

Formulation and Evaluation of Nanostructured Lipid Carriers for Enhanced Drug Solubility and Bioavailability

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ABSTRACT

The present study was undertaken to develop and optimize CTZ-loaded nanostructured lipid carriers (CTZ-NLCs) using a Quality by Design (QbD) approach for improved drug delivery. Initially, Preformulation studies were performed to evaluate the solubility of CTZ in various solid lipids, liquid lipids, surfactants, and co-surfactants. Based on the solubility results, suitable formulation components were selected. The NLCs were developed using selected lipid, surfactant, and homogenization conditions. A Box–Behnken Design (BBD) was employed to investigate the effects of three independent variables, namely lipid concentration (A), surfactant concentration (B), and homogenizer speed (C), on drug content (Y1), entrapment efficiency (Y2), and drug release (Y3). The quadratic models obtained for all three responses were found to be statistically significant. The optimized formulation F9 was selected based on the desirability criteria and evaluated for physicochemical characteristics and drug release. FTIR analysis of F9 showed characteristic absorption bands at 3322.75 cm⁻¹ (O–H), 2972.73 cm⁻¹ (aliphatic C–H), 1720–1740 cm⁻¹ (C=O), 1585.20 cm⁻¹ (C=C), 1381.75 cm⁻¹ (C–H bending), 1032.69 cm⁻¹ (C–O), and 879.38 cm⁻¹ (C–H out-of-plane bending), which were within the reported ranges. The absence of major peak shifts or disappearance indicated compatibility between CTZ and the selected excipients. In-vitro drug release was performed using the dialysis membrane method in phosphate buffer pH 6.8 for 6 h. Overall, the study demonstrated that the QbD-based approach was effective for systematic optimization of CTZ-NLCs, and the optimized F9 formulation showed satisfactory physicochemical characteristics and controlled drug release, indicating its potential as a promising lipid-based drug delivery system.

KEYWORDS: CTZ; Nanostructured Lipid Carriers; Quality by Design; Box–Behnken Design; Drug Content; Entrapment Efficiency; Drug Release; FTIR; Lipid-based Drug Delivery System.

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INTRODUCTION

The development of effective drug-delivery systems for poorly water-soluble drugs remains a major challenge in pharmaceutical research, as inadequate aqueous solubility can result in poor dissolution, limited drug availability at the absorption site, and variable therapeutic response. In recent years, nanotechnology-based delivery systems have gained considerable attention for improving the solubility, dissolution rate, bioavailability, and therapeutic efficacy of poorly soluble pharmaceutical agents.

Clotrimazole is a synthetic imidazole derivative possessing broad-spectrum antifungal activity and is widely used in the treatment of superficial fungal infections caused by susceptible species of fungi. Its antifungal action is primarily associated with inhibition of ergosterol biosynthesis, which leads to alteration of fungal cell membrane integrity and subsequent inhibition of fungal growth. However, clotrimazole is a highly lipophilic drug with very low aqueous solubility, which represents a significant formulation challenge and may adversely affect its dissolution and drug availability. [1-4]

Nanostructured Lipid Carriers (NLCs) represent a second-generation lipid nanoparticulate system developed to overcome certain limitations associated with conventional solid lipid nanoparticles. NLCs consist of a combination of solid and liquid lipids stabilized by surfactants, producing a less ordered lipid matrix with structural imperfections that can accommodate relatively higher amounts of lipophilic drugs. [5-8]

The nanosized particles provide a large surface area, which can facilitate interaction with the dissolution medium and consequently enhance the dissolution behavior of poorly water-soluble drugs. Furthermore, incorporation of Clotrimazole into the lipid matrix may improve its apparent solubility, increase drug loading, enhance entrapment efficiency, and provide modified drug-release characteristics. The lipidic composition of NLCs may also contribute to improved drug partitioning and transport across biological barriers, potentially enhancing the overall bioavailability of the incorporated drug. [9-12]

Therefore, the formulation of clotrimazole-loaded NLCs represents a rational pharmaceutical

approach for overcoming the limitations associated with its poor aqueous solubility.

