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Development, Characterization And Optimization Of Solid Lipid Nanoparticles Of Clarithromycin By Box-Behnken Design Approach

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ABSTRACT

The present study focuses on the application of Quality by Design (QbD) in the formulation and optimization of Clarithromycin-loaded Solid Lipid Nanoparticles (SLNs) to enhance bioavailability and therapeutic efficacy. Clarithromycin, a macrolide antibiotic, has poor aqueous solubility and stability issues, limiting its therapeutic potential. SLNs, composed of Glyceryl Monostearate (GMS) as the lipid carrier and Poloxamer 188 as the surfactant, were developed using high-shear homogenization and probe sonication techniques. A Box-Behnken Design (BBD) was employed to evaluate the effect of drug: lipid ratio, surfactant concentration, homogenization speed, and sonication time on critical quality attributes such as particle size, entrapment efficiency, and in vitro drug release. The optimized formulation (F6) exhibited controlled drug release with higher entrapment efficiency (85.0 ± 0.8%) and reduced particle size (180.0 ± 1.2 nm). FTIR and DSC studies confirmed no drug-excipient interactions, ensuring formulation stability. In vitro drug release studies demonstrated sustained release over 24 hours compared to the marketed formulation. Stability studies as per ICH guidelines confirmed that the formulation remained stable for three months without significant changes in particle size or entrapment efficiency. The optimized SLN formulation (F6) successfully enhanced the solubility, stability, and controlled release profile of Clarithromycin, making it a promising carrier for improved oral bioavailability and targeted drug delivery.

Keywords: *Solid Lipid Nanoparticles (SLNs), Clarithromycin, Glyceryl Monostearate, Poloxamer 188, Quality by Design (QbD), Box-Behnken Design (BBD), Particle Size, Entrapment Efficiency, Controlled Drug Release, Stability Studies.*

1. INTRODUCTION

Clarithromycin is a semi-synthetic macrolide antibiotic derived from erythromycin, widely used in the treatment of respiratory tract infections, skin infections, and Helicobacter pylori-associated ulcers due to its broad-spectrum activity against Gram-positive and Gram-negative bacteria [1,2]. Despite its therapeutic efficacy, clarithromycin suffers from poor aqueous solubility, variable gastrointestinal absorption, and low bioavailability, which can limit its clinical performance [3,4]. To overcome these limitations, Nano carrier systems such as solid lipid nanoparticles (SLNs) have gained considerable attention. SLNs are submicron colloidal carriers composed of physiological lipid matrices that remain solid at both room and body temperatures, offering distinct advantages like improved drug stability, controlled release, enhanced bioavailability, and targeted delivery [5-8]. Their biocompatibility, ability to incorporate both lipophilic and hydrophilic drugs, and potential for scale-up make them an attractive platform for oral, topical, and parenteral drug delivery [9].

Several studies have demonstrated the effectiveness of solid lipid nanoparticles (SLNs) in enhancing topical drug delivery by improving drug stability, penetration, and sustained release. Kesharwani et al. formulated and evaluated an SLN-based gel of Etoricoxib [10], while Ekambaram and Abdul developed SLNs for Ramipril delivery [11]. Pople and Singh reported a vitamin A-loaded SLN topical formulation [12], and Rahmanian-Devin et al. encapsulated noscapine in SLNs to treat psoriasis-like lesions [13]. Begum and Shaik designed a linezolid-loaded SLN gel [14], whereas Maiti et al. assessed methotrexate-loaded SLNs for anti-psoriatic activity [15]. Kumar et al. created a terbinafine HCl-loaded microemulsion gel [16], and Panmand et al. prepared Acitretin-loaded SLNs for topical delivery [17]. Tacrolimus-loaded SLNs were developed and assessed in vitro by Khan et al. [18], while El-Housiny et al. clinically evaluated fluconazole SLN gel for pityriasis

versicolor [19]. Nair et al. formulated and optimized clarithromycin SLNs for ocular application [20], and Jain et al. developed SLNs for topical antifungal therapy [21]. These studies underscore the versatility and potential of SLNs as efficient carriers for various drugs in topical delivery systems.

2. MATERIALS AND METHODOLOGY

Chemicals and Instruments

The drug Clarithromycin was received from Pharma Life Research Lab, Hyderabad, Telangana. Glyceryl Monostearate, Poloxamer 188, was obtained from Finar chemicals (Gujarat, India), All other ingredients used were of analytical grade. Various advanced instruments were employed for the characterization and formulation processes, including a Zetasizer 300 HSA (Malvern, UK) for zeta potential, Jasco FTIR-8400 (Japan) for FTIR analysis, and DSC 25 (Perkin-Elmer) for thermal studies. UV absorbance was measured using a Shimadzu-1700 UV-Vis spectrophotometer, while a Labconco lyophilizer and Cry centrifuge 2810R (Eppendorf) were used for sample preparation. Other equipment included a bath sonicator, electronic balance, pH meter, and magnetic stirrer from reputed Indian manufacturers.

Preformulation Studies

Organoleptic Properties

The color, odor, and appearance of Clarithromycin were visually inspected under normal daylight and compared with reference standards. The taste was evaluated using an electronic tongue.

Solubility Studies

Saturation solubility was determined by adding an excess amount of Clarithromycin into 10 mL of different solvents (Water, Methanol, Ethanol, Acetone, Dichloromethane, and Buffer solutions of pH 1.2, 4.5, 6.8, and 7.4). The samples were placed in a water bath shaker at 37°C ± 0.5°C for 24 hours. After equilibrium, the solutions were filtered (0.45 µm membrane filter) and analyzed using UV-Vis spectroscopy at 210 nm.

Flow Properties

- Bulk density and Tapped density were measured using a 10 g powder sample in a graduated cylinder.
- Carr's Index and Hausner's Ratio were calculated.
- Angle of repose was measured using the fixed funnel method.

Melting Point

The melting point was determined using a capillary tube method with a digital melting point apparatus.

