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Formulation, Evaluation and optimization of Lenalidomide loaded niosomes

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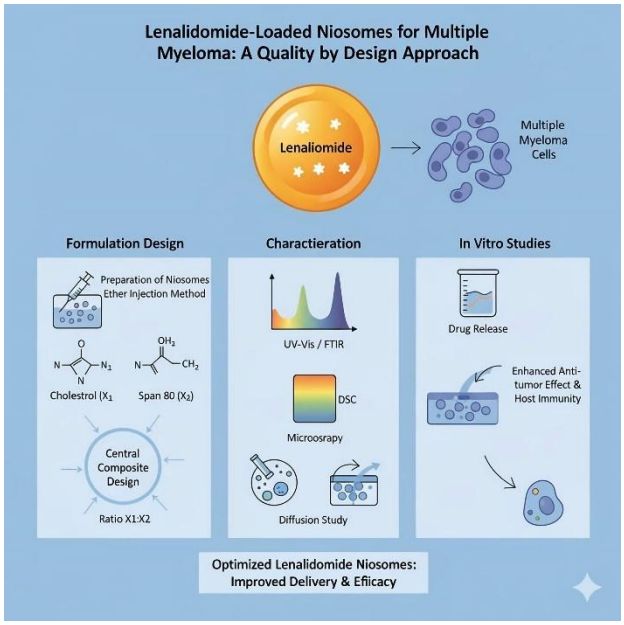
Graphical Abstract

Formulation, Evaluation and optimization of Lenalidomide loaded niosomes



Summary

The targeted and sustained delivery of anticancer drugs remains a major focus in pharmaceutical research to enhance therapeutic efficacy and reduce systemic toxicity. This study involves the formulation, evaluation, and optimization of Lenalidomide-loaded niosomes for improved drug delivery in cancer therapy. Niosomes were prepared using the ether method technique with non-ionic surfactants (Span 80 and cholesterol) in varying molar ratios. A series of formulations were developed and optimized based on particle size, polydispersity index (PDI), and entrapment
efficiency (EE%). The optimized formulation was characterized for morphology, zeta potential, in-vitro drug release, FTIR, DSC, and stability studies. Results indicated that the optimized niosomal formulation exhibited nanometric size, high entrapment efficiency, and controlled drug release over 8 hours. The findings highlight the potential of niosomal carriers to enhance the bioavailability and therapeutic performance of Lenalidomide, offering a promising approach for the effective and sustained management of multiple myeloma and related malignancies.

Keywords

Lenalidomide; multiple myeloma; niosomes; ether injection method

Introduction

Niosomes are multi lameller vesicular struc.ture of non-ionic surfactants, similar to lipos.omes and are composed of non-ionic surfactant instead of phospholipids which are the components of liposomes. [1,2] So, niosome or non-ionic surfactant vesicles are now widely studied as an alternative tool to liposome. Various types of surfactants have been reported to form vesicles, and have the capacity to entrap and retain the hydrophilic and hydrophobic solute particles [1-3]. Niosomes are vesicular nanocarriers that are stable, non-toxic, biodegradable, and reasonably priced. The instability, rapid disintegration, bioavailability,

and solubility of some drugs or natural substances may be improved by niosomes. When it comes to the targ.eted administration of antibacterial, antimicrobial, anti-inflammatory, antioxidant, and anticancer compounds, niosomes have the potential to be incredibly powerful systems. This essay will provide a summary of their makeup, the most popular methods for formulation, and their present application as delivery systems for cancer treatments. [4]

Niosomes mainly contain two types of components i.e., non-ionic surfactant and the additives. The non-ionic surfactants form the vesicular layer and the additives used in niosome preparation are cholesterol and the charged molecules.[3] The presence of the steroidal system (cholesterol) improves the rigidity of the bilayer and is important component of the cell membrane and their presence in membrane affects bilayer fluidity and permeability. This carrier system protects the drug molecules from the premature degradation and inactivation due to unwanted immunological and pharmacological effects.[5] By guaranteeing that noisome medications are distributed in a controlled manner that is customized to the needs of the patient, this innovation not only expedites the formulation process but also enhances therapeutic outcomes. [6]

In recent years, niosomes have been extensively studied for their potential to serve as a carrier for the delivery of drugs, antigens, hormones and other bioactive agents. Besides this, niosome has been used to solve the problem of insolubility, instability and rapid degradation of drugs.[7] Niosomes can be categorized into 3 groups based on their vesicle size, namely, small unilamellar vesicles (0.0.25–0.05 mm), multilamellar vesicles (>0.05 mm), and large unilamellar vesicles (>0.10 mm) [8].

Lenalidomide (prev.iously referred to as CC-.5013) is an imm.uno modul.atory drug with potent antineoplastic, anti-angiogenic, and anti-inflammatory prope.rties. It is a 4-amino-glutamyl analogue of [thali domide] and like thalidomide, lenalidomide exists as a racemic mixture of the active S(-) and R(+) forms However, Lenalidomide is much safer and potent than thalidomide, with fewer adverse effects and toxicities.[9] Thalidomide and its analogues, including Lenalidomide, are referred to as immuno modulatory imide drugs (also known as cereblon modulators), which are a class of immuno modulatory drugs that contain an imide group. Lenalidomide works through various mechanisms of actions that promote malignant cell death and enhance host immunity Available as oral capsules, Lenalidomide is approved by the FDA and EU for the treatment of multiple myeloma, myelodysplastic syndromes, mantle cell lymphoma, follicular lymphoma, and marg.inal zone lymphoma in selected patients. [10,11]

Multiple myeloma is a clonal B-cell malignancy associated with a monoclonal (M) protein in blood and/or urine, bone lesions, and immunodeficiency. It usually evol.ves from monoclonal gammopathy of undetermined significance (MGUS), with low levels of plasmacytosis and M protein without osteolytic lesions, anemia, hypercalcemia, and renal failure.[11] Multiple myeloma is characterized by genetic signatures, including frequent translocations into the immunoglobulin heavy chain switch region (IgH), oncogenes, and abnormal chromosome number[12,13]. Most patients with translocations have non-Hyperdiploid chromosome number (NHMM), while those patients lacking IgH translocations have Hyperdiploid chromosome number (HMM) with trisomies of chromosomes 3,5,7.,9,11,15,19, and 21. Importantly, patients with Hyperdiploid multiple myeloma have a better outcome with prolonged survival.[14,15].

Lenalidomide has a short plasma elimination half-life of about 3–4 hours, which necessitates frequent doses to maintain therapeutic levels and exposes patients to varying systemic exposure despite its excellent oral absorption

and bioavailability.[16] Additionally, dose-limiting toxicities including neutropenia and thrombocytopenia, which limit dose escalation and overall acceptability in multiple myeloma therapy, are correlated with increased systemic exposure to lenalidomide. These characteristics imply that, in comparison to conventional dosing, a sustained and targeted delivery platform such as niosomes, which have been demonstrated to improve drug stability, control release, and delivery to diseased tissues, may extend systemic exposure, lower off-target toxicity, and increase therapeutic efficacy [17].

