



Optimization of Semi-Solid Lipid Nanoparticle Dispersions by Quality by Design Approach for Dermal Delivery of Curcumin

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Abstract

Curcumin is an important anti-inflammatory agent for the treatment of skin disorders. However, its low water solubility, poor bioavailability, and instability limit the utilization of curcumin. Semi-solid lipid nanoparticle (SLN and NLC) dispersions, which maintain their colloidal particle size despite their high viscosity, offer a novel promising approach with high potential for dermal curcumin delivery. In this study, novel semi-solid SLN-NLC formulations of curcumin were manufactured using a one-step method, without the need to disperse the nanoparticles in an additional vehicle. Modde Pro 12 was used to examine the relationship between variables and quality attributes. QbD-based formulation optimization was successfully performed using artificial neural network program (ANN), and optimum semi-solid SLN-NLC formulations were prepared. The particle size of the optimum formulations was found to be 204.7 ± 1.5 nm for SS-SLN-Opt and 198.5 ± 0.81 nm for SS-NLC-Opt, indicating that the particle sizes were within the targeted range. The amount of curcumin released from the SS-NLC-Opt formulation was $33.72 \pm 4.99\%$ at 24th Hour, which was higher than the release obtained from the eight SS-NLC formulations entered as input into the ANN program. On the other hand, while the curcumin release percentage at the 24th Hour from the SS-SLN formulations entered into the program ranged between 11.13% and 44.31%, the release amount for the SS-SLN-Opt formulation was found to be $38.34 \pm 3.48\%$, which was within this range and close to the maximum value. Rheological characterization results indicated that the optimum semi-solid SLN and NLC formulations were more elastic than viscous. The stability of the optimum semi-solid SLN formulation at 4 °C was higher than that of the optimum semi-solid NLC after one month. In vivo studies in rats revealed that the optimum semi-solid SLN formulation exhibited higher anti-inflammatory activity than both the optimum semi-solid NLC and the conventional gel. The SS-SLN-Opt formulation effectively reduced the inflammation in rats starting from the first hour. In conclusion, the optimum semi-solid SLN formulation, which is more stable and has higher anti-inflammatory activity, is a promising alternative for the dermal delivery of curcumin.

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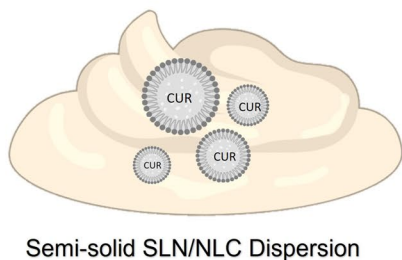
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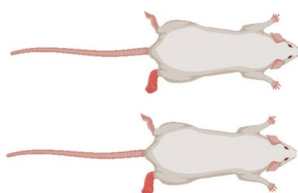
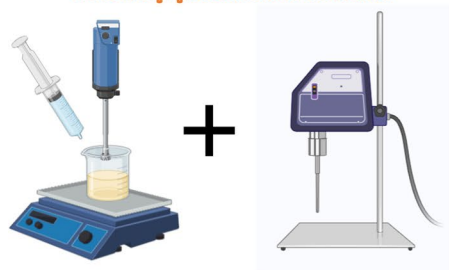
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Graphical Abstract

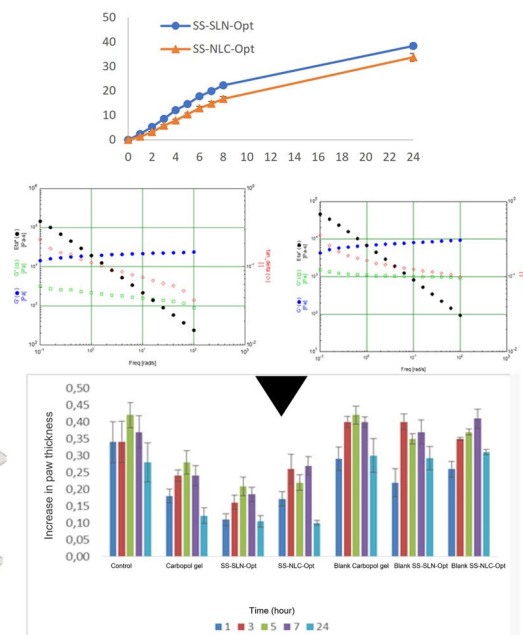
Formulation optimization using ANN by QbD approach



One step production method



In vitro characterization of curcumin loaded optimum formulations



Keywords Artificial neural network · Carrageenan · Curcumin · Quality by design · Optimization · Semi-solid SLN · Semi-solid NLC · Texture profile analysis

Introduction

Curcuma longa (Turmeric) which is also known as “golden spice” has been commonly used for centuries not only as a flavoring agent but also as herbal medicine for the treatment of several diseases such as rheumatic pain, wounds and diabetic ulcers [6, 64, 76, 102]. Curcumin is the primary polyphenolic compound found in the rhizomes of *Curcuma longa*. After it was first isolated in 1815, scientific interest towards curcumin has increased gradually due to its various beneficial effects on health [36, 76, 84]. A wide range of biological activities without toxicity have been documented over the years and curcumin has therefore been approved as “Generally Recognized as Safe” (GRAS) by FDA [91, 100, 102]. With its potent anti-oxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic activities, curcumin is a promising natural substance with a great potential for the treatment of various skin disorders such as psoriasis, dermatitis, acne, and cancers [72, 96]. The anti-inflammatory activity of curcumin is based on the inhibition of both cyclooxygenase (COX-1 and COX-2) and inducible nitric oxide synthase (iNOS), the key enzymes involved in the process

of inflammation. Curcumin also reduces the expression of various pro-inflammatory cytokines such as chemokines, interleukins and tumor necrosis factor (TNF- α) [18, 41, 73, 76].

Despite its important biological effects, some physico-chemical properties of curcumin limit its wider utilization. Low water solubility, poor bioavailability and chemical instability are the major limitations for the usage of curcumin in clinical practice [29, 74, 96, 102]. Among several strategies to overcome these limitations, drug delivery systems have an important role. Many studies have been carried out to formulate curcumin into various drug delivery systems. Solid lipid nanoparticles (SLNs) [35, 37], nanostructured lipid carriers (NLCs) [77], polymeric and lipidic nanocapsules [65], PLGA nanoparticles [24], liposomes [55, 104], niosomes [80], gel-core hyalurosomes [30], microemulsions [62] and nanoemulsions [5, 10] have been reported to increase curcumin solubility, stability and pharmacological efficiency in various skin disorders.

Lipid nanoparticles have been developed to overcome many disadvantages of traditional nanocarriers such as liposomes, polymeric nanoparticles and nanoemulsions. They

have been evaluated extensively for the delivery of active substances by different routes, specifically for dermal/transdermal applications. With biocompatible and biodegradable lipids and no organic solvents in their composition, lipid nanoparticles have attracted great attention as non-toxic carriers. They also present some other advantages such as increasing bioavailability and stability of active substances, and enhancing the skin penetration of molecules [1, 34, 54, 68]. The first generation of lipid nanoparticles, SLNs, are composed of only solid lipids and were introduced by Muller et al. around the beginning of 1990s [68]. SLNs offer advantages such as uniform particle size, increased surface area, and improved drug loading. The solid lipids that form the structure of SLNs are physiological lipids, which enhance permeation through biological membranes [63]. On the other hand, the main disadvantage of SLNs is drug expulsion because of the solidification and crystallization of lipids. Especially when the lipid matrix consisted of identical molecules, i.e. monotype solid lipids, a perfect crystal lattice with limited imperfection areas occurs. Since the drug molecules are captured in crystal gaps, high amounts of molecules cannot be loaded in the lipid matrix [1, 39]. The second generation, NLCs, were developed in the next decade, in order to obtain a higher drug loading capacity and to decrease the expulsion of drug molecules. NLCs are prepared using binary mixtures of solid and liquid lipids to create a more imperfect lipid matrix, thereby enhancing drug loading and reducing drug expulsion [34, 39, 97].

The novel approach in dermal delivery of SLNs or NLCs is introduction of semi-solid lipid nanoparticle dispersions. Semi-solid lipid nanoparticle dispersions preserve their colloidal particle size in spite of their high viscosity and offer important advantages [60]. They can be produced using a one-step method, eliminating the requirement to incorporate the lipid nanoparticle dispersion into an additional semi-solid carrier with the desired viscosity, such as a cream, hydrogel, or emulgel. This approach helps overcome several challenges, including low SLN/NLC loading in the final semi-solid formulation, time-consuming production steps and potential incompatibilities between the components of the semi-solid vehicles and lipid nanoparticle dispersions. Because of their remarkable advantages, the number of the studies related to the use of semi-solid lipid nanoparticle dispersions as dermal drug delivery systems has increased in the last 10 years [2, 11, 14, 15].

Quality by Design (QbD), a holistic methodology, aim to ensure the desired quality profile of a product throughout the lifecycle. In the last two decades, the use of the QbD approach as a tool to achieve high-quality standards in pharmaceutical development has increased significantly. The risk-based determination of the Quality Target Product Profile (QTPP), Critical Quality Attributes (CQAs), and

independent critical parameters of the process and formulation (CPPs and CMAs), along with the creation of a Design Space (DS) that ensures the achievement of the desired CQAs, form the stages of the QbD approach. Artificial intelligence methods such as RSM (Response Surface Methodology) and ANN (Artificial Neural Networks) are used for the development of predictive models. ANN methodology, with its remarkable information-processing features such as nonlinearity, high parallelism, robustness, learning, ability to process fuzzy information, and generalization capacity, mimics various parts of the human brain using different learning algorithms [7, 8, 56, 90].

The aim of this study is to develop a novel semi-solid lipid nanoparticle dispersion of curcumin which has a great potential in the treatment of inflammatory skin diseases, and to enhance its stability, skin penetration and effectiveness. The formulation optimization of curcumin-loaded semi-solid SLN and NLC dispersions were carried out using computer-based modelling methods in concept of the quality by design (QbD) approach. *In vivo* anti-inflammatory activity of optimized formulations was evaluated on rats.