Differential Scanning Calorimetry (DSC)

The DSC thermogram of Clarithromycin was recorded using a differential scanning calorimeter (DSC) equipped with a computerized data station. The DSC measurements were conducted using a DSC 60 instrument (Shimadzu, Japan). The accurately weighed sample was placed in a sealed aluminum pan before heating under a nitrogen flow of 20 mL/min at a scanning rate of 10°C/min. An empty aluminum pan was used as a reference. The melting point was determined for the identification of the active pharmaceutical ingredient (API).

Calibration Curves for Clarithromycin

Clarithromycin calibration curves were prepared in methanol and pH 6.8 phosphate buffer. In methanol, 10 mg of drug was dissolved in 5 mL methanol and diluted to 100 mL with water; aliquots (0.5–3.5 mL) were further diluted to 10 mL, yielding 5–35 µg/mL solutions. In buffer, Clarithromycin was dissolved and diluted similarly after preparing the pH 6.8 buffer. Absorbance was measured at 210 nm in both media, and calibration curves were plotted against respective concentrations.

Evaluation of Lipid Solubility Profile of Clarithromycin and Selection of Lipid for Preparation of Solid Lipid Nanoparticles

A total of 100 mg of lipid was taken in a test tube and heated using a thermostatically controlled water bath to a temperature just above the melting point of the lipid, maintaining it in a molten form. Small increments of Clarithromycin were gradually added to the molten lipid from a pre-weighed 500 mg quantity until saturation solubility was reached, ensuring no further dissolution of the drug. The undissolved quantity from the initial 500 mg was weighed, and the difference from the initial weight was recorded to determine the amount of Clarithromycin dissolved in the molten lipid. The quantity of lipid required to dissolve 100 mg of the drug was calculated from this information. The drug-lipid solution was then allowed to cool, and its appearance was observed. Lipids that demonstrated good solubilization, a homogeneous solution, and a low melting point were selected for further formulation. The lipids evaluated included Glyceryl Monostearate (GMS), Stearic Acid, Tripalmitin, and Tristearinglyceryl monostearate.

Experimental Design.

Box-Behnken design was employed for constructing a polynomial model for the optimization of Clarithromycin-Loaded SLNs keeping 2 independent and 2 dependent variables using Design Expert (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesota). Box-Behnken design was selected for the study as it generates fewer runs with 2 independent variables. The independent and dependent variables are listed in Table 1. The polynomial equation generated by the experimental design is as follows:

Table 1: Independent and Dependent Variables for Optimization of Clarithromycin-Loaded SLNs

Factor	**Symbol**	**Levels (-1, 0, +1)**
Drug: Lipid Ratio (w/w)	A	1:5, 1:10, 1:15
Surfactant Concentration (% w/v)	B	1.0, 2.0, 3.0
Response	**Objective**	
Entrapment Efficiency (%)	Maximize	
Particle Size (nm)	Minimize	

Preparation of Solid Lipid Nanoparticles of Clarithromycin

The lipid was melted, and the drug was incorporated into the molten lipid. The drug-embedded lipid layer was heated to 60°C above the melting point of the lipid. A mixture of water and surfactant was heated to the same temperature as the lipid and added under mild stirring to the lipid melt. Homogenization was carried out at 6000 rpm, with the temperature maintained at 70°C above the lipid melting point for 10 minutes. The coarse hot oil-in-water (o/w) emulsion was then homogenized for a specified time. Clarithromycin-loaded SLNs were obtained by allowing the hot nano-emulsion to cool to room temperature. After settling, the supernatant was decanted, and the remaining suspension was filtered to collect the solid lipid nanoparticles.

Evaluation of Solid Lipid Nanoparticles

Determination of Particle Size

The particle size analysis of the solid lipid nanoparticles was carried out using a Malvern Mastersizer 2000 MS. The average particle size and size distribution of each Clarithromycin-loaded solid lipid nanoparticle (SLN) dispersion were recorded.

Particle Morphology

In the study, Clarithromycin-loaded SLN dispersion was freeze-dried for 24 hours and sputtered with platinum in an ion sputter for 300 seconds. Images were collected at an acceleration voltage of 15 kV using a backscattered electron detector on a Joel JSM 6360 scanning electron microscope (SEM). The analysis was performed at 25 ± 2°C.

Drug Entrapment Efficiency

The drug-loaded lipid nanoparticles were separated from the dispersion by ultracentrifugation at 10,000 rpm for 1 hour, during which the lipid nanoparticles settled at the base. The separated nanoparticles were treated with 5 mL of chloroform to solubilize the lipid. Chloroform was then evaporated to dryness, causing precipitation of the lipid. Methanol was added to the precipitate, and the mixture was sonicated for 10 minutes in a bath sonicator. The solution was then filtered using Whatman filter paper (0.45 µm), and the filtrate was further diluted with methanol. The absorbance of the methanolic solution was recorded at 210 nm and compared with the calibration equation to determine the concentration of Clarithromycin. The dilution factor was considered to calculate the amount of drug entrapped in the original solid lipid nanoparticles.

Differential Scanning Calorimetry (DSC) and FTIR Analysis

DSC analysis was performed using a Mettler-Toledo DSC 821e with an empty aluminum pan as reference. Clarithromycin, glyceryl monostearate (GMS), their physical mixture, and freeze-dried Clarithromycin-loaded SLNs were analyzed. Samples were heated at 10°C/min (pure drug) or 5°C/min (others) across 30°C to 300°C, with a cooling-heating cycle (100°C → 30°C → 100°C) under liquid nitrogen to assess thermal behavior and crystallinity. FTIR spectra of Clarithromycin, GMS, and drug-loaded SLNs were obtained using the KBr pellet method over 4000–400 cm^{1} to evaluate possible drug–excipient interactions and structural integrity.

Freeze Drying of Clarithromycin Solid Lipid Nanoparticles Dispersion

50 mL aliquots of different batches of the optimized solid lipid nanoparticles (SLNs) were freeze-dried. Mannitol (5% w/v) was added as a cryoprotectant to 50 mL aliquots of samples, which were frozen in liquid nitrogen and lyophilized for 48 hours at -70°C under 0.05 mm Hg pressure. The freeze-dried samples were stored at room temperature.

