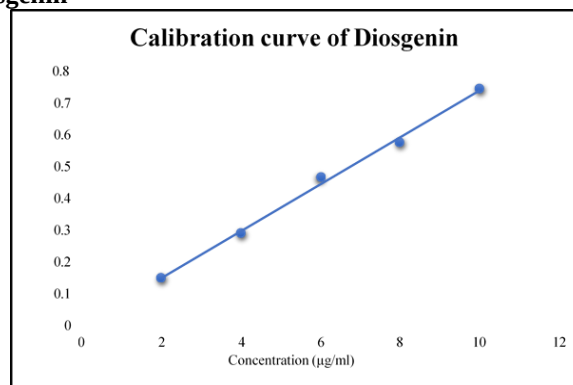
Parameter	Observation
Color	White to off-white
Odor	Odorless
Appearance	Fine crystalline powder
Nature	Solid

3.1.2 Melting point and pH

Table 3: Melting point and pH of Diosgenin.

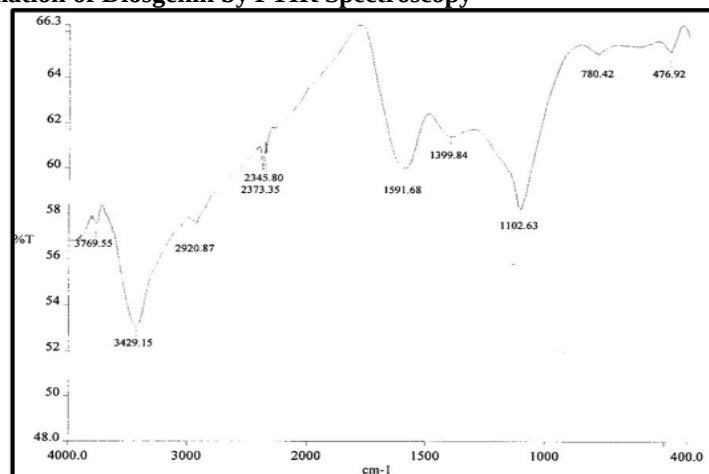
Drugs	Observed (Melting point)	Reference (Melting point)	Observed (pH)	Reference (pH)
Diosgenin	205°C	$204\text{--}207^\circ\text{C}$	6.5	6.0–7.0

3.1.3 Calibration curve of Diosgenin



Graph 1: Calibration curve of Diosgenin.

3.1.4 Structural Confirmation of Diosgenin by FTIR Spectroscopy



Graph 2: Structural Confirmation of Diosgenin by FTIR Spectroscopy.

Table 4: Interpretation of IR spectrum of Diosgenin.

Peak Obtained	Reference Peak	Functional group	Name of functional group
3769.55	4000-3000	O-H stretching	Alcohol
3429.15	3550-3200	O-H stretching	Alcohol
2920.87	3300-2500	O-H bending	Carboxylic acid
1591.68	1650-1580	N-H bending	Amine
1399.84	1400-1000	C-F stretching	Fluoro compound

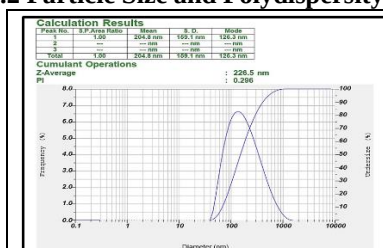
3.2 Characterization Parameters of Diosgenin loaded cubosomes formulation

3.2.1 Physical Appearance

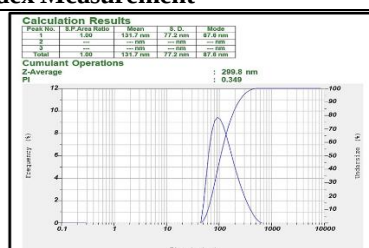
Table 5: Physical Appearance of drug Diosgenin cubosomes formulation.