The present study is therefore undertaken with the objective of formulating and evaluating clotrimazole-loaded nanostructured lipid carriers for enhancement of drug solubility and potential improvement in bioavailability. The developed formulation will be evaluated for critical quality attributes including particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, surface morphology, solid-state characteristics, and in vitro drug-release behavior.

1. MATERIAL AND METHODS

1.1 MATERIAL

Clotrimazole was obtained from Sigma-Aldrich (Merck), India. Stearic acid, glyceryl monostearate (GMS), Dynasan 114, and Pluronic F127 were procured from Cosmos Chem. Pvt. Ltd., Pune, India, through Solanki Enterprises, Pune. Phosphate-buffered saline (PBS, pH 7.4), dialysis membrane (MWCO 1000 Da), and deionized water were obtained from Cosmos Chem. Pvt. Ltd., Pune, India, through Solanki Enterprises, Pune. Methanol was procured from Research-Lab Fine Chem Industries, Mumbai, through Solanki Enterprises, Pune. All chemicals and reagents used in the study were of analytical grade and were used as received without further purification.

1.2 Screening of starting materials

1.2.1 Selection of solid lipid

The solubility in solid lipids was determined by adding Approximately 10 mg of Clotrimazole to 1 g of the lipid and then heating the obtained mixture until the lipid melted; the drug solubility in the melt lipid was visually estimated by establishing the achievement of a clear solution and the absence of drug crystals. Then, this drug lipid mixture was dissolved in methanol and filtered through 0.22 μm filter. The drug content was then analyzed by UV. [13]

1.2.2 Selection of the Liquid Lipid and Surfactant

To determine the solubility of CTZ in various liquid lipids surfactants and Co surfactants, an excessive amount of CTZ (approximately 10 mg) was added to a 2.0 mL tube containing 2 mL of each vehicle. The mixtures were shaken for 24 h at 25°C to reach an equilibrium state using a vortex shaker (50 rpm, Labline). The mixtures were then centrifuged at 1000 rpm for 15 min, and the supernatant was passed through a 0.20 μm syringe filter to remove the excess CTZ. Finally, the CTZ in the filtrates was determined using UV Spectroscopy analysis 244 and 320 nm at after appropriate dilution with Methanol. [14-15]

1.2.3 Screening of co-surfactant

Poloxamer 407 and Poloxamer 188 were selected as co-surfactants for the study. Excess amount of CTZ (Approximately 10 mg) were added to a known volume of co-surfactant (1%, 2 ml), mixed to get

saturated solution. A mechanical shaker was used for 24 h to dissolve the drug. The contents were then centrifuged at 10,000 rpm for 15–20 min. The contents of the supernatant saturated co-surfactant systems were diluted with Methanol and analyzed by UV spectrophotometer. [14-15]

1.2.4 Miscibility study

Solid and liquid lipids which identified from preliminary studies were combined in different weight ratios (90:10, 80:20, 70:30, 60:40 and 50:50 w/w) to form binary lipid mixtures. Each mixture was melted and stirred uniformly, then allowed to cool to room temperature. The formulations were evaluated for compatibility and physical stability by visual inspection for turbidity, phase separation, and precipitation. Additionally, a filter paper test was performed by smearing a small quantity of each mixture onto filter paper to detect oily stains, indicating the presence of unincorporated liquid lipid. The binary lipid composition that exhibited good miscibility, physical homogeneity, absence of turbidity or phase separation, and no oily residue on the filter paper was selected for further formulation development. [16]