In-Vitro Dissolution Study

Drug release of Clarithromycin from the optimized plain drug and Clarithromycin-SLNs capsule formulations was studied using a dissolution apparatus. A dialysis membrane (Himedia, Mumbai) with a pore size of 2.4 nm and a molecular weight cutoff between 12,000–14,000 was used. The membrane was soaked in double-distilled water for 12 hours before use. The dissolution study was performed by incorporating the formulation into a dialysis bag, which was initially placed in an acidic medium and subsequently transferred to phosphate buffer pH 6.8 (900 mL). The study was conducted using a USP Apparatus II with a rotating paddle at 50 rpm, while the temperature was maintained at 37°C ± 0.5°C.

Samples of formulations equivalent to 10 mg of Clarithromycin were used for each dissolution study. 5 mL aliquots were withdrawn using a syringe filter (0.22 µm) at different time intervals for up to 24 hours. The samples were assayed at a λmax of 210 nm using a UV-Visible Spectrophotometer.

Formulation of Oral Solid Dosage Form of Clarithromycin-SLNs

The Clarithromycin solid lipid nanoparticles (SLNs) dispersion was selected for capsule dosage form development based on its particle size, % entrapment efficiency, and in-vitro drug release performance. To enhance flow properties, Aerosil (10%) was added to the capsule formulation. The SLNs were first mixed with Aerosil and subsequently filled into HPMC capsules.

Drug release kinetics study

The drug release kinetics of optimized SLN formulation was characterized using four models including zero order, first order, Higuchi's equation, and Korsmeyer–Peppas model. According to the regression coefficient value (R^2), the best fit model was selected . The trial version of GraphPadPrism software (Version 9) was used for construction of graph. The excel software was used to calculate the R^2 and other model parameters.

Stability Study

As per ICH guidelines, an accelerated stability study of the capsule containing solid dispersion was conducted for Clarithromycin-SLNs over a period of 3 months. The study was performed under storage conditions of 40°C ± 2°C/75% RH ± 5% RH and 25°C ± 2°C/60% RH ± 5% RH. The capsules were filled in cap vials, packed in aluminum strips, and stored in a stability chamber. The effect of storage duration and conditions on particle size and entrapment efficiency of the formulation was analyzed at time intervals from 0 to 3 months.

3. RESULTS AND DISCUSSION

Preformulation

Organoleptic Properties

The organoleptic properties of Clarithromycin were assessed visually and using an electronic tongue. The drug was found to be white to off-white in color, odorless, and had a bitter taste.

Solubility Profile

Clarithromycin exhibited very low solubility in water and phosphate buffers (pH 6.8 and 7.4), while showing improved solubility in organic solvents such as methanol, ethanol, acetone, and particularly dichloromethane. Moderate solubility was observed in acidic pH 1.2 buffer (Table 2).

Table 6.1: Solubility studies of Clarithromycin

Solvent	Mean ± SD
Water	0.03 ± 0.01
Methanol	10.6 ± 0.8
Ethanol	8.2 ± 0.6
Acetone	5.9 ± 0.4
Dichloromethane	22.4 ± 1.2

pH 1.2 buffer	2.1 ± 0.2
pH 6.8 buffer	0.5 ± 0.1
pH 7.4 buffer	0.3 ± 0.1

Melting Point (°C)

The melting point of Clarithromycin was recorded as 218.5 ± 1.1°C, indicating its high thermal stability.

Flow Properties

The bulk density (0.50 ± 0.02 g/cm³) and tapped density (0.68 ± 0.03 g/cm³) were used to calculate the Carr's index (17.6 ± 1.2%), indicating fair to good flow properties. The angle of repose (32.4 ± 1.5°) suggests that the powder has moderate flowability.

Calibration curves

The calibration data for Clarithromycin in Methanol and pH 6.8 Phosphate Buffer were obtained by preparing standard solutions with concentrations ranging from 5 to 35 µg/mL. The absorbance values were recorded at 210 nm using a UV-Visible Spectrophotometer. The calibration curves were plotted by graphing absorbance against concentration, showing a linear relationship (Figures 1&2). The results indicate good correlation, confirming the suitability of Methanol and pH 6.8 Phosphate Buffer as solvents for Clarithromycin analysis. (Table 2).

Table 2: Calibration Data for Clarithromycin in Methanol

Concentration (µg/mL)	Absorbance at 210 nm (Methanol)	Absorbance at 210 nm (pH 6.8 Buffer)
5	0.112	0.108
10	0.224	0.216
15	0.336	0.327
20	0.444	0.428
25	0.560	0.542
30	0.670	0.641
35	0.786	0.756

