Ind. J. Pharm. Edu. Res., 2026; 60(4s):s1795-s1806.
https://www.ijper.org

Original Article

Optimization and Characterization of a Tofacitinib Citrate-Loaded Dermal Nanoemulgel for Rheumatoid Arthritis

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ABSTRACT

Aim: Rheumatoid Arthritis (RA) is a progressive autoimmune disorder characterized by joint inflammation and functional disability. Conventional oral therapy with tofacitinib citrate exhibits poor bioavailability as it undergoes extensive hepatic metabolism, and is associated with systemic adverse effects. The present research proposes a novel topical nanoemulgel formulation of tofacitinib to overcome the drawbacks of conventional therapy. **Materials and Methods:** Nanoemulsion was prepared with eucalyptus oil, tween 20, and labrafil M2125. A statistical design was employed to optimize drug loading and particle size. The optimized nanoemulsion was incorporated into a Carbopol 934 gel base and evaluated for physicochemical properties, rheology, spreadability, *ex vivo* permeation, and *in vivo* antiarthritic activity. **Results:** The optimized nanoemulsion exhibited a mean particle size of 180 nm, drug loading of ~71%. Surface morphology by TEM confirmed formation of stable nanosized vesicles. The nanoemulgel exhibited favourable viscosity, pH, spreadability, and sustained release profile. *Ex vivo* studies confirmed enhanced permeation compared to pure drug gel. *In vivo* results demonstrated reduced paw edema, improved mobility, and near-normal histological architecture. **Conclusion:** Therefore, nanoemulagel of tofacitinib citrate can be a promising alternative to oral therapy in RA management.

Keywords: Antiarthritic Activity, Inflammation, Nano Emulsion, Nanoemulgel, Tofacitinib Citrate, Transdermal Permeation.

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Received: 18-04-2026;
Revised: 03-06-2026;
Accepted: 27-08-2026.

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disorder that affects the joints; if left untreated, can cause severe damage to the joints and surrounding tissues. It is categorized as an autoimmune disorder that mistakenly attacks the body tissues. In severe cases, it can lead to heart, lung, or nervous system problems.[1] As per WHO, 18 million people worldwide are living with rheumatoid arthritis, among them 70% of people are women, and 55% are older than 55 years.[2]

Tofacitinib citrate is an inhibitor of Janus kinases, reported to be highly safe and effective in the treatment of arthritis over a long period.[3] It is approved by the FDA for the treatment of moderate to severe rheumatoid arthritis. The drug belongs to BCS III. It is available in the market as a tablet for oral use in a dose range of 5-22 mg, administered once or twice a day depending on the severity of the disease. Following an oral administration of the drug, peak serum concentration reaches within 0.5 and 3 hr, followed by rapid elimination.[4] The drug is extensively metabolized in the liver by CYP3A4 and CYP2C19 enzymes to its inactive metabolite and also gets eliminated through the kidneys as an unchanged drug. The half-life of the drug is reported to be 3 hr. It is reported that steady state concentration reaches in 1- 2 days with the conventional dosage regimen.[5] The drug has a high volume of distribution which is the reason for serious side effects like sudden shortness of breath, stomach pain, upper respiratory tract infections, headache, etc.[5] Therefore, to overcome the limitations of conventional oral therapy, a novel topical delivery of the drug in a colloidal particulate system is proposed for topical delivery.

Nanoemulsions (NEs) are colloidal dispersions made up of two immiscible liquids, where one phase is distributed as nanometer-sized droplets within the other and stabilized by surfactants.[6] Their nanoscale dimensions confer unique attributes such as optical clarity, large interfacial area, improved stability, and modifiable flow behavior. These features enable their wide use in areas including medicine, food technology, cosmetics, and

DOI: 10.5530/ijper.20265544

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material sciences.[7] In healthcare, NEs are particularly attractive as carriers for drugs and imaging agents because of their favorable properties like low toxicity, efficient drug encapsulation, enhanced permeation, thermodynamic stability, and excellent biocompatibility and biodegradability. Topical delivery to periarticular tissues is constrained by the stratum corneum and underlying barriers that limit drug access to the synovium. Nanoscale systems enhance follicular transport via intercellular, transcellular, and trans appendageal pathways

NEs are heterogeneous systems of 2 immiscible liquids that consist of a fine dispersion of drugs in nanodroplets. They remain both thermodynamically and kinetically stable, showing no signs of flocculation or coalescence even after prolonged storage.[6] In NEs, the deformability of the droplets is dependent on the structure and composition of the droplet interface.[8] These unique behaviors of NEs make them suitable for transdermal application. Previous studies have demonstrated that topical NSAIDs can achieve measurable concentrations in synovial fluid, supporting their therapeutic potential in joint disorders Researchers have explored the ability of NEs for deeper penetration of drugs through the skin.[9-11] The hypothesis of the study lies on the fact that the developed nanoemulgel will enhance local drug delivery through combined intercellular and trans follicular pathways, improving periarticular drug levels and minimizing systemic exposure.