1.3 FORMULATION DEVELOPMENT OF CTZ-NLC

The CTZ-loaded nanostructured lipid carrier (CTZ-NLC) was prepared using the hot homogenization-ultrasonication method. Briefly, stearic acid (solid lipid) and triglyceride (liquid lipid) were mixed at a total lipid concentration of 5% w/v and heated to approximately 5°C above the melting point of stearic acid. CTZ, equivalent to 5% w/v of the total lipid mixture, was dissolved in the molten lipid phase. Separately, an aqueous surfactant phase containing Tween 80 and Poloxamer 188 was heated to the same temperature and added dropwise to the molten lipid phase under continuous magnetic stirring at 1500 rpm for 5 min. The resulting pre-emulsion was homogenized at 20,000 rpm for 10 min using an Ultra-Turrax T25 homogenizer (WiseMix™ HG15A, Daihan Scientific, Seoul, Korea). The resulting oil-in-water (o/w) nanoemulsion was subsequently subjected to probe sonication at 60% amplitude for 10 min. Finally, the prepared CTZ-NLC dispersion was allowed to cool to room temperature before further evaluation. [16-17]

1.3.1 Experimental design

A three-factor, three-level Box-Behnken Design (BBD) was selected to evaluate the quadratic response surfaces and to investigate the effects of the predefined independent variables on the selected formulation responses. The three independent variables selected were lipid concentration (%) (A), surfactant concentration (%) (B), and homogenizer speed (RPM) (C). The lipid concentration was studied at 2, 4, and 6%, the surfactant concentration at 1, 1.5, and 2%, and the homogenizer speed at 10,000, 15000, and 20,000

RPM, corresponding to the low (−1), medium (0), and high (+1) levels, respectively. The effects of these independent variables were evaluated on three dependent responses, namely drug content (%), entrapment efficiency (%), and drug release (%), which were designated as Y1, Y2, and Y3, respectively. The constraints for all three responses were set to maximize drug content, entrapment efficiency, and drug release. The finalized independent variables, their respective levels, and the selected dependent responses with their optimization criteria are presented in Table 1

Table 1: DOE Suggested and experimental batches

Formulation Code	CT Z(mg)	Li pid (%)	Surfactant Conc (%)	Homogenizer Speed (RPM)	Aqueous Volume (mL)
F1	10	4	2	10000	30
F2	10	2	1.5	20000	30
F3	10	6	1.5	10000	30
F4	10	2	1.5	10000	30
F5	10	4	1	20000	30
F6	10	6	2	15000	30
F7	10	2	2	15000	30
F8	10	4	1	10000	30
F9	10	4	2	20000	30
F10	10	2	1	15000	30
F11	10	4	1.5	15000	30
F12	10	6	1.5	20000	30
F13	10	6	1	15000	30

1.4 EVALUATION OF CTZ-NLC

1.4.1 Drug content determination

Drug loaded NLC equivalent to 10mg was first dissolved in 10 ml methanol and was further diluted up to 10 ml with phosphate buffer pH 6.8 and stirred continuously for 2 hrs and then final colloidal suspension was ultra-centrifuged at 10,000 rpm for 30 min. Supernant was taken, appropriate dilutions were made and measured spectro photometrically at 239.2 nm to get drug content. [18]

$$\text{Drug Content (\%)} = \frac{\text{Actual drug Content}}{\text{Theoretical drug Content}} \times 100 \dots (1)$$

1.4.2 Entrapment efficiency (%)

The entrapment efficiency of CTZ loaded NLC were determined by centrifugation at 30,000 rpm for 1 h using “ultracentrifuge (Remi Instruments,

Mumbai, India)”. Then, the samples supernatant were pipetted, appropriately diluted with methanol, and then analyzed using a “UV spectrophotometer (Jasco v-630) at 296 nm. The entrapment efficiency was calculated using following equation. [19]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100 \dots (2)$$