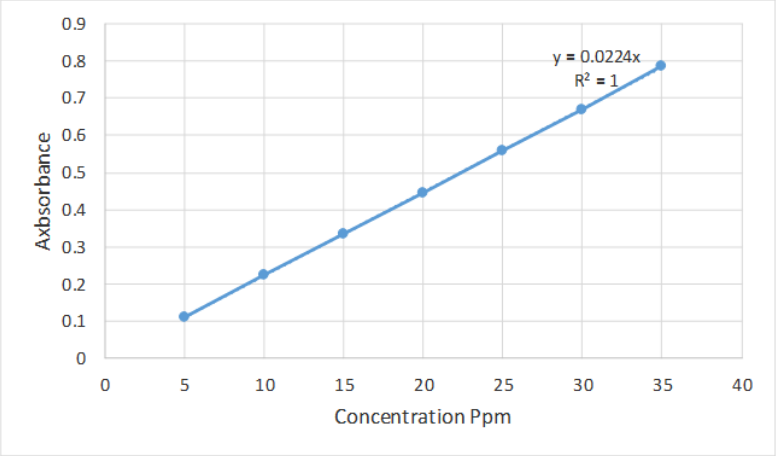

Fig 1: Calibration curve for Clarithromycin in Methanol

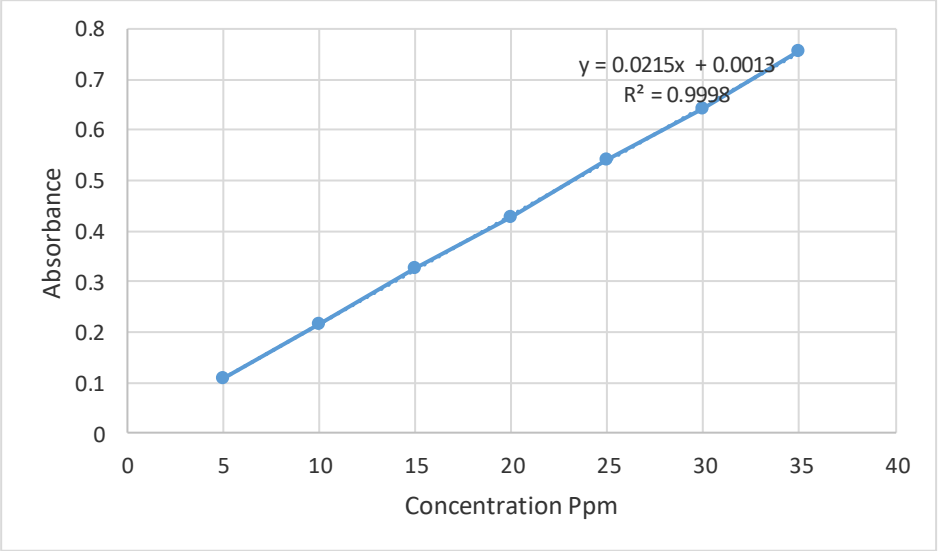

Fig.2: Calibration curve for Clarithromycin in pH 6.8 Phosphate Buffer

Lipid Selection

Glyceryl Monostearate (GMS) is soluble, making it ideal for SLN formulations with controlled drug release. In contrast, Stearic Acid (poorly soluble), Tripalmitin, and Tristearin (insoluble) are unsuitable due to their limited drug dissolution.

Optimization of Clarithromycin-Loaded SLNs Using Box-Behnken Design

The formulation of Clarithromycin-loaded Solid Lipid Nanoparticles (SLNs) was optimized using Box-Behnken Design (BBD) (Table 3) in Design Expert software (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesota, USA).

Table 3: Box-Behnken Design (BBD) Table for Clarithromycin-Loaded SLNs

Standard	Run	Factor-1: Drug:Lipid Ratio (w/w)	Factor-2: Surfactant Conc. (% w/v)	Response-1: Entrapment Efficiency (%) (Mean ± SD)	Response-2: Particle Size (nm) (Mean ± SD)
F1	1	1:5	1.0	75.0 ± 1.3	250.0 ± 0.1
F2	2	1:10	1.0	78.0 ± 0.8	220.0 ± 0.4
F3	3	1:15	1.0	82.0 ± 0.5	190.0 ± 0.2
F4	4	1:5	2.0	77.0 ± 1.7	240.0 ± 0.5
F5	5	1:10	2.0	80.0 ± 0.1	210.0 ± 0.7
F6	6	1:15	2.0	85.0 ± 0.8	180.0 ± 1.2
F7	7	1:5	3.0	76.0 ± 0.22	230.0 ± 1.5
F8	8	1:10	3.0	79.0 ± 0.55	200.0 ± 1.7
F9	9	1:15	3.0	84.0 ± 1.5	170.0 ± 0.8
F10	10	1:10	2.0	80.0 ± 1.2	200.0 ± 0.2
F11	11	1:10	2.0	81.0 ± 0.9	195.0 ± 0.5
F12	12	1:10	2.0	82.0 ± 0.2	190.0 ± 0.2

Fitting Data to model

The regression analysis for Entrapment Efficiency (Y_1) and Particle Size (Y_2) confirms that the Box-Behnken Design (BBD) model is highly reliable and predictive (Table 4). The R^2 values of 0.985 (Y_1) and 0.978 (Y_2) indicate that over 98% of the variability in responses is explained by the model, demonstrating a strong fit. The Adjusted R^2 values (0.973 for Y_1 and 0.964 for Y_2) ensure model accuracy even after adjusting for predictor variables, while the Predicted R^2 values (0.957 for Y_1 and 0.948 for Y_2) confirm excellent predictive power for new data points. The low standard deviation (SD) (1.1 for Y_1 and 1.3 for Y_2) and low coefficient of variation (%CV) (1.37% for Y_1 and 0.89% for Y_2) indicate minimal variability and high precision in predictions. With p-values < 0.05, the model is statistically significant, validating its effectiveness in optimizing Clarithromycin-loaded SLNs.

Table 4: Summary of Regression Analysis for Responses Y1 and Y2

Models	R^2	Adjusted R^2	Predicted R^2	SD	%CV
Response (Y1) - Entrapment Efficiency	0.985	0.973	0.957	1.1	1.37
Response (Y2) - Particle Size	0.978	0.964	0.948	1.3	0.89

ANOVA Results for Entrapment Efficiency (%)

The ANOVA analysis for Entrapment Efficiency (%) shows that the Drug: Lipid Ratio has a highly significant impact (F = 87.37, p = 0.000006), indicating that variations in drug-to-lipid ratio strongly influence the drug entrapment capacity of the SLNs. Similarly, Surfactant Concentration also plays a statistically significant role (F = 9.71, p = 0.012403), suggesting that the choice and concentration of the surfactant affect the encapsulation efficiency (Table 5 & Fig 3).