Results and Discussion

Pre-formulation studies

a. Physical parameters of Lenalidomide

To determine Lenalidomide's fundamental physicochemical properties, its appearance and solubility were assessed. The substance was found to be a crystalline powder that was off-white to pale yellow in colour, indicating good purity and a constant physical form. Water, methanol, ethanol, and acetone were among the solvents used for solubility tests. Lenalidomide was found to be nearly insoluble in water, but it was more soluble in acidic (low pH) solutions and organic solvents such methanol, ethanol, and diethyl ether. Using the capillary tube method, the drug's melting point was found to be 272.36 °C, which is consistent with the range reported for pure Lenalidomide and validates the drug's identity and thermal stability.

b. Calibration curve

Calibr.ation cur.ve was plotted in meth.anol at 242 nm. The calibr.ation cur.ve was plot.ted as seen in figure1

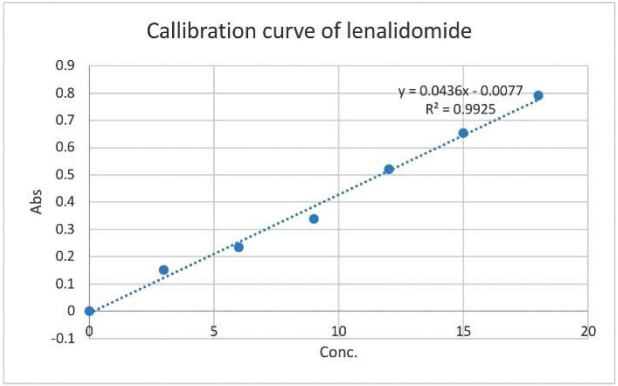


Figure1. Calibration curve of Lenalidomide in methanol

A calibration curve of Lenalidomide was prepared in methanol to determine its linearity and suitability for quantitative analysis using UV-visible spectrophotometry. The absorbance of standard solutions with concentrations ranging from 0 to 18 µg/mL was measured at 242 nm, which corresponds to the absorption maximum (λmax) of Lenalidomide in methanol. A linear relationship was observed between concentration and absorbance, indicating that the method follows Beer–Lambert's law within the tested range. The calibration curve, as shown in Figure1, exhibited a well-defined straight line with good correlation, confirming the reliability of the analytical method for determining the concentration of Lenalidomide in subsequent formulations and release studies.

Pre-formulation Screening of Surfactants

Pre-formulation trials (Table 5) comparing Span 80 and Span 60 indicated that Span 80 produced significantly smaller vesicles (106–254 nm) than Span 60 (348–488 nm) and achieved comparable drug entrapment efficiency. Therefore, Span 80 was selected for further optimization due to its lower hydrophilic–lipophilic balance (HLB = 4.3), which promotes the formation of stable, flexible bilayer vesicles with enhanced permeability. Subsequent results presented below focus on the optimization and characterization of Lenalidomide-loaded niosomes prepared using Span 80.

Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the sample was recorded using a Shimadzu instrument. The analysis reveals characteristic absorption peaks which are consistent with the structural features of Lenalidomide.

The presence of these peaks confirms the functional groups expected in the compound and supports its identity. The FTIR of the API Lenalidomide are shown in the following figure 2a.

Table 4 presents the FT-IR interpretation of Lenalidomide, comparing the reported and observed wavenumbers (cm^{-1}) for various functional groups. It confirms the presence of characteristic stretches with observed peaks closely matching reported literature values, indicating the drug's structural integrity.