There is limited evidence on tofacitinib delivery through topical nanoemulsion systems for localized rheumatoid arthritis therapy. This study addresses that gap by designing a dermally applied nanoemulsion aimed at enhancing site-specific drug concentration while potentially minimizing systemic exposure and associated adverse effects. Hence, the present study aimed to develop a topical nanoemulsion of tofacitinib for dermal application and to evaluate its efficacy in arthritic animals.

MATERIALS AND METHODS

Tofacitinib citrate was generously provided by Viatris, Hyderabad. Transcutol HP, Labrafil M2125 were procured from Gattefosse, Mumbai, while all other analytical grade chemicals were obtained from SD Fine Chemicals, Bengaluru.

Solubility Studies of the Drug in Oil, Surfactant, and Cosurfactant

Drug solubility was determined in various oils -castor, eucalyptus, and olive oil; surfactants- Transcutol HP, tween 80, tween 20, and span 80; cosurfactants-labrafil M2125, propylene glycol, ethanol, and glycerol. A quantity of 10 mg tofacitinib citrate was dispersed in 2 mL of each component in Eppendorf tubes and was kept for 24 hr in a mechanical stirrer at room temperature.[12] An aliquot of the sample was withdrawn, diluted suitably with phosphate buffer pH 7.4, and analyzed using UV spectrometry. A standard curve was developed earlier at 288 nm, with a linearity of y=0.0276x + 0.015 and a regression coefficient of R^2=0.999. The solubility was determined by using the following formula

$$Solubility = \frac{absorbance}{slope} \times \frac{dilution}{1000}$$

Preformulation study for the selection of factors and responses

The objective of the topical nanoemulgel is primarily to achieve high local drug concentrations at periarticular tissues underlying the skin over inflamed joints, thereby providing targeted anti-inflammatory action with reduced systemic exposure. Therefore, the intended outcome is predominantly local symptomatic relief and suppression of joint inflammation.

Nanoemulsion-based gels are known to enhance dermal and transdermal permeation and the presence of penetration-enhancing surfactants, enabling drugs to reach deeper tissues including synovial regions.

A knowledge space with Ishikawa diagram was created to identify the influencing factors of nanoemulsion formulation. The Ishikawa diagram is shown in Figure 1. A systematic preformulation study was carried out to evaluate the influence of formulation and process parameters-namely Hydrophilic-Lipophilic Balance (HLB), Phase Volume Ratio (PVR), Homogenization Time (HTIME), Ultrasonication Power (UPOWER), and Ultrasonication Time (UTIME)-on critical responses such as particle size and percentage drug loading. Since the drug under investigation is hydrophilic, achieving drug loading in an Oil-in-Water (o/w) nanoemulsion was challenging. To address this, different surfactant-co-surfactant combinations were explored to obtain the required HLB (RHLB), optimized in the range of 4-12 depending on drug solubility. The PVR was varied from 0.3 to 0.6 to assess its effect on emulsion stability, as a stable o/w system is desirable for developing an emulgel suitable for topical application. Other formulation variables, including the oil, surfactant, and co-surfactant composition, were fixed following preliminary solubility screening and therefore were not included in the optimization study.

Process parameters were also considered critical: homogenization time (5-10 min) and power (25-100 Hz) considerably influence globule size and emulsion stability. Ultrasonication power and time were examined for their role in refining particle size and improving drug loading. Taken together, RHLB, PVR, HTIME, UPOWER, and UTIME were identified as the critical formulation variables governing drug loading and vesicle characteristics. A two-Factor Interaction (2FI) study was used to explore complex interactions between variables and observe their combined impact on the dependent variables.[13]

Design of experiments

A Two-Factor Interaction (2FI) factorial design was employed using Design Expert version 13. The design generated 15

experimental runs, including three center points to reduce bias and improve reliability. The independent variables were coded at two levels, ranging from low (-1) to high (+1). The dependent variables considered were percentage drug loading (to be maximized) and particle size (to be minimized), which served as the key targets during model optimization. The design matrix with factor levels and corresponding responses is presented in Table 1.

