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Curcumin-loaded Nanostructured Lipid Carriers for Ocular Drug Delivery: Design Optimization and Characterization

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Abstract

The current study sought to formulate, optimize, and evaluate curcumin-loaded Nanostructured Lipid Carriers (NLCs) for their *in vitro* and *ex vivo* characteristics. NLCs, prepared using hot-melt emulsification and ultrasonication techniques, were optimized using a Central Composite Design (CCD) and evaluated for their *in vitro* physicochemical characteristics. Their stability over a 3 month period and transcorneal permeation across excised rabbit corneas (*ex vivo*) were assessed for the optimized NLCs. The optimized NLC, with a particle size of 66.8 ± 2 nm, polydispersity index of 0.17 ± 0.05 , entrapment efficiency of $96 \pm 1.6\%$, and drug loading of $3.1 \pm 0.05\%$ w/w, was chosen using CCD. The optimized NLCs showed optimum *ex vivo* stability at 4°C for the study period and demonstrated a significant increase in curcumin permeation (~2.5-fold) across the rabbit cornea in comparison to the control. Overall, these studies indicated the successful development of NLCs using the design of experiment approach; the formulation enhanced curcumin permeation across excised corneas and did not show any harmful side effects.

1. Introduction

Curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a major constituent of the curcuminoids obtained from the rhizomatous plant (*Curcuma longa*), has been widely studied as a potential therapeutic agent due to its anti-oxidant, anti-cancer, and anti-inflammatory activities as well as various other pharmacological activities that alleviate the symptoms of various acute and chronic diseases, such as cataracts, retinoblastoma, ocular infections, neurodegeneration of the optic fiber and inflammation [1–7].

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⁶Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Despite the claims in the literature about the potential clinical applications of curcumin, its utility has not been extended to clinical therapeutics. Its poor aqueous solubility, low bioavailability, and degradation of curcumin in aqueous medium constitute some of the foremost challenges associated with curcumin that hinder its transition into clinical use [8–11].

Currently, there is no marketed curcumin preparation for the treatment of ocular diseases. A couple of publications have highlighted the potential of curcumin in the prevention and treatment of various ocular diseases, such as inflammation, cataracts, age-related macular degeneration and tumors [3, 12, 13]. There have been a few reports aiming to improve the ocular bioavailability and/or stability of curcumin by encapsulating it in nanoparticulate systems, such as polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol-based micelles [14], mixed-micelles trapped in *in situ* gels [15], and chitosan-coated Nanostructured Lipid Carriers (NLCs) [16]. However, all of the formulations have been formed by a film-hydration technique; therefore, one of the major disadvantages is the necessity of an organic solvent, which not only makes the product expensive but can also lead to ocular toxicity if present in residual amounts [9, 17–19]. The report on chitosan-coated NLCs showed ~18-fold enhancement of the apparent permeability and ~6-fold enhancement of precorneal drug retention in comparison to the propylene glycol suspension. However, the study did not aim to increase the curcumin loading efficiency using optimization and evaluate the stability [16].

NLCs are being extensively investigated as vehicles for the delivery of drugs to the eye due to their enhanced drug loading and permeation characteristics as well as an acceptable safety profile [20–30]. The NLCs can carry high drug loads by virtue of the affinity of the drug towards the lipids making up the NLCs [31–35]. Additionally, lipid-based drug carrier systems, such as NLCs, protect the drugs from degradation due to the encapsulation of the drug within the lipoidal matrices of the Solid Lipid Nanocarriers (SLNs) and NLCs.

Therefore, using the above-mentioned premise, this study sought to formulate curcumin-loaded NLCs with the aim of achieving a high drug loading. Additionally, the curcumin-loaded NLCs in this study were formulated using an organic solvent-free hot-melt emulsification technique that circumvented any probable cases of residual-solvent related toxicity. Since, the lipid-based carrier systems have been reported to enhance and improve drug permeation across the corneal barrier, there was some expectation that curcumin-loaded NLCs could also show a similar trend, and hence, they were evaluated for their *ex vivo* transcorneal permeability [16, 20, 36].

2. Materials and methods

2.1 Materials

The solid lipids (Compritol™ 888 ATO, Precirol™ ATO 5, Geleol™, Dynasan™ 114, Dynasan™ 116, Gelucire™ 43/01, Gelucire™ 44/14, and Gelucire™ 50/13) and liquid lipids (Labrafilm™, Maisine™, and Capryol 90™) were gifted by Gattefossé (Weil and Rhein, Germany). The curcumin (purity > 98%), surfactants, analytical grade solvents, and all other chemicals were bought from Fisher Scientific (Massachusetts, US).

