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ORIGINAL ARTICLE

Topical Delivery of Curcumin and Caffeine Mixture-Loaded Nanostructured Lipid Carriers for Effective Treatment of Psoriasis

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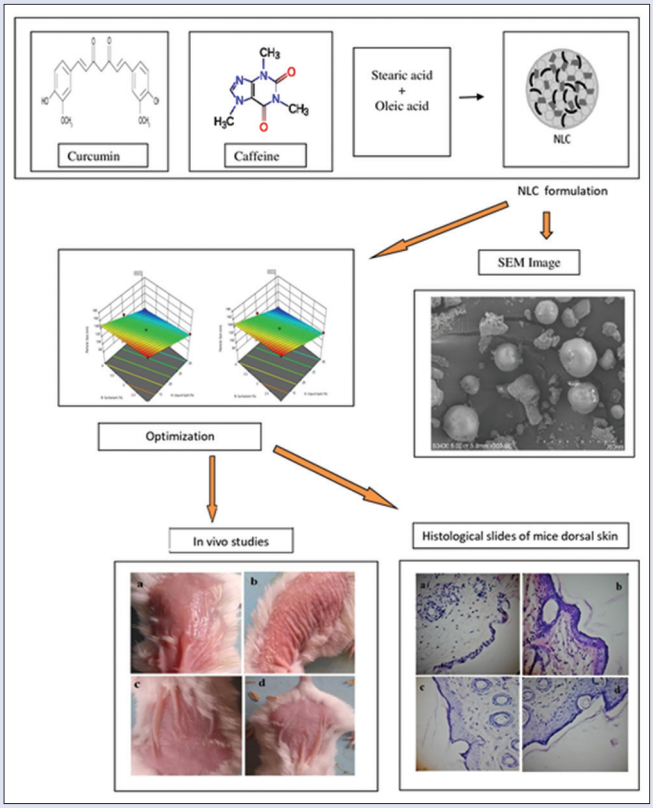
ABSTRACT

Background: Curcumin (CUR) is a well-known herbal constituent used in treating psoriasis. However, combination of CUR with other anti-inflammatory drugs such as caffeine shows amplified antipsoriatic action compared to CUR alone. **Objective:** The objective of the present study was to develop nanostructured lipid carriers (NLCs)-based topical gels of CUR and caffeine combination for facilitated treatment of psoriasis. **Materials and Methods:** Preparation of NLC's loaded with CUR and caffeine was done by hot homogenization and ultrasonication methods and incorporated in topical gels. Factorial design (3^2) was constructed in a fully randomized manner to formulate and evaluate all nine possible experimental runs. Detailed evaluation studies for NLC and NLC-based gels were conducted. *In vivo* animal studies were carried out for optimized formulation using mouse model of imiquimod-induced psoriasis. **Results:** The physical and chemical characteristics displayed by the prepared NLC's (F1–F9) and gels were found to be optimal. The optimization using experimental design approach resulted in achieving formulation F10 with 103.01 nm particle size and 61.52% entrapment efficiency. *In vivo* and histopathology studies revealed that prepared NLC-based gel exhibits promising antipsoriatic activity. **Conclusion:** The present study signified that the CUR and caffeine combination has better antipsoriatic activity. Moreover, NLC-loaded gels have provided sustained drug release till the end of 12 h.

Key words: Caffeine, curcumin, imiquimod, nanostructured lipid carriers, psoriasis, topical gel

SUMMARY

- Nanostructured Lipid Carriers (NLC's) containing Curcumin (CUR) and Caffeine (CFN) mixture developed as potential systems in treating Psoriasis. By altering their ratios, were developed. A systemic study using 3^2 factorial design was carried out which gave various NLC formulations. Evaluation studies were carried out for these NLC's. Optimized NLC was incorporated into gel system. Further studies like *ex vivo*, *in vivo* and stability studies were carried out for this optimized formulation.



Abbreviations used: CUR:Curcumin; CFN: Caffeine; NLC: Nanostructured Lipid Carriers; CD: Cyclodextrin;IMQ: Imiquimod

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INTRODUCTION

Psoriasis is a skin disease which is distinguished by massive proliferation, thick inflammatory cell infiltrates, generation of new blood vessels, modifications in lymphatic structure, and impaired differentiation of the epidermis. It is an autoimmune disorder, in which environmental and genetic components play a major function. The immune system

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releases pro-inflammatory cytokines and growth factors that accelerate the growth of skin cells, which accumulate and form thick red patches on skin of diverse parts of the body.[1]

General therapies present for treating psoriasis are systemic agents (methotrexate, cyclosporine, acitretin, biologics like adalimumab, etanercept), topical agents (corticosteroids, Vitamin D-3 derivatives, retinoids, coal tar or anthralin), and phototherapy. Despite the fact that many therapies are there in treating psoriasis, no single treatment gives complete and satisfactory cure and most of them have adverse effects. As an alternative for these drugs, phytoconstituents (curcumin [CUR], capsaicin, silymarin, beta-amyrin, etc.) have been widely used. These have better therapeutic value and have less side effects. The present work has been carried out using CUR which is one of the major phytoconstituents having antipsoriatic activity.[2,3]

CUR (Center for Responsible Nanotechnology [CRN]) is a natural polyphenolic phytochemical, extracted from rhizome of turmeric (*Curcuma longa*) having many biological and pharmacological activities such as antioxidant, antitumor, anti-inflammatory, antipsoriatic, anticarcinogenic, and free radical scavenger.[4] It is poorly water-soluble, highly photoreactive, has rapid metabolism and poor absorption that leads to poor bioavailability. It has shown some significant effect on psoriasis with properties related on numerous receptors to which CUR binds. These include 5-lipoxygenase, xanthine oxidase, thioredoxin reductase, COX-2, p-glycoprotein, glutathione S-transferase, protein kinase A (PKA), PKC, cPK, PK, Ca^{2+}-dependent protein kinase, and glutathione. Furthermore, CUR-induced suppression of phosphorylase kinase activity which correlates with the resolution of human psoriasis.[5]

Aggarwal *et al.*[6] conducted studies on clinical trials with CUR. They stated that CUR given topically to psoriasis patients has taken time of around 4 weeks to suppress psoriatic symptoms, and it was around 12 weeks when given orally.

To reduce the time taken for CUR to show its activity in treating psoriasis, an attempt has been made by combining CUR with anti-inflammatory drugs like caffeine.

Caffeine (CFN) is a methylxanthine moiety capable to hinder the phosphodiesterase (PDE) enzyme which helps in hydrolysis of cyclic nucleotides resulting in elevated concentrations of intracellular cyclic adenosine monophosphate (cAMP). Cell surface receptors inhibition for adenosine is another proposed mechanism.[7] Reduced intracellular cAMP levels are seen in cutaneous leukocytes of patients with psoriasis. Many researchers proposed that as a PDE inhibitor and methylxanthine, caffeine increases intracellular cAMP levels; which consequently suppress inflammatory pathways and psoriasis progression.[8]

To improve the solubility and stability of CUR, various studies in the form of nanoparticles including lipid-based nanospheres, nanocrystals, liposomes, and polymer-based delivery systems were reported.[9-11]

Among the available nanoparticle-based dosage forms, nanostructured lipid carriers (NLCs) conquered the lead. They are colloidal carrier systems of lipids, consisting of a mixture of solid and liquid lipids and have particle diameter range in nanometers. They have taken the lead among other nanoparticulate systems due to their apparent gains of advanced level of versatility and biocompatibility. They are considered as ideal delivery systems for pharmaceutical applications because of their exclusive properties such as constructive particle shape, solid nature, size in nanoscale range, and large definite surface. Limitations pertaining to solid lipid nanoparticles (SLN) such as elevated water content of SLN dispersions, low drug load, and drug expulsion during storage can be conquered by NLCs system and thus considered as SLN's second generation with a particular nanostructure. NLCs penetrate into mucosa or skin because of their solid lipid matrix and nanosize. Thus, prolonged drug release can be obtained. This helps in reduction of irritation caused by drug and systemic toxicity.[12]

Our exhaustive search has revealed that no studies were reported in combination of CUR and caffeine as NLC for the treatment of psoriasis till date. In the present study, an attempt has been made to develop NLC of CUR and caffeine combination as potential systems in treating psoriasis and in order to decrease time period taken for CUR alone to show its antipsoriatic activity. These NLC's were delivered in the form of topical gels which provided prolong release of drug mixture for effective treatment of psoriasis.