Preparation of nanoemulsion

Based on the solubility outcomes from preformulation studies, a nanoemulsion containing 10 mg of tofacitinib citrate was developed using the selected oil, surfactant, and co-surfactant. The oil phase, containing 25% v/v of eucalyptus oil and Labrafil M2125, was mixed. The drug was dissolved in Tween 20. This surfactant solution was then added to the oil phase under stirring to form a primary emulsion. Subsequently, the calculated volume of aqueous phase was introduced dropwise to form the coarse o/w emulsion, which was then homogenized and sonicated as per the optimized parameters. Surfactant and cosurfactant concentration was determined through allegation method as per the RHLB. This coarse emulsion was further refined using a high-speed homogenizer, with Homogenization Time (HTIME) varied according to the experimental design. The resulting emulsion was subsequently subjected to Ultrasonication, Where Sonication Power (UPOWER) and sonication time (UTIME) were optimized as per the design specifications outlined in Table 2. The amounts of surfactant and co-surfactant were determined based on the Required Hydrophilic-Lipophilic Balance (RHLB), while the oil and aqueous phase volumes were adjusted according to the designated Phase Volume Ratio (PVR).[14]

Evaluation of nanoemulsion

Determination of particle size

The globule size of the nanoemulsion formulations was measured using a Horiba SZ-100 nanoparticle analyzer. A small aliquot of the sample was diluted with millipore water and sonicated for 2 min to achieve uniform dispersion. The globule size was determined by the principle of Dynamic Light Scattering (DLS), backscatter detection at 173° at 25°C which evaluates fluctuations in light scattering intensity arising from the Brownian motion of particles. All measurements were performed in triplicate to ensure accuracy and reproducibility.[15]

Determination of drug loading

The drug loading of the nanoemulsion was evaluated by disrupting the formulation with an equal volume of methanol in an Eppendorf tube. The resulting mixture was centrifuged at room temperature for 10 min. After centrifugation, a clear supernatant layer was collected, appropriately diluted with phosphate buffer (pH 7.4), and analyzed for drug content using UV-visible spectrophotometry. All measurements were performed in triplicate to ensure accuracy and reproducibility.[14-16]

Determination of Zeta Potential

The optimized nano emulsion was diluted with Millipore water and zeta potential was determined at 25°C using Horiba SZ-100 analyzer.

Transmission Electron Microscopy (TEM)

TEM analysis of the optimized nanoemulsion was performed using the FEI Tecnai 12 instrument (Make: FEI, Model: Tecnai 12, Country: The Netherlands). The imaging was carried out at room temperature

Preparation of nanoemulgel

The gel formulation was prepared using carbopol 934 as the gelling. A predetermined quantity of carbopol 934 (0.5%, w/w) was weighed and dispersed in purified water, allowing it to soak overnight for complete hydration. The dispersion was then stirred using a magnetic stirrer and neutralized with triethanolamine to form a clear and consistent gel base of pH 5.5-6.5. Subsequently, the tofacitinib citrate-loaded nanoemulsion (5mg equivalent tofacitinib citrate/mL) was incorporated gradually into the gel

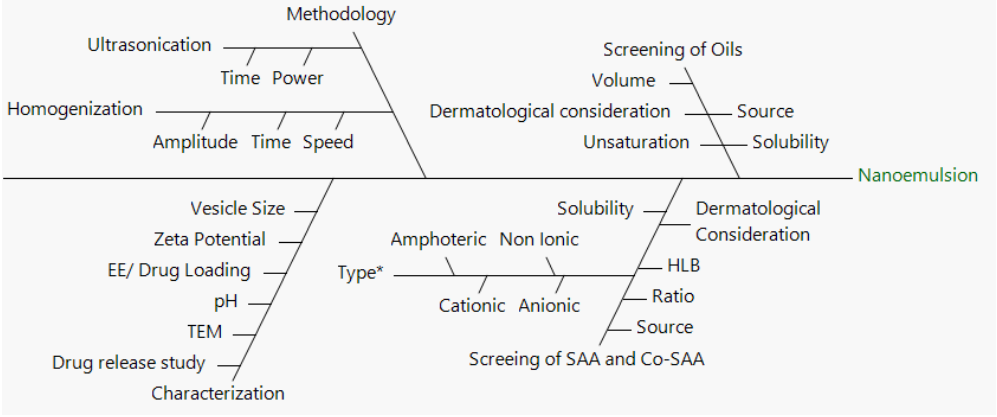


Figure 1: Ishikawa Diagram for formulation of nanoemulsion. Cause and Effect Diagram.