2.2 Lipid screening

A lipid screening test was performed to select solid and liquid lipids to form the NLCs. One gram of each lipid (Table 1) was accurately weighed and heated to 70°C in scintillation vials. To these vials, 10 mg of curcumin was added in increments (a maximum of 60 mg) with continuous stirring. The mixture was cooled to room temperature and examined for precipitation microscopically. The solid and liquid lipids that did not show any curcumin precipitation were selected for further studies.

2.3 Fabrication and optimization of curcumin-loaded NLCs

Curcumin-loaded NLCs were prepared using the formula in Table 2. Initially, curcumin was added in small portions to the lipid phase at 75°C to form homogenous lipid-curcumin phase. A coarse emulsion was formed by mixing the lipid-curcumin phase with the aqueous phase at 2000 rpm on a magnetic stirrer. This coarse emulsion was homogenized with an ULTRA-TURRAX® T-25 (IKA works INC., NC, USA) at 15000/25000 rpm to form a fine emulsion. Furthermore, the fine emulsion was ultrasonicated (Vibra-Cell Processor, Sonic and Material Inc., Connecticut, US) for 5–10 mins with a pulse of 15 sec on and 15 sec off [20]. The curcumin-loaded NLCs were optimized using the Central Composite Design (CCD), a response surface method, with 3 numeric (concentrations of olive oil, vitamin E tocopheryl polyethylene glycol succinate (TPGS), and poloxamer 188) and 2 categorical (ULTRA-TURRAX® speed and sonication time) factors.

2.4 High-Pressure Liquid Chromatography (HPLC) analysis

Curcumin was analyzed by high-performance liquid chromatography using a Waters 717 plus auto-sampler coupled with a Waters 2487 Dual λ Absorbance UV detector, a Waters 600 controller pump, and an Agilent 3395 Integrator. A Phenomenex Luna® PFP (2) column with 5 μ m packing and dimensions of 4.6 mm $\times$ 250 mm was used for the analysis. The method was previously reported and validated by Huang et. al. [1]. The mobile phase was 0.1% (v/v) glacial acetic acid and acetonitrile mixed in equal proportions. The method showed resolved peaks for all three curcuminoids (curcumin, bis-deoxy curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin). The retention time for curcumin was 16 min, with a detection wavelength (λ_{max}) of 425 nm and a 1.0 absorbance unit as the full scale. The standard curve of curcumin, ranging from 0.1 μ g/mL to 10 μ g/mL was prepared in methanol with a limit of quantification of 0.1 μ g/mL and a limit of detection of 0.05 μ g/mL.

2.5 Physicochemical characterization of the NLCs

2.5.1 Assay (curcumin content), drug loading, and entrapment efficiency—

For the total curcumin content, 10 μ L of curcumin-loaded NLCs was diluted to 1 mL with methanol and vortexed. The mixture was then centrifuged at 13000 rpm for 15 mins, and the supernatant was analyzed for the drug content using HPLC (section 2.4). The entrapment efficiency of the drug was calculated by analyzing the free un-entrapped drug (equation 2.1). The curcumin-loaded NLCs were filtered through Amicon® filters (molecular weight cut off: 100 kDa) at 5000 rpm. The filtrate was then analyzed using HPLC for the un-entrapped drug. The percentages of the entrapped drug and drug loading were calculated using equation 2.1 and equation 2.2, respectively.

$$\text{Percent Entrapment Efficiency} = \frac{W_t - W_f}{W_t} \times 100 \quad (2.1)$$

$$\text{Percent Drug Loading} = \frac{W_t - W_f}{W_1} \times 100 \quad (2.2)$$

where W_t = total curcumin content in the curcumin-loaded NLCs

W_f = curcumin in the aqueous phase

W_1 = total weight of the nanoparticles.

2.5.2 Photon correlation microscopy—The hydrodynamic particle size and Polydispersity Index (PDI) of the curcumin-loaded NLCs were determined by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, USA). From the curcumin-loaded NLCs, 10 μL was pipetted out and diluted to 1 mL with bi-distilled water. The measurements were made at 25°C in polystyrene cuvettes.