MATERIALS AND METHODS

CRN was obtained from Chaitanya Agro Herbals, Mysore, India, as a gift sample. CFN, Carbopol-934, stearic acid, and oleic acid were purchased from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade. Ultrapurified water was used for all experiments.

Methodology

Preformulation studies

Ultraviolet visible spectroscopy

The standard solutions of CUR and CFN (10 μg/ml) were separately scanned in 200–800 nm range using methanol as blank. Maximum absorbance wavelengths were determined. From this spectrum, an isosbestic point was determined which is the wavelength at which spectra of two drugs cross each other (292 nm). Dilutions were prepared and their absorbances were recorded at obtained isosbestic point wavelength and calibration curve was plotted. Analytical method validation was carried out.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectrum of CUR, CFN, drug mixture, physical mixture of total lipids, and drugs were recorded over the range of 4000–400 cm^{-1} by KBr pellet method using FTIR spectrophotometer (Shimadzu 8400 S, Tokyo, Japan).

Differential scanning calorimetry analysis

Thermograms of CUR, CFN, drug mix, physical mixture of total lipids, and drugs were recorded using Shimadzu Differential scanning calorimetry (DSC)-60 DSC where aluminum pans were used, in which samples were sealed in pans hermetically and heated at 20°C/min rate, over a range of 40°C–300°C temperature. By purging nitrogen flow at 40 mL/min rate, atmosphere which is inert was provided.

Experimental design for formulation of nanostructured lipid carriers

In the present work, a two-factor and three-level full factorial design (3^2) was used to obtain statistically significant and optimized formulation ingredients. Design-Expert software, version 11.0, purchased from Stat-Ease Inc., Minneapolis, MN, USA, was used to generate design. The two independent variables, namely, concentration of liquid lipid (A) and concentration of surfactant (B) were optimized using design of experiment (DOE) at three different levels: low (−1), medium (0), and high (+1). Particle size (nm) (R1) and entrapment efficiency (EE) (R2) were selected as response variables. Various parameters of prepared formulations were evaluated and characterized. In Table 1, the coded values of different variables have been summarized.

Formulation of nanostructured lipid carrier

Preparation of NLCs loaded with CUR and caffeine was carried out by hot homogenization and ultrasonication methods. Stearic acid and oleic acid were taken as solid lipid and liquid lipid, soya lecithin as surfactant, and polyvinyl alcohol as cosurfactant. By using a homogenizer (Heidolph,

Germany), lipid and surfactant mixtures were melted and dispersed homogeneously at 16,000–20,000 rpm, temperature was maintained at 75°C. Later, to the lipid phase, CUR and caffeine dissolved in polyvinyl alcohol (aqueous phase) were added dropwise. To form a nanoemulsion, sonication was carried out for obtained mixture for 5 min (SONOPULSHD3100, Bandelin, Germany, with amplitude 75% and titanium probe). To obtain a nanoparticle suspension this mixture was cooled down and was stored at 4°C in light-protected sealed containers.[10,11] Total nine formulations were prepared.

Characterization and evaluation of nanostructured lipid carrier

For prepared NLC's studies such as zeta potential, Polydispersity Index (PDI), particle size, EE, and % *in vitro* drug release were carried out.

Particle size and zeta potential determination

Determination of mean particle size, PDI, and zeta potential was done by dynamic light scattering using particle size analyzer (Zetasizer 3000, Malvern Instruments Worcestershire, UK) at a scattering angle of 90°. Dilution of samples to 1:10,000 ratio was done. All measurements were measured under ambient conditions in triplicate.[10]

Entrapment efficiency

Determining the drug amount embedded in NLC is of major significance because it determines the release characteristics, and consequently, the therapeutic potency. To calculate the EE, accurately weighed amount of NLC (150 mg) was dissolved in methanol, sonicated for 15 min to break the complex, and centrifuged. The obtained supernatant was then filtered, diluted suitably using 7.4 pH phosphate buffer solution (PBS), and analyzed by UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 292 nm (isosbestic point of CRN and CFN). All measurements were performed in triplicates under ambient conditions.[9] EE was calculated by using the below-mentioned formula:

$$Entrapment\ efficiency = C_a/C_{th} \times 100 \quad (1)$$

Where C_a: Actual drug content in NLC; C_{th}: Theoretical drug content

In vitro drug release study

At room temperature and pH = 7.4, studies of drug mixture release profile from the NLCs were carried out. Drug loaded NLC's equivalent to drug dose were loaded into a dialysis bag (cutoff 10 kDa). This was placed in a beaker containing 20 ml of PBS and was stirred at 200 rpm. Two milliliters of samples were withdrawn at predetermined time intervals from the incubation medium and analyzed for the drug content by UV spectrophotometry at 292 nm.[12]

Checkpoint analysis and optimization of design

Final formulation was prepared from optimum values of factors, overlay plot, and desirability of DOE. Preparation of optimized formulation (F10) was done for checkpoint analysis and evaluated for particle size (R1) and EE (R2). The optimized formulation has shown response variable of R1 = 103.01 nm and R2 = 61.52%. In the checkpoint analysis, values of observed and predicted responses were analyzed and compared. The error was calculated as given in Table 2. Desirability value of 0.976 with low relative errors was recorded.

Characterization of optimized nanostructured lipid carrier (F10) formulation

Scanning electron microscopy

Using scanning electron microscope (SEM) (Hitachi Ltd., S-3400N type II model, Tokyo, Japan), optimized NLC's surface morphology was determined. Using double-sided adhesive tape, samples were mounted on carbon mount, and at 15 KV accelerating voltage, they were scanned.[10]

Fourier transform infrared analysis of nanostructured lipid carrier (F10)

FTIR spectroscopy was carried out for F10 formulation to confirm the drug mixture entrapment in NLC.

Differential scanning calorimetry analysis of nanostructured lipid carrier (F10)

The DSC thermogram of optimized NLC formulation (F10) was recorded.

Particle size

Particle size was determined for optimized F10 formulation. This was considered as obtained particle size.

Entrapment efficiency

EE was found for F10 optimized formulation. This is taken as obtained EE and is compared with that of predicted value.