1.4.3 In Vitro Drug Release

The *in-vitro* drug release study of CTZ-NLC formulations was performed using the dialysis membrane method in phosphate buffer (pH 6.8) for 6 h. A dialysis membrane (HiMedia, India) with a molecular weight cut-off of 12,000–14,000 Da (70 DM) was used for the study. Prior to the experiment, the dialysis membrane was soaked in double-distilled water overnight to ensure adequate hydration. The release assembly consisted of donor and receptor compartments. Approximately 2 mL of CTZ-NLC formulation was placed in the donor compartment, which consisted of a boiling tube with an opening at one end and the other end covered with the pre-soaked dialysis membrane. The donor compartment was immersed in a 250 mL beaker containing 100 mL of phosphate buffer (pH 6.8) as the receptor medium. The receptor medium was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the study with continuous agitation. Approximately 2 mL of the receptor medium was withdrawn at predetermined time intervals of 1, 2, 3, 4, 5, and 6 h and immediately replaced with an equal volume of fresh release medium to maintain sink conditions. The withdrawn samples were suitably diluted and analyzed for drug content using a UV–visible spectrophotometer (Jasco V-630). The cumulative percentage of drug released was calculated at each time interval [20–21].

1.4.4 Particle Size, PDI and Zeta Potential

The particle size, polydispersity index (PDI), and zeta potential of the prepared CTZ-NLC were determined using a dynamic light scattering (DLS) instrument. The optimized batch of NLC dispersion (1ml) was suitably diluted with distilled water and analyzed at room temperature. Particle size and PDI were measured by DLS, while zeta potential was determined based on electrophoretic mobility. [22]

2.4.6 Transmission Electron Microscopy (TEM) (JEOL, 2200FS)

The surface morphology of the optimized batch was established by using Transmission Electron Microscopy (TEM).

Few microliters of diluted solution of optimized batch was put on 300 mesh copper grid film coated with copper and were air-dried at room temperature. Once it was dried completely, the sample was stained using a 2 %w/v phosphotungstic acid solution removing the excess with a filter paper. Further, the analysis and images of samples were captured by using digital micrograph and Soft Imaging Viewer Software.[24]

1.5 Lyophilization of FEN-NLC

Lyophilization of FEN-NLC was performed using trehalose as a cryoprotectant. The dispersion was pre-frozen (-80°C) for 12 h and subsequently lyophilized at a temperature of -25°C for 24 h followed by a secondary drying phase of 12 h at 20°C . [25]

1.5.1 Differential Scanning Calorimetry

Differential scanning calorimetric (DSC) measurements were carried out on a modulated DSC (Mettler Toledo, SW STARE, and USA). The optimized batch (F9) was weighed (2-8mg), the aluminum pans were used and hermetically covered with lead. The heating rate was $50-250^{\circ}\text{C}$ for sample with constant increasing rate of temperature at $10^{\circ}\text{C}/\text{min}$ under nitrogen atmosphere ($50-60\text{ml}/\text{min}$). The resultant thermograms of formulation was obtained. [111]

1.5.2 PXRD

The data obtained from PXRD (Bruker D8 Advance D8 Advance Series 1) was used to determine whether newly formed compounds are crystalline or amorphous, the following conditions were used for the measurement: target metals Cu, filter K, 40kV voltage, and 30 mA current. Optimized batch sample were scanned over a two-degree range of $10-90^{\circ}\text{C}$ with a 0.2° phase scale. [26]

2. RESULT AND DISCUSSION

2.1 Screening of starting materials

2.1.1 Screening in solid lipids

The investigated solid lipids, stearic acid exhibited the highest solubility ($1.26 \pm 0.026 \text{ mg/mL}$), followed by glycerol monostearate ($0.78 \pm 0.017 \text{ mg/mL}$), whereas Dynasan 114 showed the lowest solubility ($0.46 \pm 0.079 \text{ mg/mL}$). Based on these findings, stearic acid demonstrated comparatively better solubilizing capacity and was therefore considered a suitable lipid for further formulation development.