Materials and Methods

Materials

Curcumin was purchased from Alfa Aesar (USA). Compritol 888 ATO (glyceryl behenate) was kindly provided by Gattefossé (Saint-Priest, France). Tristearin, oleic acid and Poloxamer 188 was obtained from Sigma-Aldrich (USA). Tween 80 was purchased from Fluka (UK). Carbopol® 934 was obtained from Noveon (USA). All other chemicals were of analytical reagent grade and ultra-pure (MilliQ) water was used.

Preparation of Curcumin Loaded Semi-Solid SLN and NLC Formulations

Experimental design were created after the formulation variables and critical quality attributes (CQAs) were determined for semi-solid lipid nanoparticle formulations. Based on earlier studies in the field and preliminary studies from our laboratory, different ratios of solid lipids in binary mixtures and different total lipid concentrations were determined as formulation variables for semi-solid SLN formulations while different solid : liquid lipid ratios and different total lipid concentrations were evaluated for semi-solid NLC formulations. Since the particle size is a very important parameter in effective dermal targeting of nanoparticles applied on skin, the particle size has been identified first CQA for the optimization of the formulation [58]. On the other hand,

the amount of active substance released from nanoparticles in the target area is also important for the efficacy of the treatment. In this context, the second CQA in optimization studies were determined as the amount of active substance released at 24th hour [95].

Semi-solid lipid nanoparticle dispersions were produced using high shear homogenization and ultrasonication method. For the preparation of semi-solid SLN formulations, only Compritol 888 ATO or the mixtures of Tristearin: Compritol 888 ATO in two different ratios (25:75 and 50:50, respectively) were used as the solid lipid. The solid lipid mixture was melted at approximately 70 °C and mixed with 0.05 g of curcumin. The aqueous surfactant solution consisting of 1.5% Poloxamer 188 and 0.5% Tween 80 was heated to the same temperature. The pre-emulsion prepared using a high-speed homogenizer at 8000 rpm for 5 min (UltraTurrax T25, Germany) was then Sonicated in an ultrasonic homogenizer for 5 min with 25 W power (Vibra Cell 500, USA). Semi-solid SLN formulations were obtained by keeping the resulting nanoemulsion at 4 °C. The same method was used for semi-solid NLC dispersions. For this purpose, Compritol 888 ATO and oleic acid were used as solid and liquid lipids, respectively. Different solid : liquid lipid ratios (80:20 and 60:40, respectively) and different total lipid concentrations (8, 12, 16 and 20%) were evaluated. The mixture of 1.5% Poloxamer 188 and 0.5% Tween 80 was used as surfactant. The contents of the semi-solid SLN and NLC formulations are given in Table 1.

Table 1 The contents of semi-solid SLN and semi-solid NLC formulations

Formulation	Total lipid concentration (%)	Oleic acid (g)	Tristearin (g)	Compritol 888 ATO (g)	Curcumin (g)
SS-SLN 1	8	-	-	1.20	0.05
SS-SLN 2	8	-	0.60	0.60	0.05
SS-SLN 3	8	-	0.30	0.90	0.05
SS-SLN 4	12	-	-	1.80	0.05
SS-SLN 5	12	-	0.90	0.90	0.05
SS-SLN 6	12	-	0.45	1.35	0.05
SS-SLN 7	16	-	-	2.40	0.05
SS-SLN 8	16	-	1.20	1.20	0.05
SS-SLN 9	16	-	0.60	1.80	0.05
SS-SLN 10	20	-	-	3.00	0.05
SS-SLN 11	20	-	1.50	1.50	0.05
SS-SLN 12	20	-	0.75	2.25	0.05
SS-NLC 1	8	0.24	-	0.96	0.05
SS-NLC 2	8	0.48	-	0.72	0.05
SS-NLC 3	12	0.36	-	1.44	0.05
SS-NLC 4	12	0.72	-	1.08	0.05
SS-NLC 5	16	0.48	-	1.92	0.05
SS-NLC 6	16	0.96	-	1.44	0.05
SS-NLC 7	20	0.60	-	2.40	0.05
SS-NLC 8	20	1.20	-	1.80	0.05

In Vitro Characterization of Semi-Solid SLN and NLC Formulations

Determination of Particle Size and Zeta Potential

The particle size, polydispersity index (PDI) and zeta potential of the semi-solid SLN and NLC dispersions were determined using Malvern Zetasizer NanoZS (UK). The semi-solid lipid nanoparticle dispersions were diluted 10-fold with ultrapure water before analysis. Samples for each formulation were analyzed three times.

Determination of Drug Loading and Encapsulation Efficiency

For the determination of encapsulation efficiencies of the formulations, 150 mg of semi-solid SLN/NLC dispersion was mixed with 8 mL methanol in a tightly closed container using a magnetic stirrer at 65 °C. This was followed by the Sonication of the mixture for 15 min using an ultrasonic homogenizer and then cooled to room temperature. The supernatant obtained after the centrifugation at 57,000 rpm for 1 hour was diluted with methanol and analyzed by UV spectrophotometer at 422 nm (Thermoscientific Evolution 201, USA). The drug loading and encapsulation efficiency values of each formulation were calculated using following equations [40, 81]:

$$\text{Drug loading (\%)} = \frac{\text{Drug amount in formulation}}{\text{Amount of formulation}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Drug loading (\%)}}{\text{Theoretically drug amount (\%)}} \times 100$$

In Vitro Drug Release Studies

The dialysis bag method was carried out to determine the release profile of curcumin from semi-solid lipid nanoparticle dispersions. Weighed amount of semi-solid SLN and NLC formulations each equivalent to 2 mg of curcumin were placed into the pre-hydrated regenerated cellulose dialysis membrane (MWCO: 12–14 kDa, Spectra/Por 2®, USA). Phosphate buffer (pH 5.5): methanol (50:50) mixture was used as dissolution medium, with a total volume of 150 mL, and sink condition was provided. In vitro release studies were performed for 24 hours in an incubator shaker operated at 100 rpm. The temperature of the dissolution medium was set to 37 °C. Samples were collected at appropriate time intervals and replaced by an equal volume of fresh dissolution medium. The curcumin content of samples was analyzed at 422 nm using an UV spectrophotometer (Thermoscientific Evolution 201, USA) [67, 85].

Examination of the Effect of Formulation Parameters on Critical Quality Attributes

To investigate the multivariate relations between formulation variables and CQAs (particle size and released curcumin amount at 24th hour), all experimental studies were conducted and the results were then modeled using partial least squares (PLS) regression analysis via Modde Pro 12 (MODDE®, Umetrics) software, and the validity of the model was tested using analysis of variance (ANOVA) [50]. Then, VIP (Variable importance in the projection) and coefficient plots were examined for SLN and NLC formulations separately.

The VIP plot displays the VIP values as a column which indicates how important certain variables are for the response (Modde, User Guide, 2018). The coefficient plot which is revised values of the regression coefficients of the model equations provides a graphical depiction of the model terms to help assess the magnitude of the impact of formulation factors on the responses; the sign of the coefficient plot denotes whether the influence was positive or negative. A factor or interaction of factors is considered significant if it is far from $y=0$ and an uncertainty level that does not cross $y=0$ [17, 22, 47, 50, 69, 89].

Formulation Optimization Using ANN by QbD Approach

ANN modelling INform V.3.17 (Intelligence, UK) program was used for the QbD-based formulation optimization studies. Although INForm is referred to as a neural network software package, it includes more than just neural networks with a back-propagation algorithm type, in addition to statistical methods, fuzzy logic, genetic algorithms, and visualization tools. While the neural network element is responsible for creating the central model, pre-trained models, fuzzy logic, and genetic algorithms are employed to optimize formulation. For training of the neural network, the formulation variables and selected CQAs were entered to the ANN modelling program as input and output, respectively. After the training was complete and the models were evaluated according to R^2 values from ANOVA (Analysis of Variance), the optimization process was pursued [8].

Experiments on Optimum Semi-Solid SLN and NLC Formulations

In Vitro Characterization of Optimum Formulations

The particle size, PDI, zeta potential and encapsulation efficiency values of the optimum semi-solid lipid nanoparticle formulations were analyzed as previously described [40,

81]. In vitro release profile of curcumin from optimum formulations was also determined by dialysis bag method [67, 85].

Differential Scanning Calorimetry (DSC) Analysis

Differential Scanning Calorimetry (DSC) analyses were performed using a Shimadzu DSC-60 (Japan) instrument on the curcumin-loaded optimum semi-solid SLN and NLC formulations, optimum formulations without curcumin, solid lipids and curcumin. Before DSC analysis, optimum semi-solid lipid nanoparticle dispersions were lyophilized for avoiding the interference of the water peak at around 100 °C. The samples (5 mg) were placed in sealed aluminum Pans. DSC thermograms were obtained by heating at a rate of 10 °C/min in the range of 30–300 °C and under a nitrogen atmosphere at a flow rate of 50 ml/min [15].

Transmission Electron Microscopy (TEM) Analysis

TEM analysis of optimum semi-solid SLN and NLC formulations was performed at room temperature (Hitachi HT7800, Japan). The samples were diluted with ultra-pure water at a ratio of 1:10 and dropped onto formvar-coated grids using the dripping technique. After drying, TEM micrographs were obtained using a voltage of 100 kV [11].

In Vitro Occlusion Test

The occlusive properties of the optimum semi-solid SLN and NLC formulations and the conventional Carbopol® 934 gel containing curcumin were determined by in vitro occlusion test [26]. For this purpose, the mouths of the beakers containing 30 ml of distilled water were covered with Whatman No: 42 filter paper. 500 mg of each formulation was weighed and spread on filter paper using a spatula. The beakers were kept at 32 °C in an incubator for 48 Hours. The amount of water loss was determined by weighing the beakers at the beginning, and at 12th, 24th and 48th hours. Beakers without any formulation were used as the control group. Three parallel experiments were conducted for each group. Occlusion factor 'F' is calculated by the following equation:

$$F = \frac{A - B}{A} \times 100$$

A Water loss for the control group

B Water loss for the sample

For preparing the conventional Carbopol gel formulation, 0.5% Carbopol 934 was dissolved in distilled water, and the required amount of triethanolamine was added to achieve

neutralization, resulting in gel formation. For the preparation of curcumin-loaded gel formulations, curcumin was weighed in the same concentration as in the semisolid SLN and NLC formulations and dispersed within the gel.

pH Determination of Optimum Formulations

The pH values of the optimum semi-solid SLN and NLC formulations were measured with a pH-meter. Before measurement, the samples were diluted with water to 10%. The results were given as mean $\pm$ SD ($n=3$) [4].

