Ind. J. Pharm. Edu. Res., 2025; 59(2s):s508-s522.
https://www.ijper.org

Original Article

Nanostructured Lipid Carriers Based Gel of Methotrexate for Rheumatoid Arthritis: Development, Characterization and Optimization through Taguchi Design

Malti Arya[1, 2], Amit Porwal[3,*], Koshy Mamman Kymonil[1,4]

[1]Department of Pharmaceutics, School of Pharmacy, Babu Banarasi Das University, Faizabad Road, Lucknow, Uttar Pradesh, INDIA.
[2]Department of Pharmaceutics, Chandra Shekhar Singh College of Pharmacy, Kaushambi, Uttar Pradesh, INDIA.
[3]Department of Pharmaceutics, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, INDIA.
[4]Department of Pharmaceutics, Nova College of Pharmacy, KH. No. 74, STP Road, Khargapur, Gomti Nagar Extension, Uttar Pradesh, INDIA.

ABSTRACT

Background: The goal of this work was to develop, optimise and characterise nanostructured lipid carriers containing methotrexate for the treatment of Rheumatoid Arthritis (RA). As materials and process parameters, solid lipid, surfactant and stirring time were originally tested. The best alternative was chosen, and additional optimisation was performed using Taguchi orthogonal (L9) array design. **Materials and Methods:** Several batches of NLCs have been generated using the microemulsion process using liquid lipid (castor oil, almond oil and canola oil), solid lipid (cetyl alcohol) and surfactant. The dependent variables for optimisation were particle size, zeta potential, Entrapment Efficiency (EE) and *in vitro* release. The gel formulation was developed by combining NLCs with carbopol 940 (1% w/v) and was tested for stability, skin permeability (via goat-knee skin), skin irritation and antiarthritic properties. **Results:** The optimised NLCs (NLC9) had average particle sizes, zeta potentials and EE values of 136.2±5.4, -25.0 mV and 89.47±6.8%. Skin penetration studies revealed that NLCs may successfully penetrate the skin. The gel did not irritate the rabbits' skin, according to the Draize test (PDI=0.00). A conventional formulation was compared to a gel containing NLCs. The *in vitro* research found that NLCs gel (about 27%) and conventional tablets (about 18%) might reduce arthritic inflammation caused by intra-articular carrageenan injection. **Conclusion:** The study's findings supported NLCs gel-based formulation's promise as a stable, skin-permeable, non-irritant and patient-compliant rheumatoid arthritis administration approach.

Keywords: Methotrexate, Nanostructured Lipid Carriers, Rheumatoid Arthritis, Taguchi design, Gel, Intra-articular carrageenan injection.

Correspondence:
Dr. Amit Porwal
Assistant Professor, Department of Pharmaceutics, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah-206130, Uttar Pradesh, INDIA.
Email: amitporwal84@yahoo.co.in
ORCID: 0000-0002-7648-2232

Received: 26-10-2023;
Revised: 06-07-2024;
Accepted: 03-01-2025.

INTRODUCTION

Colloidal drug delivery systems have several advantages, including the ability to improve the bioavailability of water-insoluble drugs, enhance efficacy, promote controlled drug release, provide photo-protection and target a wide range of active substances, resulting in increased trust in these drug delivery systems.[1-3] The new generations of lipidic nanoparticles, such as Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), are attracting substantial attention as revolutionary colloidal drug carrier systems for oral, transdermal and parenteral drug delivery.[4-6] In contrast to well-ordered SLNs made from solid lipids, the crystal order is much disrupted upon the addition of liquid lipids to solid lipids in NLCs, allowing them to accommodate a greater quantity of drugs and exhibit higher stability. NLCs have higher defects in the crystal lattice and a bigger space to retain more pharmacological molecules, resulting in enhanced drug loading, less drug expulsion and better flexibility in adjusting drug release.[7-10] NLCs are composed of biodegradable and physiological lipids that have minimal or no toxicity. The nano-sized particles form an intimate bond with the stratum corneum, allowing more active substances to penetrate the skin. Due to their occlusive nature, lipidic nanoparticles can also increase skin moisture.[11-13] Many studies are being conducted on the topical and transdermal applications of lipid nanoparticles and their properties have been identified as potentially relevant for the topical administration of drugs and cosmetics.[14]

Rheumatoid Arthritis (RA) is a severe, autoimmune systemic inflammatory disease that can damage multiple organs, such as the heart, lungs, blood vessels, skin, muscles and tissues. However, it primarily attacks the joints, causing inflammatory

DOI: 10.5530/ijper.20255540

and non-supportive proliferative synovitis that commonly progresses to articular cartilage obliteration, resulting in joint ankylosis. Although the cause of RA is unknown, autoimmunity, inherited predisposition and environmental factors all play a role in the disorder's growth and development. Rheumatoid arthritis affects around 1% of the world's population, with females being affected 3-5 times more commonly than males.[15-17] Furthermore, rheumatoid arthritis and osteoarthritis are major medical challenges in the United States, with over 20 million individuals experiencing considerable difficulties at work on a regular basis. Some investigations have offered the first-ever national evaluation of arthritis prevalence for Pacific Islanders/Asian Americans (8%) and Alaska Natives/American Indians (25%), noting changes in everyday activities, work limitations and severe pain.[18,19] Intra-articular, parenteral and oral drugs have been used to treat irreversible joint injury. Despite a few potential drawbacks, administering a drug directly to a sick joint may result in increased drug concentrations at the site of action and reduced systemic toxicity.[20] Drug delivery via the dermal method has the potential to reduce the likelihood of drug-related gastrointestinal and systemic adverse effects. Because of the barrier qualities of the stratum corneum, drug delivery via the dermal route is often limited. Because of their controlled release capabilities, reduced size, biocompatibility and ability to incorporate both hydrophilic and lipophilic drugs, SLNs and NLCs have gained increased interest as dermatological preparations for drug administration.[21]

Methotrexate (MTX), a dihydrofolate reductase inhibitor, is an immunosuppressant family of Disease-Modifying Antirheumatic Drugs (DMARDs) used to treat RA due to its anti-inflammatory and immunosuppressive characteristics. Systemic administration of this drug (Trexall®, Rheumatrex® and others) causes a variety of side effects, including mouth sores (ulcerative stomatitis), lung infections (Pneumocystis jiroveci pneumonia), lung difficulties and diarrhea. The most serious is hepatic toxicity. The most common MTX side effects include hepatic enzyme increase and gastrointestinal intolerance. As a result, cutaneous administration of MTX may be advantageous in reducing unpleasant responses. However, inadequate percutaneous absorption of the drug is a significant disadvantage of this method.[16,18,22-25]

The primary benefit of NLCs is that they are made up of biodegradable and physiological lipids that are less harmful. NLCs can potentially increase the perceived solubility of implanted drug particles, resulting in a greater concentration gradient on the skin and aiding drug penetration. Their nanosize also allows for tighter contact with the stratum corneum, resulting in a greater amount of drug entering the skin in a more regulated manner. Furthermore, NLC elements like lipids and surfactants can operate as penetration enhancers by fluidizing or loosening the lipidic bilayers of the stratum corneum.[4,5]

Due to the numerous significant independent (solid lipid-liquid lipid ratio, drug-lipid ratio, type of liquid lipid and surfactant concentration) and dependent (particle size, entrapment efficiency and drug release) variables to consider, conducting several experiments and analyses would have been time-consuming and resource-intensive. The Taguchi Design of Experiment (DOE) strategy was therefore employed as a powerful tool to optimize the formulation and improve product quality with minimal testing.

The current study aimed to develop, optimize and characterize NLCs containing MTX in a gel formulation, resulting in a stable, non-irritant and patient-compliant delivery method for the treatment of RA.

MATERIALS AND METHODS

Materials

P.R. Pharma Source Pvt. Ltd., (Mumbai, India) supplied the methotrexate. S. D. Fine Chem Limited (Mumbai, India) provided the cetyl alcohol, castor oil, canola oil, almond oil, carbopol 940, propylene glycol and tween 80. The remaining solvents and reagents were of analytical grade.