2.1.2 Screening of Liquid lipid

The selected liquid lipids, triglyceride exhibited the highest solubility ($3.6 \pm 0.097 \text{ mg/mL}$), followed by castor oil ($2.7 \pm 0.123 \text{ mg/mL}$), while oleic acid showed the lowest solubility ($1.2 \pm 0.096 \text{ mg/mL}$). Based on the observed solubility, triglyceride demonstrated the highest solubilizing capacity and was considered a suitable liquid lipid for further development of the formulation.

2.1.3 Screening of Surfactant

The selected surfactants, Tween 80 exhibited the highest solubility ($2.8 \pm 0.563 \text{ mg/mL}$), followed by Tween 60 ($1.9 \pm 0.026 \text{ mg/mL}$) and Tween 40 ($1.2 \pm 0.005 \text{ mg/mL}$). Tween 20 showed the lowest solubility ($0.89 \pm 0.784 \text{ mg/mL}$). Based on the observed solubility, Tween 80 was selected as the surfactant for further formulation development.

2.1.4 Screening of co-surfactant

The selected co-surfactants, Poloxamer 188 exhibited higher solubility ($1.9 \pm 0.062 \text{ mg/mL}$) compared with Poloxamer 407 (1.3 ± 0.019

mg/mL). Based on the observed solubility, Poloxamer 188 was selected as the co-surfactant for further formulation development.

2.2 EVALUATION AND CHARACTERIZATION OF CTZ NLC

3.2.1 Optimization of EBER-Loaded Niosomes (EBER-Ns)

Table 3: Experimental runs of the Box–Behnken design (BBD) for the development and optimization of niosomal formulations, showing the observed responses: Drug Content (Y_1), Entrapment efficiency (Y_2), and Drug release (Y_3), as generated by Design-Expert® software.

Table.2: Drug Content of F1-F13

Formulation Code	Drug Content (%)	Entrapment efficiency (%)	Drug release (%)
F1	89.21 ± 0.0123	85.39 ± 0.013	88.45
F2	91.28 ± 0.031	87.62 ± 0.032	90.16
F3	90.38 ± 0.081	86.12 ± 0.095	89.16
F4	87.46 ± 0.059	84.63 ± 0.052	83.49
F5	92.97 ± 0.017	88.97 ± 0.047	90.63
F6	90.86 ± 0.095	85.93 ± 0.018	87.48
F7	81.29 ± 0.032	78.12 ± 0.093	81.95
F8	92.47 ± 0.003	88.63 ± 0.031	86.48
F9	95.36 ± 0.0263	92.46 ± 0.048	94.26
F10	83.65 ± 0.123	79.26 ± 0.082	82.17
F11	85.47 ± 0.014	80.28 ± 0.021	81.59
F12	93.45 ± 0.049	89.12 ± 0.019	89.34
F13	85.39 ± 0.078	81.77 ± 0.072	82.19

The drug content of CTZ-NLC formulations F1–F13 ranged from $81.29 \pm 0.032\%$ to $95.36 \pm 0.0263\%$. Among the formulations, F9 exhibited the highest drug content ($95.36 \pm 0.0263\%$), indicating efficient drug incorporation, whereas F7 showed the lowest drug content ($81.29 \pm 0.032\%$). Overall, the formulations demonstrated satisfactory drug content, suggesting uniform distribution of clotrimazole within the NLC formulations.

The in-vitro drug release profiles of CTZ-NLC formulations F1–F13 demonstrated a gradual increase in cumulative drug release with increasing time. After 6 h, the cumulative drug release ranged from 81.59% to 94.26%. Among all formulations, F9 exhibited the highest drug release (94.26%), whereas F11 showed the lowest release (81.59%). Formulations F2, F5, F8, and F12 also showed comparatively higher drug release, while F4, F7,

F10, F11, and F13 exhibited relatively slower release. The progressive release pattern observed in the NLC formulations indicates controlled drug release of CTZ from the lipid matrix. Based on the overall release profile, F9 may be considered a promising formulation should also consider particle size, PDI, zeta potential, drug content, and entrapment efficiency.