2.5.3 Transmission electron microscopy—A drop (20 μL) of the curcumin-loaded NLCs was placed on parafilm. A 200-mesh copper grid coated with a thin carbon film was exposed, with the film side down, to the drop of the curcumin-loaded NLCs. After 30 seconds, the grid was raised, and excess curcumin-loaded NLCs were removed using a filter paper. Immediately, the grids were placed on a drop of ultra-pure water. Then, the grid was raised, excess water was removed, and it was placed on a drop of 1% uranyl acetate to stain the nanoparticles [37]. After a minute, the grid was raised, dried completely and examined under a Zeiss Auriga microscope (Carl Zeiss, New York) operating in the Scanning Transmission Electron Microscope (STEM) mode at 30 kV.

2.5.4 Zeta potential—The zeta potential was determined using a Zetasizer Nano ZS (Malvern Instruments, US). The samples were prepared by diluting curcumin-loaded NLCs 100 times using the bi-distilled water in the folded capillary zeta cells at 25°C.

2.6 In vitro transcorneal release and corneal hydration

Freshly excised whole eye globes of Albino New Zealand rabbits were purchased from Pel-Freez® Biologicals. The eyes were stored in Hank's Balanced Salt Solution (HBSS) on ice and shipped overnight. The corneas were separated, washed with Phosphate Buffered Saline (PBS, pH = 7.4), and mounted on the modified vertical Franz diffusion cells (PermGear™) with a spherical joint (to simulate the curvature of the cornea) for transcorneal studies [38]. The water jacket around the cells was used to maintain a constant temperature ($34 \pm 0.2^\circ\text{C}$) using a water circulator. The receptor chamber (5 mL) was filled with Dulbecco's phosphate buffered saline (DPBS) with 1% Tween® 80, and the donor (1 mL) was filled with 0.1% w/v curcumin equivalent curcumin-loaded NLCs. The curcumin propylene glycol-based suspension was prepared as a control [39]. Since no marketed preparation exists, the propylene glycol-based curcumin suspension (curcumin is practically insoluble in water) is widely used as a control [16]. The NLCs and controls were evaluated for transcorneal

permeation. Aliquots of 300 μ L were drawn and replaced with the receiver solution, and the aliquots were analyzed for their curcumin contents using HPLC. The corneas were weighed accurately and extracted using 1 mL of methanol (5 mins of vortex mixing followed by 5 mins of sonication) and centrifuged for 30 mins. The supernatant was analyzed using HPLC. To determine corneal hydration, an identical experimental procedure was followed for 3 hours, after which the cornea was analyzed for its weight gain due to corneal hydration. The percent weight gain after the experiment was calculated.

2.7 Corneal histology

Freshly excised whole eye globes stored in HBSS solution on ice were procured from Pel-Freez® Biologicals. The whole eye globes were introduced in a beaker containing either blank or curcumin-loaded NLCs, ensuring that the corneal surface remained in contact with the undiluted curcumin-loaded NLCs throughout the course of the experiment. The corneas were exposed for 3 hours, and then, all of the corneas were excised, fixed in formalin solution, and shipped to Excalibur Labs Inc. (Normal, OK) for the histological analysis.

2.8 Stability of the NLCs

The optimized NLCs were evaluated for their stability upon storage for a duration of 3 months at 25°C and 4°C. The optimized curcumin-loaded NLCs were evaluated for changes in their physical and chemical characteristics such as particle size, PDI, percent entrapment efficiency, and drug loading. We also evaluated the stability of the control suspension for 3 and 6 hours to make sure the control was stable during the *ex vivo* permeation studies.

2.9 Statistical analysis

Analysis of Variance (ANOVA) was used to analyze the experimental design. At $p < 0.05$, the difference in data was considered statistically significant. The experimental design was generated and analyzed using Design Expert® version 8 (Stat-Ease INC, MN, US). The model interaction, simple effects for experimental design, and plots were obtained using the R project for statistical computing (version 3.4.4) [40].

3. Results and discussion

3.1 Development and optimization of the curcumin-loaded NLCs

The screening of lipids is a critical step to maximize the percentages of drug entrapment and loading efficiency. Capryol™ 90 and Maisine™ (liquid lipids) were the most effective in solubilizing curcumin; however, olive oil was chosen as the liquid lipid in the curcumin-loaded NLCs. This was due to the two challenges encountered during the formulation of Maisine™- and Capryol™-based NLCs. The inclusion of Maisine® in the lipid nanoparticles caused the aggregation/agglomeration of nanoparticles upon cooling, which is hypothesized to be due to the low solidification temperature of Maisine™. Capryol™ 90 is a water-insoluble surfactant-type lipid that caused the lipid to become solubilized in the micelles (approx. 20–30 nm). Therefore, olive oil and Compritol® ATO 888 were selected to form the NLCs. To further increase the drug loading, Gelucire™ 50/13 was incorporated in a lipid matrix. However, an excess of Gelucire® 50/13 elicited effects like those caused by the use of Capryol™ 90.