Parameter	Observation
Color	White to off white
Odor	Mild characteristic odor
Appearance	Homogeneous dispersion
State	Powder form

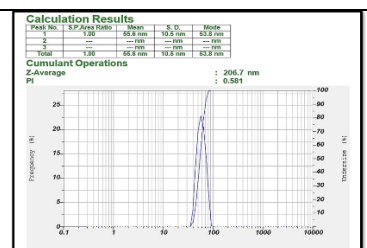
3.2.2 Particle Size and Polydispersity Index Measurement



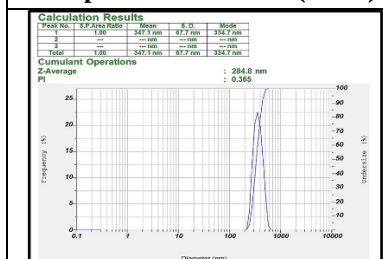
Graph 3: Particle Size (CSF1)



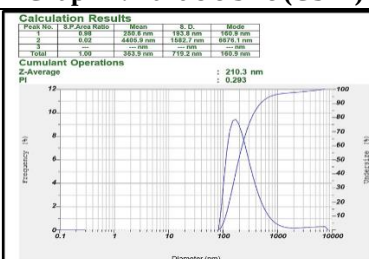
Graph 4: Particle Size (CSF2)



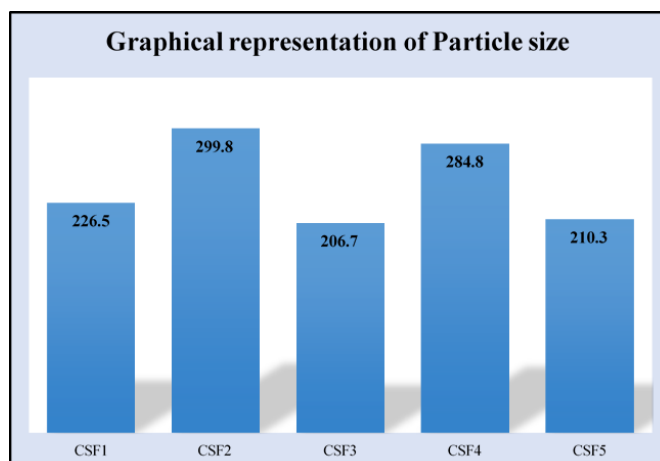
Graph 5: Particle Size (CSF3)



Graph 6: Particle Size (CSF4)

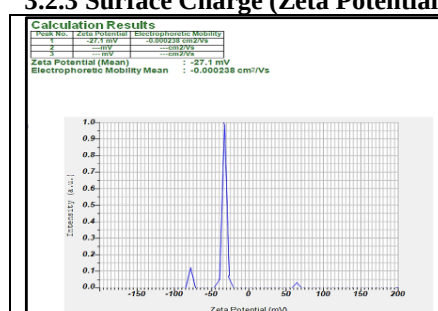


Graph 7: Particle Size (CSF5)

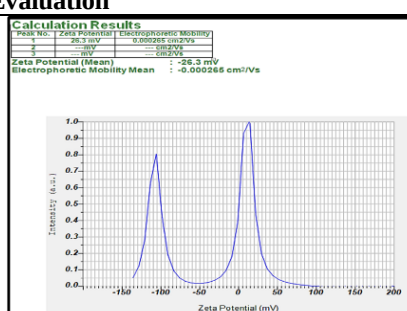


Graph 8: Graphical representation of Particle size.

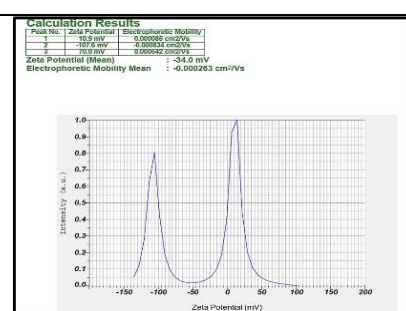
3.2.3 Surface Charge (Zeta Potential) Evaluation