ANOVA for Quadratic Model – Response 1: Drug Content (%)

The quadratic model obtained for drug content was found to be statistically significant, as indicated by the model F-value of 10.13 and p-value of 0.0413 ($p < 0.05$). This indicates that the selected quadratic model adequately represents the relationship between the independent variables and drug content. There was only a 4.13% probability that an F-value of this magnitude could occur due to experimental noise.

and the quadratic effect of homogenizer speed were the major factors influencing drug content in the developed formulation.

Final Equation in Terms of Coded Factors

The final polynomial equation for drug content (%) in terms of coded factors was obtained as:

$$\text{Drug Content (\%)} = 85.47 + 2.05A + 0.2800B + 1.69C + 1.96AB - 0.1875AC + 1.41BC - 1.02A^2 + 0.8438B^2 + 6.19C^2$$

Where, A = lipid concentration (%), B = surfactant concentration (%), and C = homogenizer speed (RPM)

ANOVA for Quadratic Model – Response 2: Entrapment Efficiency (%)

The ANOVA results indicated that the quadratic model for entrapment efficiency was statistically significant, with an F-value of 9.04 and p-value of 0.0483 ($p < 0.05$). Among the investigated terms, the quadratic effect of homogenizer speed (C^2) was found to be significant, with an F-value of 43.51 and p-value of 0.0071.

Final Equation in Terms of Coded Factors

The final polynomial equation for entrapment efficiency (%) in terms of coded factors was obtained as:

$$\text{Entrapment Efficiency (\%)} = 80.28 + 1.66A + 0.4087B + 1.68C + 1.33AB + 0.0025AC + 1.68BC - 0.5000A^2 + 1.49B^2 + 7.09C^2$$

Where A = lipid concentration (%), B = surfactant concentration (%), and C = homogenizer speed (RPM). The equation can be used to predict the entrapment efficiency at different combinations of the selected formulation variables.

ANOVA for Quadratic Model – Response 3: Drug release (%)

The ANOVA results showed that the quadratic model for drug release (%) was statistically significant, with an F-value of 44.47 and p-value of 0.0049 ($p < 0.05$), indicating a good fit of the model. Among the model terms, lipid concentration (A), surfactant concentration (B), homogenizer speed (C), AB, AC, B^2 , and C^2 were significant ($p < 0.05$). The C^2 term showed the strongest effect, with an F-value of 190.66 and p-value of 0.0008. In contrast, BC and A^2 were non-significant ($p > 0.05$). Overall, the results indicate that lipid concentration, surfactant concentration, and homogenizer speed, along with their significant interaction and quadratic effects, substantially influenced drug release.

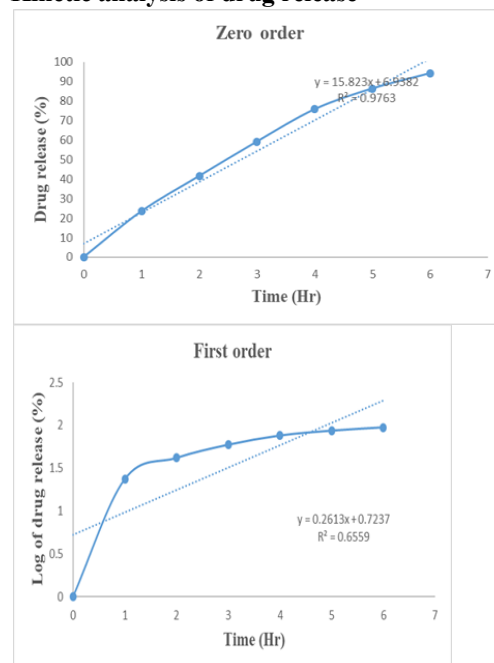
Final Equation in Terms of Coded Factors

The final polynomial equation for drug release (%) in terms of coded factors was obtained as:

