RESEARCH ARTICLE

Application of Box-Behnken Design in Optimization of Clobetasol-loaded Nanostructured Lipid Carrier for Topical Use

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ABSTRACT

The objective of this study was to optimize nanostructured lipid carriers (NLC) for clobetasol propionate using Box-Behnken design (BBD). The formulation was developed by incorporating oleic acid, compritol ATO-888, and tween 80, using the hot homogenization method. The BBD, which employed a 3-level, 3-factor design, investigated the impact of lipid ratio, surfactant concentration, and sonication time on particle size, polydispersity index, and entrapment efficiency (EE). By analyzing the models through ANOVA and diagnostic plots, the optimized formulation composition was selected using the desirability function. The results indicated that a drug-lipid ratio of 1:7.5 was optimal for all formulations. The spherical-shaped nanostructured lipid carriers had a size of 168.3 ± 0.367 µm, a polydispersity index of 0.248183 ± 0.2847, an EE of 85.4 ± 0.384%, and a desirability value of 0.979. Overall, the use of BBD proved to be a valuable tool in developing optimized NLC with desired properties.

Keywords: Nanostructured lipid carriers, Box-behnken design, Quality by design, Clobetasol propionate, Optimization.

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INTRODUCTION

Psoriasis is a persistent autoimmune skin condition characterized by inflammation affecting the skin's keratinocytes, dendritic cells, and T lymphocytes. It manifests through symptoms like accelerated epidermal keratinocyte growth, increased blood vessel development, and the presence of "T-lymphocytes, neutrophils, and other immune cells. Due to the skin's rough texture, decreased water content, and imbalanced lipids, creating an effective treatment delivery system poses challenges.[1-4] While psoriasis management varies based on its severity, topical treatments remain the primary solution. Clobetasol propionate (CP), a strong corticosteroid, is commonly employed to treat this and other skin conditions. Nanostructured lipid carriers (NLCs)" present a promising solution for topical application as they enhance drug retention in the skin, reducing potential local and systemic side effects from corticosteroids. For conditions like psoriasis, NLCs can heighten treatment effectiveness and lessen toxicity when used for transdermal delivery.[5-7] This study aimed to develop and refine solid lipid nanoparticles using specialized software and assess the optimized formulation's attributes, such as particle size (PS), zeta potential (ZP), entrapment efficiency (%EE), microscopy observations, calorimetry, and drug release patterns.

MATERIALS AND METHODS

Materials

A free sample of clobetasol propionate was offered by Orison Pharma International at Kala Amb Baddi. Sigma Aldrich was the supplier of Compritol 888ATO. In the meanwhile, we ordered oleic acid and Tween 80 from V.K Chemicals in Ambala. We only utilized high-quality analytical reagents and any supplementary materials.

Preparation of Nano-Structured Lipid Carriers

Clobetasol-loaded NLCs were created using a method that combines melt emulsification and ultrasonication. At first, the organic phase was formed by combining solid and liquid lipids on a magnetic stirrer at a temperature 5°C above the melting point of the solid lipid. An ethanolic solution containing

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50 mg of clobetasol propionate was incorporated into this organic phase. This mixture was heated until all organic solvents evaporated, ensuring the drug's inclusion in the NLC preparation. Subsequently, after stirring for 15 minutes at 800 rpm, an aqueous phase preheated with 1.5% w/v surfactant was incrementally added to the lipid mix. The blend was then subjected to probe sonication for 10 minutes, following a cycle of 30 seconds on and 5 seconds off, at 40% amplitude. The temperature was regulated between 60 and 80°C using an oil bath throughout the sonication. Post-sonication, the mixture was rapidly cooled in an ice bath for 15 minutes and remained there for a day. Finally, after adding a 2% w/v mannose cryoprotectant, the samples were freeze-dried and stored securely in a sealed container for future applications.[8-11]

Optimization of NLCs

Using Design Expert Software, we optimized our approach to assess the interplay between dependent and independent variables (Stat-Ease; MN Trial Version 11.04). To address the non-linear complexities, a quadratic polynomial model was crafted based on a 3^3 full factorial design. The study leveraged three independent factors: the drug (A), surfactant concentration (B), and sonication time (C) to determine the lipid ratio. The key outcomes, or dependent variables, of this research were particle size (PS) polydispersity index (PDI), and entrapment efficiency (%EE). Table 1 presents a detailed breakdown of each factor with its respective levels, codes, and constraints. For a deeper understanding of how independent factors influenced particle attributes, we generated perturbation plots. "These were supported by a condensed polynomial equation, supplemented by 2D contour visuals and 3D response surface graphics. The model's accuracy was gauged using R2 values, which determined how closely observed responses aligned with ideal outcomes and the overall fit of the model.