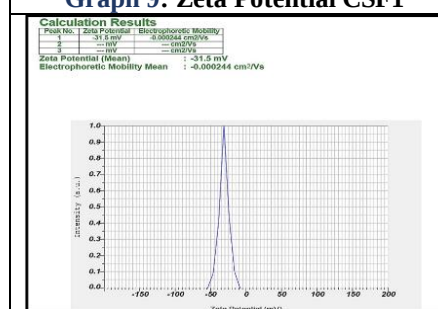
Graph 9: Zeta Potential CSF1



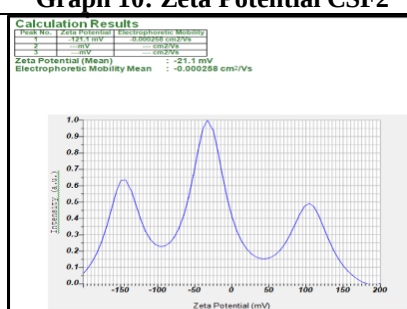
Graph 10: Zeta Potential CSF2



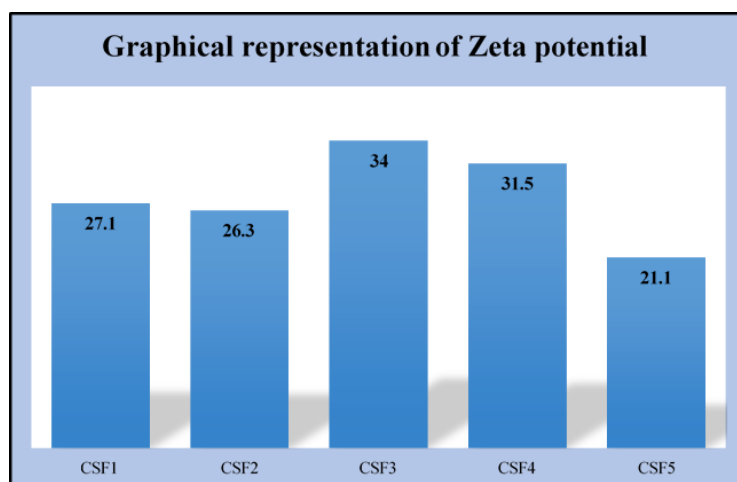
Graph 11: Zeta Potential CSF3



Graph 12: Zeta Potential CSF4

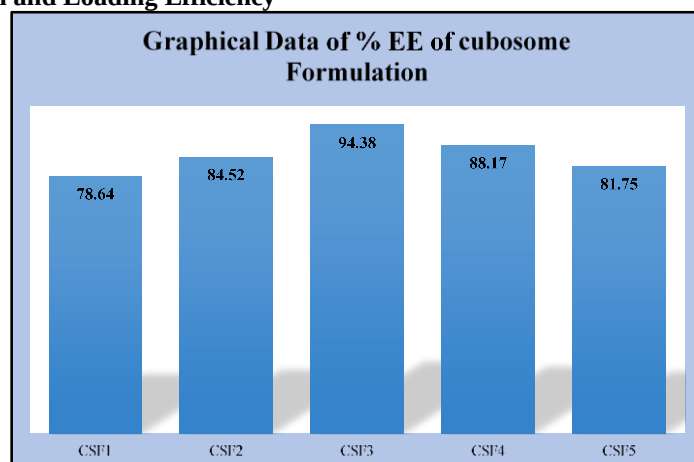


Graph 13: Zeta Potential CSF5



Graph 14: Graphical representation of Zeta potential.

3.2.4 Drug Encapsulation and Loading Efficiency



Graph 15: Graphical Data of % EE of cubosome Formulation.

Table 6: Entrapment efficacy (EE), Particle size and Zeta potential of Cubosomes Formulation.