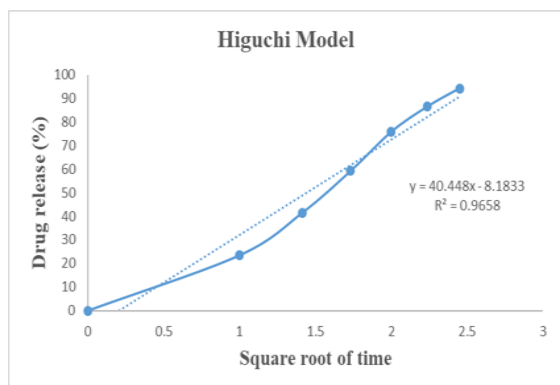
$$\text{Drug Release (\%)} = 81.59 + 1.30A + 1.36B + 2.13C + 1.38AB - 1.62AC + 0.3650BC - 0.0050A^2 + 1.86B^2 + 6.45C^2$$

Where A = lipid concentration (%), B = surfactant concentration (%), and C = homogenizer speed (RPM). The coded equation can be used to predict drug release at different levels of the selected formulation variables and to assess their relative effects on the response.

Kinetic analysis of drug release



(a)
(b)



(c)

Figure 4: (a) Zero order model of F9, (b) First order model of F9, (c) Higuchi model of F9

The release kinetics of the optimized CTZ-NLC formulation (F9) were evaluated using zero-order, first-order, and Higuchi models. The coefficient of determination (R^2) values obtained were 0.9763 for the zero-order model, 0.6559 for the first-order model, and 0.9658 for the Higuchi model. The highest R^2 value was observed for the zero-order model ($R^2 = 0.9763$), indicating that the drug release from formulation F9 followed zero-order kinetics, suggesting a relatively constant rate of drug release over the studied period.

3.2.2 Particle Size, PDI and Zeta Potential analysis

Table.27: Particle Size, PDI and Zeta potential of Optimized batch F9

Formulation code	Particle Size (nm)	PDI	Zeta potential
F9	142.5	0.185	-26.3

Calculation Results

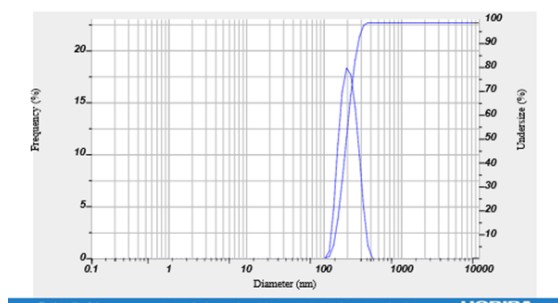
Peak No.	S.P. Area Ratio	Mean	S. D.	Mode
1	1.00	215.6 nm	25.12 nm	208.2 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	215.6 nm	25.12 nm	208.2 nm

Cumulate Operations

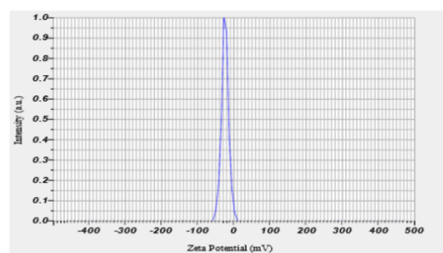
Z-Average : 142.5 nm

PI

: 0.185



Peak No.	Zeta Potential	Electrophoretic Mobility
1	-26.3 mV	-0.000187 cm ² /Vs
2	— mV	— cm ² /Vs
3	— mV	— cm ² /Vs
Zeta Potential (Mean)	-26.3 mV	
Electrophoretic Mobility Mean	-0.000187 cm ² /Vs	



(a)

(b)

Figure 5: (a) Particle Size and PDI of F9, (b) Zeta Potential of F9

The optimized CTZ-NLC formulation (F9) exhibited a mean particle size of 142.5 nm, with a PDI of 0.185, indicating a relatively narrow and uniform particle-size distribution. The zeta potential was found to be -26.3 mV, suggesting good electrostatic stability of the NLC dispersion. These results confirm the successful formation of nanosized lipid carriers with satisfactory physicochemical characteristics.