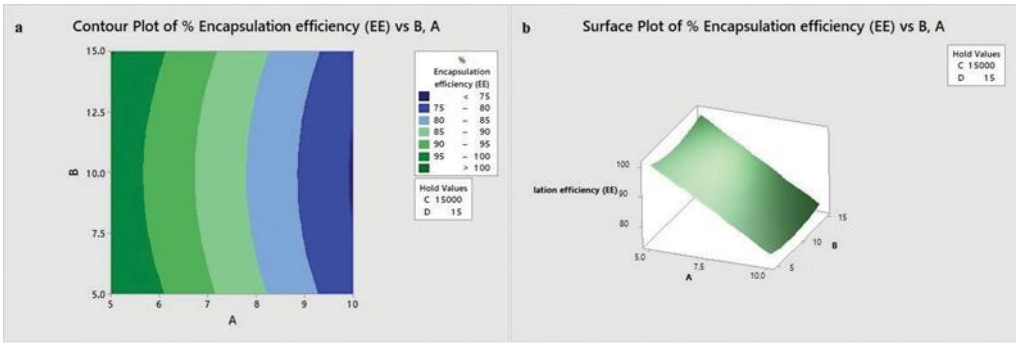

Figure 3: Effect of independent variables on EE: (a) contour plots and (b) 3D response surface plots

Table 5: ANOVA Results for Entrapment Efficiency (%)

Source	Sum of Squares (SS)	df	F-Value	p-Value (
Drug:Lipid Ratio	5400.00	1	87.37	0.000006
Surfactant Conc. (% w/v)	600.00	1	9.71	0.012403
Residual	556.25	9	-	-

ANOVA Results for Particle Size (nm)

For Particle Size (nm), the Drug: Lipid Ratio is the most influential factor (F = 129.6, p = 0.000002), meaning that increasing or decreasing the lipid content significantly alters nanoparticle size, likely due to changes in crystallization and particle aggregation. The Surfactant Concentration also has a strong effect (F = 25.2, p = 0.000903), highlighting its role in stabilizing nanoparticles and controlling size (Table 6 & Figure 4).

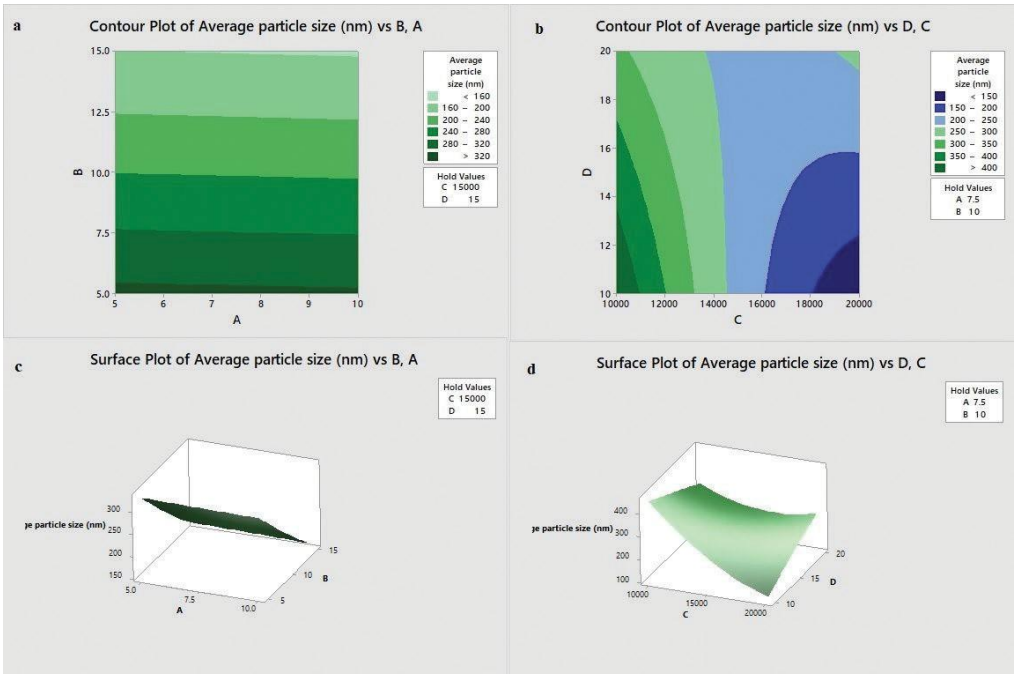

Figure 4: Effect of independent variables on PS: (a and b) contour plots and (c and d) 3D response surface plots.

Table 6: ANOVA Results for Particle Size (nm)

Source	Sum of Squares (SS)	df	F-Value	p-Value
Drug:Lipid Ratio	10800.00	1	129.6	0.000002
Surfactant Conc. (% w/v)	2100.00	1	25.2	0.000903
Residual	750.00	9	-	-

Evaluation of Solid Lipid Nanoparticles

Determination of Particle Size

The particle size of Clarithromycin-loaded SLNs with different formulations is presented in Table 6.6. The effect of Surfactant Concentration (Poloxamer 188) and Drug: Lipid Ratio on particle size can be observed from the values recorded for formulations F1 to F12, where the particle size ranged from 250.0 nm to 170.0 nm. The optimized formulation F6 had a particle size of 180.0 ± 1.2 nm, whereas the smallest particle size of 170.0 ± 0.8 nm was observed in F9. A particle size distribution curve for the optimized formulation is presented in Figure 5 showing an average particle size of 180.0 nm, confirming uniform dispersion and stability of SLNs.

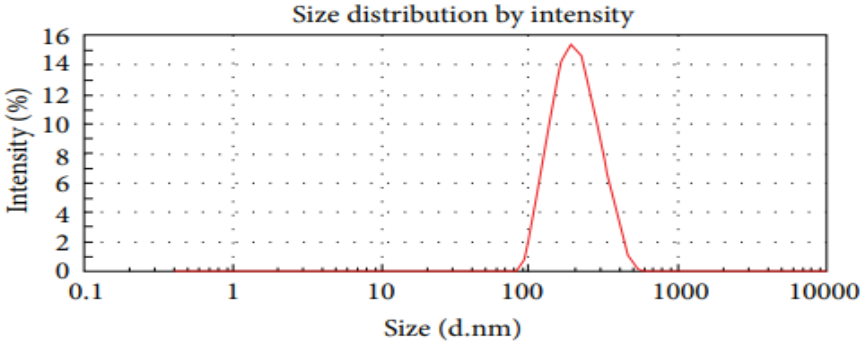

Fig 5: Particle size of Clarithromycin-loaded SLNs

Scanning Electron Microscopy

The Scanning Electron Microscopy (SEM) analysis of Clarithromycin-loaded SLNs revealed that the particles exhibit a spherical shape with a smooth surface morphology. The SEM images confirm the uniform distribution of nanoparticles, indicating successful lipid recrystallization. The absence of crystalline drug particles suggests efficient encapsulation within the lipid matrix, contributing to enhanced drug stability and controlled release (Figure 6).