Screening study

Screening of Solid Lipid

The highest amount of the drug solubilized in each lipid (compritol 888ATO, cetyl alcohol and glyceryl monostearate) was used to screen solid lipids (compritol 888ATO, cetyl alcohol and glyceryl monostearate). The maximum lipid solubilization capability for the drug was determined by dissolving the drug in small quantities in a known quantity of melted solid lipids (kept at 5°C above their respective m.p.). The drug was added, and the mixture was stirred until, based on visual examination, no more drug could be dissolved. This process was carried out 3 times.[26]

Screening of surfactants

Surfactants (span 20, tween 20 and tween 80) were chosen based on the drug's saturation solubility in a combination of lipids and surfactants. Excess drug was placed in stoppered vials (5 mL) with oils (2 mL) containing a specific surfactant, which were stirred for 72 hr using a rotary flask shaker to achieve equilibrium at room temperature. The mixture was then centrifuged at 37°C for 30 min at 5000 rpm, followed by supernatant collection, methanol dissolution and spectrophotometric analysis at 259.5 nm (UV-1700 PharmaSpec, Shimadzu). The studies were done 3 times, and the data was summarised as mean standard deviation.[27]

Optimization of stirring time

To achieve the optimal timing, the stirring times for developing NLCs were changed to 2 hr, 3 hr and 4 hr. These NLCs were evaluated for particle size and drug EE to optimise the stirring duration.[27]

Formulation: Taguchi Design

Based on the pre-optimization investigation, the Minitab software (Version 17) and Taguchi orthogonal (L9) array design was used to optimise the formulation on chosen parameters (drug-lipid ratio, solid lipid-liquid lipid ratio, type of liquid lipid, surfactant concentration) (Table 1).[28] The L9 array design formulation studies were formed and characterised for their responses (such as particle size, zeta potential, drug entrapment and *in vitro* drug release). The statistical optimisation method using ANOVA to determine significance was used to investigate the effects of critical elements on responses, with the benefit of constructing the fewest number of formulations. A graph of Signal-to-Noise ratios (S/N ratios) vs each level may be used to analyse responses (the main effects plot displays the impact of each component on responses). Each dependent variable's optimal level was established to construct the final optimised formulation. The resultant optimised formulation was validated by developing and characterising it for responses and evaluating the Taguchi design's dependability, appropriateness and robustness.[29]

Preparation of Nanostructured Lipid Carriers containing MTX

At 80ºC, cetyl alcohol was melted and a liquid lipid (castor oil, canola oil, or almond oil) was added. MTX was added to the lipid mixture, mixed for 5 min and sonicated for 60 sec at 120 W. Tween 80 was added to this mixture and it was stirred for another 2 min. The aqueous phase, which contained a co-surfactant (propylene glycol), was kept at 80ºC before being added to the melted lipid phase. Stirring at 80ºC for 15 min was followed by 5 min of sonication to create the o/w microemulsion. The microemulsion was mixed again and allowed to cool to 40ºC. The heated microemulsion was dispersed for 3 hr in a cold combination (2ºC to 4ºC) of propylene glycol and distilled water (2:8) at a constant ratio of 1:5 (microemulsion: dispersing medium). To separate the free drug from the NLCs, the suspension was centrifuged at 8000 rpm for 30 min. The NLCs suspension was kept at 4ºC until further analysis.[30]

Characterization of NLCs containing MTX

Morphology of NLCs

Scanning Electron Microscope (SEM) (FEI, Quanta 200F, Japan) BHU Varanasi, was used to examine the surface and shape morphology of the NLCs suspension.

Particle size, Polydispersity Index (PDI) and Zeta Potential

Malvern ZetaSizer Ver. 2.0, at CSIR-CDRI Lucknow, was used to characterise the optimised NLCs for average particle size and PDI. The surface charge of NLCs is measured as zeta potential by

Table 1: Taguchi Design to optimize the formulation variables.

Independent Variables	Levels		
	Low (1)	Medium (2)	High (3)
A: Solid lipid-liquid lipid ratio (SL:LL)	65:35	70:30	75:25
B: Drug-lipid ratio (D:L)	1:5	1:7	1:10
C: Type of liquid lipid (LL)	Almond oil	Castor oil	Canola oil
D: Surfactant concentration	8	10	12
Dependent variables	Goal		
(Y1): Particle size (nm)	Minimize		
(Y2): Entrapment Efficiency (%)	Maximize		
(Y3): Drug Release	Maximize		
L9 (3^4) Standard orthogonal array			

RUN	Factor A SL:LL	Factor B D:L	Factor C LL	Factor D Surf. Conc.
NLC1	75:25	1:10	Castor	8
NLC2	75:25	1:7	Almond	12
NLC3	65:35	1:7	Castor	10
NLC4	65:35	1:5	Almond	8
NLC5	65:35	1:10	Canola	12
NLC6	70:30	1:10	Almond	10
NLC7	75:25	1:5	Canola	10
NLC8	70:30	1:7	Canola	8
NLC9	70:30	1:5	Castor	12

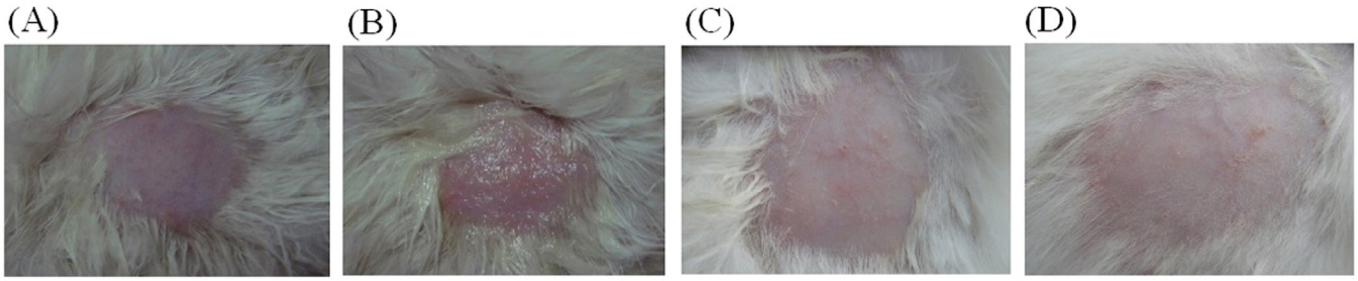

Figure 1: (A) Skin before application of gel (B) Skin after application of gel (gel containing optimized formulation NLC9) (C) Skin after 24 hr of application of gel (D) Skin after 7 days of application of gel.

utilising a zetasizer to determine the electrophoretic mobility of NLCs in a U-type tube at 25°C.

Entrapment efficiency

A volume of NLCs suspension containing 10 mg of MTX was centrifuged at 12000 rpm for 10 min at 10°C in a cold centrifuge (SIGMA 3-18K, Sartorius). A spectrophotometer was used to compare the supernatant to a reagent blank at 259.5 nm. To calculate the percentage of drug entrapment, apply the following equation:

$$\text{Entrapment Efficiency} = \frac{\text{Quantity of drug in supernatent}}{\text{Quantity of drug incorporated}} \times 100 \quad \text{Eq. (1)}$$

In vitro drug release

In vitro drug dissolution experiments were carried out in a Keshary-Chien (K-C) cell with a capacity of 25 mL and an egg membrane in Phosphate-Buffered Saline (PBS), pH 6.8. In the donor compartment, 2 mL of NLCs were poured. A flowing water bath was used to keep the receptor compartment at 37±0.5°C. At regular intervals, samples (1 mL) were taken and an equivalent volume of PBS pH 6.8 was supplied to maintain sink conditions. Filtered samples were diluted with 0.1 M sodium hydroxide and spectrophotometrically analysed at 259.5 nm against a reagent blank. Fitting the release data into several kinetic models such as Korsmeyer-Peppas, Higuchi plots, zero-order plots and first-order plots determined the drug release mechanism. The model with a correlation coefficient approaching 1 was deemed to be the best-fit model. The data was deemed to be the best appropriate model.[31]

Formulation of gel containing NLCs

Using Carbopol 940, the gel containing NLCs was produced using the optimised NLC formulation. Carbopol 940 (1% w/w) was accurately weighed and diffused in distilled water overnight.[32] The dispersion was infused with an NLCs solution containing 10 mg of MTX. The pH for gel formation was adjusted by gently stirring in triethanolamine. The gel was left overnight to release the trapped air.[33,34] The prepared gel was further evaluated for pH, drug content, viscosity, spreadability, extrudability, *ex vivo* permeation, skin irritation and an antiarthritic study.

Characterization of NLCS Based Gel of Optimized Formulation

pH determination

The pH of the gel was measured using a digital pH metre (Systronics, 361-micro pH metre). A sample of gel (1 g) was solubilized in distilled water (25 mL) and then the electrode was immersed in the gel preparation for half an hour until a constant value was obtained.[23]

Drug content

In 10 mL of ethanol, 100 mg of optimised gel preparation were dissolved. 1 mL of this mixture was diluted to 10 mL and used as a control, while another 1 mL was mixed with 10 mL of 0.1 M sodium hydroxide, sonicated for 2 min and centrifuged at 6000 rpm. The supernatant was collected and spectrophotometrically analysed for drug concentration at 259.5 nm.

Viscosity

It is an important metric to consider when analysing gels since it effects drug release, extrudability and spreadability. The viscosity (cps) of the gel formulation was determined using a Brookfield digital viscometer with spindle no. 7 operated at 10-100 rpm.

Spreadability

The gel spreadability was determined using slightly modified equipment proposed by Mutimer *et al*. The device is made from a wooden block with a pulley at one end. A rectangular glass slide was mounted on the wooden block. An excess of gel (about 2 g) was placed on the ground glass slide under examination, over which another glass slide with equal dimensions to the ground glass slide was placed and fastened with a hook.[35] For 5 min, a 300 g weight was placed on the upper slide to eliminate air and establish a homogenous gel coating between the slides. The extra gel was scraped away from the margins. The top slide was then subjected to a 30 g weight pull-off via a line attached to the hook and the time (in sec) required for the upper slide to move across a 10cm distance was recorded.[36] The spreadability was calculated using the following formula:

$$S = m \times l/t \quad \text{Eq. (2)}$$

Table 2: Effect of formulation variables on particle size, zeta potential, drug entrapment and release characteristics according to Taguchi design.