Characterization of NLCs

Evaluation of mean particle size, polydispersity index, and zeta potential

The NLCs containing clobetasol were assessed for their average particle size, polydispersity index, and zeta potential using a particle size analyzer (Malvern Zetasizer ZS 90, UK). To mitigate the opalescence, the NLCs were sufficiently dispersed in distilled water. The measurements were conducted at a 90º scattering angle".

Percentage entrapment efficiency (%EE)

Measurement of the amount of free drug (unencapsulated Clobetasol) in the water phase of the NLCs dispersion was used to infer the entrapment effectiveness of the dispersion.

"%EE = (Total amount of Clobetasol - Amount of Free Clobetasol)/(Total amount of Clobetasol) 100 was used in the calculation.

A chilled centrifuge and millipore filtration assembly were used to separate the free clobetasol. The NLCs were centrifuged for 10 minutes at 11,000 rpm while kept at 4°C after being suspended in water. After centrifugation, a UV-visible spectrophotometer was used to examine the supernatant (the top liquid layer) at a wavelength of 239 nm.[12,13]

Table 1: Factors used formulation design

Type of variable (Independent Variable)	*Low (-1)*	*Medium (0)*	*High (+1)*
Drug: Lipid (% w/w)	1:5	1:7.5	1:10
Surfactant conc (% w/v)	1	1.5	2
Sonication time (min)	5	10	15
Dependent Variables			
Particle Size	Minimum		
Polydispersity Index	Minimum		
Entrapment Efficiency	Maximum		

Differential scanning calorimetry study

Differential scanning calorimetry study (DSC) analysis was performed to see whether the medicine and excipients were compatible with one another. By progressively raising the temperature from 25 to 350°C at a rate of 10°C per minute, thermograms of both the blank and drug-infused NLCs were collected. The American company Perkin-Elmer Instruments supplied the apparatus used to conduct the DSC analysis. For the purpose of this study, CP-NLC samples were weighed precisely and stored in airtight aluminum pans. The frying pans were then heated to a constant 25°C in an isothermal environment for 10 minutes. The baseline for this assessment was a sealed, empty aluminum baking pan.

Transmission electron microscopy

Transmission electron microscopy was used to look at the CP-NLCs' surface morphology (TEM; Philips, Tecnai 20, Holland). A diluted sample was put on a carbon-coated copper grid and stained negatively for 30 seconds using an aqueous solution of phosphotungstic acid at a concentration of 1% (w/w). After removing the surplus stain, the grid was allowed to air dry. Subsequently, Transmission electron microscopy (TEM) images of the sample were captured using the microscope.

In-vitro release study

The release characteristics and mechanisms of two formulations, the NLCs loaded with clobetasol and the pure clobetasol suspension, were investigated. A pretreatment dialysis membrane from HIMEDIA, Mumbai, India, with specifications MWCO: 12,000 to 14,000 Da and pore size: 2.4 nm, was used in conjunction with a locally constructed Franz diffusion cell.[14] The membrane underwent a 24-hour soaking in distilled water before the setup".

The receptor section of the diffusion cell was filled with a phosphate buffer (pH 7.4) to act as the release medium. Concurrently, the donor section was loaded with an NLCs suspension equivalent to a 2 mg/mL concentration of clobetasol. Throughout the study, maintained at a temperature of 37 ± 0.5°C, the suspension was continuously agitated using a magnetic stirrer at 200 rpm. At predetermined time points "(0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours), samples were

extracted from the receptor section and replenished with an equal volume of the release medium. Post-appropriate dilution, these samples were analyzed using a UV spectrophotometer set at 239 nm. A plot was constructed representing the relationship between elapsed time and the determined percentage of drug release. To decipher the drug release mechanism from the NLCs formulation, cumulative percentage drug release values over time (Qt) were computed. The data was then aligned with Higuchi release kinetics, first-order kinetic, and zero-order release models. The drug release data was presented as the mean and standard deviation (SD) of three separate trials.[15,16]

Ex-vivo diffusion study

Ex-vivo diffusion studies were conducted using sheepskin sourced from a local slaughterhouse in Tirupati, Andhra Pradesh. The goal was to evaluate the diffusion properties of clobetasol propionate (CP) when delivered both through CP-infused NLC solution and a regular CP suspension. This method was integrated with the use of Franz diffusion cells. Each skin sample was individually subjected to a test formulation containing a 400 mg dose of the drug and was left in contact for six hours. At the end of this period, the skin samples were carefully removed from the Franz cells and cleaned. The epidermal and dermal layers of the skin were then precisely separated using forceps. The CP was extracted from each skin layer with the aid of a sonicator and 10 mL of methanol. The concentrations of these extracted samples were then measured using UV spectroscopy set at 239 nm.