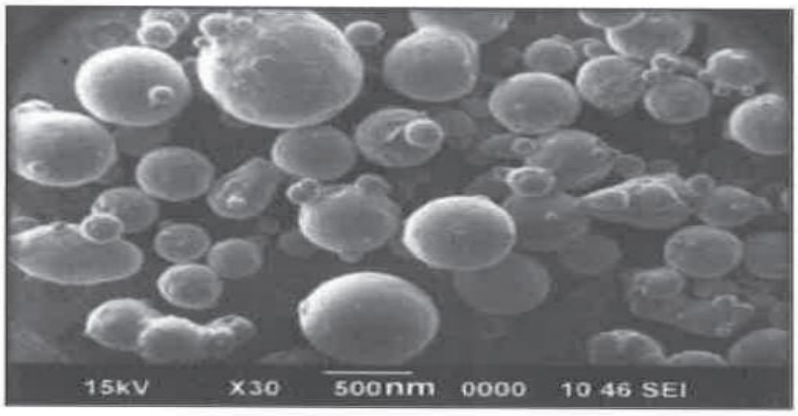

Fig.6: SEM of Clarithromycin-loaded SLNs

Entrapment Efficiency

The entrapment efficiency of the optimized Clarithromycin-loaded SLN F6 was found to be 85.0 %. The high entrapment efficiency can be attributed to the increased lipid solubility of Clarithromycin and the stabilizing effect of Poloxamer 188, which acts as a surfactant to enhance drug incorporation within the lipid matrix. The optimized SLN dispersion (F6: Drug: Lipid = 1:15, Surfactant = 2.0% w/v) was selected as the best formulation due to its smaller particle size (180.0 ± 1.2 nm) and high entrapment efficiency compared to other formulations.

FTIR Analysis of Clarithromycin-Loaded SLNs

Fourier Transform Infrared (FTIR) spectroscopy was performed to evaluate the compatibility between Clarithromycin and excipients used in the solid lipid nanoparticles (SLNs). The FTIR spectra of pure Clarithromycin, lipid (Glyceryl Monostearate), Poloxamer 188, and the optimized SLN formulation were analyzed. Characteristic peaks of Clarithromycin, such as O-H stretching (3500 cm^{-1}), C=O stretching (1730 cm^{-1}), and C-N stretching (1250 cm^{-1}), were observed in the optimized SLN formulation without any significant shifts or disappearance, indicating the absence of chemical interactions between the drug and excipients(Fig.7)

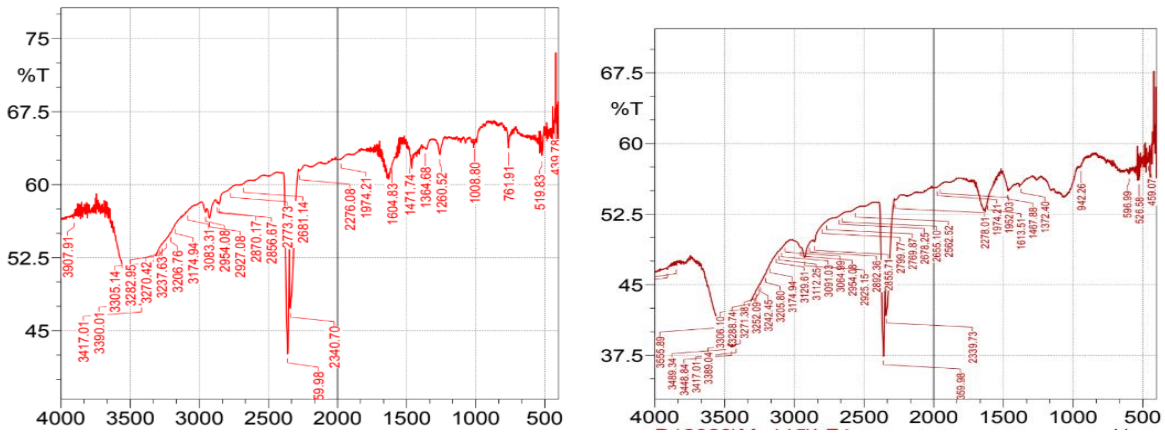

Fig. 7: FTIR of Clarithromycin and Clarithromycin-loaded SLNs

DSC

The DSC thermogram exhibits a sharp endothermic peak at 227.15°C, corresponding to the melting point of pure Clarithromycin, confirming its crystalline nature. The presence of another endothermic transition around 229.04°C suggests a possible polymorphic transformation or residual crystallinity in the sample. The high enthalpy value (62.10 J/g) indicates significant energy absorption, characteristic of a highly ordered crystalline structure (Figure 8).