Formulation Code	Entrapment Efficiency (%)	Particle size (nm)	Zeta potential (mV)
CSF1	78.64	226.5 nm	-27.1 mV
CSF2	84.52	299.8 nm	-26.3 mV
CSF3	94.38	206.7 nm	-34.0 mV
CSF4	88.17	284.8 nm	-31.5 mV
CSF5	81.75	210.3 nm	-21.1 mV

3.2.5 Morphological Analysis by Scanning Electron Microscopy (SEM)

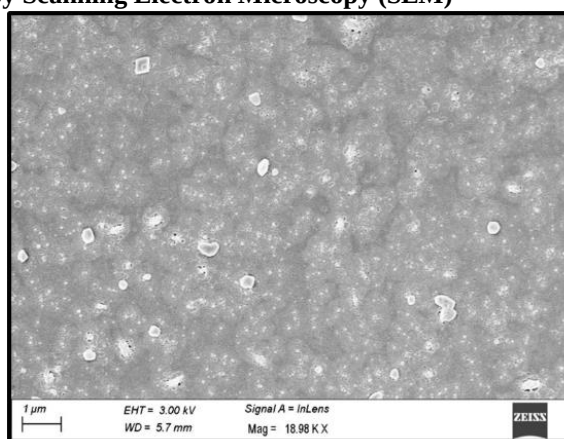


Figure 1: Scanning Electron Microscope (SEM).

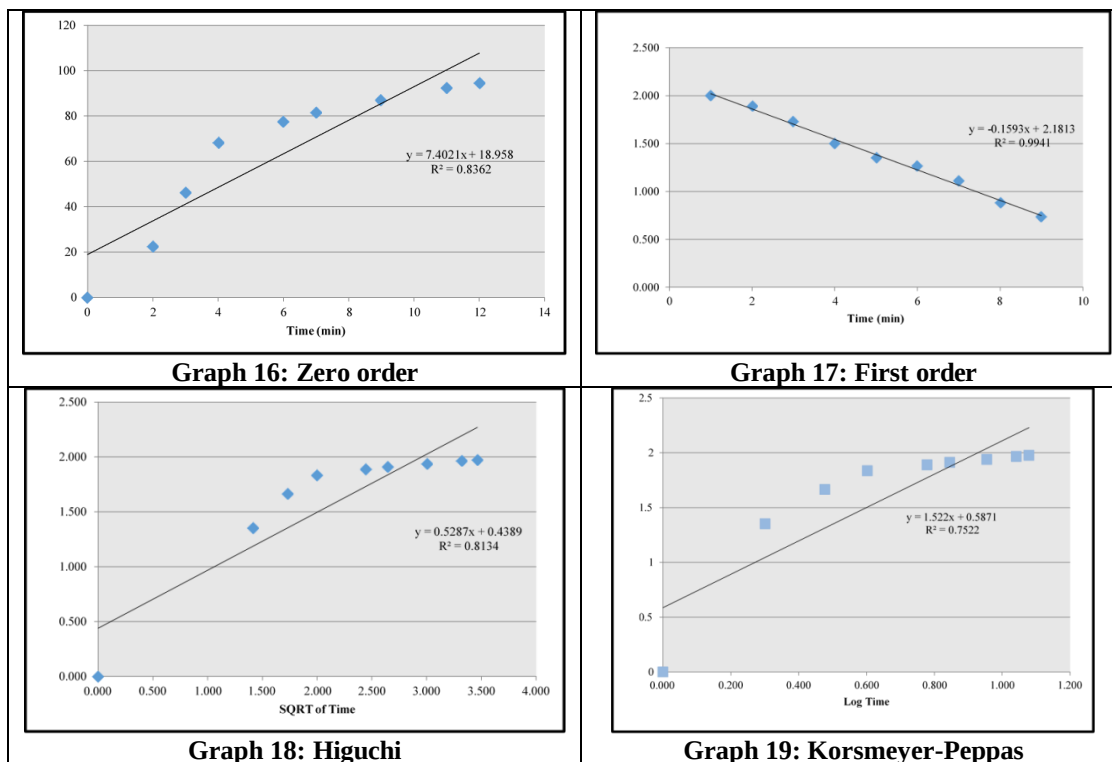
3.3 In-vitro Drug Release STUDY of Cubosome Formulation

Table 7: In-vitro Drug Release Studies.