Formulations	Parameters				Particle Size (nm)	Zeta Potential (mV)	Drug entrapment (%)	*In-vitro* drug release (%)
	A	B	C	D				
NLC1	3	3	2	1	197.3±0.5	-15.60	70.83±0.2	75.37±1.3
NLC2	3	2	1	3	207.9±5.1	-21.30	79.97±7.2	78.26±1.2
NLC3	1	2	2	2	199.6±3.4	-22.98	83.87±4.9	76.38±1.3
NLC4	1	1	1	1	135.5±4.6	-28.70	77.60±6.4	82.17±1.4
NLC5	1	3	3	3	139.8±7.4	-27.50	78.13±2.4	84.19±0.5
NLC6	2	3	1	2	178.8±2.5	-27.10	86.23±5.0	85.64±2.3
NLC7	3	1	3	2	187.9±5.6	-26.80	83.13±5.4	64.86±0.8
NLC8	2	2	3	1	154.7±4.1	-23.60	86.23±7.8	69.42±0.7
NLC9	2	1	2	3	136.2±5.4	-25.00	89.47±6.8	88.04±1.6

Where S denotes spreadability, l is the length in cm of the glass slide, m is the weight in grams attached to the upper slide and t is the time taken in sec.

Extrudability

A collapsible tube was used to estimate the amount of gel released from the tube when a constant weight was applied to measure gel extrudability. A steady weight was used to apply pressure to the crimped end of the collapsible tube containing 1 g of gel. When the cap was removed, the gel extruded until the pressure was released. The extruded gel was measured and collected. Extrudability is assessed in gram/force applied (Newton) per minute.

Ex vivo Skin Permeation Study through Goat Knee Skin

Drug penetration through the skin was investigated using a Keshary-Chein cell with a capacity of 25 mL in PBS pH 6.8 over excised goat knee skin (obtained from a nearby abattoir). The donor compartment was filled with NLCs gel (equal to 5 mg of drug), while the receptor compartment was filled with PBS pH 6.8 and kept at 37±0.5°C. At predetermined intervals, samples (1 mL) were extracted and replaced with an equivalent volume of PBS pH 6.8 to maintain the sink condition. Filtered samples were diluted with 0.1 M sodium hydroxide and spectrophotometrically analysed at 259.5 nm (UV-1700 PharmaSpec, Shimadzu) against a reagent blank.

Skin irritation test

For skin irritation studies, three healthy New Zealand white male rabbits weighing 2.5-3.0 kg were used. They were fed a standard diet and water *ad libitum* and kept under standard laboratory conditions. The *in vitro* investigations were carried out following the protocol authorised by the Pinnacle Biomedical Research Institute's Institutional Animal Ethical Committee (PBRI/IAEC/PN-16044). The animals were treated by applying a 0.5 g gel formulation to a 9 cm^2 region of the rabbit's abdomen skin. For the next 7 days, the rabbits were visually inspected for criteria such as sensitization (allergic response), excessive redness and oedema in both test and control animals (Figure 1). In comparison to the irritation index categories, the main Dermal-Irritation index (PDI) was determined using the following formula. For the report, the appropriate response type was specified.

$$PDI = \frac{\text{Combined index for 1, 24, 48 and 72 hours}}{4} \quad \text{Eq. (3)}$$

Antiarthritic study

To develop arthritis, 10 mg of carrageenan diluted in 1 mL of saline was administered intra-articularly (on days 0, 7 and 14) in the left knee of New Zealand white rabbits (weighing 2.5-3 kg) for three weeks. Arthritic inflammation was measured by measuring the diameter of the femorotibial joint using Vernier Callipers. The evaluation was performed on day 0 (before to each intra-articular injection) as well as on days 7, 14, 21, 28, 35 and 45. The treatment of the test and standard began on the 15th day, when the highest score of arthritic inflammation was detected. The MTX NLCs gel was applied topically in a weekly split dosage of 7.5 mg, while the equal amount of the commercial formulation was given once a week. Using Tukey's multiple comparison tests, the data was statistically assessed to see if there was a significant difference between groups.

Stability

According to ICH recommendations, the optimised preparations of MTX NLCs gel were stored at 5±1°C (refrigerator) and 25±1°C (room temperature) for 90 days. Every 15 days, the samples were analysed for physical properties such as appearance, precipitate formation, dispersibility and so on, as well as residual drug concentration.

RESULTS

Screening study

The drug MTX was most soluble in cetyl alcohol and Tween 80. A 3 hr stirring period resulted in NLCs with reduced particle sizes and maximal drug entrapment.

Formulation of MTX loaded NLCs

As shown in Table 2, formulation parameters such as drug-lipid ratio, solid lipid-liquid lipid ratio, type of liquid lipid and surfactant concentration were optimised based on particle size, zeta potential, drug entrapment and *in vitro* drug release.

Optimization and Response analysis

All the trials that were carried out according to plan yielded positive findings for particle size, EE and drug release. For each trial, the S/N for the dependent responses Y1, Y2 and Y3 was computed and analysed to determine the relevance of each element to select the optimum formulation.

Response analysis: Particle Size and Zeta Potential Measurements

The particle size and zeta potential were calculated using a zeta-sizer. PDI was used to determine the size distribution of the nanoparticles. As seen in Figure 2(I), the S/N ratio for particle size falls as the concentration of solid lipids increases. As the quantity of lipids increases, the S/N ratio decreases drastically, then modestly, showing that the lowest particle size is at the lowest level of the drug lipid ratio. However, when the kind of liquid lipid was altered, the S/N ratio of particle size did not vary considerably. As the surfactant concentration increases, the S/N ratio decreases at the intermediate level and subsequently increases at the higher level. Table 3 shows the ANOVA results for the S/N ratio for particle size. This trend can be attributed to the complex interplay between surfactant concentration and its effects on the physicochemical properties of the nanoparticles. At intermediate surfactant levels, the increased concentration may lead to factors like increased membrane permeability, drug leakage and changes in the packing of the lipid bilayer, which can contribute to the decrease in S/N ratio. However, at higher

Table 3: ANOVA calculations of the S/N ratio for particle size, entrapment efficiency and drug release.

Particle Size							
Sl. No.	**Factors**	**Degree of freedom (f)**	**Sum of Squares (S)**	**Variance (V)**	**F- Ratio (F)**	**Pure Sum (S')**	**Percent P (%)**
1	SL:LL	2	8.54	4.27	-	8.54	45.75
2	Drug: Lipid	2	4.79	2.39	-	4.79	25.67
3	Liquid lipid	2	1.045	0.52	-	1.05	5.62
4	Surfactant concentration	2	4.29	2.14	-	4.29	22.97
Total		8	18.66				100.00
Entrapment efficiency							
Sl. No.	**Factors**	**Degree of freedom (f)**	**Sum of Squares (S)**	**Variance (V)**	**F- Ratio (F)**	**Pure Sum (S')**	**Percent P(%)**
1	SL:LL	2	1.51	0.754	-	1.51	53.15
2	Drug :Lipid	2	0.53	0.264	-	0.53	18.60
3	Liquid lipid	2	0.05	0.024	-	0.048	1.70
4	Surfactant conc.	2	0.75	0.374	-	0.75	26.40
Total		8	2.84				100.00
Drug release							
Sl. No.	**Factors**	**Degree of freedom (f)**	**Sum of Squares (S)**	**Variance (V)**	**F- Ratio (F)**	**Pure Sum (S')**	**Percent P(%)**
1	SL:LL	2	1.75	0.87	-	1.75	27.84
2	Drug:Lipid	2	0.89	0.44	-	0.889	14.14
3	Liquid lipid	2	1.97	0.98	-	1.97	31.36
4	Surfactant concentration	2	1.68	0.84	-	1.68	26.66
Total	8						100.00