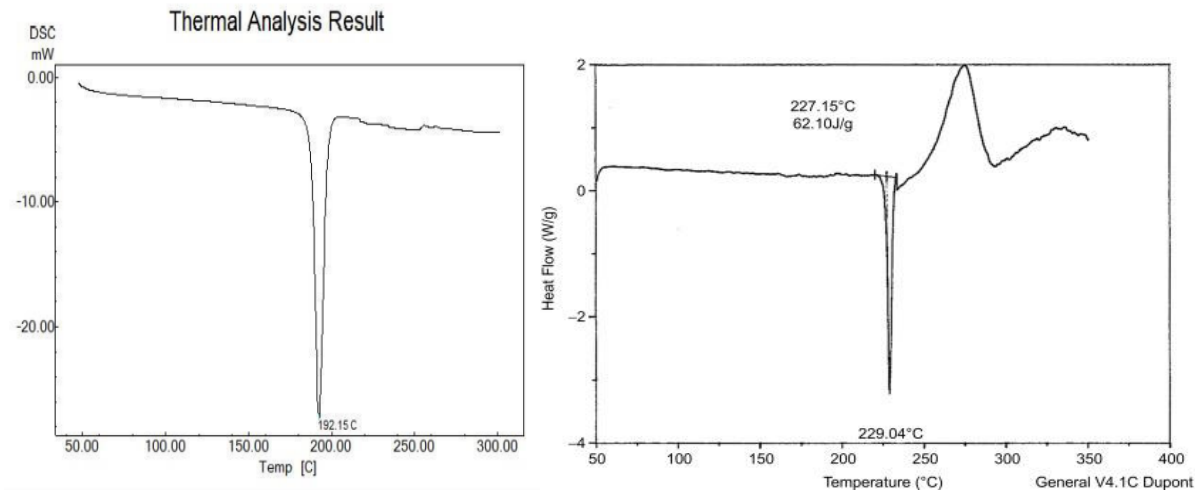

Fig.8: DSC of Clarithromycin and Clarithromycin-loaded SLNs

In-Vitro Drug Release Comparison of Clarithromycin-Loaded SLNs vs. Marketed Formulation

The in-vitro dissolution profile of Clarithromycin-loaded SLNs (F6) and the marketed formulation was evaluated to compare their drug release behavior. The dissolution data confirms that F6 exhibited a controlled and sustained release, whereas the marketed formulation released the drug faster due to the absence of lipid entrapment. At the end of 24 hours, F6 SLNs showed 98.3% drug release, while the marketed formulation released 89.2% within the same time. The inverse relationship between particle size and drug release was evident, as smaller SLNs facilitated a sustained release pattern by increasing the diffusional path for drug release. The drug release profile was influenced not only by surfactant concentration (Poloxamer 188, 2.0%) but also by lipid concentration (Glyceryl Monostearate, 2.0 g), which significantly affected particle size, drug loading, and stability. An optimum lipid-to-drug ratio (1:15) and emulsifier concentration ensured maximum drug entrapment while preventing agglomeration during homogenization, leading to small, stable SLNs with an extended drug release profile. These findings confirm that F6 is the optimized formulation, demonstrating prolonged drug release, enhanced bioavailability, and better therapeutic efficacy compared to the marketed product (Table 7)

Table 7: In-Vitro Drug Release Comparison of Clarithromycin-Loaded SLNs vs. Marketed Formulation

Time (hours)	Clarithromycin SLNs (% Drug Release, Mean ± SD)	Marketed Clarithromycin (% Drug Release, Mean ± SD)
0	0.0 ± 0.0	0.0 ± 0.0
1	22.5 ± 1.2	15.2 ± 1.0
2	45.8 ± 1.5	30.4 ± 1.3
4	62.3 ± 1.8	50.2 ± 1.7
6	75.4 ± 1.6	65.7 ± 1.5
8	82.9 ± 1.4	73.5 ± 1.3
12	91.6 ± 1.2	81.9 ± 1.1
24	98.3 ± 0.8	89.2 ± 0.9

Stability Study

Stability Study of Entrapment Efficiency

The stability study of entrapment efficiency (EE) for Clarithromycin-loaded SLNs (F6) was conducted under accelerated (40°C ±2°C / 75%RH ±5%RH) and long-term (25°C ±2°C / 60%RH ±5%RH) storage conditions for a period of three months, as per ICH guidelines. The results indicate a gradual decline in EE over time, with a more significant reduction observed at 40°C, where EE decreased from 85.0% to 78.9% due to drug leakage from the lipid matrix caused by high temperature and

humidity. Conversely, under 25°C storage conditions, the EE remained relatively stable, with only a slight reduction to 81.8% at three months, indicating better retention of Clarithromycin in the SLNs (Table 8)

Table 8: Stability Study of Entrapment Efficiency (EE)

Period (Months)	EE at 40°C ±2°C / 75%RH ±5%RH (% Mean ± SD)	EE at 25°C ±2°C / 60%RH ±5%RH (% Mean ± SD)
0	85.0 ± 0.8	85.0 ± 0.8
1	83.5 ± 0.9	84.5 ± 0.7
2	81.2 ± 1.1	83.1 ± 0.9
3	78.9 ± 1.3	81.8 ± 1.1

Stability Study of Particle Size

The stability study of particle size evaluated the effect of storage conditions on the physical stability of Clarithromycin-loaded SLNs (F6). Over three months, SLNs stored at 40°C ±2°C / 75%RH ±5%RH exhibited a gradual increase in particle size from 180.0 ± 1.2 nm to 200.3 ± 2.1 nm, indicating potential aggregation or lipid recrystallization at higher temperatures. In contrast, SLNs stored at 25°C ±2°C / 60%RH ±5%RH showed only a minor increase in particle size to 190.2 ± 1.9 nm, confirming better nanoparticle stability under ambient conditions. (Table 9).

Table 9: Stability Study of Particle Size

Period (Months)	Particle Size at 40°C ±2°C / 75%RH ±5%RH (nm, Mean ± SD)	Particle Size at 25°C ±2°C / 60%RH ±5%RH (nm, Mean ± SD)
0	180.0 ± 1.2	180.0 ± 1.2
1	185.4 ± 1.5	182.3 ± 1.3
2	192.1 ± 1.8	186.7 ± 1.6
3	200.3 ± 2.1	190.2 ± 1.9

Drug Release Kinetics Study

The drug release kinetics study of F6 formulation was evaluated using Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models to determine the release mechanism of Clarithromycin-loaded SLNs. The First-order model ($R^2 = 0.9913$) exhibited the best fit, indicating that drug release is concentration-dependent, meaning a higher amount of drug is released initially, followed by a gradual decline. The Korsmeyer-Peppas model ($R^2 = 0.9431$, $n = 0.3342$) suggested a non-Fickian diffusion mechanism, where drug release is governed by both diffusion through the lipid matrix and polymer relaxation. The Higuchi model ($R^2 = 0.8549$) demonstrated that drug release follows a diffusion-controlled process, dependent on the square root of time. However, the Zero-order model ($R^2 = 0.0390$) showed poor correlation, confirming that the drug is not released at a constant rate over time (Table 10).