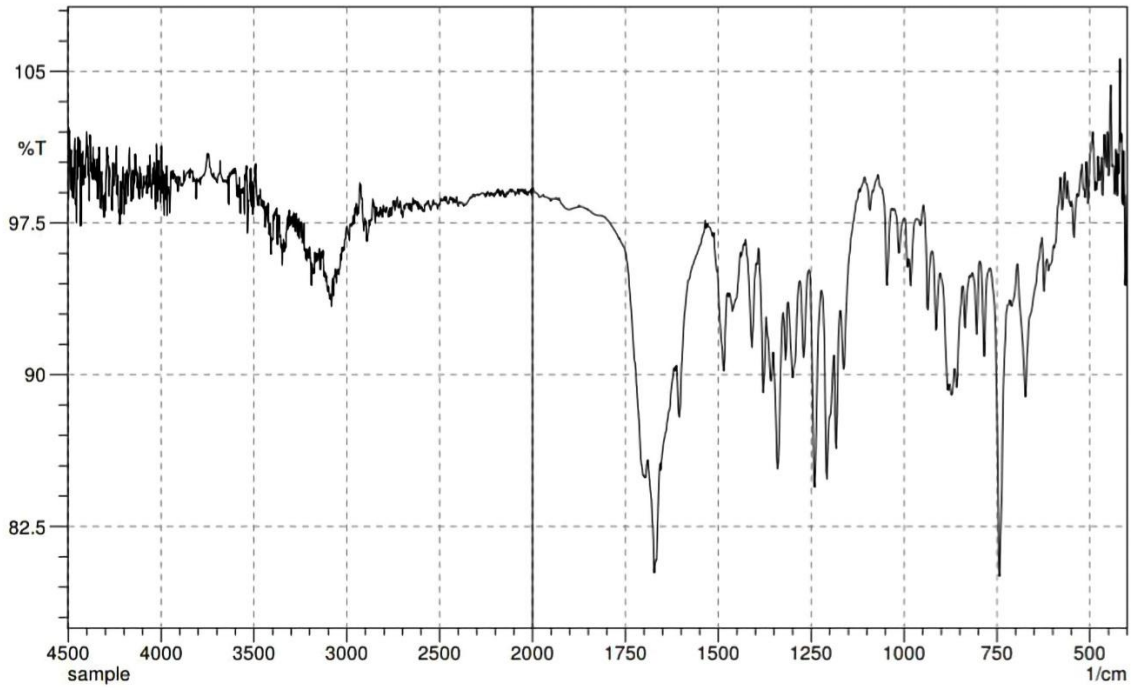


Figure2a. FTIR Spectrum of Lenalidomide

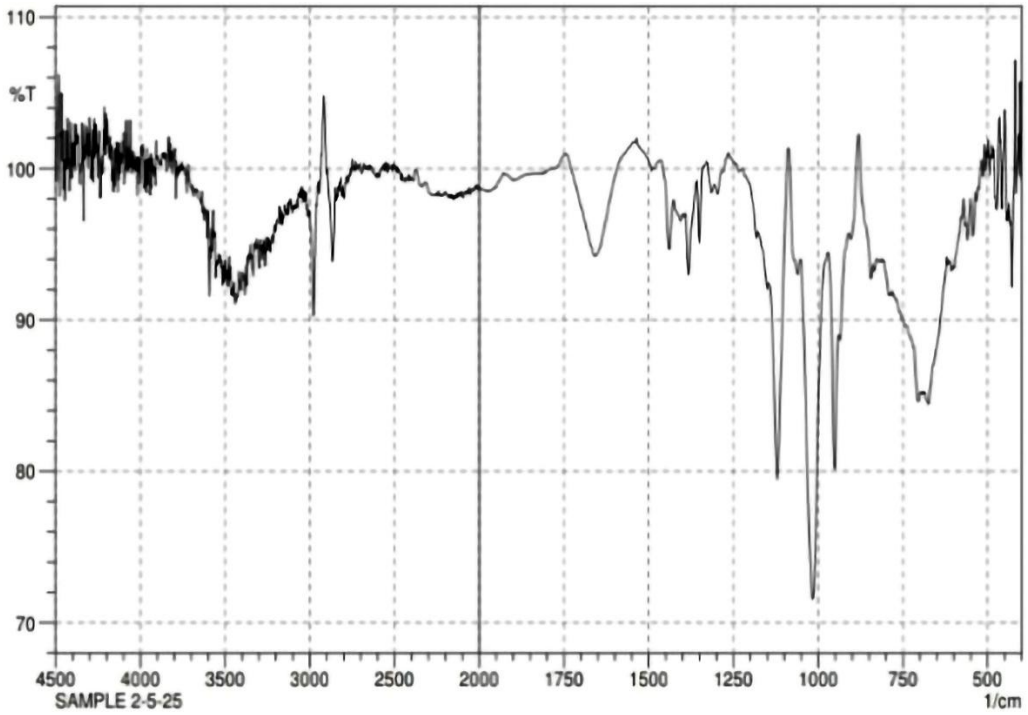


Figure2b. FTIR Spectrum of Lenalidomide loaded niosomes

Figureure. 2b illustrates Fourier-transform infrared (FT IR) spectroscopy of drug-loaded niosomes show the existence of fundamental functional groups and evidence successful incorporation of the drug into the vesicular formation.

Sr. no.	Stretching	Functional Group	
		Reported (cm^{-1})	Observed (cm^{-1})
1	C=O	1700-1750	1690
2	C=C	1450-1600	1500
3	C-O	1000-1300	1079.81
4	C-H	700-900	755.34
5	C-N	1200-1350	1311.59
6	N-H	3300-3500	3365.48

Table1 FTIR spectra readings

Sr. no.	Stretching	Functional Group	
		Reported (cm^{-1})	Observed (cm^{-1})
1	C=O (Amide)	1650-1750	1655
12	C=C	1450-1600	1595
3	C=O (Ester)	1720-1750	1732
4	C-H	2850-2950	2920
5	C-O-C	1050-1250	1100
6	N-H	3300-3500	3410

Table 2. FTIR spectra readings

Tab.le 1 and 2 presents the FT-IR interpretation of Lenalidomide, comparing the reported and observed wavenumbers (cm^{-1}) for various functional groups. All these peaks combined indicate that the active chemical properties of Lenalidomide and the components of niosomes are intact and properly introduced, with no significant chemical or chemical interaction or shift in peaks and evidence supporting the physical entrapment mechanism process, as opposed to any sort of covalent processing of the formulation.

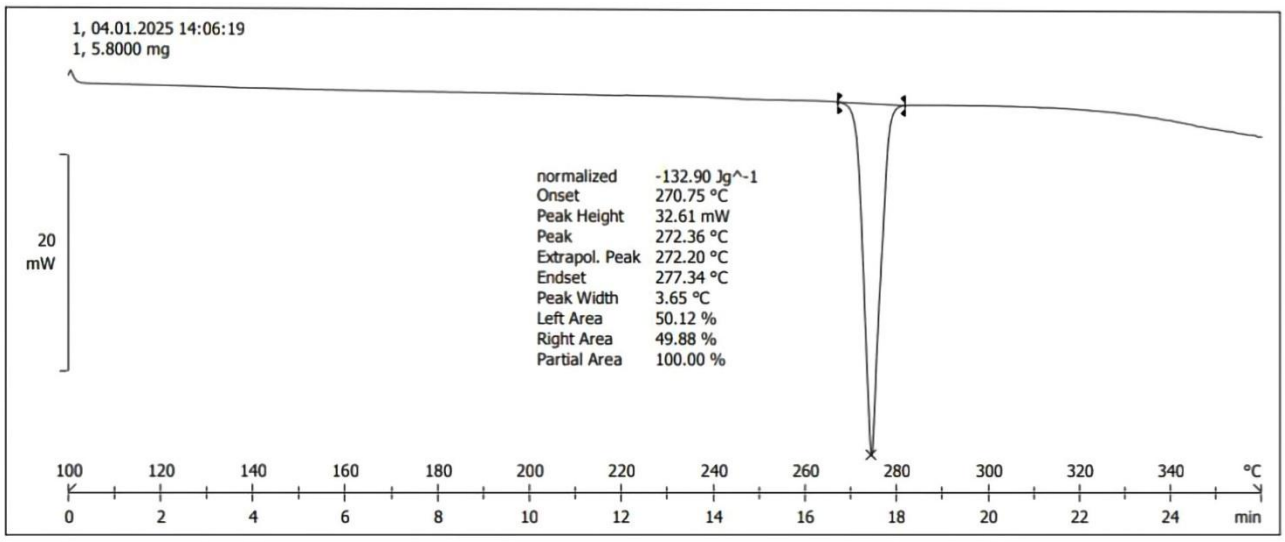


Figure3a shows the sharpness and narrow peak width (3.05 °C) suggest that the API is thermally pure with minimal impurities. The enthalpy value represents the energy absorbed during melting. A single, sharp peak with a narrow temperature range between onset and endset further confirms the homogeneity of the API. The sample exhibits a distinct and sharp melting endotherm at 272.36 °C, indicating high purity and crystalline nature. This information can be useful in confirming the identity of the compound and its suitability for formulation development.