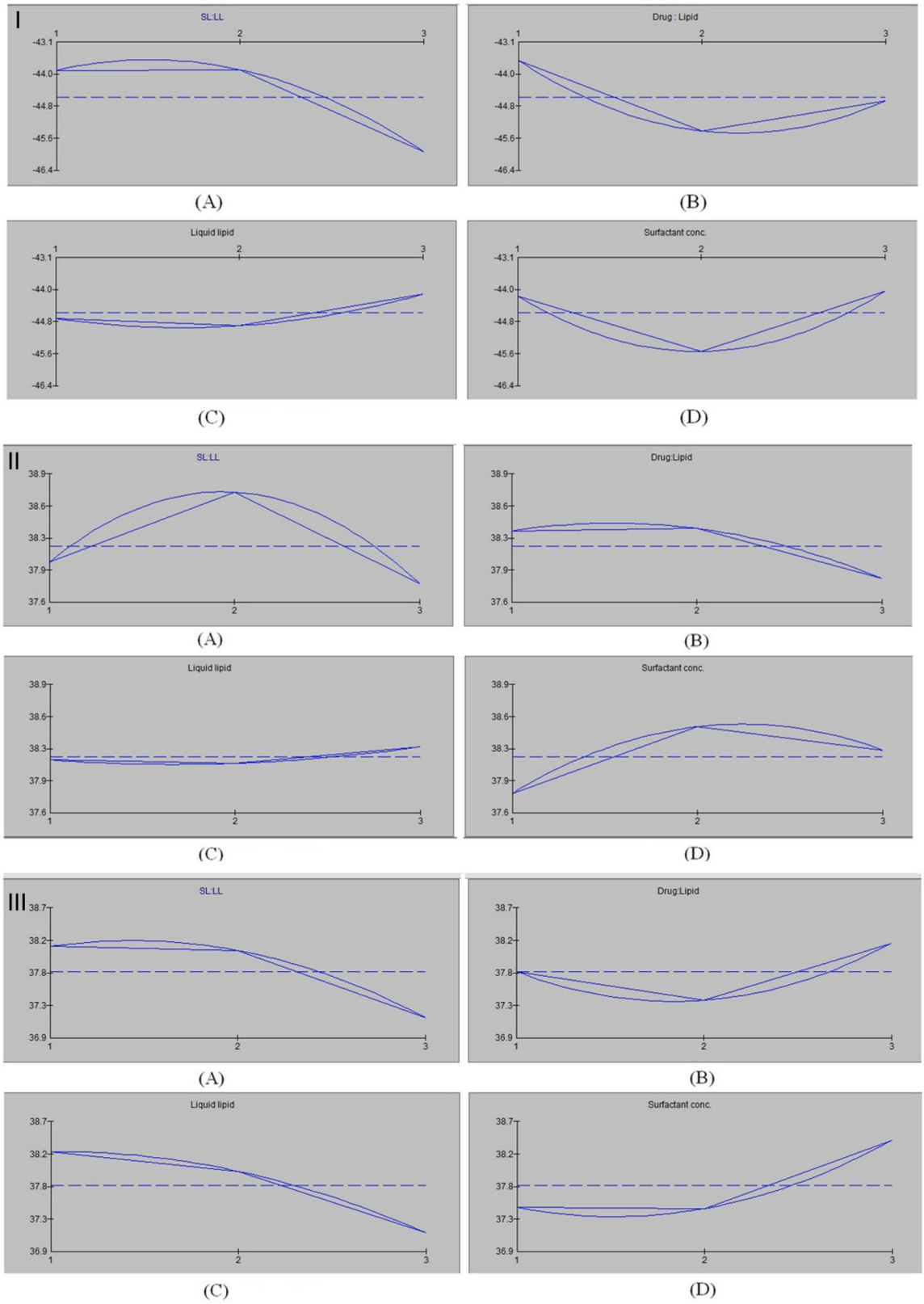

Figure 2: I: Smaller is best main effects plot for particle size (A) Solid lipid: Liquid Lipid (SL:LL), (B) Drug:Lipid (C) Concentration of liquid lipid and (D) Concentration of surfactant. II: Higher is best main effects plot for entrapment efficiency (A) Solid lipid: Liquid Lipid (SL:LL), (B) Drug: Lipid (C) Liquid lipid concentration and (D) Surfactant Concentration. III: Higher is best main effects plot for drug release (A) Solid lipid: Liquid Lipid (SL:LL), (B) Drug:Lipid (C) Concentration of liquid lipid and (D) Concentration of surfactant.

surfactant concentrations, the increased charge and reduced aggregation of the nanoparticles can enhance the stability and entrapment efficiency, leading to an increase in the S/N ratio.

As the quantity of lipids increases, the S/N ratio decreases drastically at first and then more modestly. This trend can be explained by the relationship between lipid concentration and nanoparticle size. Higher lipid concentrations generally result in the formation of larger nanoparticles, which can lead to decreased drug entrapment efficiency and increased drug leakage, causing a decrease in the S/N ratio. However, the rate of decrease in S/N ratio slows down at higher lipid levels, as the nanoparticle size reaches an optimal range for drug entrapment and stability.

Response analysis: Drug Entrapment Efficiency

The percent drug entrapment ranged from 70.83±2.2 to 89.47±6.8% for different NLC formulations (Figure 2(II)). Table 3 displays the results of the ANOVA used to compute the S/N ratio for entrapment efficiency.

Response analysis: *in vitro* drug release study in PBS pH 6.8

Figure 2(III) main effect graphs show how different formulation factors impact nanoparticle dispersion. The S/N ratio showed that formulations including canola oil (NLC5, NLC7 and NLC8) had lower drug release. Liquid lipids (almond oil and castor oil) had comparable impacts on drug release. Figure 3 displays the *in vitro* release profile. Table 5 shows the ANOVA results for the S/N ratio for drug release.

Selection of optimized formulation

The optimised formulation was NLC 9, which had the smallest particle size (136.2 nm), the best entrapment effectiveness (89.47±6.8%) and the highest drug release (88.04%). It has an

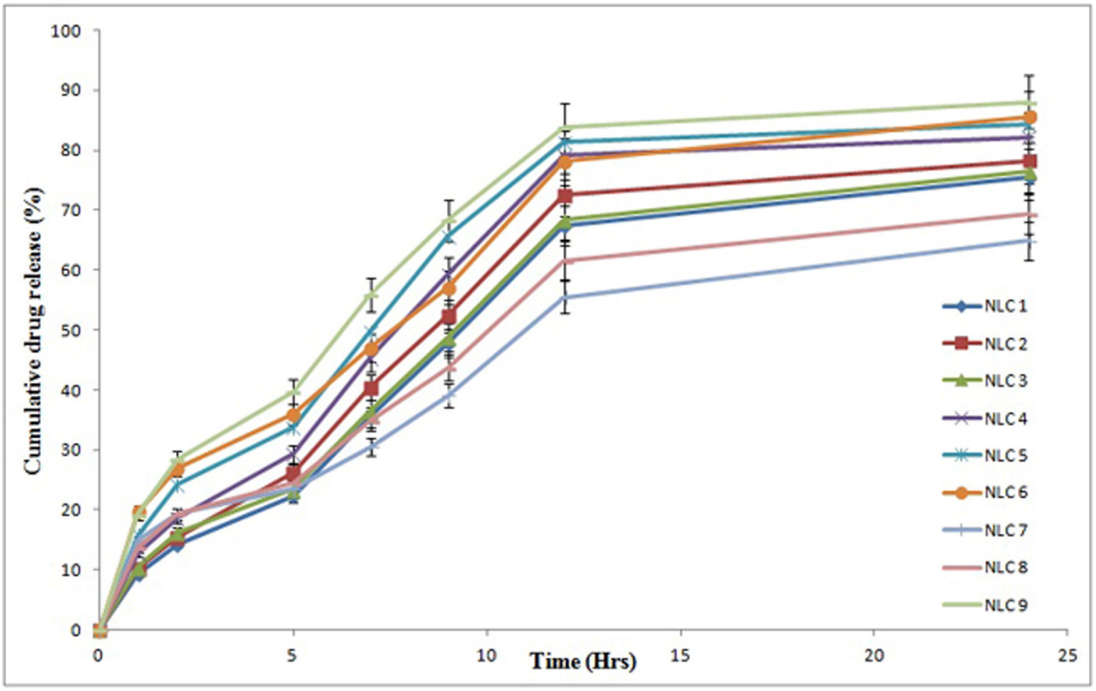

Figure 3: *In vitro* drug release of Methotrexate loaded NLCs, values mentioned in mean±SD, *n*=3.

Table 4: Compilation of observed primary skin irritation scores of MTX NLCs gel.

Animal no.	Time Period after gel application					
	Day 1		Day 2		Day 7	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	0	0	0	0	0	0
2	0	0	0	0	0	0
Total	0	0	0	0	0	0
Mean	0.00	0.00	0.00	0.00	0.00	0.00
Combined index	0.00		0.00		0.00	
PDI	0.00 (Zero)					

intermediate SL:LL ratio (70:30), a lower drug:lipid ratio (1:5), castor oil as a liquid lipid and a greater surfactant content (12%).

Release kinetics

The release kinetics of the optimised batch NLC9 matched the Higuchi model, as the figure with the best linearity (R^2=0.942) demonstrated. To investigate the diffusion mechanism, the data were fitted into the Korsmeyer-Peppas equation. The optimised batch's Korsmeyer-Peppas release exponent 'n' was 0.987.

Surface morphology

SEM micrographs revealed that the MTX-NLCs particles were spherical with smooth surfaces (Figure 4).

pH

It was found to be 6.8 for the gel-containing optimized batch NLC9.

Drug content

The drug content was found to be 83±0.43% for the optimized gel formulation.

Viscosity measurement

The viscosity of the gel preparation was evaluated using a Brookfield viscometer and reported to be 29800 cps.