To ensure the stability of the formulated nano-lipid carriers under various environmental conditions, stability tests were conducted in accordance with ICH guidelines (Q1AR2). The NLCs were stored for a total of 180 days under both standard conditions (25 ± 2°C, 60 ± 5% RH) and accelerated conditions (40 ± 2°C, 75 ± 5% RH). Throughout this period, various parameters such as PS, ZP, PDI, and EE of the NLCs were periodically evaluated.[17]

RESULTS AND DISCUSSION

Screening of Components

Table 2 presents the solubility of clobetasol in various solid and liquid lipids. Among the lipids examined, the drug demonstrated the highest solubility in oleic acid. Additionally, a solid lipid named compritol 888 ATO, frequently utilized in topical applications, also exhibited considerable solubility for clobetasol. A ratio between liquid and solid lipids was formulated to ensure a suitable melting point, maintain a solid/semi-solid state at room temperature, and optimize drug-loading capacity. While a larger proportion of liquid lipid was favored to enhance drug entrapment efficiency, maintaining the consistency of the lipid mixture was essential.

During melting point evaluations, it was observed that a solid-liquid lipid combination with a ratio up to 70:30 could sustain a stable melting point (70.0–80.0°C). Increasing the quantity of oil beyond this predefined threshold led to a reduction in the melting point of the mixture. Based on smear test outcomes, a binary lipid matrix with a weight ratio of 70:30

Table 2: Solubility of clobetasol in various solid and liquid lipids

Component	*Solubility (mg/mL)*
Glyceryl monostearate	4.10 ± 0.23
Compritol 888 ATO	6.11 ± 0.04
Stearic acid	3.26 ± 0.31
Coconut oil	2.43 ± 0.42
Oleic acid	5.74 ± 0.53
Isopropyl Myristate	3.21 ± 0.27

Solubility of clobetasol in various solid and liquid lipids"

between solid and liquid lipids was selected for nanocarrier development. "A surfactant blend of Tween 80 (1.5% w/v) and Poloxamer (Stabilizer) (1% w/v) was employed to achieve optimal stability, as it reduced interfacial tension and increased interface fluidity, thereby enhancing the entropy of the system. Consequently, this ratio was chosen for the formation of the NLC dispersion.

Refinement of NLCs Production Technique

Initial investigations showed that a number of variables, including the total lipid-to-drug ratio, surfactant concentration, and sonication time, greatly impacted particle characteristics. "To go further, we used MN Stat-Ease Trial Version 11.04 to create combinations of these variables while staying within the parameters we defined. These parameters included a sonication duration range of 5 to 15 minutes, a lipid-to-drug ratio of 5:1–10:1, and a surfactant concentration of 1 to 2% w/v. The amplitude (40%) and the solid-to-liquid lipid ratio (70:30) were constant throughout.

Were optimized using a three-factor, three-level Box-Behnken (BBD) design. Independent variables were PVA concentration (A), ethanol concentration (B), and stirring rate (C). Table 3 shows how the aforementioned factors affect particle size (Y1) and the percentage of encapsulation effectiveness (Y2). A total of seventeen different formulations (MS1–MS17) were developed following the BBD's guidelines". Optimal %EE and small particle size were shown by the most efficient batch of nanostructured lipid carriers (NLCs) (PS). Visualizations of response surfaces in three dimensions, perturbation analysis, and two-dimensional contour plots were used to examine how external variables affected the results. "Equation 1 depicts a second-order polynomial expression that describes the relationship between the independent and dependent variables.

Model Analysis

Effect on size

By comparing the model summary statistics, model summary sum of squares, model summary statistics, and fit summary, "a quadratic model was found to be the most appropriate for evaluating the impact of variables on particle size. Particle size was modeled using the drug-to-lipid concentration, surfactant concentration, and ultrasonication time as independent variables.

Table 3: 3^3 Full factorial design showing the values of dependent variables of possible NLC formulations"