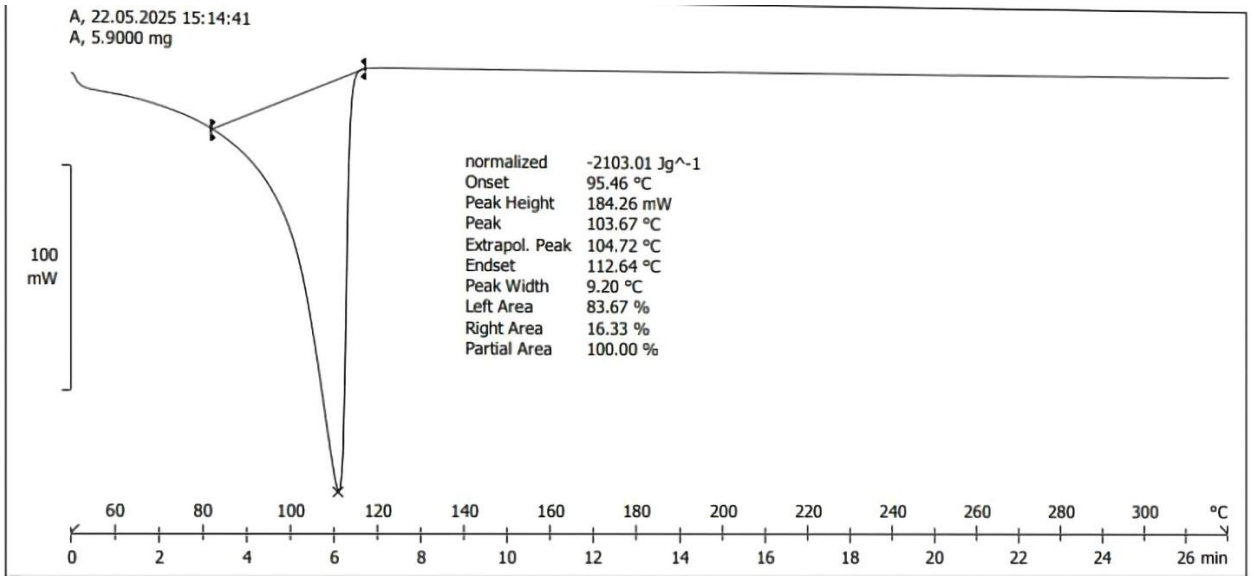


Figure3b. DSC of Lenalidomide loaded niosomes. The DSC thermogram of the niosomal formulation showed a completely different thermal behavior. The characteristic melting peak of pure Lenalidomide was absent, and instead, a new broad endothermic peak was observed at 103.57 °C, with an onset at 95.46 °C and an endset at 112.46 °C. The absence of the melting peak of pure Lenalidomide in the niosomal formulation suggests that the drug was successfully encapsulated within the niosomal bilayer. The shift in the thermal peak indicates that Lenalidomide may be present in an amorphous or molecularly dispersed form, rather than in its original crystalline state. The new thermal event around 103.5 °C could be attributed to the phase transition of the surfactant/cholesterol bilayer or interactions

between the drug and lipid matrix. The drug-excipient interaction may lead to improved solubility and bioavailability, which is often desirable in pharmaceutical formulations.

Evaluation of parameters of all batches

All prepared batches of niosomes were evaluated for parameters such as particle size, zeta potential and drug content. Results are shown in table 3.

FORMULATION	PARTICLE SIZE (in nm)	ZETA POTENTIAL (in mV)
F1	220.17	-30.71
F2	318.41	-32.87
F3	254.18	-31.69
F4	177.1	-30.5
F5	250.84	-31.23
F6	250.84	-31.23
F7	273.57	-30.55
F8	252.28	-29.73
F9	318.41	-32.87
F10	232.67	-30.87
F11	252.28	-29.73
F12	273.57	-30.55
F13	228.74	-28.23
F14	220.17	-30.71

Table 3. Evaluation of optimized batches

Above results indicates that batch B4 shows acceptable results, here selected as Optimized formulation.

3^2 Full factorial design:

Response 1: Particle size

The visualization of interactive effects of Cholesterol & Span 80 on particle size are shown in figure4a and 4b,

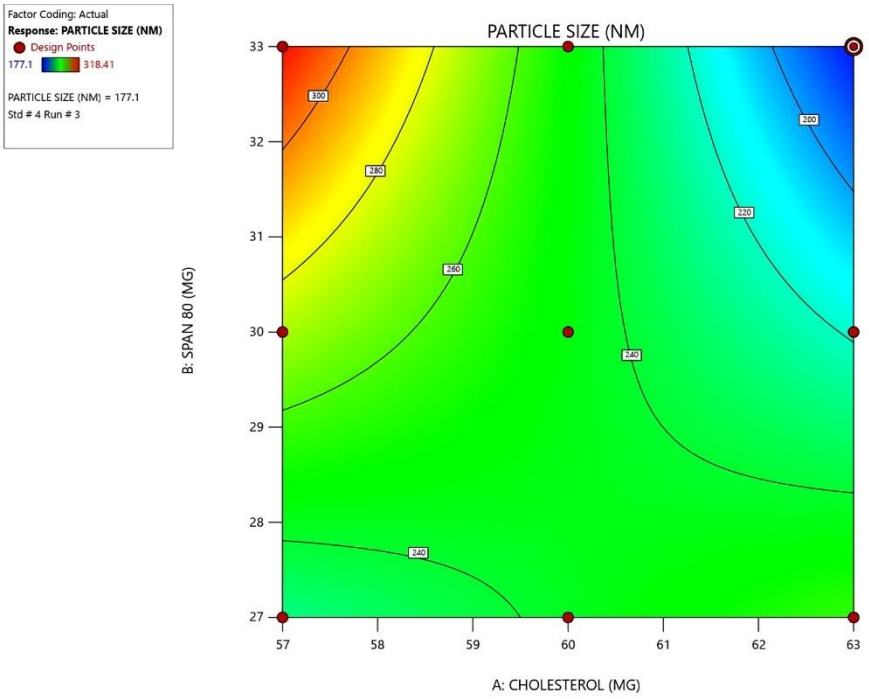
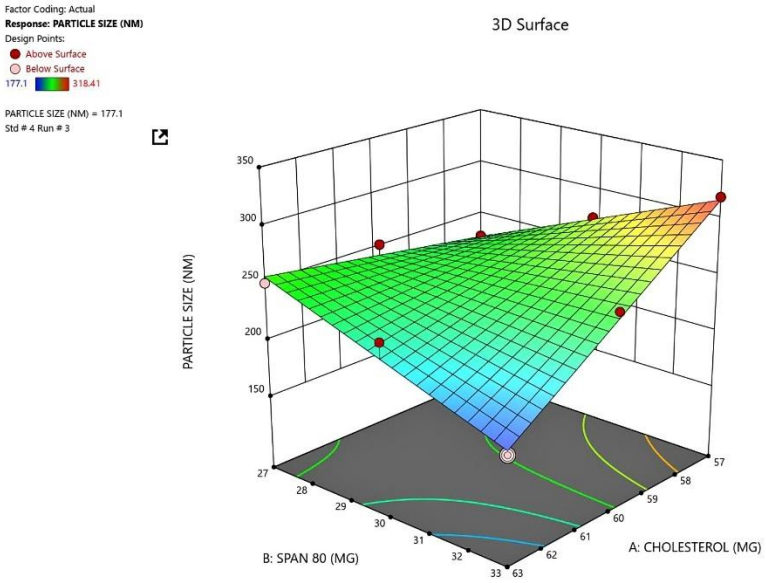