Spreadability

The spreadability of the gel formulation was estimated utilizing modified apparatus and was found to be 4.347±0.39 g.cm/sec.

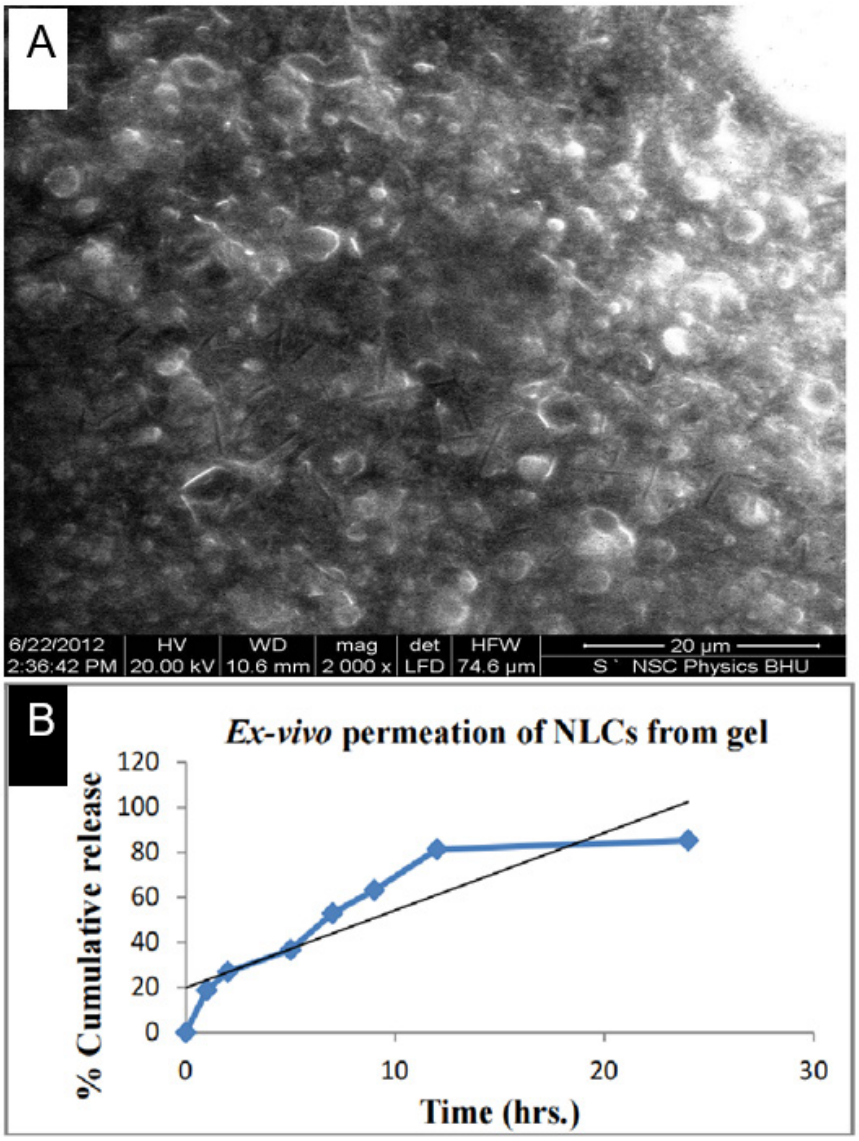

Figure 4: (A): SEM image of optimized formulation NLC9 and (B): *Ex vivo* permeation of NLCs from gel.

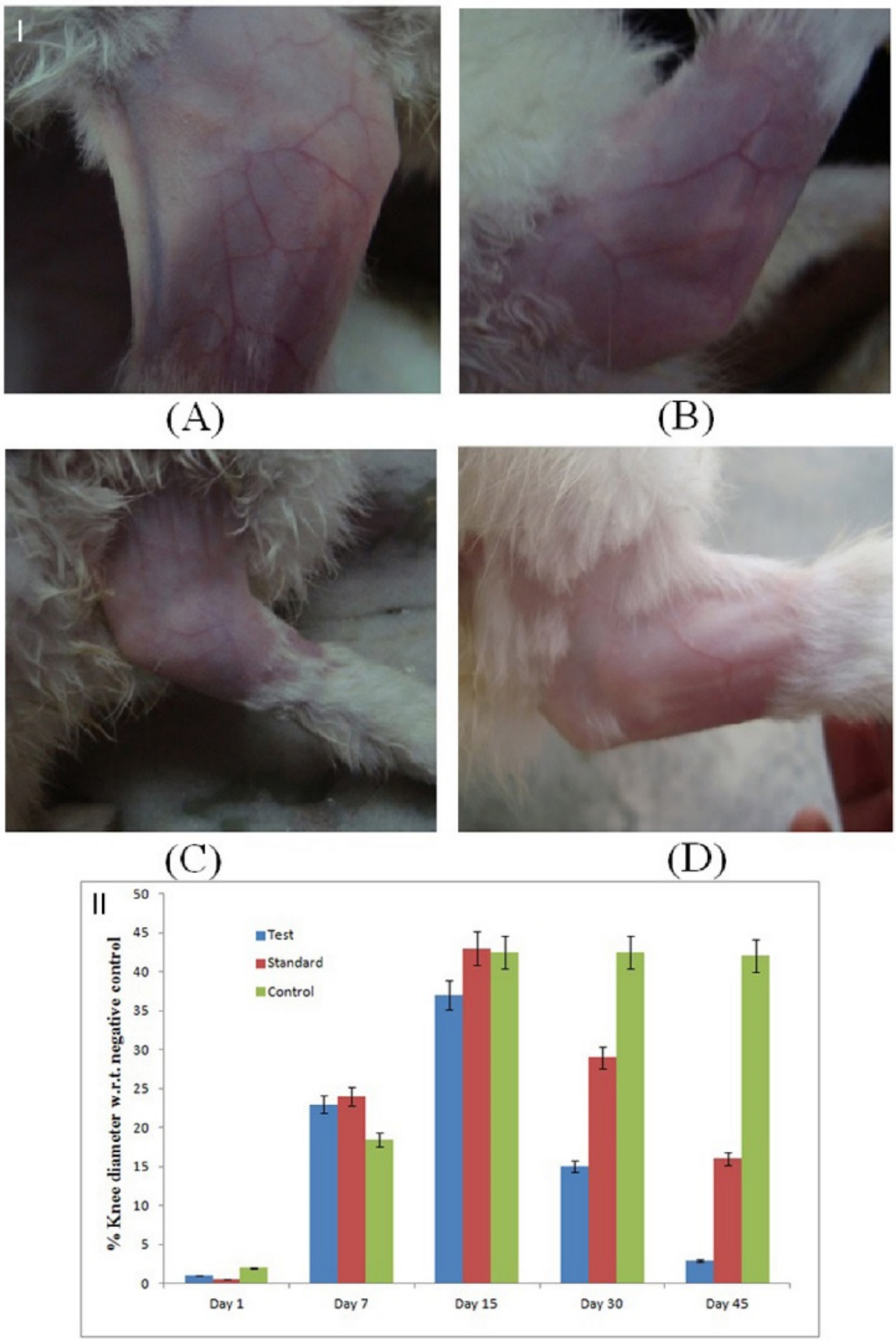

Figure 5: I: (A) and (B) shows knees after inflammation and (C) and (D) shows knees after treatment. II: Bar graph indicating inflammatory responses of various animal groups w.r.t. negative control, values mentioned in mean±SD, *n*=3.

Extrudability

The extrudability of optimized MTX-NLC gel was found to be 7.05 g/cm^2 on applying a weight of 100 g.

Ex vivo Skin Permeation Study through Goat Knee Skin

Ex vivo skin permeation study of gel formulation was performed for 24 hr and release was found sustained, as displayed in Figure 4(B).

Skin irritation test

The individual skin scores and the Primary Dermal Irritation (PDI) index of the MTX NLCs gel formulations were as mentioned in Table 4.

Antiarthritic study

Maximum inflammation was found at day 15, as indicated in Figure 5(I) (A) and (B) for various rabbits. The maximum arthritic score was seen in all groups between the second and third weeks. The formulation application for the test and standard groups began on the 15th day. The diameter of the joint was observed to be reduced after the formulation was applied, as shown in Table 5 and Figure 5(I) (C) and (D). Non-treated animals (control group) continued almost as intensely till the end of the research. Figure 5(II) shows the % knee diameter of the control and treatment groups in relation to the negative control group at various time intervals. After the formulations were applied (15th day), the knee diameter of the treated groups was significantly decreased. In the test group, Rabbit 1 showed a reduction in the inflamed diameter from 7.6 mm to 5.7 mm, Rabbit 2 showed a reduction from 6.9 mm to 5.3 mm and Rabbit 3 showed a reduction from 7.2 mm to 5.3 mm, all towards the normal diameter (Table 5). The reduction in knee diameter was greater in the test group than in the control group. The positive control group, on the other hand, showed no significant decrease in knee diameter.