Formulation of nanostructured lipid carrier-based nanogels

The obtained F10 formulation was delivered in the form of a gel. Various gel formulations were prepared by altering carbopol 934%. Carbopol 934 (0.5%, 1%, 1.5%) was soaked in water (around 5 ml) for 2 h. By stirring, it was then neutralized with triethanolamine. Optimized NLC formulation (F10) was dissolved in preweighed and appropriate amount of propylene glycol. This was transferred to carbopol container and mixed further for 20 min. The dispersion was

Table 1: 3^2 full factorial design layout and responses noted for nanostructured lipid carriers-based gel formulations

Formulation run	X1# (%)	X2# (%)	R1# (nm)*	R2# (%)*
F1	−1	+1	148±0.52	50.15±1.59
F2	0	−1	143±0.81	53.82±2.09
F3	−1	+1	157±0.15	47.56±1.57
F4	0	+1	130±0.37	54.42±1.59
F5	0	0	135±0.31	52.58±1.63
F6	−1	−1	169±0.16	44.14±1.72
F7	+1	+1	98±0.24	63.92±1.06
F8	+1	−1	121±0.53	55.36±1.05
F9	+1	0	107±0.32	59.84±1.64
Factors and their coded levels	**Low (−1)**	**Medium (0)**	**High (+1)**	
X1	10	20	30	
X2	2	3	4	

#X1: Concentration of liquid lipid; X2: Concentration of surfactant; R1: Particle size; R2: EE; *Mean±SD, *n*=3. EE: Entrapment efficiency; SD: Standard deviation

Table 2: Checkpoint analysis of optimized nanostructured lipid carriers formulation (F10)

Formulation F10	R1 (nm)	R2 (%)	Desirability
Predicted	103.01	61.52	0.976
Observed	103.89	62.31	
Relative error	0.88	0.79	

Table 3: Formulation chart of gel preparations

Ingredients	Formulation		
	N1	N2	N3
Carbopol-934 (%)	0.5	1.0	1.5
Propylene glycol (%)	0.5	0.5	0.5
Propyl paraben (%)	0.02	0.02	0.02
TEA (ml)	0.1	0.1	0.1

TEA: Triethanolamine

kept aside for 60 min for hydrating and swelling. Before performing viscosity studies, all the samples were allowed to equilibrate for at least 24 h at room temperature.[13] Table 3 represents prepared gel formulations.

Evaluation of nanostructured lipid carrier-based nano gels

Evaluation studies such as homogeneity, pH, viscosity, spreadability, *in vitro* drug release, *ex vivo* permeation, and *in vivo* studies were carried out for prepared carbapol gels. The results have been represented in Table 4.

Determination of pH

Digital pH meter was used to determine the pH of the gels. Glass electrode was completely placed into the gel system.[14,15]

Homogeneity

After placing the gels in the container, all formulations were tested for homogeneity (appearance and presence of any aggregates) by inspecting visually.

Spreadability studies

Spreadability signifies the area of affected part or skin on which formulation spreads easily when applied. This determines the therapeutic efficacy of formulation. Spreadability studies were carried out by placing calculated amount of gel between two slides and applying specific load on them, which leads to spreading of gel. It is expressed in terms of time (in seconds). Superior spreadability is indicated by minimum time required for separation of slides. Below formula was used to calculate spreadability.

$$S = ML/T \quad (2)$$

Where S = spreadability, M = weight (in g) tide to upper slide, L = length (in cm) of glass slides, and T = time (in sec) taken to separate the two slides completely from each other.

Viscosity studies

All measurements were carried out by viscometer (Brookfield Engineering Laboratories, Inc., MA, USA) with spindle No. 6 at 10 rpm at temperature of 37°C ± 0.5°C. The rheological properties of the formulated NLC-based gels were studied at different rpm and the viscosity was calculated in cP.

In vitro drug diffusion studies

NLC-based gels were permeated through an artificial cellophane membrane (dialysis membrane) with molecular weight cutoff: 12,000 Dalton and 200 µm in thickness was used as a artificial membrane for preliminary *in vitro* studies because of simplicity, homogeneity, and uniformity. In the donor compartment of the Franz diffusion apparatus (Perme Gear Inc., Bethlehem, PA, USA), 0.5 g of NLC-based gel was placed. The receptor medium consists of pH 7.4 PBS. To simulate the human skin condition during the experiment, temperature was maintained at 37°C ± 0.5°C. Five milliliters of sample aliquots were withdrawn at 0.5, 1, 2, 4, 8, and 12 h and replaced with equal volume of fresh receptor media. Collected samples were spectrophotometrically analyzed at 292 nm and the amount of drug released from gel was calculated.

From the above results, formulation with high *in vitro* drug diffusion was chosen for *in vivo* studies, *ex vivo* permeation studies, estimation of drug retained in the skin layers, and stability studies.

Table 4: Linearity table of drug mix (curcumin and caffeine)

Concentration	Absorbances
0	0
5	0.135
10	0.276
15	0.412
20	0.586
25	0.708

In vivo studies

Mice grouping and treatment

8–11-week-old healthy BALB/c mice of either sex were purchased from Adita Biosys Pvt. Ltd., Tumkuru, India. Experimental study procedure and handling of the experimental animals were approved by Institutional Animal Ethics Committee, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysore (approval No. P12-282/2018), and was in accordance with the guidelines set out by the Committee for the Purpose of Control and supervision on experiments on animals, Animal Welfare Division, Ministry of Environment and Forests, Government of India, New Delhi, India.

Obtained mice were allowed to acclimate for 7 days prior to the start of the experiments. They were divided into four groups: Group 1 (G1) is the control group which is treated with vaseline. Group 2 (G2) is negative control group, Group 3 (G3) is test group, and Group 4 (G4) is marketed group. A topical dose of 62.5 mg Imiquad® (IMQ marketed cream; Glenmark Pharmaceuticals Ltd., India) was applied daily to the shaved dorsal skin/region of mice of G2, G3, and G4 groups for 8 consecutive days for development of psoriasis-like skin features. No treatment was given to mice in G2, mice in G3 were treated with NLC, and those in G4 were treated with marketed CRN product.

Visual inspection

In specified groups, after applying IMQ marketed gel for 8 days, psoriasis-like flakes and minor lesions were observed. After treatment with test gel sample and marketed product, visual inspection was done on daily basis and observations were noted.

Skin irritation studies

Acute skin irritation was evaluated in mice, after they have been shaved. Gel samples were applied to the skin and the appearance of edema and/or erythema was evaluated at 1st, 24th, 48th, and 72nd h after application.[16,17]

Histopathology studies

In 10% neutral-buffered formalin, selected tissue samples were fixed which was followed by dehydration in a graded alcohol series. It is then embedded in paraffin blocks. Sections of the embedded tissues were taken at 4-µm thickness using a rotary microtome and were stained with hematoxylin and eosin (HE) stains. These stained tissues were then observed under an optical microscope equipped with a digital camera system and images were captured.[16,17]

Scoring severity of skin inflammation

An ideal scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI), to score the inflammation severity of the back skin. Erythema and scaling were taken as two parameters and were independently scored on a scale from 0 to 4 as: 0 – none, 1 – slight, 2 – moderate, 3 – marked, and 4 – very marked.

Ex vivo permeation studies

Animals were purchased from Adita Biosys Pvt. Ltd., Tumkuru, India. Skins of healthy BALB/c mice were collected by sacrificing them and were used for *ex vivo* permeation studies. Topical gels containing pure CRN- and NLC-based gel (N1) were prepared and permeated through dorsal skin of mouse. Procedure similar to that of *in vitro* drug diffusion studies was followed.