Figure4a Factorial design for response 1 particle size



Figureure4b 3-D plot for response 1 particle size

The visualization of interactive effects of Cholesterol & Span 80 on zeta potential are shown in figure5a and 5b

Response 2: Zeta potential

The visualization of interactive effects of Cholesterol & Span 80 on zeta potential are shown in figure5a and 5b

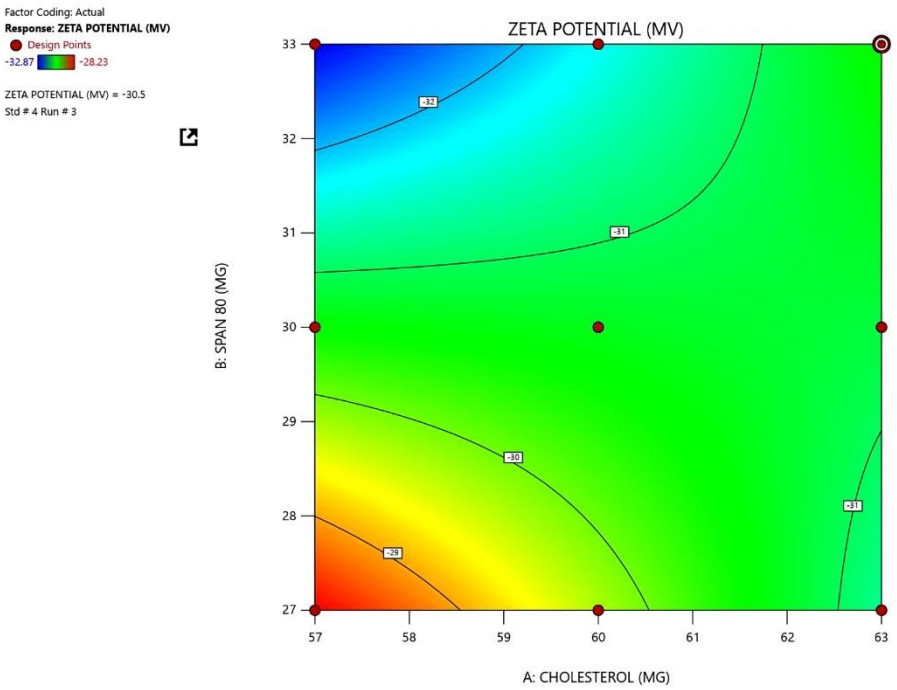
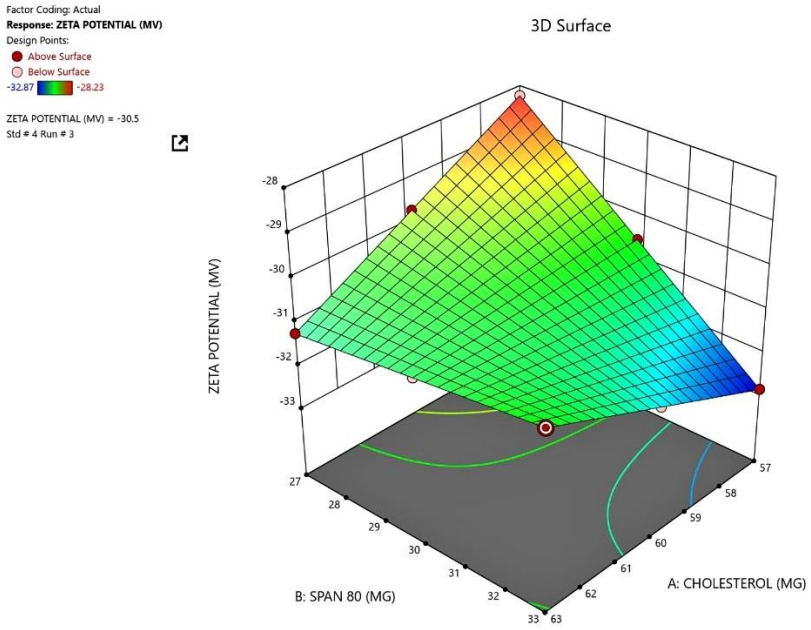


Figure5a Factorial design for response 2 zeta potential



Figureure5b 3-D plot for response 2 zeta potential

Optimization of Niosomal Formulation Using 3² Full Factorial Design

A **3² full factorial design** was utilized to study the combined influence of Span 80 and cholesterol concentrations on the physical characteristics of niosomes. The experimental outcomes for all nine formulations (F1–F14) are presented in Table 3.

Response 1: Particle Size

As shown in the contour and 3D response surface plots (Figure. 4a and 4b), an **increase in Span 80 concentration (X_1)** resulted in a **decrease in particle size**, suggesting that higher surfactant content enhances vesicle flexibility and reduces interfacial tension, thereby promoting the formation of smaller niosomes. Conversely, **increasing cholesterol concentration (X_2)** caused a **slight increase in particle size**, as the rigid bilayer formed by excess cholesterol limits curvature and expansion, producing larger vesicles. Therefore, an optimal Span 80-to-cholesterol ratio is necessary to maintain vesicle stability and uniform nanometric size.

Final equation in terms of coded factors is as follows

Particle Size = +245.33+28.24*A[1]-3.12*A[2]1.38*B[1]-3.19*B[2]-43.46*A[1]B[1]+11.45*A[2]B[1]+3.19A[1]B[2]-18.85*A[2]B[2]

Response 2: Zeta Potential

The response surface plots for zeta potential (Figure. 5a and 5b) indicated that **both Span 80 and cholesterol significantly influenced surface charge**. A moderate increase in Span 80 led to a more negative zeta potential, improving electrostatic stabilization of vesicles. Cholesterol also enhanced zeta potential up to an intermediate level, beyond which no substantial improvement was observed—likely due to charge shielding within the bilayer.

Among all batches, **formulation B4 (33 mg Span 80 and 63 mg cholesterol)** showed the **optimal combination**, achieving the **smallest particle size (177.10 nm)** and **high zeta potential (–30.50 mV)**, indicating excellent physical stability and bilayer integrity.

Final equation in terms of coded factors is as follows:

Zeta potential = -30.71+0.16*A[1]-1.11E-003*A[2]+0.98*B[1]-1.111E-003*B[2]+1.34*A[1]B[1]+1.111E-003*A[2]B[1]+1.111E-003*A[1]B[2]+1.111E-003*A[2]B[2]

Evaluation of Optimized batch:

Visual Appearance:

Dispe.rsion of niosomes was visually inspected and its appearance was seen as white turbid solution .