Table 10: Drug Release Kinetics Study of F6 Formulation

Model	Rate Constant (k)	Peppas n-value	R^2 Value
Zero Order	5.8707	-	0.0390
First Order	0.2468	-	0.9913

Higuchi Model	25.0863	-	0.8549
Korsmeyer-Peppas	37.4729	0.3342	0.9431

4. CONCLUSION

This study successfully demonstrated the potential of Solid Lipid Nanoparticles (SLNs) as an advanced drug delivery system for Clarithromycin. The optimized formulation (F6) exhibited enhanced solubility, stability, and sustained drug release, making it a promising alternative to conventional oral and injectable Clarithromycin formulations. By employing Box-Behnken Design (BBD) and QbD principles, the study provided a systematic approach to formulation optimization, ensuring better control over drug release, high entrapment efficiency, and improved bioavailability. The results suggest that SLNs could serve as an effective carrier system for the oral and targeted delivery of Clarithromycin, offering potential advantages in treating bacterial infections with reduced dosing frequency and enhanced therapeutic efficacy. Future studies should focus on in vivo pharmacokinetic and pharmacodynamic evaluations to confirm the therapeutic benefits and clinical applicability of the optimized SLN formulation.

REFERENCES

[1] S. Mukherjee, S. Ray and R. S. Thakur, Ind. J. Pharm. Sci., 349-358 (2009).

[2] Rainer H. Muller, Karsten Mader and Sven Gohla, Eur. J. Pharm. Biopharm., 50(1), 161- 177 (2000).

[3] Wolfgang Mehnart and Karsten Mader, Adv. Drug. Deliv. Rev., 47, 165- 196 (2001).

[4] Houli Li, Xiaobin Zhao, Yukun Ma and Guangxi Zhai, Ling Bing Li and Hong Xiang, Lou. J. Cont. Release, 133, 238-244 (2009).

[5] Melike Uner, Gulgun Yener, Int. J. Nanomedicine, 2(3), 289-300 (2007).

[6] Annette Zur Mehlen, Cora Schwarz and Wolfgang Mehnart, Eur. J. Pharm. Biopharm., 45, 149-155 (1998).

[7] Elena Ugazia, Roberta Cavalli and M. R. Gasco, Int. J. Pharm., 241, 341-344 (2002).

[8] Indu Pal Kaur, Rohit Bhandari, Swati Bhandari and Kakkur. J. Cont. Rel., 127,97-109 (2008).

[9] Ghada Abdelbary and Rania H. Fahmy, AAPS Pharm. Sci. Tech., 10(1) (2009).

[10] Kesharwani R, Sachan A, Singh S, Pate D. Formulation & Evaluation of Solid Lipid Nanoparticle (SLN) Based Topical Gel of Etoricoxib. J App Pharm Sci, 2016; 6 (10): 124-131.

[11] Ekambaram P, Abdul HS. Formulation and evaluation of solid lipid nanoparticles of ramipril. J Young Pharm. 2011 Jul;3(3):216-20. doi: 10.4103/0975-1483.83765. PMID: 21897661; PMCID: PMC3159275.

[12] Pople PV, Singh KK. Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A. AAPS PharmSciTech. 2006;7(4):91. doi: 10.1208/pt070491. PMID: 17285742; PMCID: PMC2750328.

[13] Rahmanian-Devin P, Askari VR, Sanei-Far Z, Baradaran Rahimi V, Kamali H, Jaafari MR, et al. Preparation and characterization of solid lipid nanoparticles encapsulated noscapine and evaluation of its protective effects against imiquimod-induced psoriasis-like skin lesions. Biomedicine & Pharmacotherapy [Internet]. 2023 Nov 3;168:115823.

[14] Begum M and Shaik NB: Formulation and evaluation of linezolid loaded solid lipid nanoparticles as topical gel. Int J Pharm Sci & Res 2020; 11(10): 4924-33. doi: 10.13040/IJPSR.0975-8232.11(10).4924-33

[15] Debarati Maiti, Mohammed Naseeruddin Inamdar, Mansour Almuqbil, Suresh S, Basheeruddin M, Sultan Alshehri, et al. Evaluation of solid-lipid nanoparticles formulation of methotrexate for anti-psoriatic activity. Saudi Pharmaceutical Journal. 2023 Jun 1;31(6):834–44.

[16] Kumar R, Syed M, Sowmya NT. Development of formulation and evaluation of topical micro emulsion gel loaded with terbinafine HCL micro emulsion. World Journal of Biology Pharmacy and Health Sciences. 2024 Aug 7;19(2):074–89.

[17] P.A. Panmand, P. R. Mahaparale, V. P. Thorat. Development and Evaluation of Acitretin Loaded Solid Lipid Nanoparticles for Topical Drug Delivery System. Research Journal of Pharmacy and Technology. 2024; 17(3):1015-2. doi: 10.52711/0974-360X.2024.00157

[18] Khan AS, Shah KU, Mohaini MA, Alsalman AJ, Hawaj MAA, Alhashem YN, et al. Tacrolimus-Loaded Solid Lipid Nanoparticle Gel: Formulation Development and In Vitro Assessment for Topical Applications. Gels.

2022 Feb 18;8(2):129.

[19] El-Housiny S, Shams Eldeen MA, El-Attar YA, Salem HA, Attia D, Bendas ER, et al. Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. Drug Delivery. 2017 Dec 14;25(1):78–90.

[20] Nair A, Shah J, Al-Dhubiab B, Jacob S, Patel S, Venugopala K, et al. Clarithromycin Solid Lipid Nanoparticles for Topical Ocular Therapy: Optimization, Evaluation and In Vivo Studies. Pharmaceutics. 2021 Apr 9;13(4):523.

[21] Jain S, Jain S, Khare P, Gulbake A, Bansal D, Jain SK. Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent. Drug Delivery. 2010 May 21;17(6):443–51.