Time (Hr)	Cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
2	22.55	77.45	1.414	1.889	0.301	1.353
3	46.27	53.73	1.732	1.730	0.477	1.665
4	68.32	31.68	2.000	1.501	0.602	1.835
6	77.5	22.5	2.449	1.352	0.778	1.889
7	81.6	18.4	2.646	1.265	0.845	1.912
9	87.14	12.86	3.000	1.109	0.954	1.940
11	92.39	7.61	3.317	0.881	1.041	1.966
12	94.56	5.44	3.464	0.736	1.079	1.976

Table 8: Correlation value (R^2 value).

Formulation	Model	Kinetic parameter values
CSF3 Formulation	Zero Order	$R^2 = 0.836$
	First Order	$R^2 = 0.994$
	Higuchi	$R^2 = 0.813$
	Korsmeyer-Peppas	$R^2 = 0.752$



3.4 Stability Study

Table 9: Stability Study of Optimized Formulation (Cubosomes).

Time (Days)	Physical Appearance	Particle Size (nm) at 25°C ± 2°C & 60 ± 5% RH	Entrapment Efficiency (%) at 25°C ± 2°C & 60 ± 5% RH	Particle Size (nm) at 40°C ± 2°C & 70 ± 5% RH	Entrapment Efficiency (%) at 40°C ± 2°C & 70 ± 5% RH
0	Milky white, homogeneous dispersion, no aggregation	206.7 ± 2.14	94.38 ± 0.52	206.7 ± 2.14	94.38 ± 0.52
20	No visible change, homogeneous dispersion	208.5 ± 2.35	93.96 ± 0.61	213.2 ± 2.48	92.84 ± 0.74
50	Stable appearance, no phase separation observed	210.8 ± 2.62	93.42 ± 0.68	218.6 ± 2.91	91.75 ± 0.83
70	Slight increase in turbidity, no aggregation	213.6 ± 2.84	92.87 ± 0.72	224.3 ± 3.16	90.62 ± 0.91
90	Homogeneous dispersion maintained, no visible instability	216.4 ± 3.05	92.31 ± 0.85	231.7 ± 3.42	89.48 ± 1.02

DISCUSSION

The present study successfully demonstrated the development and evaluation of Diosgenin-loaded cubosomes as a lipid-based delivery system. Preformulation studies confirmed the crystalline nature and acceptable physicochemical characteristics of Diosgenin, while its poor aqueous solubility supported the selection of cubosomes as a suitable carrier for improving drug dispersion and delivery. Among the five formulations, CSF3 showed the most favorable characteristics, with the smallest particle size of 206.7 nm, highest negative zeta potential of -34.0 mV, and maximum entrapment efficiency of 94.38%. The nanosized particles and higher negative surface charge indicated good dispersion and reduced aggregation, while the high entrapment efficiency confirmed efficient incorporation of the lipophilic drug within the lipidic cubic structure. SEM findings further supported the formation of nearly spherical, smooth, and minimally aggregated cubosomal nanoparticles.

The optimized CSF3 formulation demonstrated sustained drug release, achieving 94.56% cumulative release after 12 h. Release kinetic analysis showed the best fit with the First-order model ($R^2 = 0.994$), indicating concentration-dependent drug release. The sustained release behavior may be attributed to the highly ordered lipidic cubic structure of cubosomes, which can act as a barrier to rapid drug diffusion and prolong drug release.

Stability studies conducted for 90 days demonstrated satisfactory stability of the optimized formulation under both storage conditions. Only a slight increase in particle size and reduction in entrapment efficiency were observed, indicating that the cubosomal system maintained its physical integrity and drug-retention capacity during storage. Overall, CSF3 exhibited desirable nanoscale characteristics, high drug entrapment, satisfactory stability, and prolonged drug release, demonstrating the potential of Diosgenin-loaded cubosomes as a promising advanced drug-delivery system. Further *in vivo* studies are required to confirm its pharmacokinetic and therapeutic advantages.