Determination of Rheological Properties

The viscosity values of the optimum semi-solid SLN and NLC formulations were measured using a rotational viscometer (Brookfield DV-II, USA) with TF 96 spindle at 10 rpm. The ambient temperature was set to 25 °C before measurements ($n=3$).

ARES rheometer (TA Instruments, USA) with parallel plate geometry was used to perform the rheological characterization of the optimum semi-solid lipid nanoparticle formulations by oscillation frequency sweep test. The operating conditions were set as parallel plate diameter of 40 mm, inter-plate space of 1 mm and ambient temperature of 25 °C. An oscillation frequency sweep test was performed in the frequency range of 0.1–100 rad/s and at a constant strain (1%). As a result of the analysis, storage module (G'), loss module (G'') and complex viscosity (η^*) values were obtained for both formulations [15].

Determination of Mechanical Properties

Texture profile analysis (TPA) was performed in order to determine the mechanical properties of optimum semi-solid SLN and NLC formulations (TA-XT Plus Texture Analyzer, Stable Microsystems, UK). TPA analysis was performed in two-cycle compression mode and the ambient temperature was set at 25 ± 1 °C. Optimum formulations without air bubbles were placed in cylindrical glass containers. During analysis, the cylindrical perspex probe with a diameter of 10 mm was immersed twice into each sample 8 mm in depth and withdrawn. The delay period allowed between two compressions was 15 s. The parameters of TPA were set as; pre-test, test and post-test speed: 2 mm/s, load cell: 5 kg and trigger force: 0.05 N. The analysis was repeated 5 times for each sample and the hardness, compressibility, cohesiveness, adhesiveness and elasticity values of the optimum formulations were calculated using the force-time curves [46].

Stability Studies

The stability of optimum formulations was examined. Particle size, PDI, zeta potential and encapsulation efficiency values of the samples stored at 4 °C and 25 °C for 1 month were determined and statistically evaluated (SPSS 16) (ICH Topic Q 1 A R2) [45]. The data was considered to be significant at $p < 0.05$.

In Vivo Evaluation of the Anti-Inflammatory Activity of Optimum Formulations

In vivo anti-inflammatory activity experiments were carried out on adult male Sprague-Dawley rats weighing 250 g. The animals were kept in metal cages, at 22–24 °C and 55% Humidity, in a 12-hour light/12-hour dark environment, with water and feed given ad libitum. All in vivo experiments were approved by the Local Animal Ethics Committee of Ankara University (Approval no: 2018-19-126).

Carrageenan-induced paw edema test was performed to determine anti-inflammatory activity of optimum formulations on rats [48, 66, 70]. The rats were divided into 7 groups, with 6 animals in each group and the formulations were topically applied to the right hind paw of the anesthetized rats. The formulations administered to these seven animal groups are as follows: the control group, curcumin-containing SS-SLN-Opt, SS-NLC-Opt, and Carbopol®934 gel groups, as well as the active ingredient-free (blank) SS-SLN-Opt, SS-NLC-Opt, and Carbopol®934 gel groups. Carbopol® 934 gel formulations with or without curcumin were prepared and evaluated for comparison. Following the application of formulations, 0.1 ml 1% w/v solution of lambda-carrageenan in 0.9% NaCl was injected into the subplantar region of the right hind paw of the rats. No treatment was applied to the rats in the control group except for carrageenan injection. The increase in paw thickness was measured using calipers for 24 hours, immediately after carrageenan injection and at certain time intervals.

Statistical Analysis

Results were statistically analyzed using Student's *t* Test to determine the significance between two groups and using One-Way ANOVA followed by Tukey's Post Hoc test for multiple comparisons (SPSS- 26). The data were considered to be significant at $p < 0.05$.

Results and Discussion

In Vitro Characterization of Semi-Solid Lipid Nanoparticle Dispersions

Particle size, PDI and zeta potential analysis results of semi-solid SLN and NLC formulations are given in Table 2.

The formulations labeled as SS-SLN 1, SS-SLN 4, SS-SLN 7 and SS-SLN 10 were prepared only with Compritol® at different total lipid concentrations. An increase in Compritol concentration resulted in an increase in the particle size of the lipid nanoparticles. Similar results were obtained for the formulations prepared with Tristearin: Compritol ratio of 50:50, except for SS-SLN 2 and SS-SLN 5. As opposed our expectation, the particle sizes of SS-SLN 2 (8% total lipid) and SS-SLN 5 (12% total lipid) were similar ($p > 0.05$). The increment in lipid concentration also resulted in an increase in the particle size of the formulations consist of Tristearin: Compritol (25:75); SS-SLN 3, SS-SLN 6, SS-SLN 9 and SS-SLN 12. These results were attributed to the enhanced viscosity of the disperse phase with increasing total lipid concentration in all formulations. Since the surfactant concentration and homogenization rate were constant for all formulations, the increase in disperse phase viscosity resulted in the formation of larger droplets in the

emulsification process. Similar findings were obtained in various studies related to SLNs [57, 82, 93].

The effect of the Compritol ratio in lipid mixture on the particle size of SLNs was observed more clearly in those formulations prepared at high lipid concentrations. The particle size decreased when the Compritol ratio in the lipid mixture decreased for the formulations with 20% total lipid content (SS-SLN 10, SS-SLN 11 and SS-SLN 12, $p < 0.05$). For the semi-solid SLN formulations with 16% total lipid content (SS-SLN 7, SS-SLN 8 and SS-SLN 9), when the Compritol ratio was reduced from 100 to 75% the particle size decreased. However, the decrease in particle size was not statistically significant ($p > 0.05$) when the Compritol ratio was reduced to 50%. For SS-SLN 4, SS-SLN 5 and SS-SLN 6 (12% total lipid), the reduction in the amount of Compritol from 100 to 75% resulted in an increment in the particle size from 305.5 nm to 325.6 nm, contrary to previous findings. However, when the Compritol ratio was reduced to 50%, the particle size was 301.7 nm. On the other hand, changing the Compritol percentage in the lipid mixture did not create a significant change on the particle size for the formulations prepared at 8% total lipid concentration (SS-SLN 1, SS-SLN 2 and SS-SLN 3, $p > 0.05$).

When the particle sizes of SS-NLC 1 and SS-NLC 2 (8% total lipid) were evaluated, the particle size decreased from 310.1 nm to 253 nm as a result of increasing the amount of oleic acid from 20 to 40%. Similarly, for the SS-NLC formulations with 12%, 16% and 20% total lipid concentrations, increasing the ratio of liquid lipid resulted in a decrease in particle size ($p < 0.05$). These results can be attributed to the formation of smaller droplets due to the decrement in the viscosity of the disperse phase by increasing the amount of oleic acid used as liquid lipid. Similar findings were reported in the literature [38, 49]. It was observed that the particle size significantly increased depending on the enhancement in total lipid concentration for the SS-NLC 1, SS-NLC 3, SS-NLC 5 and SS-NLC 7 prepared with 80:20 (solid: liquid) lipid ratio ($p < 0.05$). When the solid: liquid lipid ratio was 60:40, the particle size generally increased as the total lipid concentration increased, except for the SS-NLC 8. On the other hand, the difference between the particle sizes of SS-NLC 4 and SS-NLC 6 were not significant ($p > 0.05$).

As it is well known, the polydispersity index (PDI) value is used to define the particle size distribution for nanoparticulate systems. The PDI values of all semi-solid SLN and NLC formulations were below 0.5. Hence, it was concluded that the formulations have an acceptable narrow particle size distribution [21, 42, 101].

Zeta potential represents the surface charge of nanoparticles and provides information about the long-term stability

Table 2 Particle size, PDI and zeta potential analysis results of semi-solid SLN and semi-solid NLC formulations ($n = 3$)

Formulation	Particle size (nm) (mean $\pm$ SD)	PDI (mean $\pm$ SD)	Zeta potential (mV) (mean $\pm$ SD)
SS-SLN 1	284.5 $\pm$ 1.9	0.373 $\pm$ 0.070	-26.47 $\pm$ 0.83
SS-SLN 2	295.1 $\pm$ 2.6	0.349 $\pm$ 0.054	-24.97 $\pm$ 0.29
SS-SLN 3	288.8 $\pm$ 3.6	0.327 $\pm$ 0.040	-24.80 $\pm$ 0.35
SS-SLN 4	305.5 $\pm$ 0.7	0.394 $\pm$ 0.019	-21.70 $\pm$ 0.10
SS-SLN 5	301.7 $\pm$ 14.2	0.404 $\pm$ 0.020	-26.57 $\pm$ 0.12
SS-SLN 6	325.6 $\pm$ 4.5	0.330 $\pm$ 0.060	-23.93 $\pm$ 0.31
SS-SLN 7	380.4 $\pm$ 2.8	0.330 $\pm$ 0.002	-28.20 $\pm$ 0.26
SS-SLN 8	350.0 $\pm$ 1.5	0.252 $\pm$ 0.011	-24.50 $\pm$ 0.10
SS-SLN 9	362.8 $\pm$ 1.2	0.313 $\pm$ 0.028	-22.30 $\pm$ 0.26
SS-SLN 10	463.4 $\pm$ 4.9	0.461 $\pm$ 0.015	-25.27 $\pm$ 0.06
SS-SLN 11	371.1 $\pm$ 3.3	0.369 $\pm$ 0.056	-30.83 $\pm$ 0.15
SS-SLN 12	418.6 $\pm$ 2.9	0.414 $\pm$ 0.021	-22.33 $\pm$ 0.31
SS-NLC 1	310.1 $\pm$ 5.1	0.382 $\pm$ 0.047	-31.10 $\pm$ 0.10
SS-NLC 2	253.0 $\pm$ 2.2	0.368 $\pm$ 0.045	-26.33 $\pm$ 0.12
SS-NLC 3	401.1 $\pm$ 2.0	0.404 $\pm$ 0.008	-29.70 $\pm$ 0.10
SS-NLC 4	267.9 $\pm$ 3.2	0.244 $\pm$ 0.010	-30.30 $\pm$ 0.20
SS-NLC 5	453.5 $\pm$ 5.7	0.481 $\pm$ 0.020	-31.13 $\pm$ 0.15
SS-NLC 6	272.9 $\pm$ 2.4	0.296 $\pm$ 0.022	-29.73 $\pm$ 0.06
SS-NLC 7	524.8 $\pm$ 6.3	0.398 $\pm$ 0.024	-29.80 $\pm$ 0.10
SS-NLC 8	259.3 $\pm$ 1.4	0.257 $\pm$ 0.003	-27.13 $\pm$ 0.15