The curcumin-loaded NLCs were optimized using CCD, a response surface method proposed by G. E. P. Box and K. B. Wilson, 1951 [41]. The adjusted R^2 , proposed by Wherry, 1931, penalizes R^2 for adding non-significant predictors to the model [42]. The adjusted R^2 , sequential p-value, and lack-of-fit test were considered for the comparison and selection of a model [43]. The aim of the experimental design was to study the effects of variations in the surfactants, liquid lipids and homogenization parameters (ULTRA-TURRAX® speed and ultrasonication time) on the physicochemical characteristics (particle size, zeta potential, PDI, drug loading, and entrapment efficiency) of the curcumin-loaded NLCs (Table 3).

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_5x_1x_2 + \beta_6x_1x_3 + \beta_7x_1x_4 + \beta_8x_2x_3 + \beta_9x_2x_4 + \beta_{10}x_3x_4 + \beta_{11}x_1^2 + \beta_{12}x_2^2 + \beta_{13}x_3^2 + \beta_{14}x_4^2 \quad (2.3)$$

Equation 2.3 is a general quadratic equation with two-way interactions for 4 predictors. The coefficients of the predictors are obtained by performing ANOVA on the experimental runs. The vitamin E TPGS, poloxamer 188, and olive oil contents were the numeric variables, and the ULTRA-TURRAX™ speed and sonication time were the categorical factors (two-level). The experiment was a full factorial CCD design (includes the center points), and star points were included to determine the curvature. The linear, two-factor interaction and quadratic models were compared with respect to the goodness of fit, sequential p-value, and adjusted R^2 (2.3). The positive unstandardized coefficients (β) represent an increase in the response variable with a unit increase in the predictor (2.3). The reverse applies to coefficients with a negative value [44]. The interaction is represented as a product of two factors in the equation, and it implies that the effect of a factor on the dependent variable is moderated by another independent variable [43]. A statistically significant model implies that a linear relationship between the dependent and independent variable exists (Table 4).

3.1.1 Particle size—The particle size ranged from 37–1275 nm for the experimental runs. The inverse square-root ($1/\sqrt{y}$) transformation was applied to the particle size (Box-Cox transformation). A simple linear model without interaction was chosen based on the lack-of-fit test and sequential p-value. The curcumin-loaded NLCs' particle size was robust to changes in the olive oil, vitamin E TPGS and poloxamer 188 concentrations within the levels tested in the experimental design. The ULTRA-TURRAX® speed and ultrasonication time significantly reduced the particle size (Equation 2.4, Figure 1, Table 4). Therefore, to achieve optimized curcumin-loaded NLCs, the sonication time and ULTRA-TURRAX® speed were set to the highest level.

The ultrasonication and ULTRA-TURRAX® causes the breakdown of the particles due to sound energy and high-shear, respectively, thus reducing the particle size. During sonication, there is a significant buildup of temperature, which is known to cause a variation in the particle size and distribution (PDI) responses. Therefore, these variations in responses remain unexplained by the predictors, leading to an inflation in the model variance [9, 45].

$$\frac{1}{\sqrt{(\text{Particle Size})}} = 0.084 + 0.002x_1 + 0.003x_2 + 0.003x_3 + 0.009x_4 + 0.03x_5 \quad (2.4)$$

3.1.2 PDI—The PDI ranged from 0.14 to 1. From the Box-Cox transformation, logarithmic transformation for the PDI was inferred. The variation in the PDI was significantly dependent on homogenization parameters (Equation 2.5, Figure 2, Table 4). At a high ULTRA-TURRAX® speed and sonication time, the PDI was significantly reduced. Therefore, the PDI was inversely proportional to the changes in the homogenization parameters. The ULTRA-TURRAX® speed and ultrasonication causes the breakdown of the particles, which over time become uniform to give a narrow particle distribution [20, 45, 46].

$$\text{Ln(PDI)} = -1.06 + 0.019 * x_1 + 0.019 * x_2 + 0.037 * x_3 - 0.13 * x_4 - 0.57 * x_5 \quad (2.5)$$