Estimation of drug retained in the skin layers

The skin was removed from the diffusion apparatus after the completion of experiments. The surface of skin specimens was washed ten times with 1 mL distilled water and the drug content in the washings was determined using UV spectrophotometer. By the heat method, epidermis and dermis layers were effectively separated. The skin specimen was placed in a sealed bag and placed in water maintained at 52°C for 30 s. After 30 s, the dermis and epidermis were separated by peeling.[18] The separated dermis and epidermis layers are minced with a surgical sterile scalpel and placed in 10 mL methanol and vortexed for 5 min. The tissue suspensions were then centrifuged at 10,000 rpm for 15 min and the supernatant was filtered. Then, filtered supernatant tissue suspension of dermis and epidermis was further extracted with methanol and filtered. The filtrate was analyzed by serial dilutions if necessary using UV-visible spectrophotometry.

Stability studies

Stability studies were conducted for 6 months for optimized formulation according to the ICH guidelines. The storage requirements were 25°C/60% RH, 30°C/60% RH, and 40°C/75% RH. Changes in physical appearance and drug content of formulations were observed at standard time intervals.

RESULTS

Preformulation studies

Ultraviolet visible spectroscopy

Maximum absorbance wavelengths (λ_{max}) of CUR and CFN in methanol are 419 and 272 nm, respectively, which is observed. Reported wavelengths of CUR and CFN are 421 and 273 nm, respectively. Overlay plot of two drugs is shown in Figure 1. From the overlay plot, isosbestic point of two drugs was found to be 292 nm. Calibration curve of drug mix was shown in Figure 2. 0.9985 was found to be the regression coefficient with 0.0287 as slope value and 0.0064 as Y-intercept value.

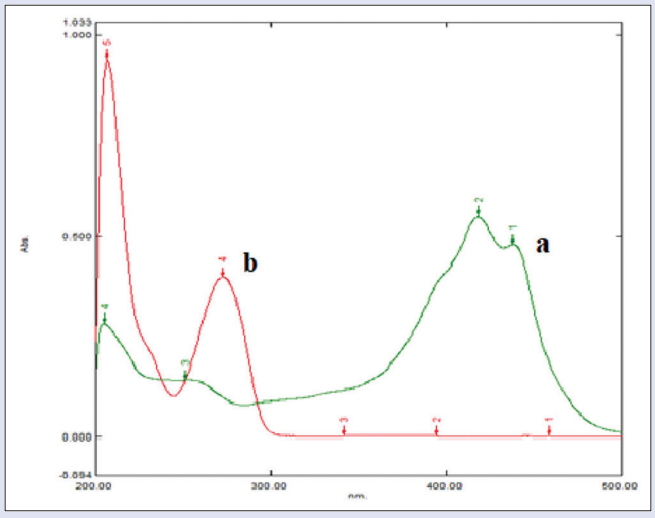

Figure 1: Overlay spectrum of curcumin (a) and caffeine (b) mixture in methanol

Analytical method validation

Linearity, precision, determination of limit of detection and limit of quantitation, and robustness were taken as parameters. Results obtained are given in Tables 4-8. From the results, it was found that all the parameters were within limits.

Fourier transform infrared analysis

The peaks which are prominent in drug mixture are shown in Figure 3. The prominent peaks of drug mixture, i.e., alkane C-H stretching (2918.40/cm); alkene C-H bending (1464.02/cm); amine C-N-stretching (1291.39/cm); and amide N-H-bending (1654.01/cm) were noticed in the physical mixture FTIR spectra.

Differential scanning calorimetry analysis

The DSC thermograms of drugs (CUR and CFN) drug mixture and drug-total lipids physical mixture are shown in Figure 4. The endotherm A that appeared at around 175.37°C represents the melting point of CUR (183°C theoretically). The second endotherm B at 227.40°C represents the melting point of CFN (235°C theoretically). Drug mixture exhibited endothermic peaks of CUR ad CFN at 171°C and 225°C, respectively. Endotherm D shows melting point of total lipids at 53.67°C.

Experimental design for formulation of nanostructured lipid carriers

From the experimental design used nine formulations were obtained from chosen responses and are given in Table 1.

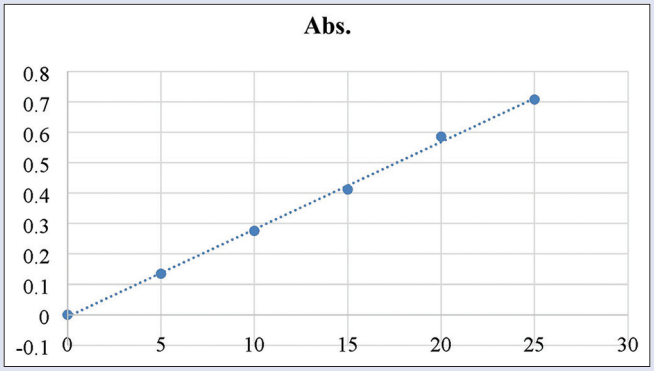

Figure 2: Calibration curve of drug mix in 7.4 pH buffer

Table 5: Intraday method precision table

Concentration	Absorbances	Concentration	Absorbances	Concentration	Absorbances
5	0.135	15	0.412	25	0.708
5	0.137	15	0.405	25	0.693
5	0.131	15	0.418	25	0.713
5	0.133	15	0.412	25	0.704
5	0.135	15	0.401	25	0.713
5	0.132	15	0.398	25	0.717
Average	0.1338	Average	0.4076	Average	0.7081
SD	0.0020	SD	0.0069	SD	0.0078
Percentage relative SD	1.52	Percentage relative SD	1.70	Percentage relative SD	1.11

SD: Standard deviation

Table 6: Interday method precision table

Concentration	Absorbances	Concentration	Absorbances	Concentration	Absorbances
5	0.136	15	0.418	25	0.697
5	0.132	15	0.412	25	0.715
5	0.131	15	0.417	25	0.703
5	0.135	15	0.404	25	0.713
5	0.136	15	0.414	25	0.709
5	0.133	15	0.398	25	0.712
Average	0.1338	Average	0.4105	Average	0.7082
SD	0.0020	SD	0.0072	SD	0.0063
Percentage relative SD	1.46	Percentage relative SD	1.76	Percentage relative SD	0.89

SD: Standard deviation

Table 7: Determination of limit of detection and limit of quantification

Concentration	Absorbances	Theoritical Absorbances	Residuals
0	0	0	0
5	0.135	0.137	0.0021
10	0.276	0.281	0.0046
15	0.412	0.424	0.0121
20	0.586	0.568	−0.0184
25	0.708	0.711	0.0031
SD			0.01018
Slope			0.0064
LOD			5.25
LOQ			15.91

SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of quantification

Characterization and evaluation of nanostructured lipid carriers

Particle size and zeta potential analysis

The average particle size of prepared NLC formulations was found to be in the range of 98 to 170 nm with a PDI in range of 0.23 ± 0.038–0.58 ± 0.026 representing uniformity in distribution of particle size. Particle sizes of all prepared formulations are given in Table 1.

Zeta potential of the prepared NLC's was in − 13.02 ± 0.20 mV––30.81 ± 0.19 mV range. Hot homogenization followed by melt ultrasonication method was followed which is a modified one.

Entrapment efficiency

EE of the NLC dispersion was found to be in the range of 44.14% ± 1.72%–63.92% ±1.06%. It reflects that 63.92% of drug is encapsulated in NLC's. EE of all formulations is given in Table 1.

In vitro drug release studies

All the formulations exhibited initial burst release followed by prolonged release of drug.