of dispersion. Therefore, the larger the zeta potential value, the greater the repulsive force between the particles, and the more stable the nanoparticulate system [71]. In our study, the zeta potential values of semi-solid SLN formulations were between -21.70 mV and -30.83 mV, while the zeta potentials of semi-solid NLC formulations were between -26.33 mV and -31.13 mV. Since the zeta potential of all formulations was greater than -20 mV, it was concluded that the semi-solid lipid nanoparticle dispersions were stable. On the other hand, encapsulation efficiency of all semi-solid lipid nanoparticle dispersions were higher than 94% (Table 3). These high encapsulation efficiency values can be attributed to the very low water solubility of curcumin [20, 27].

The in vitro released amounts of curcumin (%) at 24th hour were given in Table 3 and release profiles were also shown in Supplementary Fig. 1 and Supplementary Fig. 2 (Online Resource 1). When the release profiles of SS-SLN 1, SS-SLN 4, SS-SLN 7 and SS-SLN 10 were examined, the released amount of curcumin from the SS-SLN 1 (8% total lipid) was higher compared to other formulations ($p < 0.05$). The amount of curcumin released at the 24th Hour from SS-SLN 4, SS-SLN 7 and SS-SLN 10 were similar despite increasing total lipid concentrations ($p > 0.05$). Among the formulations prepared using the mixture of Tristearin and Compritol (50:50), the highest curcumin release was obtained from the SS-SLN 2 formulation (8% total lipid),

while the active substance release was the lowest from the SS-SLN 11 formulation with 20% total lipid content. On the other hand, the difference between the released amount of curcumin from SS-SLN 5 (12% total lipid) and SS-SLN 8 (16% total lipid) was not statistically significant ($p > 0.05$). A similar situation was observed for the SS-SLN 3, SS-SLN 6, SS-SLN 9 and SS-SLN 12 which were prepared using the Tristearin: Compritol ratio of 25:75. It was found that the curcumin release from the SS-SLN 3 formulation prepared with 8% total lipid concentration was the highest, while the active substance release from the SS-SLN 12 formulation containing 20% total lipid was the lowest. The difference between the amounts of active substance released from SS-SLN 6 and SS-SLN 9 was not statistically significant ($p > 0.05$). Lipid concentration is an important factor which affects the release profile of drugs. The highest curcumin release from formulations with the lowest total lipid concentration (8% total lipid) and the decrease in curcumin release as the total lipid concentration increased is consistent with the literature findings. In the literature, it has been reported that as the lipid concentration in the lipid nanoparticle matrix increases, the release of the active substance decreases [25, 87, 92].

The release profiles of SS-SLN 4, SS-SLN 5 and SS-SLN 6 (12% total lipid) showed that the amount of curcumin released at the 24th Hour was highest for SS-SLN 4 prepared only with Compritol. There was no difference between the amounts of curcumin released at the 24th Hour from the SS-SLN 5 and SS-SLN 6 where the Tristearin: Compritol ratio was 50:50 and 25:75, respectively ($p > 0.05$). Similar results were obtained for SS-SLN 10, SS-SLN 11 and SS-SLN 12 with 20% total lipid content. When SS-SLN 7, SS-SLN 8 and SS-SLN 9 (16% total lipid) were evaluated, the amount of curcumin released at the 24th Hour from SS-SLN 7, which was prepared only with Compritol, was higher than the other two formulations, but the difference was not statistically significant ($p > 0.05$). On the other hand, while the amount of curcumin released at the 24th Hour was the same from the SS-SLN 1 (Compritol only) and SS-SLN 2 (Tristearin: Compritol, 50:50), released amount of curcumin from SS-SLN 3 (Tristearin: Compritol, 25:75) was lower ($p < 0.05$). These findings demonstrate that, as mentioned in the literature, the type of lipid is an important factor influencing the drug release from lipid nanoparticles [87].

When the curcumin release profiles of SS-NLC 1 (8% total lipid and 20% liquid lipid) and SS-NLC 2 (8% total lipid and 40% liquid lipid) were compared, it was observed that the increase in liquid lipid content results in an increase in the release of the active substance ($p < 0.05$). Similar results were obtained for SS-NLC 3 and SS-NLC 4 formulations containing 12% total lipid ($p < 0.05$). The amount of curcumin released at the 24th Hour also increased due to the

Table 3 Encapsulation efficiency and in vitro release study results of SS-SLN and SS-NLC formulations ($n=3$)

Formulation	Encapsulation efficiency (%) (mean $\pm$ SD)	Released amount of curcumin (%) at 24th hour (mean $\pm$ SD)
SS-SLN 1	99.06 $\pm$ 3.15	44.26 $\pm$ 7.83
SS-SLN 2	103.31 $\pm$ 0.16	44.31 $\pm$ 10.40
SS-SLN 3	98.24 $\pm$ 2.70	28.51 $\pm$ 4.15
SS-SLN 4	98.58 $\pm$ 0.83	32.79 $\pm$ 6.29
SS-SLN 5	102.36 $\pm$ 0.55	18.46 $\pm$ 3.60
SS-SLN 6	103.39 $\pm$ 2.37	20.50 $\pm$ 4.34
SS-SLN 7	97.85 $\pm$ 8.78	26.42 $\pm$ 3.82
SS-SLN 8	95.76 $\pm$ 0.58	21.76 $\pm$ 2.28
SS-SLN 9	97.80 $\pm$ 5.87	20.18 $\pm$ 1.11
SS-SLN 10	98.28 $\pm$ 3.43	28.64 $\pm$ 3.06
SS-SLN 11	97.93 $\pm$ 11.54	11.13 $\pm$ 2.08
SS-SLN 12	97.19 $\pm$ 1.38	14.60 $\pm$ 2.35
SS-NLC 1	101.43 $\pm$ 2.15	20.25 $\pm$ 7.78
SS-NLC 2	101.59 $\pm$ 0.43	30.23 $\pm$ 2.17
SS-NLC 3	98.04 $\pm$ 1.50	19.62 $\pm$ 8.03
SS-NLC 4	94.28 $\pm$ 0.42	26.96 $\pm$ 2.59
SS-NLC 5	97.43 $\pm$ 10.84	21.37 $\pm$ 2.98
SS-NLC 6	99.52 $\pm$ 7.33	24.60 $\pm$ 0.98
SS-NLC 7	98.98 $\pm$ 7.65	15.09 $\pm$ 1.29
SS-NLC 8	97.80 $\pm$ 4.09	19.28 $\pm$ 3.38

increase in the percentage of liquid lipid for the SS-NLC 5 and SS-NLC 6 formulations with 16% total lipid content and the SS-NLC 7 and SS-NLC 8 formulations with 20% total lipid content. However, this increase was not significant ($p > 0.05$). Because of the higher percentage of liquid lipid, the crystal deficiency areas within the lipid matrix increased and the diffusion of the active substance from this structure was enhanced [44, 103].

There was no significant difference between the active substance release profiles from SS-NLC 1, SS-NLC 3, SS-NLC 5 and SS-NLC 7 which were prepared with Compritol: Oleic acid ratio of 80:20 and with different total lipid concentrations ($p > 0.05$). On the contrary, for the SS-NLC 2, SS-NLC 4, SS-NLC 6 and SS-NLC 8 (Compritol: Oleic acid, 60:40 and different total lipid concentrations) the amount of curcumin released at the 24th hour decreased as the total lipid concentration increased [25, 92].

Examination of the Effect of Formulation Parameters on Critical Quality Attributes

To demonstrate the effect of variables on responses, VIP plots and coefficient plots were created after PLS modelling of the experimental design using Modde Pro 12 (Sartorius). The R^2 was found 0,57 for particle size and 0,67 for released amount at 24th hour for developed models of SLN formulations. According to VIP plot (Fig. 1) which shows the importance of formulation variables on quality attributes, the most effective variable on particle size is Compritol amount, while the Tristearin amount is the most effective variable for released amount of curcumin at 24th hour. Despite the model terms has uncertainty level extend $y=0$ line, Compritol amount in the formulation has positive

effect on particle size but its interaction with Tristearin shows decreasing effect. Both Tristearin and Compritol amounts used in SLN formulation results in decreasing of the released amount at 24th hour, while increasing tristearin amount has more negative effect on release (Fig. 2). It is important to choose the proper amount of lipids in the formulation, to obtain intended particle size and maximum release percentage.

A better fit to the developed models were obtained for both responses ($R^2 > 90$) for NLC formulations. According to VIP plot for NLC formulations, most effective variable is Compritol amount on particle size and released amount at 24 hours (Fig. 3). As seen in coefficient plot for NLC formulation, oleic acid amount has decreasing effect on particle size while it positively influences the released amount at 24th Hour. Otherwise, its quadratic term has a negative effect on 24th hour release (Fig. 4). This negative effect on release results is related to the increase in total lipid concentration. On the other hand, a vice versa effect was detected on both particle size and release with Compritol. The interactive effect between Compritol and Oleic acid on particle size is also negative. Model coefficients show that using Compritol and Oleic acid together at a proper level is important to adjust the particle size and released amount at 24th hour.