Particle size

Figure6 displays particle size measurement results of the prep.ared niosomes. The particle size of the samples ranged between 177.10 and 318.41 nm. The PS of niosomes was affected by many factors, such as the cholesterol amount in the formula. The vesicles formation and properties are well kno.wn to be affected by the HLB of the used surfactant.

Polydispersity index (PDI) of niosomes ranged between **0.2 and 0.6**, indicating that the produced niosomes were uniform in size and homogeneous. A PDI.≤0.5 is regarded as suitable for drug delivery applications because it demonstrates a relatively homogeneous and uniform distribution of nanocarriers. To provide proper particle distribution, the optimal formul.ation must have a PDI valu.e≤0.5.

Zeta potential

Niosomes were physically stable since the ZP values of all formulations ranged from **– 32.87 to – 28.23 mV**. Cholesterol had a sig.nificant eff.ect on ZP with a p val.ue of 0.0.094. Zeta

potential increased, as an absolute value, by increasing the cholesterol amount leading to enhancing niosomes stability. As a result, cholesterol is an important excipient in the preparation of niosomes because it enhances the stability of niosomes bilayers and reduces drug leakage due to the retarded solute permeability of these vesicles' aqueous core.

Entrapment Efficiency

After the vesicles were broken up with Triton X-100 (or propanol) and quantitative drug analysis was performed, the entrapment efficiency (EE%) of lenalidomide-loaded niosomes was found to be 80.20±2.53%. The data were expressed as mean ± standard deviation to account for experimental variability, and this value is the mean entrapment efficiency derived from several independent measurements (n = 3). The reliability of the niosome preparation procedure is confirmed by the comparatively low variability seen across duplicates.

The composition of the formulation is responsible for the high entrapment efficiency. The addition of Span 80, a non-ionic surfactant with a low hydrophilic–lipophilic balance (HLB), promotes the development of persistent, hydrophobic bilayers, which are especially useful for encasing drugs that are poorly soluble in water, such lenalidomide. Additionally, adding cholesterol to the niosomal membrane reduces drug leakage and membrane permeability by increasing bilayer stiffness and packing density. Additionally, cholesterol strengthens the bilayer's hydrophobic environment, which facilitates more lenalidomide sequestration and adds to the noted rise in EE%. (Mokale V. Niosomes as an ideal drug delivery system. J Nanosci Res Reports SRC/JNSRR-126. 2021.)

There are a number of reasons why about 20% of the medication was not caught. These include (i) lenalidomide's restricted solubility and partitioning during vesicle formation, which leaves a portion in the external aqueous phase; (ii) the bilayer domain becoming saturated, beyond which more drug cannot be added without compromising vesicle stability; and (iii) unanticipated process-related losses during the hydration, separation, or purification stages.

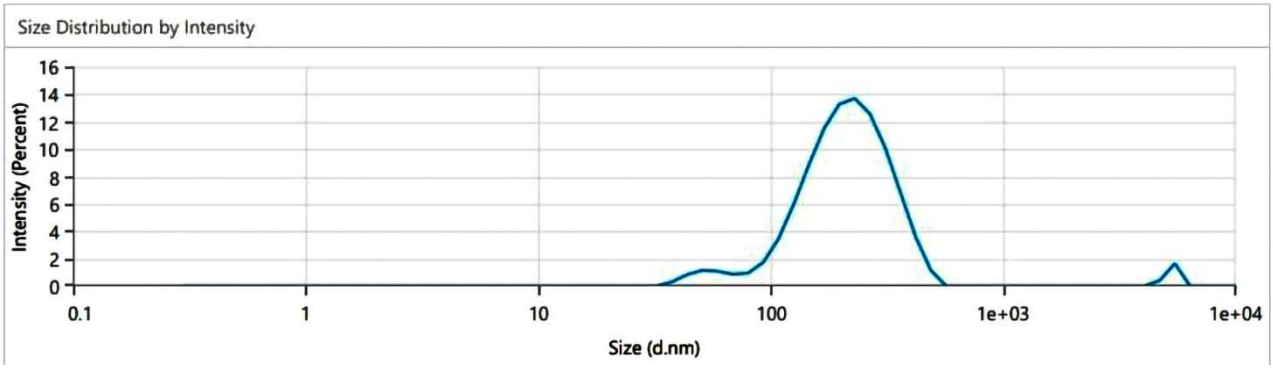


Figure6 Particle size distribution

For niosomal systems, an entrapment efficiency of about 80% is regarded as high, especially for hydrophobic medications. Depending on the kind of surfactant, cholesterol level, and preparation technique, reported EE% values for comparable niosome-based formulations usually fall between 60% and 90%. As a result, the EE% attained in this investigation is at the upper end of the reported range, suggesting that the developed formulation's efficiency is supported and drug loading is successfully enhanced by the optimised combination of Span 80 and cholesterol.[18]

Drug Content

Drug content was determined for all the batches. It was found in the range of

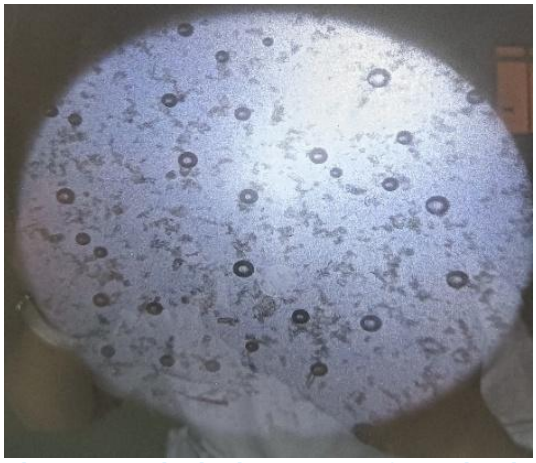


Figure7. Optical microscopy of LEN loaded niosomes

78.18±1.5 to 86.36±2.34. The maximum drug release was found to be 86.36 for F4 batch.

The micro.scopy ima.ges reve.aled from figure 7 that the nios.omes were predominantly spherical to slightly oval in shape, and they appe.ared as well-.formed, discrete vesi.cles. The vesicles showed uniform distribution without any noticeable aggregation, indicating good formulation stability. The spherical morphology is suggestive of successful vesicle formation and appropriate self-assembly of the surfactant and cholesterol components.

In vitro drug release studies

The lenalidomide-loaded niosomal formulation and plain lenalidomide differ significantly in the in vitro drug release profile (Figure8). The niosomal formulation had a higher and more sustained release, achieving virtually entire drug release by the completion of the investigation, whereas the plain drug showed a comparatively slower and partial release, reaching roughly 65–70% over 12 hours. As is typical of vesicular drug delivery systems like niosomes, the release pattern from the niosomes was biphasic, with an initial, quicker release followed by a longer, regulated phase [19,20].