3.2.4 TEM

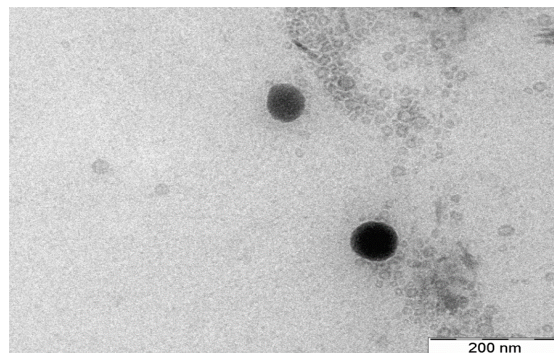


Figure 7: TEM Image of F9

TEM image of formulation F9 shows discrete, nearly spherical nanoparticles with a relatively uniform and well-defined morphology. The particles appear individually distributed with limited aggregation, indicating good colloidal stability and effective nanoparticle formation.

3.2.5 DSC

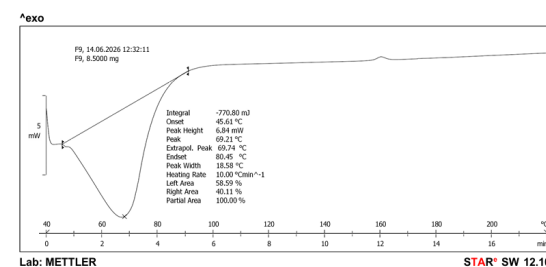


Figure 8: DSC Thermogram of F9

The DSC thermogram of optimized formulation F9 showed a broad endothermic peak at 69.21 °C, with an onset temperature of 45.61 °C and an extrapolated peak temperature of 69.74 °C. The

shift and broadening of the characteristic clotrimazole melting peak compared with the pure drug (147.47 °C) indicate that clotrimazole is incorporated within the formulation matrix in a less crystalline or molecularly dispersed state. The absence of the sharp drug-melting peak at 147.47 °C suggests possible interaction with the formulation components and/or reduced drug crystallinity. Overall, the DSC result indicates successful incorporation of clotrimazole into F9 without evidence of a distinct crystalline drug phase.

3.2.6 PXRD

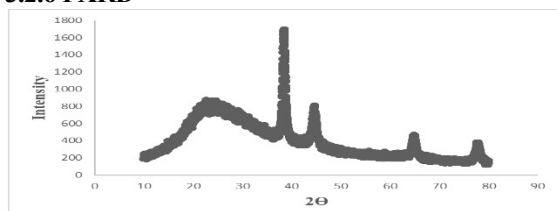


Figure 9: Diffractogram of F9

The X-ray diffraction (XRD) diffractogram of optimized formulation F9 shows a broad diffraction pattern with characteristic peaks at approximately $2\theta = 38.5^\circ$, 44.5° , 64.5° , and 77.5° . The broad halo observed in the range of approximately $20\text{--}35^\circ$ indicates the presence of an amorphous or less crystalline lipid matrix, while the distinct diffraction peaks may be attributed to the crystalline components of the formulation.

CONCLUSION

CTZ-loaded nanostructured lipid carriers were successfully developed and optimized using a Quality by Design (QbD)-based Box–Behnken design. The developed quadratic models demonstrated significant effects of the formulation and process variables on drug content, entrapment efficiency, and drug release. The optimized F9 formulation exhibited satisfactory performance with respect to the evaluated responses. FTIR analysis confirmed the retention of characteristic functional groups without significant spectral alterations, indicating good compatibility between CTZ and the selected excipients. Overall, the findings support the successful application of the QbD approach for systematic optimization of CTZ-NLCs and establish F9 as a promising optimized formulation for further pharmaceutical development.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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