3.1.3 Percentages of entrapment efficiency and drug loading—The entrapment efficiency ranged from 88–99%; however, for most of the experimental runs the entrapment was ~99%. Therefore, the model was statistically insignificant, with all of the variables having no impact on the entrapment efficiency. This could be due to the presence of Gelucire® 50/13 in the lipid phase, which effectively solubilizes the drug in the lipid phase. The drug loading ranged from 2–4% w/w. The variation in drug loading was due to the olive oil, as a change in the olive oil concentrations causes changes in the denominator of the drug loading equation (Equation 2.2). Generally, a high surfactant (vitamin E TPGS) concentration in the aqueous phase reduces the drug entrapment [35, 47], but in this study, the relationship between the above two factors and the drug loading were poor (Pearson's correlation coefficient: 0.2) and statistically insignificant; therefore, the particle size and PDI were the important parameters to obtain optimized curcumin-loaded NLCs.

3.2 Optimization

The optimized independent variables were obtained by feeding constraints (desired values of the dependent variables) into the Design Expert® 8 software. The constraints were the minimum particle size and PDI. The optimized independent variables are presented in Table 5, with a desirability of 0.977. The particle size and shape of the curcumin-loaded NLCs were confirmed by STEM imaging (Figure 3). The drug loading for the optimized curcumin-loaded NLCs was found to be $3.1 \pm 0.05\%$, and the entrapment efficiency was found to be $96 \pm 1.6\%$ (Table 6).

3.3 Stability of curcumin-loaded NLCs

The particle size, PDI, entrapment efficiency, and drug loading were analyzed to infer the stability of the curcumin-loaded NLCs. The particle size and PDI changes are significant indicators of the stability of nanoparticles [48]. The optimized NLCs were stable for 3 months at 4°C. However, NLC aggregation and curcumin degradation were observed at 25°C with a significant drop in the assay and increase in the particle size as well as PDI (Figure 4). At room temperature, the curcumin in the aqueous phase degrades causing the

curcumin from the nanoparticles to diffuse out (maintains equilibrium), which eventually degrades. Lipid nanoparticles significantly reduce the rate of degradation at room temperature but are unable to prevent it. However, the reduction in the rate of degradation can be ascribed to the entrapment of curcumin in the lipid matrix. This ascertains a minimum or absence of curcumin on the surface of nanoparticles. The stability of the curcumin-loaded NLCs can be further increased by freeze-drying the curcumin-loaded NLCs and storing them at 4°C, preventing hydrolytic degradation of the curcumin caused by its contact with the water and light. The control formulation was stable for the duration of 6 hours; therefore, it can be used for the *ex vivo* transcorneal studies.

3.4 In vitro transcorneal study

The transmembrane permeabilities of the curcumin suspension (control) and curcumin-loaded NLCs were found to be $0.66 \pm 0.4 \times 10^{-7}$ and $1.66 \pm 0.5 \times 10^{-7}$ cm/s, respectively (Figure 5), whereas, the fluxes were found to be 0.005 and 0.002 µg/min/cm² for the curcumin-loaded NLCs and control, respectively (Figure 5). The transcorneal permeability and flux for curcumin-loaded NLCs were significantly higher (~2.5-fold) in comparison to the control curcumin suspension. The NLCs enhance permeation either by phagocytosis/endocytosis dissociation of the drug from the NLCs or collision-mediated transfer of the drug from NLCs to the cornea, thereby increasing the permeability of the curcumin in comparison to the control suspension [9, 15, 16, 39, 49, 50]. The EC₅₀, determined by the DPPH assay, for curcumin's antioxidant activity is 8 ± 2 µM (2.9 ng/µL) [51]. Since the flux of curcumin-loaded NLCs is higher in comparison to the control, higher concentrations of curcumin would reach the aqueous humor and lens, thereby effectively preventing cataracts.

Damaged epithelium causes an uncontrolled amount of water to reach the stroma, causing the swelling of proteoglycans and indicating corneal damage [52, 53]. A percent corneal hydration in the range of 76 to 83% indicates that the corneal integrity was intact [54]. Therefore, we can conclude that the cornea did not lose integrity upon contact with the curcumin-loaded formulation for 3 hours. Therefore, from this study, we infer that the curcumin-loaded NLCs did not have any adverse effects on the cornea; however, the long-term effects still need to be studied.