Formulation Optimization Using ANN by QbD Approach

Particle size is a very important parameter for effective targeting of nanoparticles into the skin layers. When nanoparticles are applied by dermal route, they penetrate into different layers of skin depending on their particle size and release the

Fig. 1 VIP graphs of SLN formulation for responses **A:** Particle Size **B:** Released amount at 24 hours

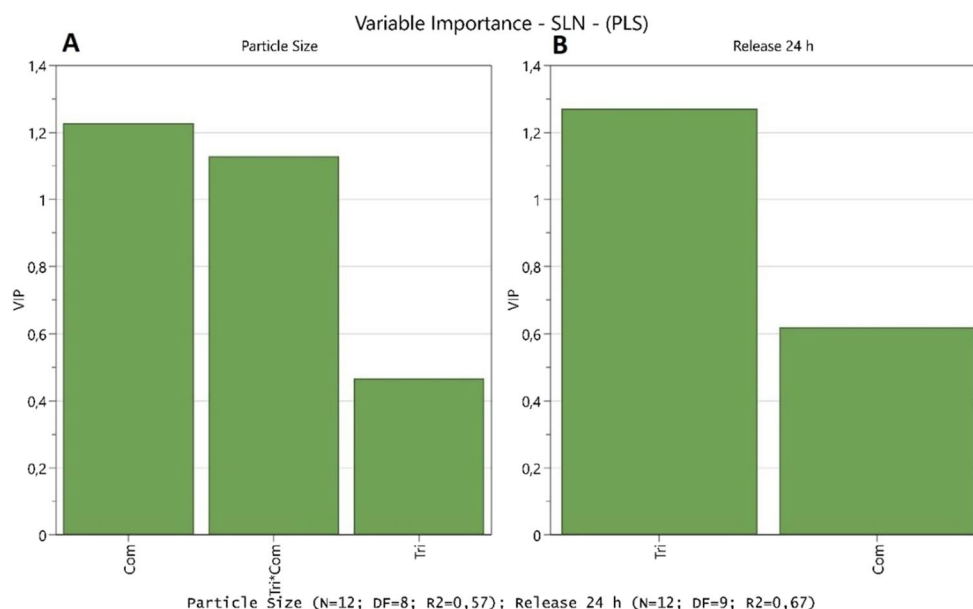


Fig. 2 Coefficient plots for SLN Formulation **A**: Particle Size **B**: Released amount at 24 hours

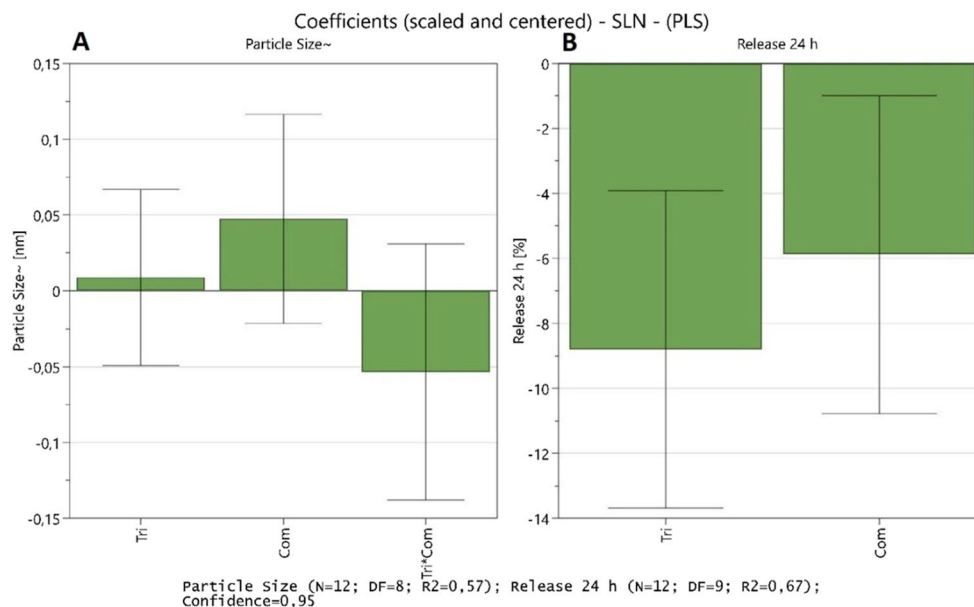
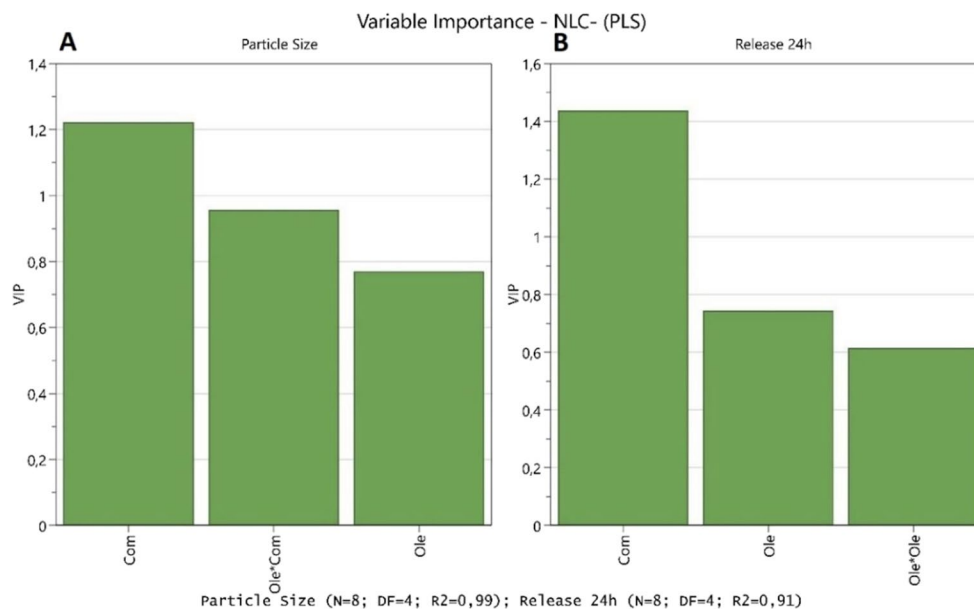


Fig. 3 VIP graphs of NLC formulation for responses **A**: Particle Size **B**: Released amount at 24 hours



active substance at the target skin layer. Therefore, particle size has been identified as the first CQA for the optimization of the formulation. It has been shown that nanoparticles with a particle size of around 300 nm can penetrate into deeper skin layers through hair follicles [58]. To provide an effective treatment for inflammatory skin diseases, the particle size of the nanocarrier should be to be around at 300 nm in order to deliver anti-inflammatory agents to the lower epidermis and dermis layers where inflammation reactions occur. However, there is always a risk of toxicity associated with nano-size [52]. In consequence, the particle size of the

optimum SS-SLN and SS-NLC formulations prepared using biodegradable lipids was aimed to be larger than 100 nm to minimize the nanotoxicological risk and to be smaller than 300 nm to ensure effective targeting to the lower epidermis and dermis layers. The ANN program was directed to offer the optimum formulation which has the smallest particle size within the specified limits.

The released amount of active substance from the nanoparticles at targeted skin layer is another important parameter for the effectiveness of treatment. For this reason, the released amount of curcumin at 24th hour was

Fig. 4 Coefficient plots for NLC Formulation **A**: Particle Size **B**: Released amount at 24 hours

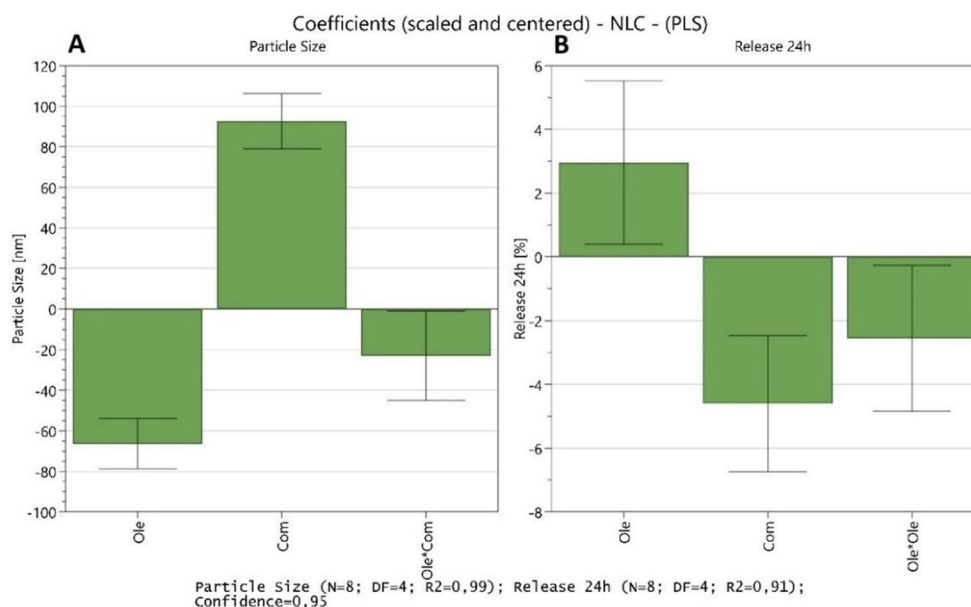


Table 4 r^2 values of the models and formulation parameters of the optimum semi-solid SLN and NLC formulations suggested by the ANN modeling INform V.3.17 program

Formulation	r^2 values of models		Formulation parameters			
	Particle size	Released Amount of Curcumin at 24th hour (%)	Tristearin (g)	Compritol 888 ATO (g)	Oleic acid (g)	Curcumin (g)
SS-SLN-Opt	0,98	0,88	0.123	0.825	-	0.05
SS-NLC-Opt	0,99	0,95	-	0.777	0.971	0.05

determined as the second CQA for optimization studies and the ANN program was directed to offer the optimum formulation that provides the highest drug release percentage.