4. CONCLUSION

The present investigation successfully developed and optimized a Diosgenin-loaded cubosomal drug delivery system using lipid-based nanotechnology. Preformulation studies confirmed that Diosgenin possessed acceptable physicochemical properties, including white to off-white color, odorless nature, melting point of 205°C , pH of 6.5, λ_{max} of 211 nm, and characteristic FTIR peaks confirming its structural integrity. The poor aqueous solubility observed in water and PBS further justified the selection of cubosomes as a suitable carrier system.

Among the five formulations prepared, CSF3 was identified as the optimized formulation based on its superior physicochemical characteristics. It exhibited the

smallest particle size (206.7 nm), highest negative zeta potential (-34.0 mV), and maximum entrapment efficiency (94.38%), indicating excellent nanoparticle stability and efficient drug incorporation. SEM analysis confirmed the formation of well-defined, nearly spherical nanoparticles with uniform morphology. The optimized formulation demonstrated sustained drug release, achieving 94.56% cumulative release within 12 hours, and followed First-order release kinetics ($R^2 = 0.994$), indicating concentration-dependent drug release predominantly governed by diffusion through the cubosomal matrix. Stability studies confirmed that the formulation maintained its physical appearance, nanosize, and high entrapment efficiency over 90 days, with only minimal increases in particle size (206.7 to 216.4 nm at 25°C and 206.7 to 231.7 nm at 40°C) and slight reductions in entrapment efficiency (94.38% to 92.31% and 89.48%, respectively).

Based on these findings, the optimized CSF3 formulation demonstrated superior physicochemical stability, high drug encapsulation, controlled drug release, and excellent storage stability. Therefore, Diosgenin-loaded cubosomes can be considered a promising lipid-based nanocarrier for improving the therapeutic performance of Diosgenin. Further *in vivo* pharmacokinetic, pharmacodynamic, and clinical studies are recommended to validate its therapeutic efficacy and support its future pharmaceutical application.

REFERENCES

1. Chaudhary K, Sharma D. Cubosomes: a potential drug delivery system. Asian journal of pharmaceutical research and development. 2021 Oct 15; 9(5): 93-101.
2. Umar, H., Wahab, H. A., Gazzali, A. M., Tahir, H., & Ahmad, W. Cubosomes: design, development, and tumor-targeted drug delivery applications. *Polymers*, 2022; 14(15): 3118.
3. Chaudhary K, Sharma D. Cubosomes: a potential drug delivery system. Asian journal of pharmaceutical research and development. 2021 Oct 15; 9(5): 93-101.
4. Sivadasan D, Sultan MH, Alqahtani SS, Javed S. Cubosomes in drug delivery—a comprehensive review on its structural components, preparation techniques and therapeutic applications. *Biomedicines*. 2023 Apr 7; 11(4): 1114.
5. Rao SV, Sravya BN, Padmalatha K. A review on cubosome: The novel drug delivery system. *GSC Biol. Pharm. Sci.*, 2018; 5(1): 1-5.
6. Jyotishi C, Patel M, Prajapati S, Gupta R. Therapeutic potential of diosgenin in hepatocellular carcinoma through molecular mechanisms and nanodelivery strategies. *Discover Oncology*. 2026 Jan 16; 17(1): 282.
7. Wu FC, Jiang JG. Effects of diosgenin and its derivatives on atherosclerosis. *Food & function*. 2019 Nov 1; 10(11): 7022-36.