RUN	*X1*	*X2*	*X3*	*Y1*	*Y2*	*Y3*
1	5	1.5	5	201.8 ± 0.234	0.285251 ± 0.248	79.8 ± 0.251
2	7.5	1.5	10	97.3 ± 0.327	0.157007 ± 0.332	84.4 ± 0.241
3	5	1.5	15	147.1 ± 0.432	0.333917 ± 0.245	54.6 ± 0.284
4	7.5	1	5	159.3 ± 0.387	0.242927 ± 0.532	74.3 ± 0.481
5	7.5	1	15	140.2 ± 0.274	0.337675 ± 0.213	57.3 ± 0.004
6	7.5	1.5	10	93.2 ± 0.412	0.14906 ± 0.003	87.4 ± 0.043
7	10	1.5	5	203.2 ± 0.287	0.331478 ± 0.347	79.5 ± 0.423
8	5	2	15	168.3 ± 0.367	0.248183 ± 0.2847	85.4 ± 0.384
9	7.5	2	5	243.3 ± 0.247	0.339932 ± 0.002	60.5 ± 0.391
10	10	2	10	197.2 ± 0.179	0.251945 ± 0.043	75.3 ± 0.201
11	10	1	10	187.2 ± 0.034	0.253637 ± 0.058	81.3 ± 0.384
12	10	1.5	15	181.1 ± 0.372	0.356108 ± 0.324	69.2 ± 0.324
13	7.5	1.5	10	97.3 ± 0.384	0.168416 ± 0.351	83.3 ± 0.732
14	5	1	10	146.2 ± 0.297	0.203879 ± 0.227	68.3 ± 0.047
15	7.5	1.5	10	96.2 ± 0.127	0.1458 ± 0.104	86.9 ± 0.138
16	7.5	1.5	10	96.8 ± 0.007	0.14788 ± 0.211	85.4 ± 0.432
17	5	2	10	170.3 ± 0.187	0.276658 ± 0.307	62.2 ± 0.327

Average particle sizes in the tests varied from 93.200.412 to 243.300.247 nm across all batches. Equation (2) is a potential quadratic model for estimating particle size.

Formula for PS = 12.9125 A + 25.025 B + 14.6125 C - 3.525 + 8.15 + 0.4755 BC + 35.545 A + 43.52 + 51.59 C

The size of NLCs was modeled using a quadratic equation so that researchers could examine the effect of different factors. Analysis of variance (ANOVA) and fit statistical analysis, with significance set at (P 0.0500), validated the model's applicability and efficacy in investigating the impacts of these factors. Measured values for the produced samples were within the allowable range (as shown in Figure 1(a)–(c)), as shown by the diagnostic graphs highlighting the model's fit quality.

Particle size was shown to be favorably impacted by factor A (the ratio of lipid to drug), with a higher ratio producing bigger particles. Particle size was positively affected by surfactant concentration (factor B), but negatively by sonication time. Particle size was shown to be jointly affected by the lipid-to-drug ratio and the surfactant concentration, rather than by each factor acting alone. Larger particles formed due to an increase in the lipid percentage and a decrease in the surfactant concentration. This may be because of the creation of a poorly structured surfactant coating at the nano dispersion's interface and the increased viscosity resulting from a high lipid-drug ratio. However, smaller particles might be achieved in a nanodispersion by combining a greater surfactant concentration with a lower lipid fraction."

The F-value of 11.14 and the *p-value* of 0.0022 (p 0.0500) in the ANOVA analysis, as well as the lack of fit value of 0.0001 (p 0.0500), "all provide support to the sufficiency of the model. The signal-to-noise ratio of 11.77 in the fit statistical assessment demonstrates outstanding accuracy (a value greater than 4 is preferred for navigating the design space). The residuals' normal distribution, constant variance adherence, and the tight alignment between experimentally observed and predicted particle sizes were all confirmed by diagnostic illustrations such as the normal plots of residuals (Figure 1(a)), the residuals against predicted values (Figure 1(b)), and the comparison of predicted vs. actual plots (Figure 1(c)).

Last but not least, the NLCs' particle size decreased with continued sonication, most likely because to the shear energy introduced by the sonication".

Effect of formulation variables on PDI

The impact of various variables on the overall encapsulation efficiency was calculated using a quadratic model (percent EE). The fit summary, the sum of squares in the sequential model, the statistics from the model's summary, and the exhaustive information from the fit summary all played a role in this evaluation (comprising PredR2, AdjR2, F-value, p-value, PRESS, and df). The equation we generated shows that the encapsulation effectiveness is affected by three factors: the quantity of PVA (factor A), the amount of ethanol (factor B), and the time we agitate the mixture (factor C) (factor C).

The results of the studies showed that the PDI may be anywhere from 0.14580.104 to 0.33990.002. From these data, the quadratic model for PDI prediction shown in the equation was proposed (3).

An appropriate PDI formula is: PDI= 0.011683A +0.021075B +0.20787C -0.01862AB -0.00601AC -0.02412BC +0.052453 A2 + 0.040044B2 +0.120603 C2

The polynomial equation revealed associations between the lipid-to-drug ratio, surfactant concentration, and sonication period that contributed to the PDI. Sonication duration was positively correlated with the lipid-to-drug ratio, but the lipid-to-drug ratio was not positively correlated with the surfactant concentration. The PDI was negatively affected by the relationship between surfactant concentration and sonication time.