As previously described, different solid : liquid lipid ratios and different total lipid concentrations were determined as formulation variables for SS-NLC formulations, while different ratios of solid lipids in binary mixtures and different total lipid concentrations were determined for SS-SLN formulations. The experimental data set of formulation variables and CQAs has been processed by INform V.3.17 program which examines the interactions between variables and non-linear relationships between inputs and outputs by artificial neural network (ANN) technology.

The r^2 value obtained at the end of the training phase, i.e. establishment of the relationship between the inputs and outputs, is an indicator for the reliability of the model generated by the program. If the r^2 value is greater than 0.70, the model is reliable. After the training phase of the program, the data optimization by “Genetic Algorithm” was completed. The r^2 values of the models and formulation parameters suggested by INform at the end

of the data optimization are given in Table 4. Optimum semi-solid lipid nanoparticle formulations were prepared using the lipids in the amounts recommended by the program and characterization studies were carried out.

Experiments on Optimum Semi-Solid SLN and NLC Formulations

In Vitro Characterization of Optimum Formulations

The particle sizes of SS-SLN-Opt and SS-NLC-Opt formulations were 204.7 ± 1.47 nm and 198.5 ± 0.81 nm, respectively ($p < 0.05$) (Table 5; Fig. 5). It can be concluded that the particle sizes of the optimum formulations suggested by the ANN program were in the targeted range of 100–300 nm and were smaller than the particle sizes of the SS-SLN and SS-NLC formulations entered to the program as data set. PDI values of both optimum formulations are below 0.5 which indicates a narrow particle size distribution. Since the zeta potential values of the optimum formulations were

Table 5 In vitro characterization results of optimum SS-SLN and SS-NLC formulations ($n=3$)

Formulation	Particle Size (nm) (Mean $\pm$ SD)	PDI (Mean $\pm$ SD)	Zeta Potential (mV) (Mean $\pm$ SD)	Encapsulation Efficiency (%) (Mean $\pm$ SD)	Released Amount of Curcumin at 24th hour (%) (Mean $\pm$ SD)
SS-SLN-Opt	204.7 $\pm$ 1.5	0.361 $\pm$ 0.023	-26.23 $\pm$ 0.23	100.71 $\pm$ 2.66	38.34 $\pm$ 3.48
SS-NLC-Opt	198.5 $\pm$ 0.8	0.231 $\pm$ 0.007	-37.83 $\pm$ 0.23	103.37 $\pm$ 0.34	33.72 $\pm$ 4.99

found to be greater than -20 mV, it was concluded that the optimum formulations were stable.

While the amount of curcumin released from the SS-SLN-Opt formulation at 24th Hour was $38.34\pm 3.48\%$, the released amount of the active substance from the SS-NLC-Opt formulation was $33.72\pm 4.99\%$ ($p<0.05$). It was observed that the release percentage of curcumin from SS-NLC-Opt was higher than the SS-NLC formulations entered into the INform program. On the other hand, while the curcumin release percentage at the 24th Hour from the SS-SLN formulations entered into program was between 11.13% and 44.31%, the release percentage for SS-SLN-Opt formulation was within this range and close to the maximum value (Table 5). It is safe to emphasize that the particle size and the curcumin release of SS-SLN-Opt and SS-NLC-Opt, the parameters set as CQA for optimization studies, were within the targeted range. In the light of these results, we conclude that the formulation optimization study was carried out successfully.

Kinetic evaluation of the in vitro release profiles obtained from the SS-SLN-Opt and SS-NLC-Opt formulations was performed, and the fit to first-order and Higuchi kinetics was assessed. The highest r^2 (coefficient of determination) and F (goodness-of-fit value), along with the low RMS (residual mean square) values, were

obtained with the Higuchi kinetic model for the SS-SLN-Opt formulation, whereas the first-order kinetic model provided the best fit for the SS-NLC-Opt formulation. In other words, while curcumin release from the SS-SLN-Opt formulation followed the Higuchi kinetic model, the release of the active substance from the SS-NLC-Opt formulation, due to the presence of liquid lipids in the structure, approached first-order release kinetics rather than matrix-based release kinetics. In vitro release profiles of optimum formulations are given in Supplementary Fig. 3. The results of the kinetic evaluation for the release profiles are presented in Supplementary Tables 1 and Supplementary Fig. 4 (Online Resource 1).

DSC Analysis on Optimum Formulations

As seen in Fig. 6, an endothermic melting peak at 178°C , which is compatible with the literature findings, was obtained in the DSC thermogram of curcumin. The melting peaks of Tristearin and Compritol were observed at 61.48°C and 75.32°C , respectively. The melting peaks of solid lipids were also compatible with literature reports [3, 13, 32]. Small shifts and intensity reductions in the melting peaks of pure lipids were observed in the DSC thermograms of blank and curcumin-loaded optimum

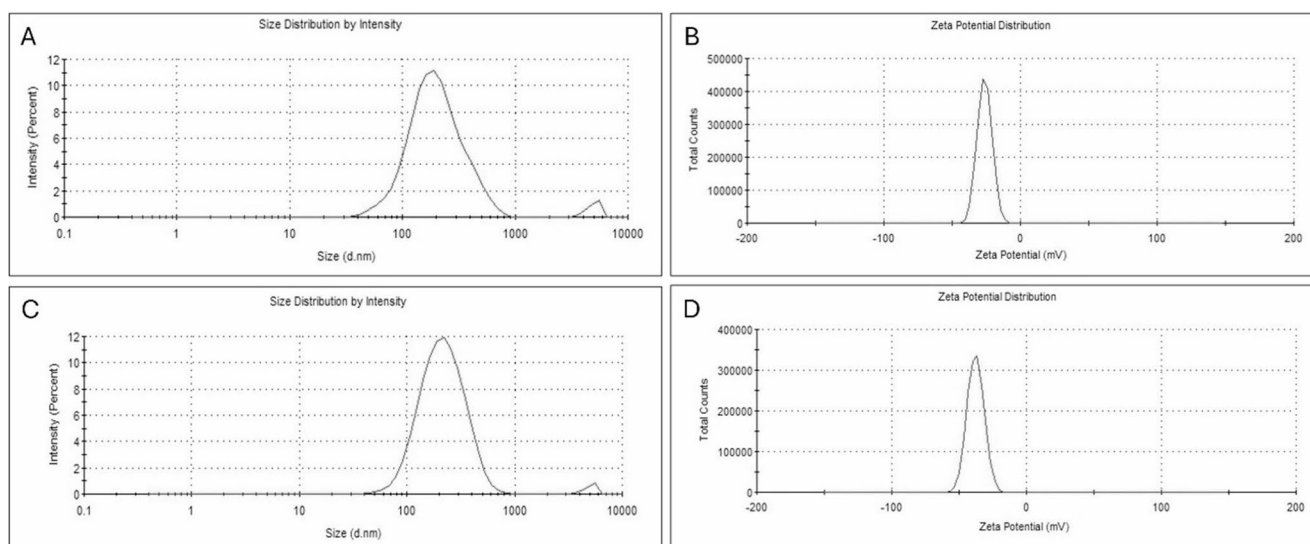


Fig. 5 Particle size (A: SS-SLN Opt and C: SS-NLC-Opt) and zeta potential (B: SS-SLN-Opt and D: SS-NLC-Opt) graphics of optimum formulations

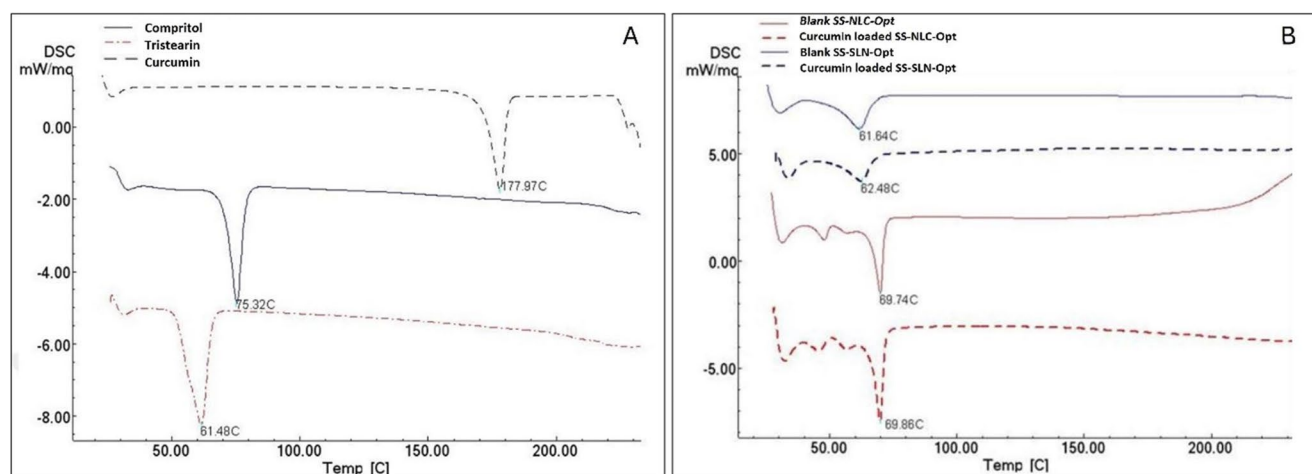


Fig. 6 DSC thermograms of pure materials (A) and formulations (B)

formulations. This phenomenon can be explained by the lipids in the nanoparticles having a less ordered arrangement compared to the bulk lipids. Additionally, it is possible that the oleic acid present in the NLC formulation's composition contributes to the small shifts in the melting point of the solid lipids [43, 99]. No melting peak of curcumin was observed in the DSC thermograms of the optimum formulations containing the active ingredient. These findings show that curcumin is in an amorphous state in the lipid matrix [9, 79].