The Higuchi and Korsmeyer-Peppas models showed a stronger correlation with the release data when fitted to different dissolution kinetic models than with zero-order kinetics, suggesting that the release of lenalidomide from the niosomes was primarily diffusion-controlled with a contribution from bilayer relaxation or reorganisation [21, 22]. This behaviour implies that the surfactant–cholesterol bilayer modulates drug release in a sustained way by acting as a diffusional barrier.

In vitro permeation studies

There is progressive and uniform increase in the amount of the drug permeation with time. Lenalidomide loa.ded niosomes sho.wed significantly greater permeation i.e. 66.83% than plain Lenalidomide at 8 hours, this indicates enhanced permeation profile. Figureure. 8 shows the graphical presentation of % cumulative percent permeability of Lenalidomide loaded niosomes.

The purpose of the in vitro permeation investigation was to assess the niosomal formulation's capacity to improve drug transport through a biological membrane model. Even though lenalidomide is mostly taken orally, measuring membrane permeation is important to investigate the possibility of using niosomes to enhance drug absorption across lipidic biological barriers and to look into other delivery methods, like transdermal or transmucosal administration, particularly for patients who might not tolerate oral therapy. When comparing the niosomal formulation to the plain medication, the penetration data showed a significant improvement.

Lenalidomide's cumulative permeation from the niosomes reached roughly 66.8% at 8 hours, while the plain drug's permeability was only 42.3%, suggesting a notable improvement in membrane transport. The presence of the non-ionic surfactant (Span 80), which is known to interact with membrane lipids, increase membrane fluidity, and decrease diffusional

resistance, thereby facilitating drug permeation, and the smaller particle size of the niosomes, which increases surface area and contact with the membrane, are both responsible for this enhancement [20, 23, 24].

But it's important to recognise the permeation model's limitations. The complex structure and barrier characteristics of human skin or intestinal mucosa are not fully represented by the egg membrane employed in this study, which functions as a simpler surrogate membrane. Therefore, additional research utilising more physiologically relevant membranes and in vivo models is necessary to confirm the clinical and translational relevance of these findings, even though the observed increase in permeation offers a strong preliminary indication of the ability of niosomes to enhance lenalidomide permeability [25].

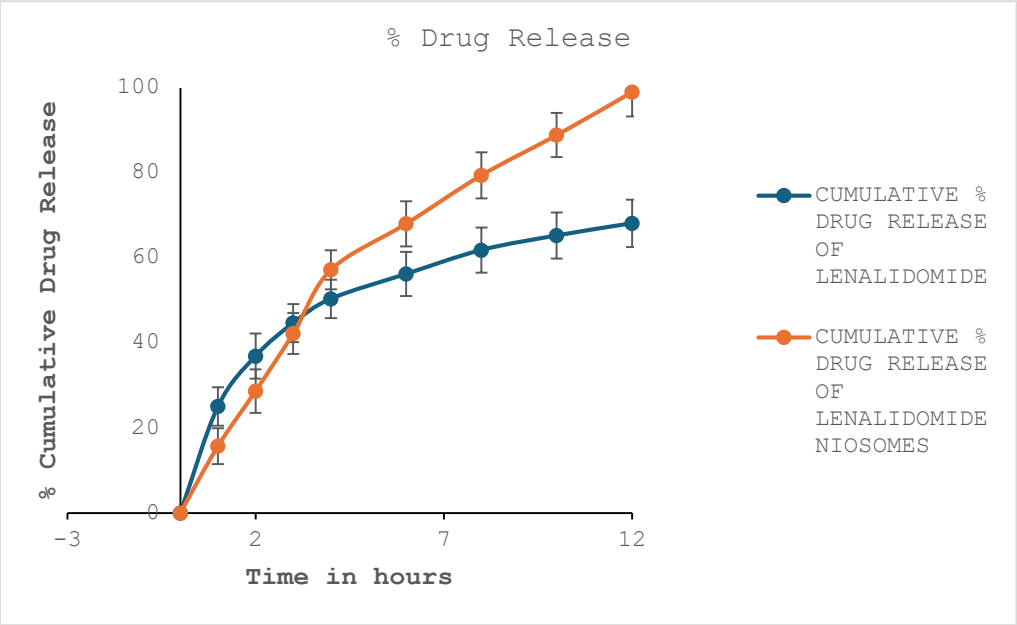


Figure8. Time vs Cumulative % drug release of plain Lenalidomide and Lenalidomide niosomes

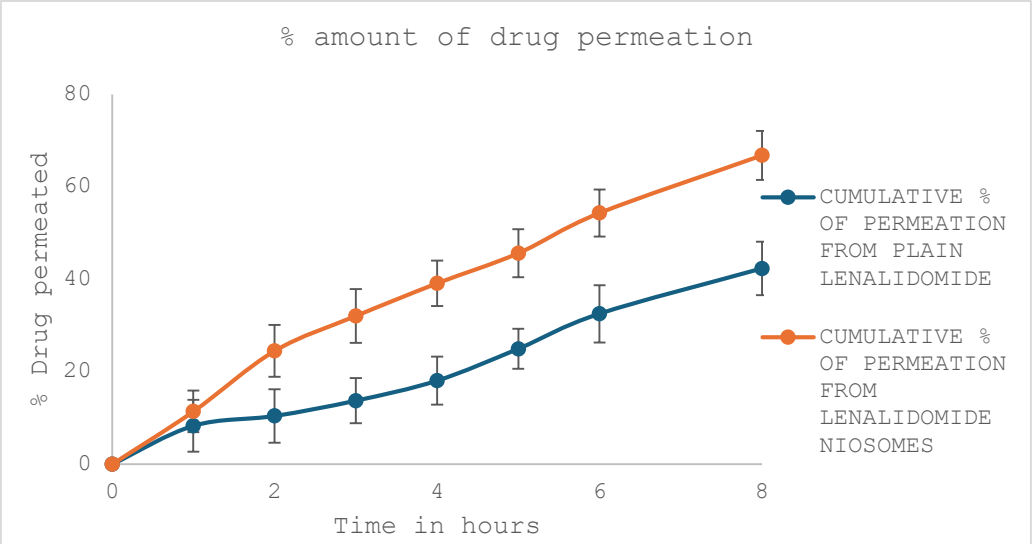


Figure9. Time vs Cumulative % Permeability of Lenalidomide niosome

Conclusion

The current investigation sought to develop, optimise and assess niosomes loaded with lenalidomide. The final formulation (F4) was chosen based on the design space generated through a factorial design approach for optimisation. In comparison to previous batches, the optimised formulation (F4) showed favourable properties such as ideal particle size and homogeneity, excellent entrapment effectiveness, and an improved release profile, suggesting its potential to increase bioavailability and decrease dosage frequency With a particle size of roughly 177.10 nm, niosomes made with Span 80 confirmed their nanoscale range and suggested increased bilayer flexibility that might aid drug penetration through biological membranes. Additionally, after niosomal encapsulation, the release profile showed a higher release rate.