The addition of 2% w/w tween 80 led to an increase in PDI because of the buildup of excess surfactant particles on the surface of the lipid matrix. A persistent lipid matrix was created during sonication, and the alkyl chain of the surfactant

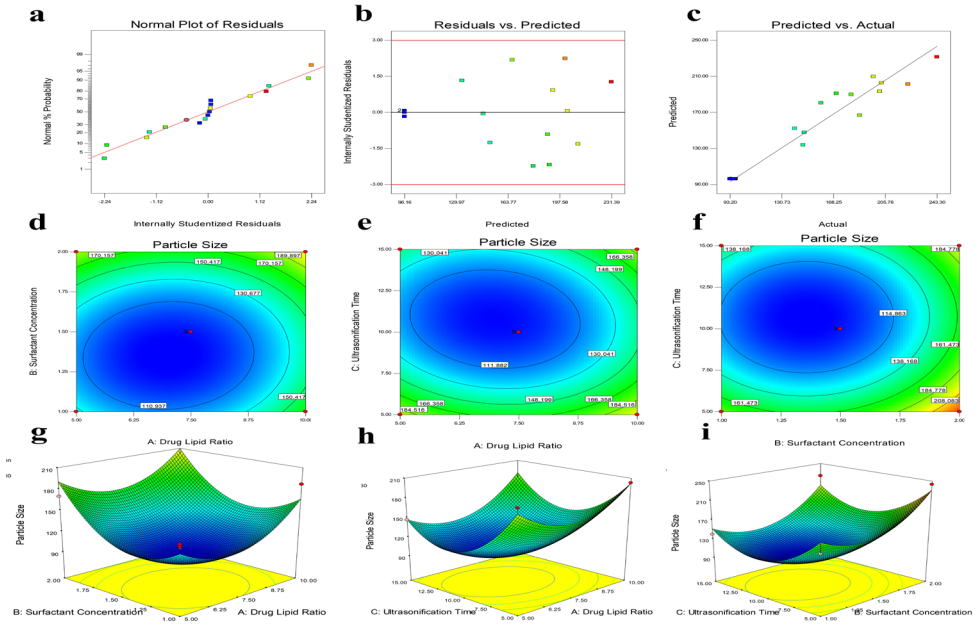

Figure 1: "Effect of different variables on particle size. (a) normal plot of residuals, (b) residual vs predicted plot, (c)predicted vs actual plot, (d-f) 2D contour plots and (g-i) response surface 3D plots

molecule interacted hydrophobically with the surface of the lipid particle. The response surface plots show that the addition of surfactant messed with this equilibrium, resulting to a higher PDI (Figure 2).

Analysis of variance was used to examine the used model for PDI effect assessment (ANOVA). The significance of the F-value of the ANOVA, which was 106.87, the *p-value*, which was 0.0001 (p 0.0500), and the lack of fit values, which were 0.00212 (p 0.0500), all lent credence to the accuracy and utility of the model. With a signal-to-noise ratio of 34.82, the statistical analysis of the fit showed outstanding accuracy, crucial for navigating the design space (values above 4 are preferred). Diagnostic plots were used to further verify the model's fit quality (Figure 2 a-c). Most spots on the normal residual plots were quite near to the straight line representing the standard probability (Figure 2 a). Comparison of residuals to expectations revealed that all %EE measurements were within the allowed range and followed a random distribution (Figure 2b), lending credence to the hypothesis of constant variance. Particle size as measured experimentally agrees well with expected values, as seen in the plot of predicted against actual values (Figure 2 c)".

Effect of formulation variables entrapment efficiency

Between 55.40.384 to 87.40.043, entrapment efficiency was measured. The quadratic model, represented by equation 4, was found to be the most appropriate for assessing the effects of the variables on entrapment efficiency after a thorough review of the fit summary, sequential model sum of squares, model summary statistics, and detailed fit summary (including PredR2, AdjR2, F-value, *p-value*, PRESS, and df) (%EE). The effectiveness was affected by the PVA concentration (A), ethanol concentration (B), and mixing time (C).

EE (%) = 85.48+5.05A – 3.475B -3.475B-7.2 C+ 0.025 AB+ 3.725 AC +2.975 BC-2.4025A^2 -11.3025 B^2 -12.3025 C^2

In order to assess the impact on entrapment efficiency (percent EE), an analysis of variance was used to the model (ANOVA). F-value = 60.23, p = 0.0001 (p 0.0500), and lack of fit = 0.0212 (p 0.0500) all indicate that the model is appropriate and trustworthy. With a signal-to-noise ratio of 25.867, the statistical analysis of the fit demonstrated impressive accuracy, crucial for the investigation of design space (values greater than 4 are preferred).