3.5 Corneal histology

The corneal epithelium was found to be intact, appeared healthy and was found to be attached to Bowman's membrane (in some focal areas the epithelium was disrupted, but it appeared to be caused by mechanical separation rather than any toxic event or exposure) (Figure 6). The stroma was not found to be swollen and had even layers. The endothelium was intact, looked healthy and was attached to Descemet's membrane (in some focal areas the endothelium was missing, but it appeared to be caused by mechanical separation rather than any toxic event or exposure). The histology of corneas exposed to curcumin-loaded NLCs was similar to that of blank NLCs. Hence, exposing the corneas to the undiluted curcumin-loaded NLCs did not cause any toxicity *ex vivo*. Therefore, extrapolating results from the *ex vivo* test of the undiluted curcumin-loaded NLCs, the curcumin-loaded NLCs were less likely to have an adverse effect on the cornea when the cornea was exposed to 50 µL of the curcumin-loaded NLCs and further would get diluted by the tear fluid [55].

4. Conclusion

A quality-based design approach was used to optimize curcumin-loaded NLCs with a high curcumin loading and stability over 3 months at 4°C. The transcorneal permeation and flux of the optimized curcumin NLCs were approximately 2.5-fold higher than that of the control curcumin propylene glycol-based suspension. Curcumin-loaded NLCs were safe based on the results from the corneal histology and hydration test. Therefore, the curcumin-loaded NLCs have potential uses in various anterior segment diseases; however, further *in vivo* evaluation is needed.

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Figure 1:
Perspective plots illustrating effect of high level of the categoric factors.

Figure 2:
Perspective plots for effect of predictors on the PDI.

Figure 3:
Transmission electron microscopy images of the optimized curcumin formulation.

Figure 4: Bar graphs representing particle size (a), PDI (b), and assay(c) for stability at 4C and 25C for 3 months.

Figure 5:
Mean permeability and the flux of the curcumin loaded NLC and the control curcumin formulation.

Figure 6:
Histology of cornea exposed to a) Blank NLC, b) Curcumin loaded NLC.

Table 1:

Various solid and liquid lipids screened for curcumin solubility.

Solid lipid	Liquid lipid
Compritol™ 888 ATO	Labrafilm™ M 1944 CS
Precirol™ ATO 5	Maisine™
Geleol™	Capryol™ 90
Dynasan™ 114	Olive Oil
Dynasan™ 116	Sesame Oil
Gelucire™ 43/01	Soy bean oil
Gelucire™ 44/14	Captex™ 200
Gelucire™ 50/13	Captex™ 355
	Oleic acid
	Castor Oil

Table 2:

NLC formulation composition.

Ingredients	Amount (% w/v)
Lipid Phase	
Compritol™ ATO 888	3
Olive oil	0.5–2
Gelucire™ 50/13	1.5
Curcumin	0.3
Aqueous phase	
Vitamin E TPGS	0.4–1.0
Poloxamer 188	0.3–0.5
Glycerin	2.5
Water	QS

Table 3:

Design factors.

Factors/Independent variables/Predictors					
Coded Level	Poloxamer 188 % (x_1)	TPGS % (x_2)	Olive Oil % (x_3)	ULTRA-TURRAX speed Rpm (x_4)	Sonication time (min)(x_5)
Low (-1)	0.3	0.4	0.5	15000	5
Medium (0)	0.4	0.7	1.0		
High (1)	0.5	1	2	20000	10

Table 4:

Model summaries.

Parameters	Particle Size	Polydispersity Index	Drug Loading
	Unstandardized Coefficient	Unstandardized coefficient	Unstandardized coefficient
Intercept	0.084	-1.06	3.69
X1	2.153E-003	0.019	-
X2	2.582E-003	0.019	0.58
X3	2.770E-004	0.037	-
X4	9.378E-003	-0.13	0.24
X5	0.030	-0.57	
X2*X5	-	-	0.36
X2 ²	-	-	0.69
R2	0.8736	0.79	0.26
Adj. R2	0.8650	0.78	0.22
F value	94 (df=5,1)	57.24 (df=5,1)	6.45 (df=4,1)
Model p-value	<0.0001	< 0.0001	<0.0001

Table 5:

Optimized value of the predictor variables.

Variables	Optimized value (%)
Poloxamer 188	0.3
TPGS	1.0
Olive oil	1.5

Table 6:

Observed and predicted values for the response variable of the optimized formulation.

Variables	Observed	Predicted
Particle Size	66.09±2 nm	75.83
PDI	0.17±0.05	0.21