Statistical modeling and optimization

The results obtained for chosen responses gave nine formulations and are given in Table 1. The Particle size (nm) ranged between 98 and 170 nm. The EE (%) ranged between 44.14% ±0.87% and 63.92% ±1.21%.

Regression analysis

The regression equations depicted relative effect of the independent variables X1: Concentration of liquid lipid (%) and X2: Concentration of surfactant (%) on R1: Particle size (nm) and R2: EE (%) which is dependent variables.

The optimization resulted in achieving formulation F10 with 103.01 nm particle size and 61.52% entrapment efficiency. The R^2 values were found to be 0.988 and 0.936 for particle size and EE, respectively.

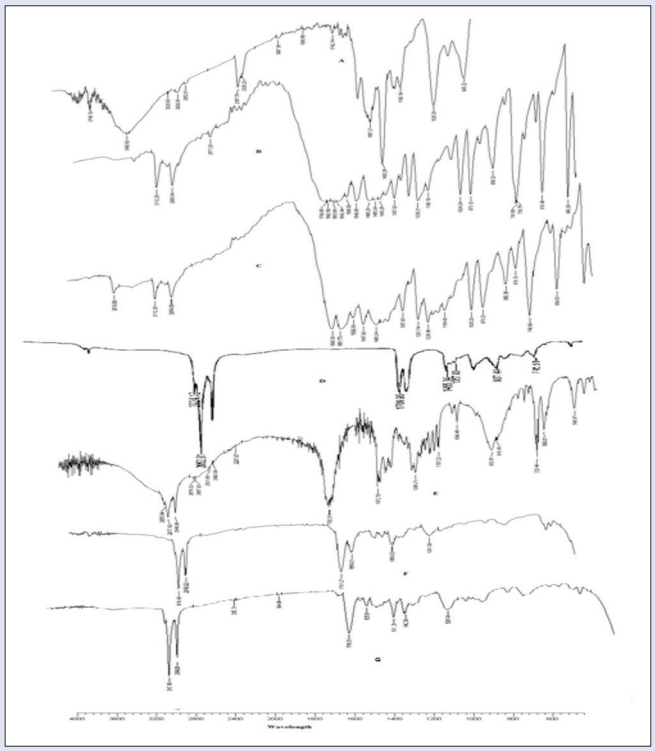

Figure 3: Fourier transform infrared spectra of (a) Curcumin; (b) Caffeine; (c) Drug mixture; (d) Oleic acid; (e) Stearic acid; (f) Drug + Total lipids physical mixture; (g) F10 formulation

Final equation in terms of actual factor

To make predictions about the response for given levels of each factor, the equation in terms of actual factors can be used. The equation for response R1 has showed that both the factors have negative effect on particle size.

$$R1 = +134.22 - 24.67 \times A - 9.50 \times B \quad (3)$$

The equation for response R2 has showed that both the factors have positive effect on entrapment efficiency

$$R2 = +69.72 - 5.36 \times A + 256.33 \times B \quad (4)$$

Response-surface analysis

The objective of optimization is to discover the levels of optimization of various variables which affect the method, so that production of product with required qualities can be achieved easily and reproducibly. Identification of the optimum region was possible using selected responses response-surface with constraint (particle size and entrapment efficiency). Figure 5 shows three-dimensional relationship between factors and responses.

Table 8: Robustness data table

As such wave length	292 nm	As such absorbance	0.708
Increase in wavelength (+1 nm-293 nm)	0.718	Decrease in wavelength (−1 nm-291 nm)	0.697
Increase in wavelength (+2 nm-294 nm)	0.723	Decrease in wavelength (−2 nm-290 nm)	0.683
Average	0.7205	Average	0.6960
SD	0.003	SD	0.010
Percentage relative SD	0.35	Percentage relative SD	1.47

SD: Standard deviation

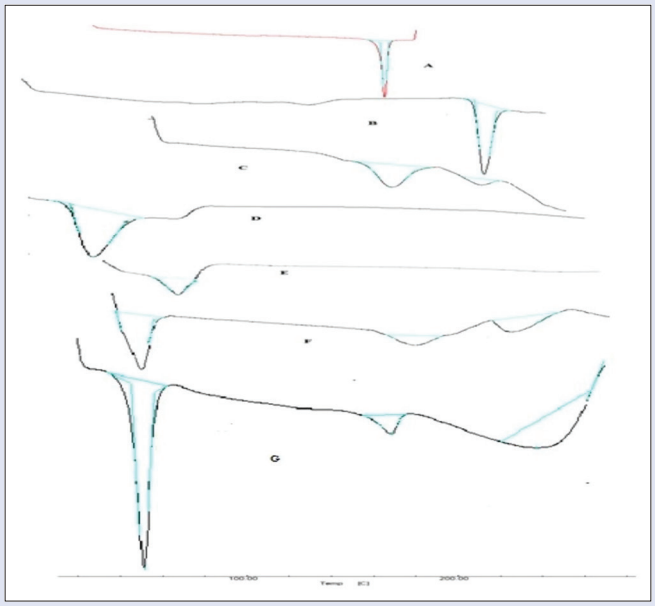

Figure 4: Differential scanning calorimetry thermograms of (a) Curcumin; (b) Caffeine; (c) Drug mixture; (d) Oleic acid; (e) Stearic acid; (f) Drug + Total lipids physical mixture; (g) F10 formulation

Analysis of variance

Analysis of variance shows that particle size and EE results obtained have shown statistically significant difference ($P < 0.05$).

Checkpoint analysis and optimization of design

Final formulation was prepared from optimum values of factors, desirability, and overlay plot of DoE. Preparation of optimized formulation (F10) was done for checkpoint analysis and evaluated for *ex vitro* drug release (%cumulative drug release) up to 12 h and *in vivo* studies. F10 showed response variable as R1 = 103.01 nm and R2 = 61.52%. Desirability value of 0.976 with small relative errors was seen. Reliability of the optimization procedure to prepare formulation as per 3^2 factorial designs which is followed in the present study was confirmed by the above value. A and B factors with the composition of 28.802% and 4.0% are proper for drug delivery as NLC-based topical gel. Figures 6 and 7 show counterplots representing desirability and overlay plots.

Characterization of optimized nanostructured lipid carriers (F10) formulation

Scanning electron microscopy

SEM for the prepared formulations was carried out to check the morphology for the NLC. They had spongy nature and were roughly spherical in shape. Figure 8 shows SEM photograph of optimized NLC (F10) formulation.

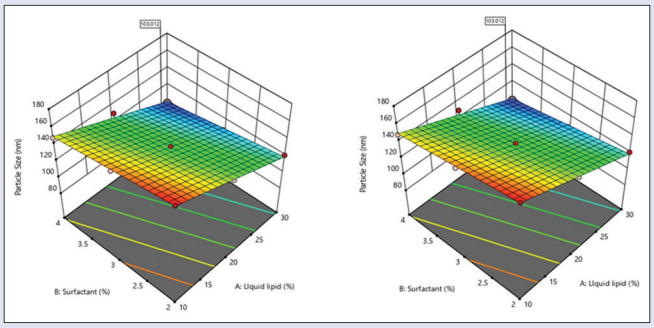

Figure 5: Three-dimensional surface plots of (a) R1: Particle size and (b) R2: Entrapment efficiency as a function of liquid lipid concentration and surfactant concentration

Fourier transform infrared analysis of nanostructured lipid carriers (F10)

To confirm the drug mixture entrapment in NLC, FTIR spectroscopy was carried out for F10 formulation. As shown in Figure 3, the major peaks of drug mix, i.e., 3510.56/cm to phenolic OH group stretch, 3112.26/cm to amine N-H stretch, and 740.69/cm to alkene = C-H bonding were not found in the FTIR spectra of F10 formulation.