The quality of the model fit was further confirmed by diagnostic plots (Figure 3 a-c). Response data analysis was reliable since most locations in the normal residual plots tracked closely with the standard probability straight line (Figure 3a). All values of %EE were found to be within the predetermined range when comparing residuals to predictions. Studentized residuals (shown in Figure 3b) were randomly distributed, further supporting the hypothesis of constant variance. Furthermore, a close agreement was seen between expected and experimentally observed particle size values when comparing predicted vs. actual values (Figure 3c).

Coefficient estimates showed that a positive value for term A, which stood for the lipid-to-drug ratio, indicated a clear correlation with entrapment efficiency. On the other hand, surfactant concentration and sonication time had detrimental effects. However, results improved when the lipid:drug ratio was combined with increased surfactant concentration and longer sonication times. In particular, when the lipid-to-drug ratio was 7.5:1, formulations with an EE of 85.4% showed improved drug entrapment. Due to the increased lipid content, the %EE is higher since there is less surface area for drug molecules to interact with. Increased lipid content may cause

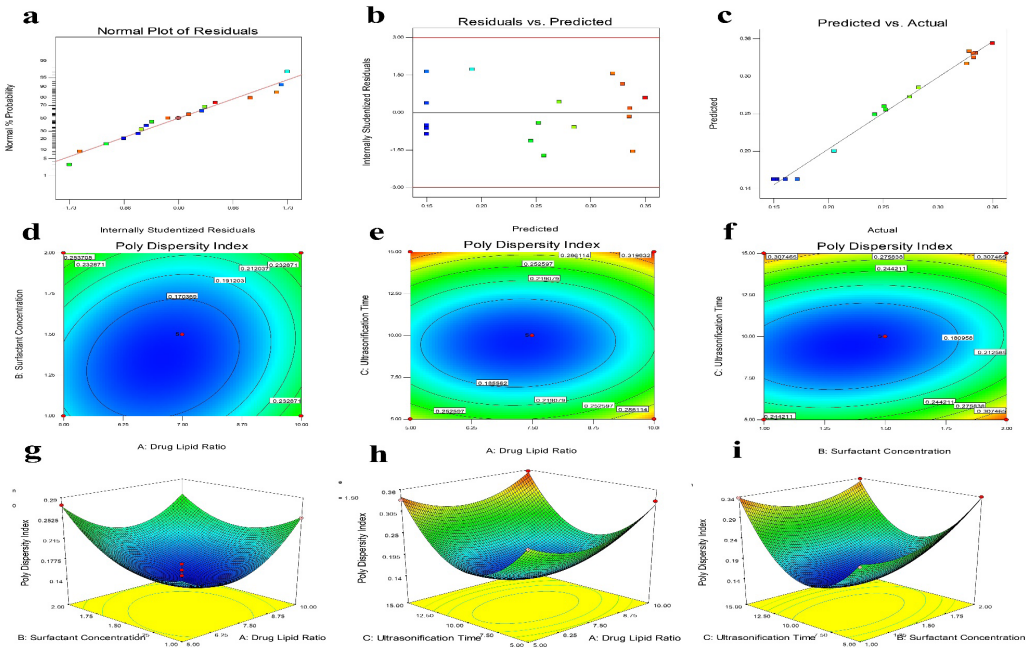

Figure 2: "Effect of different variables on polydispersity index (a) normal plot of residuals, (b) residual vs predicted plot, (c) predicted vs actual plot, (d-f) 2D contour plots and (g-i) response surface 3D plots.