TEM Analysis on Optimum Formulations

The TEM micrographs of the optimum formulations are presented in Fig. 7. The TEM image of the SS-SLN-Opt formulation reveals that the particles exhibit a spherical shape. In the case of the SS-NLC-Opt formulation, due to particle aggregation, it is difficult to make a definitive statement regarding the individual particle morphology. However, based on the limited number of distinguishable non-aggregated particles, it can be inferred that the particles possess a nearly spherical shape.

Determination of in Vitro Occlusion Factor for Optimum Formulations

One of the most important features of lipid nanoparticles is their occlusive effect due to the formation of a film layer on the skin. The occlusive effect reduces water loss from the skin, resulting in increased skin moisture and penetration of active ingredients through the skin. The increase in the occlusion factor (F) value that obtained by the in vitro occlusion test indicates a high occlusive effect. As seen in Table 6, F values of the SS-NLC-Opt formulation at all time points were found higher than the SS-SLN-Opt formulation, although the difference was not statistically significant ($p > 0.05$). On the other hand, the F value of the Carbopol®934 gel formulation of curcumin which was prepared for comparison was significantly lower than the optimum semi-solid lipid nanoparticle formulations ($p < 0.05$). Submicron-sized lipid particles exhibit adhesive properties upon contact with skin, forming a film that results in varying degrees of occlusion depending on the particle size. This monolayer film, being hydrophobic in nature, prevents moisture loss from the skin through its occlusive effect [88].

Fig. 7 TEM micrographs of SS-SLN-Opt (a) and SS-NLC-Opt (b) formulations

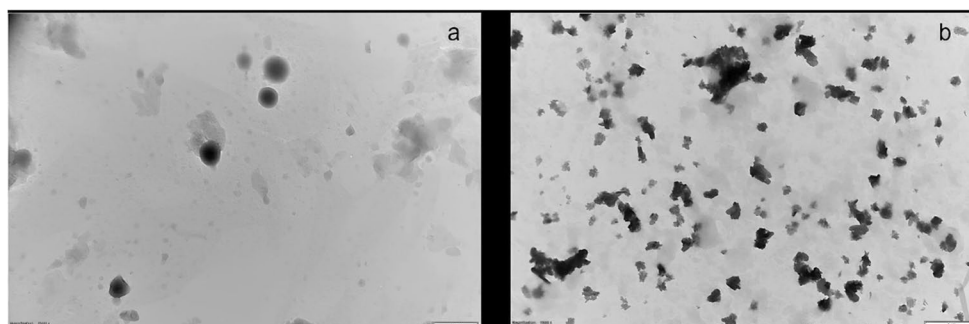


Table 6 F values of SS-SLN-Opt, SS-NLC-Opt and conventional gel formulations ($n=3$)

Formulation	F 12th hour (Mean $\pm$ SD)	F 24th hour (Mean $\pm$ SD)	F 48th hour (Mean $\pm$ SD)
SS-SLN-Opt	33.64 $\pm$ 2.01	57.68 $\pm$ 1.51	61.21 $\pm$ 1.57
SS-NLC-Opt	37.38 $\pm$ 5.79	62.71 $\pm$ 3.83	66.21 $\pm$ 4.08
Carbopol gel	3.43 $\pm$ 1.54	12.57 $\pm$ 0.99	14.53 $\pm$ 1.48

pH Analysis of Optimum Formulations

While the pH value of the SS-SLN-Opt formulation was 6.45 ± 0.18 , the one of the SS-NLC-Opt formulation was 6.06 ± 0.35 ($n=3$). The pH values of the formulations were found compatible with the pH of skin.

Rheological Characterization of Optimum Formulations

As a result of the measurements performed with a rotational viscometer (Brookfield DV-II, USA) at a shear rate of 10 rpm, while the viscosity of the SS-SLN-Opt formulation was 24.33 ± 5.69 Pa.s, the viscosity of the SS-NLC-Opt formulation was 97.33 ± 10.21 Pa.s ($n=3$).

The rheological properties of a semi-solid dosage form are of great importance as it is a parameter that affects all steps of the production process and determines the quality of the final product. On the other hand, the release of the active substance from the semi-solid dosage form also depends on the rheological properties of the product. Viscoelastic measurements are based on the measurement of the mechanical properties of materials that exhibit the elastic behavior of solids and the viscous behavior of liquids. Oscillation tests are dynamic methods used to determine the viscoelastic properties of the material without disturbing its static structure. The oscillation frequency sweep test measures the response of the system as a function of frequency at constant stress amplitude and gives storage module (G'),

loss module (G'') and complex viscosity (η^*) values. While the storage module (G') defines the elastic character of the product, the loss module (G'') is a measure of the viscous response. The ratio G'/G'' defines the tendency of the product to be more elastic, i.e. its ability to recover from deformation. It is reported that the dominant elastic property contributes to the stabilization of the product [59, 78].

The graphics obtained as a result of the oscillation frequency sweep test performed on optimum semi-solid SLN and NLC dispersions are given in Fig. 8. As shown in this figure, the storage module (G'), i.e. the elastic component, was significantly higher than the loss module (G''), i.e. the viscous component, throughout the whole frequency range for both formulations. These results indicate that the optimum semi-solid lipid nanoparticle formulations have a gel-like structure and show more elastic properties than viscous properties in the frequency range. On the other hand, as the frequency increased, an indistinct increase in the storage module (G') and a non-significant decrease in the loss module (G'') value were observed. In other words, both parameters show weak dependence on the applied frequency. Additionally, complex viscosity (η^*) values decrease as the frequency increases. These findings are specific for viscoelastic structures [59, 61].

Determination of the Mechanical Properties of Optimum Formulations

TPA analysis was carried out to determine the mechanical properties such as hardness, compressibility, adhesiveness, cohesiveness and elasticity of the optimum semi-solid lipid nanoparticle formulations. The results of TPA analysis were given in Table 7.

Hardness is defined as the strength that must be applied to obtain a certain deformation. The maximum strength (F_{max}) value obtained in the first compression period of

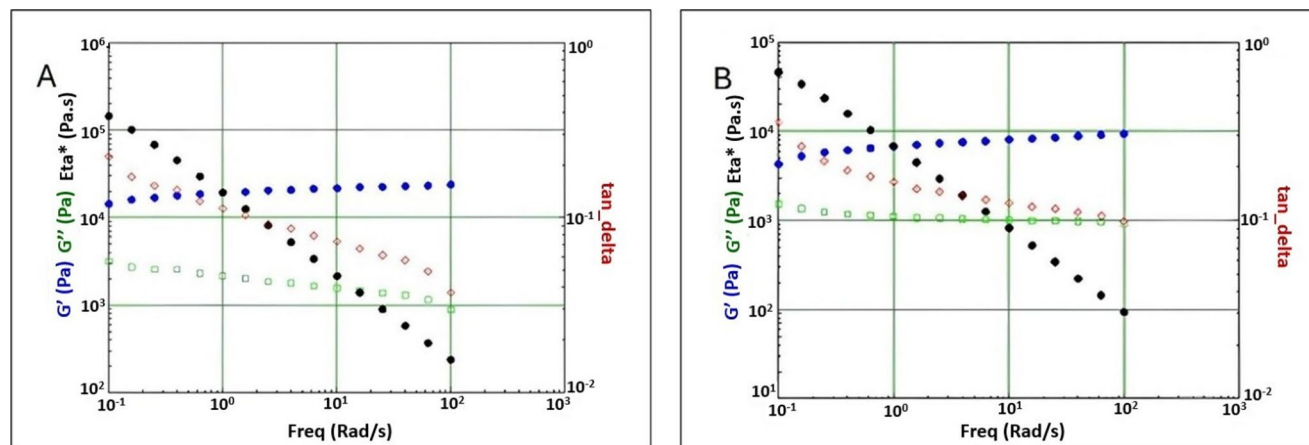
**Fig. 8** Oscillation frequency sweep test graphic of SS-SLN-Opt (A) and SS-NLC-Opt (B) formulation

Table 7 Mechanical properties of optimum semi-solid SLN and NLC formulations ($n=5$)

	SS-SLN-Opt	SS-NLC-Opt
Hardness (N) (mean $\pm$ SD)	0.043 $\pm$ 0.003	0.373 $\pm$ 0.042
Compressibility (N.mm) (mean $\pm$ SD)	0.117 $\pm$ 0.016	0.951 $\pm$ 0.362
Adhesiveness (N.mm) (mean $\pm$ SD)	0.061 $\pm$ 0.014	0.462 $\pm$ 0.080
Cohesiveness (mean $\pm$ SD)	0.768 $\pm$ 0.050	0.824 $\pm$ 0.093
Elasticity (mean $\pm$ SD)	0.978 $\pm$ 0.011	1.014 $\pm$ 0.038

TPA analysis gives the hardness value of the semi-solid product. A small hardness value, which indicates the applicability of the semi-solid preparation to the target area, is preferred for ease of application. TPA analysis showed that while the hardness value of the SS-SLN-Opt formulation was 0.043 N, the hardness value of the SS-NLC-Opt formulation was 0.373 N. The hardness value of the SS-SLN-Opt formulation was significantly smaller ($p<0.05$) compared to the optimum semi-solid NLC formulation. On the other hand, the hardness values obtained for both optimum formulations were suitable for easy application of the product [12, 94].

Compressibility is defined as the work required for deforming the product. The area under the curve (AUC1) value obtained in the first compression period of TPA analysis gives the compressibility value of the product. The compressibility value is required to be low so that the semi-solid product can be easily taken from the package and spread onto the skin. In our study, the compressibility value obtained for the SS-SLN-Opt formulation was significantly smaller than the value obtained for the SS-NLC-Opt formulation ($p<0.05$). Compressibility and hardness values are related to the viscosity value of the semi-solid product [98]. As mentioned before, the viscosity of the SS-NLC-Opt formulation was higher than the viscosity of the SS-SLN-Opt formulation. The hardness and compressibility values of the SS-NLC-Opt formulation, which has higher in viscosity, were also higher. On the other hand, the compressibility values of both optimum formulations were suitable for easy removal of the semi-solid product from the package and provide ease of application to the skin [12, 23].