Differential scanning calorimetry analysis of nanostructured lipid carriers

The DSC thermogram of optimized NLC formulation (F10) is shown in Figure 4. An endothermic peak at 58.7°C was seen.

Particle size

The obtained particle size was almost similar to that of predicted particle size value from DoE. Table 2 explained that predicted and observed values of particle size in NLC were almost similar and very low relative error of 0.88 was found between them. In Figure 9, particle size distribution of NLC formulation has been explained.

Entrapment efficiency

The obtained EE was almost similar to that of predicted value from DoE. Table 2 explained that predicted and observed values of drug entrapment in NLC were almost similar and very low relative error of 0.79 was found between them.

Evaluation of carbopol gels

pH determination

pH values of all gels were found to in the range of 6.5–7.3, which states that the values were within the range near to that of skin pH. Increase in the pH values may lead to skin irritation.

Homogeneity

For homogeneity, gels were evaluated visually. No aggregates were seen in prepared formulations, and they were clear. The values were represented in Table 9.

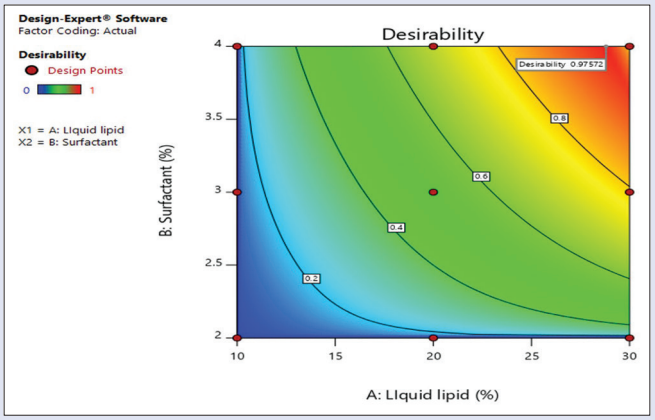

Figure 6: Contour plots represent the overall desirability function of optimized formulation (F10)

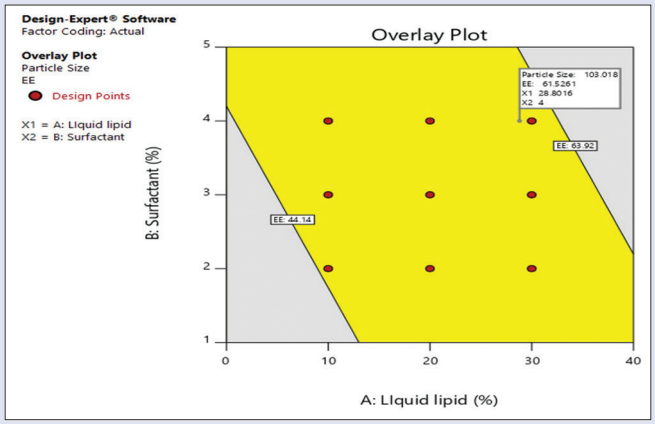

Figure 7: Overlay plot for optimization of nanostructured lipid carrier-based smart gel

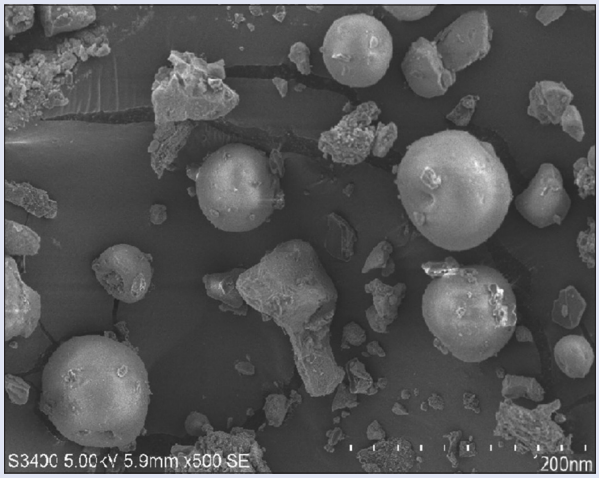

Figure 8: Scanning electron microscope photograph of F10 formulation

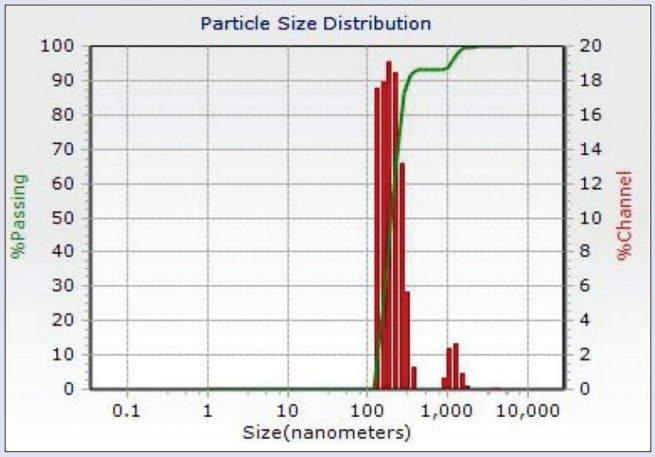

Figure 9: Particle size for F10 formulation

Table 9: Evaluation parameters for nanogels

Formulation	N1	N2	N3
pH	6.8	7.1	6.9
Homogeneity	Clear, transparent	Clear, transparent	Clear, transparent
Spreadability[#]	+++	+++	+++
Viscosity (cp)	9914	10542	12879
In vitro release studies (%)	74.56±0.93	71.24±1.14	67.62±1.02

[#],+: Poor; ++: Intermediate; +++: Good

Viscosity

Viscosity of carbopol gel was measured 37°C ± 0.5°C representing the body temperature. Different NLC samples viscosity was obtained and results are given in Table 9.

Spreadability

Spreadability is inversely proportional to the viscosity of prepared gel. Spreadability decreased as the polymer and carbopol amounts increased. The spreadabilities of the prepared carbopol gels were in the range. All the formulations have shown good spreadability.

In vitro drug release study

These studies supply essential data regarding formulation's imitative action during *in vivo* conditions. The results are shown in Figure 10.

From the results, N1 formulation was found to have high *in vitro* release and was used for further studies.

In vivo studies

Visual inspection

Representative images of the dorsal skin of mice treated with IMQ and those treated with the test gel samples are shown in Figure 11.

Kang *et al.*[19] has conducted work on CUR where they have conducted *in vivo* studies on mouse models. In their study, CUR was applied topically. The results stated that it took around 20 days to observe decrease in psoriatic lesions in mice when topical CUR was applied.

Skin irritation studies

After application of the optimized formulation onto the skin, signs for appearance of edema and/or erythema were observed at the end of 1, 24, 48, and 72 h. No signs of edema or erythema were observed at the end of these time intervals.

Histopathology studies

After the initiation of the *in vivo* studies, IMQ-treated mice's dorsal back skin started to show signs of very mild thickening, erythema, and scaling between days 2 and 4 after the IMQ treatment. From day 4 onward, inflammation was noticeable and got constantly increased in severity up

to day 8. These symptoms gradually reduced in groups treated with test sample (G3) and marketed formulation (G4). Maximum reduction of signs was seen in 8 days in case of G3, whereas it took around 20 days to get similar results in the group G4. The results are given in Figure 12.