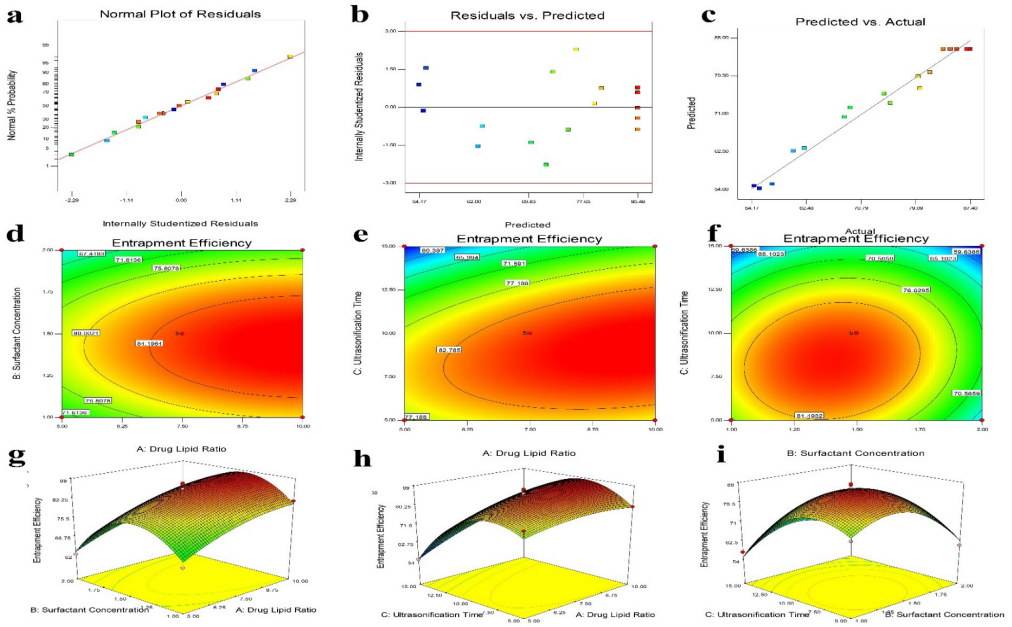

Figure 3: "Effects of several factors on entrapment efficiency (a) normal residual plot (b) residual vs expected plot (c) predicted vs actual plot (d) 2D contour plots (e) and (f) response surface 3D plots (g-i).

a greater %EE compared to other formulations because the diffusion rate of drug molecules is slowed by the more viscous lipidic phase. The formulation's efficacy was clearly shown by the correlation between the lipid-to-drug molar ratio and the %EE.significant dependency on this ratio.

Optimization and Validation

To determine the best formulation, we used the Design-Expert program. Nanostructured lipid carriers have their particle size, polydispersity index, and medication entrapment efficiency optimized for maximum benefit to the patient (NLCs). The software's output was scored according to how desired it was, and the top-ranked formulation was selected for further refinement.

By contrasting the predicted values with the actual values and computing the relative error, the accuracy of the projections might be evaluated. The relative error was found to be less than 5%, demonstrating the high degree of uniformity between batches (as presented in Table 4).

Desirability function

"The formulation's optimal composition was determined using the desirability function. This was done to achieve a reduced size, low PDI, and maximum %EE, all of which were predetermined goals for the end product's quality. A desirability close to one is desired for an optimum formulation composition.

The desirability study resulted in an optimum formulation composition of 1:7.4 drug to lipid, 1.38 wt% w/v surfactant, and 9.58 minutes of ultrasonication. The expected parameters were all present in this formulation: a size of 94 nm, a PDI of 0.1356, and an EE of 85.35. The formulation's remarkable desirability value was 0.979.

Transmission electron microscopy (TEM) was used to look at the nanoparticles' size and shape after they had been optimized. In the transmission electron microscopy pictures, the nanoparticles seemed to be round or oval, and the lack of aggregation suggested a consistent size distribution. Results from a Malvern particle size analyzer were found to be in agreement with the size measurements acquired from these photos.

Also used in the research was a technique called differential scanning calorimetry (DSC). Different melting events were represented in the DSC thermograms for the different samples, including pure CP, Compritol 888 ATO, a physical combination of CP and Compritol 888 ATO, and CP-loaded NLCs (Figure 4). The melting point of Compritol 888 ATO was determined to have a sharp endothermic peak at 75.41°C. Crystallinity in pure CP was shown by a sharp endothermic peak at its melting point of 196.24°C. The CP signal was clearly visible in the physical combination of CP and Compritol 888 ATO, but it was completely missing in the lyophilized CP-loaded NLCs.

The absence of an endothermic peak during drug synthesis in CP SLN formulations has been used as evidence supporting the amorphous nature of the drug in the past. This indicates that the medication was spread uniformly throughout the nanocarrier in a non-crystalline, amorphous condition".

"A 24-hour *in-vitro* drug release assay was performed on both the CP suspension and the CP suspension loaded with NLCs. Three independent experiments were performed on each sample, and the results are shown in Figure 6. In under 7 hours, the standard CP suspension showed a 94.56 1.23% release of the

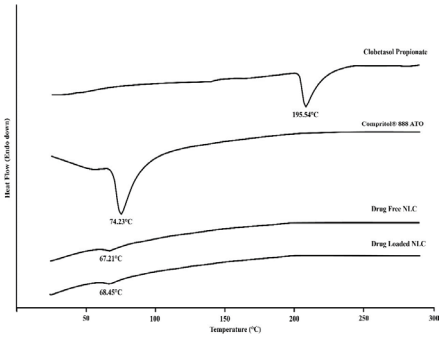

Figure 4: DSC analysis of drug and excipients

Table 4: Responses for the optimal CP loaded NLC formulation, predicted and observed"

Factors	*Predicted value*	*Response*	*Observed Value*	*Predicted Value*	*Relative Error*
Drug: lipid	1:7.4	Particle Size (nm)	94.3 ± 1.23	93.77 ±1.23	0.59
Surfactant conc % (w/v)	1.38	Polydis persity Index	0.1356 ± 2.43	0.1489 ± 0.45	0.1353
Ultrasonic ation time (min)	9.58	Entrapment Efficiency (%)	85.35 ± .036	86.04 ± 0.653	0.69

medication. During the same period of time, 75.23 1.55% of the medicine was released from the CP-loaded NLCs suspension. The CP-loaded NLCs suspension exhibited a biphasic release pattern characterised by a quick onset of activity followed by a more gradual decline in activity. Evaluation of release kinetics showed that the optimal CP-loaded NLCs formulation followed Higuchi release kinetics (r2 = 0.9838), whereas the plain CP suspension followed zero-order release kinetics (r2 = 0.9904). The outcomes from the novel and conventional approaches were similar.