Stability

Under all test situations, the physical features of the delivery mechanism remain unchanged. Furthermore, stability experiments demonstrated that MTX-NLCs were stable at 5±0.5°C and room temperature for 90 days, with no significant variations in the formulation's residual drug content or particle size.

DISCUSSION

Drug solubility in lipids must be high for increased drug entrapment effectiveness, necessitating research into drug solubility in diverse lipids. The drug MTX dissolved completely in cetyl alcohol, forming a homogeneous liquid that did not divide into layers upon cold and congealing. Surfactant screening is critical for topical distribution since certain surfactants can induce skin irritation and enhance bacterial translocation. Tween 80 (HLB value 15), a non-ionic surfactant, demonstrated the greatest MTX solubility and was thus chosen as the surfactant. It also gives the delivery mechanism steric stability, resulting in electrostatic repulsion between nanoparticles. Optimising stirring time is also critical since it influences particle size, drug entrapment and production time.[27] The microemulsion process was used to create the MTX-NLCs, which were then optimised using the Taguchi design.

DoE is a powerful statistical technique used to screen and optimize formulations by systematically varying multiple factors and analyzing their effects on desired responses. The Taguchi orthogonal array design, specifically used in this study, is a type of DoE that allows for the investigation of critical factors with a minimal number of experiments. In this study, the Taguchi L9 orthogonal array design was employed to get an optimized formulation of methotrexate MTX loaded NLCs. The main effects of each factor on the responses were analyzed using S/N ratios. The S/N ratio measures the variability in the response due to uncontrolled factors, with a higher S/N ratio indicating a more robust formulation. The optimal levels of each factor were determined based on the highest S/N ratios for the desired responses. ANOVA was used to determine the significance of each factor on the responses. The ANOVA results provided information on the percentage contribution of each factor to the overall variability in the responses.

By using DoE, the researchers identified the critical factors affecting the performance of the MTX-NLCs and optimized the formulation with a minimal number of experiments. This approach is more efficient and cost-effective compared to the traditional one-factor-at-a-time method, where only one factor is varied while others are kept constant. All-in-all, DoE is a valuable tool for formulation screening and optimization as it allows for the systematic investigation of multiple factors and their interactions, leading to the development of robust and reliable pharmaceutical products.

Even though all the studies carried out as planned showed good results for particle size, EE and drug release, the optimised batch was discovered using statistical techniques. Therefore, the S/N for the dependent responses Y1, Y2 and Y3 for each trial was computed and analysed to determine the relevance of each element to select the optimum formulation. The S/N ratio measures the dependability of data derived from the effect of certain independent variables or factors on dependent answers. The S/N ratio is an appropriate analytical criterion for determining the optimal level for each experimental condition. The sort of S/N ratio used is determined by the desired attribute. The "main effects plots" were also generated by plotting the S/N ratio for each component on the y-axis and the levels on the x-axis. These graphics illustrate how various variables influence S/N ratios over a variety of responses. The optimum compromise

Table 5: Arthritic inflammation of joint after carrageenan injection, (diameter in mm), values mentioned in mean±SD, *n*=3.

Test group was treated by prepared NLCs based gel of MTX from day 15 to day 45.	**Rabbit**	**Day 0**	**Day 1**	**Day 2**	**Day 7**	**Day 15**	**Day 30**	**Day 45**
	1	5.6	5.6	5.7	6.8	7.6	6.1	5.7
	2	5.1	5.1	5.1	6.1	6.9	6.2	5.3
	3	5.3	5.3	5.3	6.6	7.2	5.9	5.3
Mean±SD, *n*=3		5.3± 0.2	5.3±0.2	5.4±0.2	6.5±0.3	7.2 ±0.2	6.1±0.3	5.4± 0.2
Standard group was treated by standard preparation of MTX from day 15 to day 45.	**Rabbit**	**Day 0**	**Day 1**	**Day 2**	**Day 7**	**Day 15**	**Day 30**	**Day 45**
	1	5.2	5.2	5.1	6.2	7.3	6.9	6.0
	2	5.5	5.5	5.7	6.8	7.6	6.9	6.2
	3	5.2	5.2	5.2	6.6	7.8	6.6	6.2
Mean±SD, *n*=3		5.3±0.1	5.3±0.1	5.3±0.2	6.5±0.2	7.6±0.2	6.8±0.1	6.1±0.1
Positive Control group (disease induced, no treatment given).	**Rabbit**	**Day 0**	**Day 1**	**Day 2**	**Day 7**	**Day 15**	**Day 30**	**Day 45**
	1	5.2	5.2	5.3	6.0	7.3	7.3	7.3
	2	5.5	5.5	5.5	6.3	7.6	7.6	7.5
	3	5.5	5.5	5.6	6.5	7.7	7.7	7.7
Mean±SD, *n*=3		5.4±0.1	5.4±0.1	5.5±0.1	6.3±0.2	7.5±0.2	7.5±0.2	7.5±0.1
Negative Control group (treated with normal saline only).	**Rabbit**	**Day 0**	**Day 1**	**Day 2**	**Day 7**	**Day 15**	**Day 30**	**Day 45**
	1	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	2	5.3	5.3	5.3	5.3	5.3	5.3	5.3
	3	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Mean±SD, *n*=3		5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2

between three replies (Y1, Y2 and Y3) was obtained based on the plots to find the level for the best factor combinations (X1, X2 and X3) as an optimised formulation.

The motion of the particles in the dynamically dispersed laser light is used to calculate particle size and zeta potential.[37-39] The dynamic laser particle size analyzer provides the bulk population's average diameter and the PDI value to examine the distribution range of 0.0 to 0.5. When applied to the skin, lipid nanoparticles are said to have occlusion, adhesiveness and skin hydration effects. When the size of the nanoparticles was less than 200 nm, they displayed an adhesive effect by forming a film over the skin. Because of the lipidic nature of this monolayer film, it had an occlusive effect on the skin and retarded moisture loss, resulting in evaporation inhibition and loosening of corneocyte packing and opening of inter-corneocyte gaps, allowing for deeper penetration of the drug into the skin.[40,41]

As seen in Figure 2(I), the S/N ratio for particle size falls as the concentration of solid lipids increases. The increase in particle size with the increase in solid lipid content was caused by an increase in dispersion viscosity, which causes a stronger resistance to emulsification when introduced to the external phase and results in the generation of bigger particles. Furthermore, higher viscosity inhibits the dispersion of the lipid phase in water from getting finer during mechanical stirring. The S/N ratio is greatest at high surfactant concentrations, suggesting a favourable reaction. This demonstrates that the lowest particle size was at the greatest surfactant level because it promoted particle coating and stabilisation.[26] Charged particles with high zeta potentials (-20 mV to -50 mV) are less likely to aggregate due to electric repulsion. This is consistent with the broader literature, which states that increasing zeta potential leads to improved physical stability. Aggregation is facilitated by lower zeta potential. The impact plot (Figure 2(II)) shows that the highest levels of entrapment were seen at medium levels of surfactant concentration and SL:LL ratio (high S/N ratio at intermediate levels of SL:LL signals increased entrapment). However, the drug-to-lipid ratio and the kind of liquid lipid have no discernable effect on drug entrapment.

The results revealed that as the Solid Lipid (SL) concentration increased, it led to a higher viscosity of the nanoparticle dispersion, which in turn delayed the drug release. This effect was more pronounced as the solid lipid concentration was raised. Furthermore, the data showed that greater drug release was observed at lower and intermediate SL:LL (liquid lipid) ratios. Additionally, higher drug:lipid ratios (resulting in higher S/N ratios) and higher surfactant concentrations (also leading to higher S/N ratios) were found to enhance the drug release from the nanoparticle formulations. These findings suggest that the balance between the solid and liquid lipid components, as well as the optimization of the drug:lipid ratio and surfactant concentration, are critical factors in modulating the drug release kinetics from the nanoparticle systems. However, whereas formulations including canola oil (NLC5, NLC7 and NLC8) indicated lower drug release as measured by the S/N ratio, liquid

lipids (almond oil and castor oil) had similar effects. The S/N ratio was used to evaluate and statistically analyse the responses to the Taguchi L9 array design formulation experiments. S/N ratios were submitted to an ANOVA analysis to establish the significance of each factor's effect on individual responses. The drug:lipid ratio and surfactant concentration were the next two most important parameters for particle size after the SL:LL, according to the S/N ratio and degree of variation (Table 3). The SL:LL ratio is the most critical factor in drug entrapment, followed by particle size and surfactant concentration. This is because the liquid lipid lowers the index of crystallinity of the mixture's solid lipids, resulting in vacuum areas where more drugs can be trapped. However, surfactant concentration and liquid lipid type had equivalent impacts on drug release in SL:LL. To create an optimised batch, the ideal levels of each element must be determined once their relevance has been established. It was done by looking at "main effect plots" of the S/N ratio of all the components at each level and doing an ANOVA on the variance of each factor.

The optimised batch NLC9 release kinetics followed the Higuchi model, indicating a diffusion-controlled mechanism. Following the Korsmeyer-Peppas, which indicates non-fickian (anomalous) diffusion, drug release includes more than one mechanism, namely diffusion and swelling-determined release.