Scoring severity of skin inflammation

Based on the clinical PASI score, erythema and scaling were independently scored on 0–4 scale as mentioned in methods section. The scoring results noted for diverse groups are given in Table 10.

Table 10: Psoriasis Area and Severity Index score observations

Days	Group 1*	Group 2*	Group 3*	Group 4*
0	0	0	0	0
2	0	4	4	4
4	0	4	3	3
6	0	4	2	3
8	0	4	1	2
10	0	4	0	2
15	0	4	0	1
20	0	4	0	0

*0: None; 1: Slight; 2: Moderate; 3: Marked; 4: Very marked

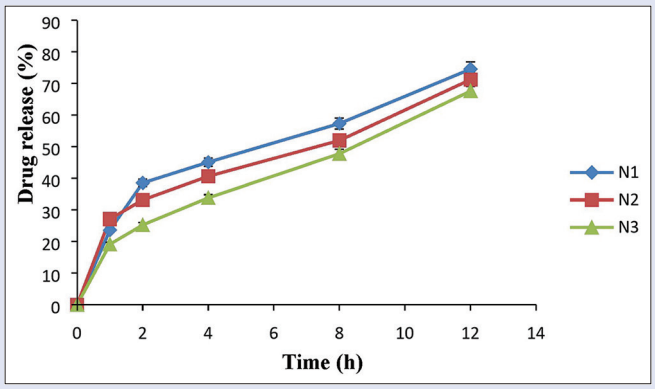

Figure 10: *In vitro* drug release profiles of formulations N1–N3

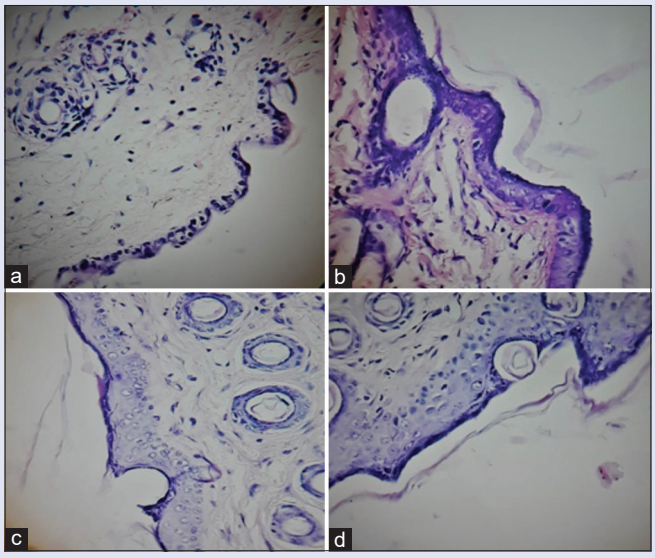

Figure 12: H and E staining of the mouse dorsal skin of different treatment groups. (a) Control; (b) IMQ-treated skin; (c) IMQ-induced skin treated with nanostructured lipid carrier test sample; (d) IMQ-induced skin treated with marketed sample

Ex vivo permeation study

Marketed CRN gel has shown drug release for 8 h, and after that, no release was noticed; whereas N1 formulation has shown drug release till 12 h (max 66.48 ± 0.93). The percent drug release of marketed formulation was noted to be around 63.74% at the end of 8 h and after that no release was seen. Obtained results in *ex vivo* release studies were plotted as percent cumulative drug release versus time and shown in Figure 13.

Estimation of drug retained in the skin layers

The drug release profiles obtained for drug retention in skin layers are presented in Table 11. The concentration of drug in skin layers was found to be less.

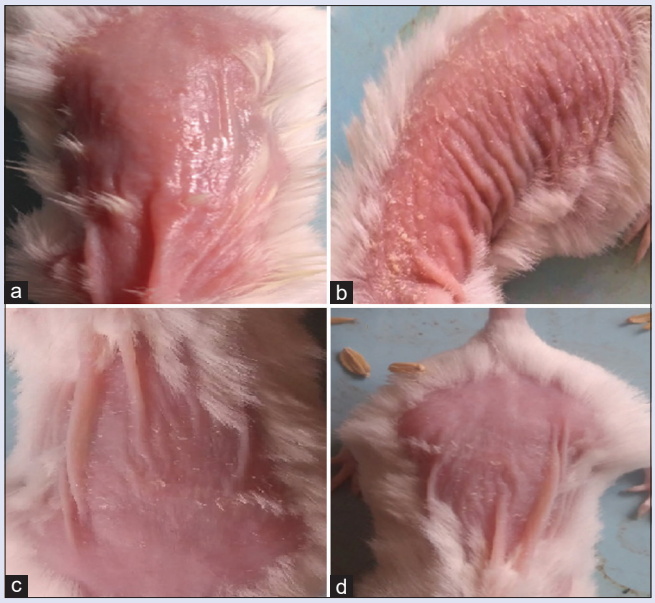

Figure 11: Images of dorsal skin of mice (a) Treated with vaseline (control); (b) Treated with Imiquimod (IMQ); (c) Reduction in scaling after treatment with nanostructured lipid carrier; (d) Reduction in scaling after treatment with marketed formulation

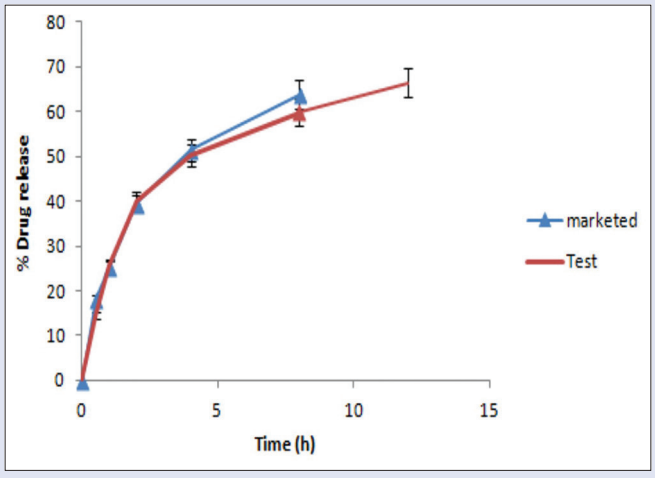

Figure 13: Drug release profiles of marketed Center for Responsible Nanotechnology formulation and optimized nanostructured lipid carrier-based gel formulation obtained during *ex vivo* permeation studies

Stability studies

Results obtained are shown in Table 12, which indicated there was no significant change in appearance and drug content of NLC formulation after subjection to stress testing for 6 months period.

DISCUSSION

Preformulation studies

Fourier transform infrared analysis

Functional groups obtained for physical mixture of drugs and total lipids were found to be in correlation with pure drug mixture peaks. Obtained results state that no interactions between drug mixture and total lipids occurred because no change in the peaks was seen. Hence, drug mixture (CUR and CFN) and lipids had compatibility.

Differential scanning calorimetry analysis

The physical mixture of drugs and lipids exhibited the endothermic peak of and disappearance of drug peaks. This shows that drug is encapsulated in lipids system, indicating their compatibility with each other.