In further research we will concentrate on in vivo studies, including pharmacokinetic and pharmacodynamic assessments in appropriate animal models of multiple myeloma, as the current study was restricted to in vitro evaluations. These investigations would be necessary to establish the Lenalidomide-loaded niosomal formulation's improved bioavailability, therapeutic efficacy, and safety, thereby validating its promise as a clinically feasible drug delivery system.

Materials And Methods

Materials

Lenalidomide was purchased from J. K. Chemicals Gujarat India Cholesterol span 60 and span 80 was purchased from Analab fine Chemicals The all other reagents were of analytical grade.

Pre-formulation studies

Pre-formulation studies involves the physical parameters (Appearance, solubility, melting point), Ultra-violet spectroscopy, FT-IR (Fourier transform- Infrared) studies and DSC (Differential scanning calorimetry) to check the physical parameters and compatibility of drug and excipients.

Formulation of Lenalidomide loaded niosomes

The drug was dissolved into an organic phase i.e. methanol, then it was mixed until compl.etely dissolved. Next, sorbitan mono stearate (Span 80), cholesterol, and lip.id were added into the solution and mixed using a magnetic spin bar in a 20 mL glass beaker. In a separate 50 mL glass beaker, purified water was heated at various temperatures using a hot plate with magnetic stirring. The temperature of the water phase was selected based on the design requirement. The organic phase was filled into a 10 mL syringe with a 26 G needle. The organic phase mixture was injected into the preheated aqueous phase using predetermined parameters based on the experimental design. Mixing was carried out based on the values identified from the design of experiment (DoE). In the last step of the process, the batch was cooled down to RT and the formulation was sto.red in a suit.able glass storage container.

Formulation trial batches of different ratios of span 80 and Span 60 were prepared as seen in table 4.

Sr. no	Surfactant type	Surfactant to drug ratio	Particle size (nm)	Drug content (in %)
1.	Span 80	1:0.5	189..20	82.26
2.	Span 80	1:1	106.0	85.23
3.	Span 80	1:2	254..40	79.18
4.	Span 60	1:0.5	435..77	84.49
5.	Span 60	1:1	348..03	81.86
6.	Span 60	1:2	488.0	83.76

Table 4. Trial batches formulation table

Factor	Symbol	Low (–1)	Medium (0)	High (+1)
Conc. of Span 80 (mg)	X_1	27	30	33
Conc. of Cholesterol (mg)	X_2	57	60	63

Table 5 Independent parameters and levels

The dependent variables (responses) were particle size (Y_1 , nm) and zeta potential (Y_2 , mV). The experimental design generated nine formulation trials (F1–F14), each representing a unique combination of the two independent variables (Table 6).

The statistical analysis, model fitting, and generation of contour and 3D surface response plots were performed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, USA). The optimized batch was selected based on achieving the smallest vesicle size, high absolute zeta potential, and satisfactory entrapment efficiency.

Different experimental runs F1 to F14 were prep.ared as shown in table 6

Table 6. Experimental runs

Sr. No.	Batches	Cholesterol Conc.	Span 80
1	F1	0	0
2	F2	-1	+1
3	F3	0	+1
4	F4	+1	+1
5	F5	+1	-1
6	F6	+1	-1
7	F7	-1	0
8	F8	0	0
9	F9	-1	+1
10	F10	+1	0
11	F11	0	-1
12	F12	-1	0
13	F13	-1	-1
14	F14	0	0

Visual Appearance

Dispersion of niosomes was visually inspected to determine its appearance, turbidity and to see the presence of flocculation and phase separation by taking dispersion in transparent container.

Drug content

10 mg equivalent was taken from prepared niosomal suspension and dissolved in 10 ml of methanol. These were kept for the sonication and to check absorbance in the UV spectrophotometer.

Optical Microscopy

The ultrastructure of the niosomal vesicles was observed using the microscope. A slide of a dispersion was applied on to a glass slide and this was covered with a cover slip. The eye piece magnification set to 10X and the objective lens to 40X was used.

Entrapment

The efficiency of entrapping (EE%), can be regarded as the percentage that represents that segment of the amount of the drug used and gets trapped within the niosomes. Centrifugation can be applied to eliminate free drug which has not been encapsulated in the niosomal solution by applying dialysis procedure. In the phase the vesicles destroyed enable the drug that is loaded in niosomes to be released. Niosomal suspension niosomes could be destroyed by addition of 0.1 per cent Triton X-100 or 50 percent propanol. The loaded and free concentration of the drug can be measured by the UV spectrophotometer. In order to measure the entrapment efficiency, it is calculated as follows:

$$\textbf{Entrapment Efficiency (EE\%)} = \frac{\text{Entrapped drug}}{\text{Total amount of added drug}} \times 100$$

Dispersion of size, Poly Dispersity Index and Measurement of Zeta potential:

To obtain a Size Dispersion, and Poly Dispersity Index (PDI), Dynamic light scattering was done. Zeta potential of dispersion value is noted by the exposure of dispersion into electric field. The zeta potential will have a proportionate relationship to the speed of the particles of the dispersion that will be inclined to stray to the electrode of opposite polarity.

***In vitro* release studies**

In vitro release of Lenalidomide from the niosomes was studied in phosphate buffer pH 6.8 for 8 h using United States Pharmacopeia (USP) type II Paddle type apparatus using volume 900 mL, at 100 rpm and 37°C. Samples (5 mL) were withdrawn through pipette at different time intervals and were assayed at 242 nm for Lenalidomide content spectrophotometrically.

***In vitro* permeation study:**

The in vitro permeation study of the prepared niosomes was carried out using Franz diffusion cell through egg shell membrane because the egg shell membrane resembles human stratum corneum as it consists mainly of keratin [26]. The membrane was accordingly prepared before use [27]. The water in the outer jacket of the cell was warmed and set at 37 ± 1◦C throughout the experiments to provide a skin surface temperature. Phosphate buffer solution of pH 6.8 was used as dissolution medium in the receptor compartment. A 10mg equivalent of niosomes was taken and applied over the mounted membrane in diffusion cell. After that, the samples were withdrawn from the receptor compartment at regulated intervals. The sampling schedule was at 0, 1 hour, 2 and then it was at every hour interval till 8th hour of release. One mL of the receptor solution was collected as sample each time and simultaneously one mL of phosphate buffer solution was added back to the receptor cell for maintaining the same initial volume of the receptor cell solution. The collected samples were analysed using UV-Vis spectrophotometer [28].

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Conflict Of Interest

The authors declare no conflict of interest

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