The ability of both the newly formulated medication formulation and the pure drug solution to be transported *via* sheepskin was studied in an *ex-vivo* skin distribution study. The concentration of CP in the epidermis, dermis, and receptor layers was determined using UV spectroscopy. A total of 142.21 ng/mL of medication was transported to the receptor compartment within 6 hours from the simple CP solution, as measured by the greatest concentration in the 1-cm^2 diffusion regions. In contrast, the epidermis (51.23 g/mL) was the primary site of drug delivery for the CP-loaded NLCs solution, which is where keratinocyte hyperproliferation is thought to begin in psoriasis. The dermis (16.19 g/mL) and the receptor compartment (3.99 g/mL) contained much less. This shows that the drug-loaded NLCs successfully blocked the steroid from escaping into the body's circulatory system".

To determine the reliability and safety of the CP-loaded NLCs, a stability study was carried out. Particle size was observed as 91.2 2.37 nm, PDI as 0.173 0.035, ZP as -34.7 1.49 mV, and %EE as 85.4 2.24% for the newly formulated NLCs. "The particle size, PDI, ZP, and %EE of the NLCs formulation were determined to be 97.52 3.17 nm; 0.186 0.019; -32.26 0.57 mV; and 81.78 1.59% after 180 days of storage at 4°C. These little shifts over the course of 180 days demonstrate the NLCs' high degree of physical stability.

SUMMARY AND CONCLUSION

The major purpose of this research was to use the Quality by Design (QbD) strategy to analyse how different factors affect the quality characteristics of clobetasol-loaded NLCs. A formulation with the desired properties was derived using a desirability function. Initially, FTIR spectroscopy was used to check that the medicine and excipients were compatible with one another (as shown in Figure 1).

Using a 3-factor, 3-level Box-Behnken design (BBD), the formulation of clobetasol-loaded NLCs was optimized, considering factors such as the drug-to-lipid ratio, surfactant concentration, and ultrasonication duration. Based on the BBD, seventeen distinct formulations (F1–F17) were developed, each with varying compositions and ultrasonication durations (as detailed in Table 1)". The resulting formulations were assessed for size, PDI, and %EE. The synthesized NLCs exhibited a particle size range from 93.2 nm up to 243.3 nm. The %EE values varied between 55.4% and 87.4%, while the PDI oscillated from 0.1458 to 0.339932. Microscopic analysis revealed the NLCs to be spherical with a porous exterior (refer to Figure 3)

The overarching goal was to design an efficient topical clobetasol-loaded NLC formulation that could reduce the frequency of administration, mitigate irritation reactions, and sidestep the side effects associated with conventional oral formulations. The study pinpointed the significant impacts of factors such as lipid-to-drug ratio, surfactant concentration, and ultrasonication time on the NLC's size, PDI, and %EE. By fine-tuning these variables, the formulation can be tailored to achieve the desired attributes. The application of the QbD methodology proved instrumental in achieving an optimized NLC formulation suitable for topical delivery, characterized by its size, PDI, and %EE.

In this endeavor, compritol 888 ATO (a solid lipid) and oleic acid (a liquid lipid) were amalgamated through a melt emulsification and ultrasonication process to construct a clobetasol-infused NLC delivery system. Employing a 3x3 full factorial design, the study optimized parameters such as drug-to-lipid ratio, surfactant concentration, and homogenization duration, aiming for minimal particle size, a concise PDI, and maximal %EE. This systematic formulation approach facilitated the attainment of desired outcomes swiftly and with a reduced number of experimental runs. The NLCs showcased a pronounced anionic nature, with a zeta potential reaching up to -32.26 mV, signifying their physical stability.

The DSC analyses ascertained the absence of interactions between clobetasol and the employed lipids. TEM imaging portrayed the spherical morphology of the CP-loaded NLCs. Notably, compared to its pure form, the drug exhibited a sustained release pattern when encapsulated in the NLC dispersion. The Higuchi model aptly described this drug release behavior from the NLC matrix. Stability assessments revealed notable alterations in particle size upon storage at ambient conditions.

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