Characterization and evaluation of nanostructured lipid carriers

Particle size and zeta potential analysis

Size of a particle is a very important parameter in NLC performance, because drug release rate, extend, and drug absorption are majorly affected by it. As the particle size decreases, interfacial area available for drug diffusion increases and thus improve in drug release can be seen. The Ostwald ripening probability was conquered because of narrow size distribution.[20]

In the interaction of formulation with biological system, zeta potential plays a significant role, and it has been reported in various studies.[21] It gives the charge type present on the NLC surface and the prepared formulation stability. As the surfactant concentration increases, zeta potential also increases. It may be due to globule size reduction that leads to elevated surface area and further increased zeta potential. These results state that selected excipients ratio helped in forming nanoemulsion which further resulted in nanosize NLC formation which are uniformly distributed.

Table 11: Retention of drug in skin layers

Formulation	Epidermis (percentage of drug retained)	Dermis (percentage of drug retained)	Diffused drug (%)
Optimized F10	55.18±0.61	32.61±0.75	16.91±0.82
Marketed	84.12±0.91	8.76±0.75	7.08±0.83

Entrapment efficiency

Encapsulation of remaining drug may occur in micelles of surfactant present in dispersion media. Thus, the drug solubilized in the dispersion would be useful as loading dose and drug entrapped in NLC acts as maintenance dose for prolonged release.

In vitro drug release studies

Drug release from NLC does occur due to NLC's degradation gradually and drug's diffusion simultaneously into the external polymer matrix. For immediate control of disease symptoms, optimum concentration is provided from initial burst release, and concentration required for overall treatment is provided by prolonged drug release.

Statistical modeling and optimization

Regression analysis

From the equations, it was clearly established that increase in the concentration of liquid lipid and surfactant concentrations particle leads to decrease in size and increase in entrapment efficiency.

Characterization of optimized nanostructured lipid carriers (F10) formulation

Scanning electron microscopy

From the results, it can be concluded that no particle aggregation was seen. Hence, NLC in SEM image was having a clear shape.

Fourier transform infrared analysis of nanostructured lipid carriers (F10)

Thus drug mixture entrapment in the NLC lipids systems can be concluded. Furthermore, other characteristic peaks such as alkane C-H stretch, alkene C-H bending, amine C-N-stretch, and amide N-H bending which are present in the pure drug mix and drug and lipids physical mixture were found in the F10 formulation. This indicates the presence of drug in the NLC.

Differential scanning calorimetry analysis of nanostructured lipid carriers

No prominent peaks pertaining to drugs were noticed which shows that drug mixture is encapsulated in the lipids system, which shows their compatibility with each other. Endothermic peak obtained at 58.7°C indicates the presence of lipids mixture.

Table 12: Stability study data of nanostructured lipid carrier-based gel formulation

Stability testing conditions	Sampling interval (months)	Physical appearance	Percentage drug content of NS-based gel formulation*
25°C 60%±5% RH	0	No change	92.65±0.47
	3	No change	91.12±0.34
	6	No change	90.32±0.27
30°C/60%±5% RH	0	No change	91.87±0.32
	3	No change	90.67±0.26
	6	No change	89.12±0.82
40°C/75%±5% RH	0	No change	92.65±0.47
	3	No change	89.54±0.28
	6	No change	88.43±0.56

*Mean±SD, *n*=3. SD: Standard deviation

Evaluation of carbopol gels

Viscosity studies

From the results, it has been portrayed that packing and micellar arrangement results in gel formation, and at greater polymer concentrations, the gel is more entangled. Due to these micelle entanglements, they cannot be readily separated from each other, thereby causing rigidity and high viscosity of gel at higher polymer concentrations.[22]

In vitro drug release study

The results suggested that sustained drug release was obtained. Initial burst release was exhibited by all formulations followed by prolonged release of drug. In initial hours, some amount of drug was unentrapped in the gel matrix, which leads to initial burst release. Later, remaining amount of entrapped in NLC core gives prolonged release. Due to NLC's continuous degradation and simultaneous diffusion of drug into the external polymer matrix drug release from NLC does occur. For immediate control of disease symptoms, optimum concentration is provided from initial burst release and concentration required for overall treatment is provided by prolonged drug release. The conclusions drawn from the study were supported by the findings of Joshi *et al.*[23]

In vivo studies

Visual inspection

In Figure 11a, image of mice treated with vaseline (control) is seen. In Figure 11b, image of mice treated with IMQ can be seen with visible, clear white flakes on dorsal back skin of mice. However, in Figure 11c and Figure 11d, marked reduction in that scaling was observed after treatment with test NLC gel sample (after 10 days) and marketed CUR gel (after 20 days). Flakes formed in Figure 11b are indicative of skin scaling as happens in psoriatic condition. Curative action in Figure 11c can be attributed to the presence of CRN and CFN loaded NLC in gel; that inhibited the IMQ-induced flakes formation and thickening of both the epidermal and subcutaneous tissue. Hence, potency of prepared and optimized NLC-based topical gel in reducing major signs and interventions of psoriasis were visually established.

Skin irritation studies

Results of the study have proved the compatibility and non-irritant nature of the prepared optimized NLC-based gel.

Histopathology studies

Histopathological slides stained with HE stain were observed and assessed taking help of an adept pathologist. When compared with the control group [Figure 12a], the IMQ-treated dorsal back skin section exhibited amplified epidermal and subcutaneous tissue thickness [Figure 12b]. Hyperplasia of basal and suprabasal keratinocytes is the reason behind this increased thickness. Furthermore, abnormal differentiation of keratinocyte with evident parakeratosis (nuclei in the stratum corneum) was seen. CRN and CFN NLC-based gel inhibited the thickness increased due to IMQ induction in both epidermal and subcutaneous tissues [Figure 12c]. In the control group, no abnormal phenotype was noticed. The keratinocyte layer thickness in each group stained skin section is indicated with the arrows in Figure 12. Since Group 1 was treated only with vaseline and no other chemicals were used, no unusual phenotype was noticed in the control group. In case of Group 2, marked changes were observed since the animal subjects were treated with IMQ. In Groups 3 and Group 4 slight changes were observed since treatment with test sample and marketed product was given after treating with IMQ. These results further confirmed that prepared test sample containing CRN and CFN NLC-based gel inhibited the thickness which increased due to IMQ induction in both epidermal and subcutaneous tissues.

Scoring severity of skin inflammation

From the results, it can be concluded that G4, i.e., group treated with marketed formulation has taken around 20 days to show its action, while G3, i.e., group treated with test formulation (NLC) has taken around 8 days to show its action. This may be due to enhanced antipsoriatic activity due to combination of CUR and CFN.

Ex vivo permeation study

Since NLC gel is a nanoformulation, sustained release of drug is noticed, the release time of this formulation was prolonged.

Estimation of drug retained in the skin layers

Obtained results clearly state in case of optimized N1 formulation, most of the drug was found to be in the epidermal layer of the skin. In the dermis layer, very minimal amount of drug was present. In case of marketed formulation, more amount of drug diffused into dermis layer.

Stability studies

From the results obtained, it can be concluded that the optimized CUR-CFN NLC-based gel was stable during study period.

CONCLUSION

NLC's of CRN and CFN were successfully synthesized and characterized in great detail. Inclusion of CRN and CFN NLC in topical gel has shown therapeutically better effects in treating psoriasis compared with the conventional marketed formulation. Sustained drug release was achieved till the end of 12 h by prepared optimized NLC-based topical gel. Moreover, *ex vivo* permeation studies and *in vivo* studies revealed improved potential of NLC-based gel to lighten psoriasis. The prepared gel consisting NLC might be proposed as a potential delivery system for an effective and superior local treatment of psoriasis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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