Adhesiveness is defined as the work required to overcome the force of attraction between the surfaces of the probe and the semi-solid sample in TPA analysis. The AUC2 value obtained in the first withdrawal phase of the probe gives the adhesiveness value. Adhesiveness value expresses the ability of the semi-solid product to adhere to the application area. The adhesiveness of the optimum semi-solid NLC formulation was significantly higher than that of the SLN formulation ($p<0.05$). These results were compatible with the in vitro occlusion test findings. As explained previously, the F values obtained for the SS-NLC-Opt formulation at all-time points were greater than the F values of

the SS-SLN-Opt formulation. In other words, the F value of the SS-NLC-Opt formulation, which was determined to have higher adhesive properties, is also higher. As stated before, dermal application of lipid nanoparticle dispersions increases the occlusive effect due to the adhesive film layer formation on the skin.

The cohesiveness value, which is calculated by dividing the area under the curve (AUC3) obtained in the second compression period to the area under the curve (AUC1) obtained in the first compression period, is important to determine the restructuring ability of the product after application. The high cohesiveness value increases the performance of the product in the application area by providing complete structural recovery following the application of the semi-solid. In other words, the product can maintain its structure for a long time after application. The cohesiveness values of SS-SLN-Opt and SS-NLC-Opt formulations were 0.768 and 0.824, respectively, and there was no difference between the cohesiveness values of the formulations ($p>0.05$). In light of the literature findings obtained in similar studies, it was concluded that both optimum formulations are suitable for topical application in terms of their cohesive properties [51, 70, 98].

Elasticity is defined as the ratio of the time required for the maximum structural deformation obtained in the second compression period to the time required for the maximum structural deformation obtained in the first compression period. The numerically small elasticity values indicates that the elasticity of the product is high since elasticity refers to the return of the semi-solid product to its previous form after deformation. The elasticity values of SS-SLN-Opt and SS-NLC-Opt formulations were 0.978 and 1.014, respectively, and they were similar ($p>0.05$). The elasticity values of the optimum formulations around one were interpreted as high elasticity properties suitable for topical application [16, 94].

When the results of the TPA analysis and the organoleptic evaluation of the optimum formulations were interpreted together, we concluded that the new optimum semi-solid lipid nanoparticle formulations had appropriate mechanical properties in terms of product performance and patient compliance.

Stability Studies on Optimum Formulations

The results of the stability studies on the optimum formulations are given in Table 8. For SS-SLN-Opt formulation, no difference was found between the particle sizes, PDI values and encapsulation efficiencies measured at the beginning and at the end of the first month at 4 °C ($p>0.05$). When the SS-SLN-Opt formulation was stored at 25 °C for one month, the particle size increased from 204.7 nm to

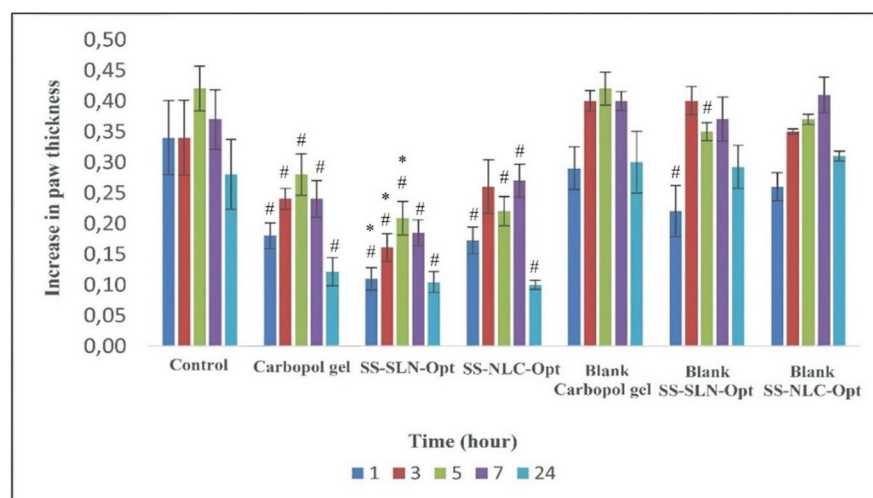
Table 8 Stability of optimum semi-solid SLN and semi-solid NLC formulations after 1 month ($n=3$)

	4 °C		25 °C	
	T=0	1 st month	T=0	1 st month
Optimum semi-solid SLN				
Particle size (nm) (mean±SD)	204.7±1.5	206.5±1.7	204.7±1.5	292.9±2.3
PDI (mean±SD)	0.361±0.023	0.344±0.040	0.361±0.023	0.422±0.065
Zeta potential (mV) (mean±SD)	-26.23±0.23	-24.10±0.12	-26.23±0.23	-23.10±0.26
Encapsulation efficiency (%) (mean±SD)	100.71±2.66	99.75±0.44	100.71±2.66	98.66±0.99
Drug loading (%) (mean±SD)	0.302±0.008	0.299±0.001	0.302±0.008	0.296±0.003
Optimum semi-solid NLC				
Particle size (nm) (mean±SD)	198.5±0.8	231.8±0.2	198.5±0.8	295.1±6.7
PDI (mean±SD)	0.231±0.007	0.243±0.090	0.231±0.007	0.373±0.024
Zeta potential (mV) (mean±SD)	-37.83±0.23	-32.00±0.26	-37.83±0.23	-30.30±0.10
Encapsulation efficiency (%) (mean±SD)	103.37±0.34	103.70±0.40	103.37±0.34	102.90±0.21
Drug loading (%) (mean±SD)	0.310±0.001	0.311±0.001	0.310±0.001	0.308±0.001

292.9 nm ($p<0.05$). On the other hand, there was no change in the PDI value of the sample stored at 25 °C ($p>0.05$). For the SS-NLC-Opt formulation stored at 4 °C for one month, there was no statistically significant difference in PDI and encapsulation efficiency values ($p>0.05$). However, the particle size of the sample stored at 4 °C increased from 198.5 nm to 231.8 nm at the end of the first month, resulting in a significant increase ($p<0.05$). On the other hand, when the SS-NLC-Opt formulation was stored at 25 °C for one month, the particle size and PDI values increased from 198.5 nm to 295.1 nm and from 0.231 to 0.373, respectively ($p<0.05$). It was also observed that the first month zeta potential values of both of the optimum semi-solid SLN and NLC formulations were greater than -20 mV. When all

these findings of stability studies were evaluated together, the stability of the SS-SLN-Opt formulation at 4 °C was higher than the SS-NLC-Opt formulation.

The significant increase in particle size observed in semi-solid lipid nanoparticle dispersions stored at 25 °C can be attributed to the reduction in microviscosity, a property of the emulsifier, due to the effect of temperature. Microviscosity, which can be described as the high film rigidity of the emulsifier, prevents aggregation after particle contact. The increase in temperature leads to a decrease in microviscosity, resulting in particle aggregation. Furthermore, the increase in temperature enhances the kinetic energy of the lipid nanoparticles, in combination with a reduced zeta potential, and leads to the

Fig. 9 Results of the in vivo anti-inflammatory activity experiments

: Represents the difference from the Control group at a 95% confidence interval.

* : Represents the difference of optimum formulations from the Carbopol gel group at a 95% confidence interval.

overcoming of electrostatic repulsion and the formation of aggregates [31, 83].

In Vivo Evaluation of the Anti-Inflammatory Activity of Optimum Formulations

In vivo anti-inflammatory activity of curcumin loaded optimum formulations were investigated by carrageenan-induced paw edema test based on the assessment of anti-edema effect. Carrageenan-induced paw edema test which has a wide application area is an appropriate method in order to evaluate the efficiency of anti-inflammatory drugs [28, 33, 53, 75, 86].

The experiments on rats showed that the optimum SS-SLN formulation with curcumin had higher anti-inflammatory activity in comparison with the conventional Carbopol® 934 gel formulation. Figure 9 clearly shows that the optimum SS-SLN formulation effectively reduced the inflammation starting from the first hour. The enhanced anti-inflammatory effect observed with SS-SLN-Opt has been attributed to the increased curcumin penetration, which was promoted by the occlusive effect [19]. The reduction in paw edema of rats achieved with the SS-SLN-Opt formulation was greater than the reduction with the SS-NLC-Opt formulation at all-time points, except for the 5th and 24th Hours. The difference between the anti-edema effect of both formulations at the 5th and 24th hours was similar ($p > 0.05$).

Conclusion

In this study, the novel semi-solid SLN and NLC formulations of curcumin, the compound with a significant anti-inflammatory potential, were prepared by one-step production method. QbD-based formulation optimization was successfully carried out using the artificial neural network (ANN) and optimum SS-SLN and SS-NLC formulations were prepared using the formulation parameters suggested by the program. The CQA values of the optimum formulations were found within the targeted range. In light of the findings of in vitro characterization studies, it was concluded that the optimum semi-solid SLN and NLC formulations have suitable properties in terms of product performance and patient compliance. According to the stability test results, the optimum SS-SLN formulation was more stable than the optimum SS-NLC formulation. In vivo studies conducted on rats revealed that the optimum SS-SLN formulation had a higher potency and anti-inflammatory activity compared with the optimum SS-NLC and conventional gel formulations. Thus, it is concluded that the SS-SLN-Opt formulation, with its higher stability, higher curcumin release in 24 hours and better anti-inflammatory activity,

can be accepted as a suitable formulation for the dermal application of curcumin. The developed optimum SS-SLN formulation has been considered to possess potential worthy of further in vivo evaluations.

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Author Contributions U.B. performed the experiments, administrated the project and wrote the main manuscript text A.S. performed the experiments and wrote the main manuscript text G.Y. performed the formulation optimization studies and wrote the manuscript text B.G.C. performed in vivo experiments B.A. performed the formulation optimization studies and reviewed & edited the manuscript text A.O.B. performed the in vivo experiments and reviewed & edited the manuscript text.

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Data Availability Data is provided within the manuscript or [Supplementary information](#) files.

Declarations

Ethical Approval All in vivo experiments on animals were approved by the Local Animal Ethics Committee of Ankara University (Approval no: 2018-19-126).

Competing Interests The authors declare no competing interests.

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