The Carbopol microgels' rheological behaviour did not alter significantly in the pH range of 5.0-8.0, which is within the permissible range for dermatological preparations. As a result, these gels can be used as excellent dermatological basis for cutaneous applications.

Viscosity measurement has played an important role in the creation of controlled-release preparations, particularly cutaneous preparations. The goal is to determine the flowability, viscosity and elastic properties of the produced dosage form. Viscosity was measured at various shear rates and it was discovered that when shear rate increased from 10 to 100 rpm, viscosity reduced continuously. The shear-thinning behaviour of the MTX-NLC gel is shown here, demonstrating pseudoplastic nature. The gel's viscosity was suitable for a dermal preparation.[42,43] This characteristic is caused by the colloidal network in the polymeric matrix, which aligns itself when shear is applied, resulting in a decrease in viscosity as the rate of shear increases. It may be deduced that the created NLC gel will require a significant amount of force to be withdrawn from the tube. Spreadability is another crucial feature needed for spreading gel uniformly to the skin and making the product appealing, which improves patient compliance. Using adapted apparatus, the spreadability of the gel formulation was assessed, revealing optimal spreadability for cutaneous application. The force required to evacuate the gel from the tube is measured using this approach. When some weight is introduced to a gel formulation, maximum extrudability is required for easy removal from the tube. The extrudability result indicated that the gel may be easily removed with minimum force.[27,36] *Ex vivo* skin permeation study of gel formulation was found sustained for 24 hr. The natural skin surface pH is typically in the range of 4.1-5.8, with some variations across different body sites. However, the use of a pH 6.8 buffer in the receptor compartment of the *ex vivo* skin permeation study was still found physiologically relevant, as it helps maintain the appropriate pH gradient across the skin barrier. The pH 6.8 buffer was used in the receptor compartment to maintain sink conditions for the drug being studied. Maintaining sink conditions is crucial to accurately determining the steady-state flux and permeability coefficients across the skin.[44-46]

In some cases, the pH 6.8 buffers may have been chosen to better mimic the physiological pH conditions that the drug would encounter in vivo after topical application.

The MTX NLCs gel was "non-irritant" (PDI=0.00) to rabbit skin, suggesting that the animals were healthy and active. Aside from skin discomfort, there were no symptoms of aberrant behaviour, unfavourable pharmacologic effects, or gross toxicity. Arthritis has been produced in all animals and is visible at the end of the first week as a result of frequent intra-articular carrageenan injections. After the research was completed (on the 45th day), the data was statistically analysed using Tukey's multiple comparison tests for statistical significance. There was a significant difference ($p<0.05$) between the treated and untreated groups, demonstrating that the administered formulation had a genuine impact. In addition, there was a significant difference ($p<0.05$) in knee diameter between the test and control groups. Nonetheless, there was no significant difference ($p>0.05$) between the test and the negative control group. The stability investigations demonstrated that excellent dispersibility was found without the formation of any precipitate over the period of the experiment. The greater physical stability of the dosage form may be due to the smaller size of NLCs, stronger zeta potential and steric action of Tween 80. There were no significant changes in the appearance, pH, or residual drug content of the MTX-NLC gel. MTX-NLC gel also showed no grittiness, precipitate formation, or phase separation during the research. As a result, it is advised that the created formulations be stored at room temperature or at room temperature for a longer shelf life.

CONCLUSION

Finally, using the microemulsion process, an optimised batch of methotrexate-loaded nanostructured lipid carriers with smaller particle sizes and long-term physical stability was successfully created. The Taguchi design was discovered to be a potential approach for attaining the best conditions while simultaneously minimising the number of experimental sets. The NLC gel provided satisfactory characterization findings. When compared to the usual formulation, the NLC gel was found to be much more efficient in progressively lowering the symptoms of carrageenan-induced arthritis. The optimised NLCs formulation

gel is expected to keep the drug at the location and might be useful for delivering pharmaceuticals to a specific place through the skin. Thus, based on the research, it can be concluded that NLC formulations have the potential to be used as promising delivery methods for the topical application of methotrexate for the treatment of arthritis rather than conventional dose forms.

ETHICS APPROVAL

Institutional Animal Ethical Committee of Pinnacle Biomedical Research Institute, Bhopal, India, no. PBRI/IAEC/PN-16044.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NLCs: Nanostructured lipid carriers; **RA:** Rheumatoid Arthritis; **EE:** Entrapment Efficiency; **SLNs:** Solid Lipid Nanoparticles; **MTX:** Methotrexate; **DMARDs:** Disease-Modifying Antirheumatic drugs; **DOE:** Design of Experiment; **ANOVA:** Analysis of variance; **S/N ratios:** Signal-to-noise ratios; **PDI:** Polydispersity index; **PBS:** Phosphate-Buffered Saline; **SL:LL:** Solid Lipid-Liquid lipid ratio; **D:L:** Drug-Lipid ratio; **LL:** Liquid Lipid.

AUTHORS CONTRIBUTION

MA performed bench-work and wrote the main manuscript, KMK and AP conceptualized, coordinated and supervised the activities. All authors reviewed the manuscript.