8. Eldeeb AE, Salah S, Ghorab M. Formulation and evaluation of cubosomes drug delivery system for treatment of glaucoma: Ex-vivo permeation and in-vivo pharmacodynamic study. *Journal of drug delivery science and technology*. 2019 Aug. 1; 52: 236-47.
9. Aslam R, Bhatt V, Pandey P, Dutta D. Development and Assessment of Cubosomal Nanoformulation Loaded with β -Sitosterol for Improving Antioxidant Activity. In *International Symposium on Global Trends in Health, Technology and Management* 2025 Feb 15: 487-501. Cham: Springer Nature Switzerland.
10. Mahapatra AK. Basics on Pre-formulation Research: For Formulation Scientists. *Research Journal of Pharmacy and Life Sciences: Volume*. 2023 Sep; 4(3): 12-34.
11. Saada M, Ürgüp NS, Bakirtas F, Çirban G. Effects of the tablets" organoleptic properties on patients" compliance. *ACTA Pharm. Sci.*, 2024; 62(3).
12. Knopp MM, Tajber L, Tian Y, Olesen NE, Jones DS, Kozyra A, Lobmann K, Paluch K, Brennan CM, Holm R, Healy AM. Comparative study of different methods for the prediction of drug-polymer solubility. *Molecular pharmaceutics*. 2015 Sep 8; 12(9): 3408-19.
13. Frankaer CG, Hussain KJ, Dorge TC, Sørensen TJ. Optical chemical sensor using intensity ratiometric fluorescence signals for fast and reliable pH determination. *ACS sensors*. 2018 Dec 28; 4(1): 26-31.
14. Liu Y, Tu KN. Low melting point solders based on Sn, Bi, and In elements. *Materials Today Advances*. 2020 Dec. 1; 8: 100115.
15. Qiao L, Mu Y, Lu B, Tang X. Calibration maintenance application of near-infrared spectrometric model in food analysis. *Food Reviews International*. 2023 Apr 3; 39(3): 1628-44.
16. Deepak, Gokhe, S., & Khan, A. (2025). Development and characterization of β -cyclodextrin-based nanosponge gel containing herbal extract using a quality by design (QbD) approach and its antimicrobial activity.
17. Azadeh M, Gorovits B, Kamerud J, MacMannis S, Safavi A, Sailstad J, Sondag P. Calibration curves in quantitative ligand binding assays: recommendations and best practices for preparation, design, and editing of calibration curves. *The AAPS journal*. 2017 Dec 27; 20(1): 22.
18. Rosi F, Cartechini L, Sali D, Miliani C. Recent trends in the application of Fourier Transform Infrared (FT-IR) spectroscopy in Heritage Science: From micro-to non-invasive FT-IR. *Physical Sciences Reviews*. 2019 Nov. 26; 4(11): 20180006.
19. Gaballa SA, El Garhy OH, Abdelkader H. Cubosomes: composition, preparation, and drug delivery applications. *Journal of advanced biomedical and pharmaceutical sciences*. 2020 Jan 1; 3(1): 1-9.
20. Chaiken S. Physical appearance and social influence. In *Physical appearance, stigma, and social behavior* 2022 Nov 1: 143-178). Routledge.
21. Fennelly KP. Particle sizes of infectious aerosols: implications for infection control. *The lancet respiratory medicine*. 2020 Sep 1; 8(9): 914-24.
22. Lunardi CN, Gomes AJ, Rocha FS, De Tommaso J, Patience GS. Experimental methods in chemical engineering: Zeta potential. *The Canadian Journal of Chemical Engineering*. 2021 Mar; 99(3): 627-39.
23. Mohammed A, Abdullah A. Scanning electron microscopy (SEM): A review. In *Proceedings of the 2018 international conference on hydraulics and pneumatics HERVEX, Băile Govora, Romania 2018 Nov 7 (Vol. 2018: 7-9)*.
24. Lv Y, He H, Qi J, Lu Y, Zhao W, Dong X, Wu W. Visual validation of the measurement of entrapment efficiency of drug nanocarriers. *International journal of pharmaceutics*. 2018 Aug 25; 547(1-2): 395-403.
25. Paswan SK, Saini T. Comparative evaluation of in vitro drug release methods employed for nanoparticle drug release studies. *Clin. Trials*. 2021 Nov; 14(17): 10-4227.
26. Mieloszyk J, Tarnowski A, Tomaszewski A, Goetzendorf-Grabowski T. Validation of flight dynamic stability optimization constraints with flight tests. *Aerospace Science and Technology*. 2020 Nov 1; 106: 106193.