REFERENCES

1. Singh S, Sharma N, Behl T, Sarkar BC, Saha HR, Garg K, *et al.* Promising Strategies of Colloidal Drug Delivery-Based Approaches in Psoriasis Management. Pharmaceutics. 2021;13(11):1978. doi: 10.3390/pharmaceutics13111978. PMID: 34834393; PMCID: PMC8623849.
2. Raza K, Singh B, Lohan S, Sharma G, Negi P, Yachha Y, *et al.* Nano-lipoidal carriers of tretinoin with enhanced percutaneous absorption, photostability, biocompatibility and anti-psoriatic activity. Int J Pharm. 2013;456(1):65-72. doi: 10.1016/j.ijpharm.2013.08.019, PMID 23973754.
3. Beloqui A, Solinís MA, Delgado A, Evora C, del Pozo-Rodríguez A, Rodríguez-Gascón A. Biodistribution of nanostructured lipid carriers (NLCs) after intravenous administration to rats: influence of technological factors. Eur J Pharm Biopharm. 2013;84(2):309-14. doi: 10.1016/j.ejpb.2013.01.029, PMID 23461861.
4. Elmowafy M, Al-Sanea MM. Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. Saudi Pharm J. 2021;29(9):999-1012. doi: 10.1016/j.jsps.2021.07.015. Epub 2021 Jul 21. PMID: 34588846; PMCID: PMC8463508.
5. Fan X, Chen J, Shen Q. Docetaxel-nicotinamide complex-loaded nanostructured lipid carriers for transdermal delivery. Int J Pharm. 2013;458(2):296-304. doi: 10.1016/j.ijpharm.2013.10.036, PMID 24177313.
6. Beloqui A, Solinís MÁ, des Rieux A, Préat V, Rodríguez-Gascón A. Dextran-protamine coated nanostructured lipid carriers as mucus-penetrating nanoparticles for lipophilic drugs. Int J Pharm. 2014;468(1-2):105-11. doi: 10.1016/j.ijpharm.2014.04.027, PMID 24746410.
7. Agrawal Y, Petkar KC, Sawant KK. Development, evaluation and clinical studies of acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. Int J Pharm. 2010;401(1-2):93-102. doi: 10.1016/j.ijpharm.2010.09.007, PMID 20858539.
8. Tian Z, Yi Y, Yuan H, Han J, Zhang X, Xie Y, *et al.* Solidification of nanostructured lipid carriers (NLCs) onto pellets by fluid-bed coating: preparation, *in vitro* characterization and bioavailability in dogs. Powder Technol. 2013;247:120-7. doi: 10.1016/j.powtec.2013.07.010.
9. Zheng M, Falkeborg M, Zheng Y, Yang T, Xu X. Formulation and characterization of nanostructured lipid carriers containing a mixed lipids core. Colloids Surf a Physicochem Eng Aspects. 2013;430:76-84. doi: 10.1016/j.colsurfa.2013.03.070.
10. Ranpise NS, Korabu SS, Ghodake VN. Second generation lipid nanoparticles (NLC) as an oral drug carrier for delivery of lercanidipine hydrochloride. Colloids Surf B Biointerfaces. 2014;116:81-7. doi: 10.1016/j.colsurfb.2013.12.012, PMID 24445002.
11. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int J Pharm. 2008;346(1-2):124-32. doi: 10.1016/j.ijpharm.2007.05.060, PMID 17651933.
12. Elmowafy M, Al-Sanea MM. Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. Saudi Pharm J. 2021;29(9):999-1012. doi: 10.1016/j.jsps.2021.07.015. Epub 2021 Jul 21. PMID: 34588846; PMCID: PMC8463508.
13. Chauhan I, Yasir M, Verma M, Singh AP. Nanostructured Lipid Carriers: A Groundbreaking Approach for Transdermal Drug Delivery. Adv Pharm Bull. 2020;10(2):150-65. doi: 10.34172/apb.2020.021. Epub 2020 Feb 18. PMID: 32373485; PMCID: PMC7191226.
14. Tang CH, Chen HL, Dong JR. Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) as Food-Grade Nanovehicles for Hydrophobic Nutraceuticals or Bioactives. Appl. Sci. 2023;13:1726. https://doi.org/10.3390/app13031726.
15. Robbins CK. Basic pathology. 7th ed. New Delhi: Harcourt Publishers India Pvt Ltd; 2001. p. 285-6.
16. Chauhan K, Jandu JS, Brent LH, *et al.* Rheumatoid Arthritis. [Updated 2023 May 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441999/
17. Zhou HF, Yan H, Senpan A, Wickline SA, Pan D, Lanza GM, *et al.* Suppression of inflammation in a mouse model of rheumatoid arthritis using targeted lipase-labile fumagillin prodrug nanoparticles. Biomaterials. 2012;33(33):8632-40. doi: 10.1016/j.biomaterials.2012.08.005, PMID 22922023.
18. Hanoodi M, Mittal M. Methotrexate. [Updated 2023 Aug 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556114/
19. Hamed KM, Dighriri IM, Baomar AF, Alharthy BT, Alenazi FE, Alali GH, *et al.* Overview of Methotrexate Toxicity: A Comprehensive Literature Review. Cureus. 2022;14(9):e29518. doi: 10.7759/cureus.29518. PMID: 36312688; PMCID: PMC9595261.
20. Butoescu N, Jordan O, Doelker E. Intra-articular drug delivery systems for the treatment of rheumatic diseases: a review of the factors influencing their performance. Eur J Pharm Biopharm. 2009;73(2):205-18. doi: 10.1016/j.ejpb.2009.06.009, PMID 19545624.
21. Khurana S, Bedi PM, Jain NK. Preparation and evaluation of solid lipid nanoparticles based nanogel for dermal delivery of meloxicam. Chem Phys Lipids. 2013;175-176:65-72. doi: 10.1016/j.chemphyslip.2013.07.010, PMID 23994283.
22. Schnabel A, Gross WL. Low-dose methotrexate in rheumatic diseases-efficacy, side effects and risk factors for side effects. Semin Arthritis Rheum. 1994;23(5):310-27. doi: 10.1016/0049-0172(94)90027-2, PMID 8036521.
23. Bhowmik BB, Nayak BS, Chatterjee A. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. Int J Pharm Pharm Sci. 2009;1(1):240-57.
24. Javadzadeh Y, Hamishehkar H. Enhancing percutaneous delivery of methotrexate using different types of surfactants. Colloids Surf B Biointerfaces. 2011;82(2):422-6. doi: 10.1016/j.colsurfb.2010.09.015, PMID 20951009.
25. Webb CJ, Waters GT, Thomas AJ, Liu GP, Thomas C. The use of the Taguchi design of experiment method in optimizing splicing conditions for a Nylon 66 yarn. J Text Inst. 2007;98(4):327-36. doi: 10.1080/00405000701489255.
26. Alam T, Khan S, Gaba B, Haider MF, Baboota S, Ali J. Adaptation of quality by design-based development of isradipine nanostructured-lipid carrier and its evaluation for *in vitro* gut permeation and *in vitro* solubilization fate. J Pharm Sci. 2018;107(11):2914-26. doi: 10.1016/j.xphs.2018.07.021, PMID 30076853.
27. Fatima N, Rehman S, Nabi B, Baboota S, Ali J. Harnessing nanotechnology for enhanced topical delivery of clindamycin phosphate. J Drug Deliv Sci Technol. 2019;54:101253. doi: 10.1016/j.jddst.2019.101253.
28. Csányi E, Bakonyi M, Kovács A, Budai-Szűcs M, Csóka I, Berkó S. Development of topical nanocarriers for skin cancer treatment using quality by design approach. Curr Med Chem. 2019;26(35):6440-58. doi: 10.2174/0929867325666181116143713, PMID 30444194.
29. Sonam H, Chaudhary H, Kumar V. Taguchi design for optimization and development of antibacterial drug-loaded PLGA nanoparticles. Int J Biol Macromol. 2014;64:99-105. doi: 10.1016/j.ijbiomac.2013.11.032, PMID 24315945.
30. Misra A, Kalariya M, Padhi BK, Chougule M. Methotrexate-loaded solid lipid nanoparticles for topical treatment of psoriasis: formulation and clinical implications. Drug Deliv Technol. 2004;4:8.
31. Porwal A, Dwivedi H, Pathak K. Gastroretentive bilayer film for sustained release of atorvastatin calcium and immediate release of amlodipine besylate: pharmaceutical, pharmacokinetic evaluation and IVIVC. Pharm Dev Technol. 2020;25(4):416-31. doi: 10.1080/10837450.2019.1705486, PMID 31852330.
32. Pivetta TP, Silva LB, Kawakami CM, Araújo MM, Del Lama MPFM, Naal RMZG, *et al.* Topical formulation of quercetin encapsulated in natural lipid nanocarriers: evaluation of biological properties and phototoxic effect. J Drug Deliv Sci Technol. 2019;53:101148. doi: 10.1016/j.jddst.2019.101148.

33. Joshi M, Patravale V. Formulation and evaluation of nanostructured lipid carrier (NLC)-based gel of valdecoxib. Drug Dev Ind Pharm. 2006;32(8):911-8. doi: 10.1080/03639040600814676, PMID 16954103.
34. Bhalekar M, Upadhaya P, Madgulkar A. Formulation and evaluation of adapalene-loaded nanoparticulates for epidermal localization. Drug Deliv Transl Res. 2015;5(6):585-95. doi: 10.1007/s13346-015-0261-z, PMID 26483036.
35. Mutimer MN, Riffkin C, Hill JA, Glickman ME, Cyr GN. Modern ointment base technology II. Comparative evaluation of bases. J am Pharm assoc am Pharm assoc. J Am Pharm Assoc Am Pharm Assoc. 1956;45(4):212-8. doi: 10.1002/jps.3030450406, PMID 13319092.
36. Madan J, Singh R. Formulation and evaluation of Aloe vera topical gels. Int J Phys Sci. 2010;2(2):551-5.
37. Porwal A, Dwivedi H, Pathak K. Predicting pharmacokinetic parameters by convolution: an *in vitro* approach for investigating bifunctional capsulated dosage form. J Drug Deliv Sci Technol. 2020;60:102078. doi: 10.1016/j.jddst.2020.102078.
38. Kanoujia J, Kushwaha PS, Saraf SA. Evaluation of gatifloxacin pluronic micelles and development of its formulation for ocular delivery. Drug Deliv Transl Res. 2014;4(4):334-43. doi: 10.1007/s13346-014-0194-y, PMID 25787066.
39. Sharma G, Thakur K, Raza K, Singh B, Katare OP. Nanostructured lipid carriers: a new paradigm in topical delivery for dermal and transdermal applications. Crit Rev Ther Drug Carrier Syst. 2017;34(4):355-86. doi: 10.1615/CritRevTherDrugCarrierSyst.2017019047, PMID 29199589.
40. Desai P, Patlolla RR, Singh M. Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol Membr Biol. 2010;27(7):247-59. doi: 10.3109/09687688.2010.522203, PMID 21028936.
41. Schneider M, Stracke F, Hansen S, Schaefer UF. Nanoparticles and their interactions with the dermal barrier. Dermato-Endocrinology. 2009;1(4):197-206. doi: 10.4161/derm.1.4.9501, PMID 20592791.
42. Puro D, Athawale R, Pandya A. Design, optimization and characterization of nanostructured lipid carriers of raloxifene hydrochloride for transdermal delivery. NANOASIA. 2020;10(1):57-67. doi: 10.2174/2210681208666181106124337.
43. Singh SK, Banala VT, Gupta GK, Verma A, Shukla R, Pawar VK, *et al.* Development of docetaxel nanocapsules for improving *in vitro* cytotoxicity and cellular uptake in MCF-7 cells. Drug Dev Ind Pharm. 2015;41(11):1759-68. doi: 10.3109/03639045.2014.1003220, PMID 25686725.
44. Segger, D, Aßmus, U, Brock, M, Erasmy, J, Finkel, P, Fitzner, A, *et al.* Multicenter study on measurement of the natural pH of the skin surface. International Journal of Cosmetic Science, 2008;30(1):75.
45. Lambers, H, Piessens, S, Bloem, A, Pronk, H, and Finkel, P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. International journal of cosmetic science, 2006;28(5):359-70.
46. Varela-Fernández, R, García-Otero, X, Díaz-Tomé, V, Regueiro, U, López-López, R, González-Barcia, M, *et al.* Lactoferrin-loaded nanostructured lipid carriers (NLCs) as a new formulation for optimized ocular drug delivery. International Journal of Pharmaceutics, 2022;615:121487.

Cite this article: Arya M, Porwal A, Kymonil KM. Nanostructured Lipid Carriers Based Gel of Methotrexate for Rheumatoid Arthritis: Development, Characterization and Optimization through Taguchi Design. Indian J of Pharmaceutical Education and Research. 2025;59(2s):s580-s522.