base (10%v/v) under continuous stirring to ensure uniform distribution, resulting in the formation of a homogeneous nanoemulgel.[17]

Evaluation of gel

Appearance

The tofacitinib citrate-loaded nanoemulgel was tested for appearance, odour, and feel upon application such as thickness, smoothness, stiffness, and greasiness.

pH

To evaluate the pH of the nanoemulgel, approximately 0.5 g of the formulation was accurately weighed into a 200 mL glass beaker, and the volume was made up to 100 mL with distilled water. The mixture was stirred gently to ensure uniform dispersion. A digital pH meter, previously calibrated with phosphate buffer, was used to measure the pH. Readings were taken in triplicate, and the average value was recorded as the final pH of the formulation.

Viscosity

The viscosity of the optimized nanoemulsion was determined using a Brookfield viscometer. A small quantity of the formulation was transferred into a clean beaker and allowed to stabilize at room temperature for 30 min to ensure thermal equilibrium.[18] The measurement was conducted at various rotational speeds (180-600 rotations per sec), using LV2 spindle and the viscosity value was recorded at 32±0.1°C.[19] The rheological behavior of the gel was further evaluated for consistency and flow index using the following equation

$$\tau = k r^n$$

Where τ= shear stress, r=shear rate, k=consistency index, and n=flow index. A log graph was plotted (Log τ vs. Log r), from the slope of the linear portion and intercept, flow and consistency index were determined, respectively

Spreadability

The spreadability of the formulation was evaluated by placing a known quantity of gel between two glass slides. A weight of approximately 100 g was carefully placed on the upper slide to facilitate uniform spreading. After allowing sufficient time for the gel to spread, the weight was gently removed, and the length of the spread area was measured.[18]

$$\text{Spreadability} = \frac{mass \times \text{length}}{time}$$

In vitro drug diffusion study

In vitro drug diffusion studies were performed using a dialysis membrane method for pure drug gel and nano gel of tofacitinib in Franz diffusion cell, using dialysis membrane (Hi media-molecular weight cut-off (MWCO) of 12-14 kDa). Prior experimentation the dialysis membrane was soaked in Phosphate buffer pH7.4 for overnight. The receptor cell was filled with 40 mL phosphate buffer pH 7.4 and temperature was maintained at 32±1°C. A quantity of 0.25 g sample from each formulation were studied in triplicate, drug diffusion was measured at a predetermined interval for 24 hr by withdrawing aliquot of sample from the receptor flask and replenishing with equal volume of fresh buffer, measured the amount of drug diffused by spectrophotometrically.[20]

Ex vivo diffusion

The *ex vivo* skin permeation study was performed using excised Wistar albino rat skin, obtained following approval from the Institutional Animal Ethical Committee (Approval No. KCP/IAEC/PCOL/PCEU/145/APR-2024). The animals were euthanized using excess carbon dioxide, adhering to ethical guidelines. The abdominal skin was carefully excised, cleaned thoroughly with saline solution, and subsequently washed with phosphate buffer (pH 7.4) to remove any residual contaminants.

The study was conducted using a Franz diffusion cell, with the receptor compartment containing phosphate buffer pH 7.4 and maintained at 32±1°C to simulate physiological skin conditions. The tofacitinib citrate-loaded nanoemulgel (0.25 g) was applied on the skin and mounted on the donor compartment. The drug diffusion study was carried out for 24 hr and the entire assembly was kept at minimal stirring throughout the experiment.

At specific time points, 2 mL of the medium was collected from the receptor chamber and replenished with an equal volume of fresh buffer and all measurements were performed in triplicate. The collected samples were then analyzed using a UV spectrophotometer at 288 nm to determine the amount of drug permeated through the skin.[21]

The cumulative amount of drug permeated per unit area of skin was plotted against time to evaluate transdermal delivery. The steady-state flux (Jss, expressed in mg/cm²/h) was determined from the slope of the linear segment of this plot. The lag time (Tlag) was obtained by extending the linear portion of the

Table 1: Design Parameters.

Variables	Ranges	
Independent variables	**Low (-1)**	**High (+1)**
RHLB	4	12
PVR	0.3	0.6
HTIME (min)	5	10
UPOWER (Hz)	25	100
UTIME (min)	5	10
Dependent variable	Target to achieve	
Particle size (R1) (nm)	Minimize	
Drug Loading (%) (R^2)	Maximize	

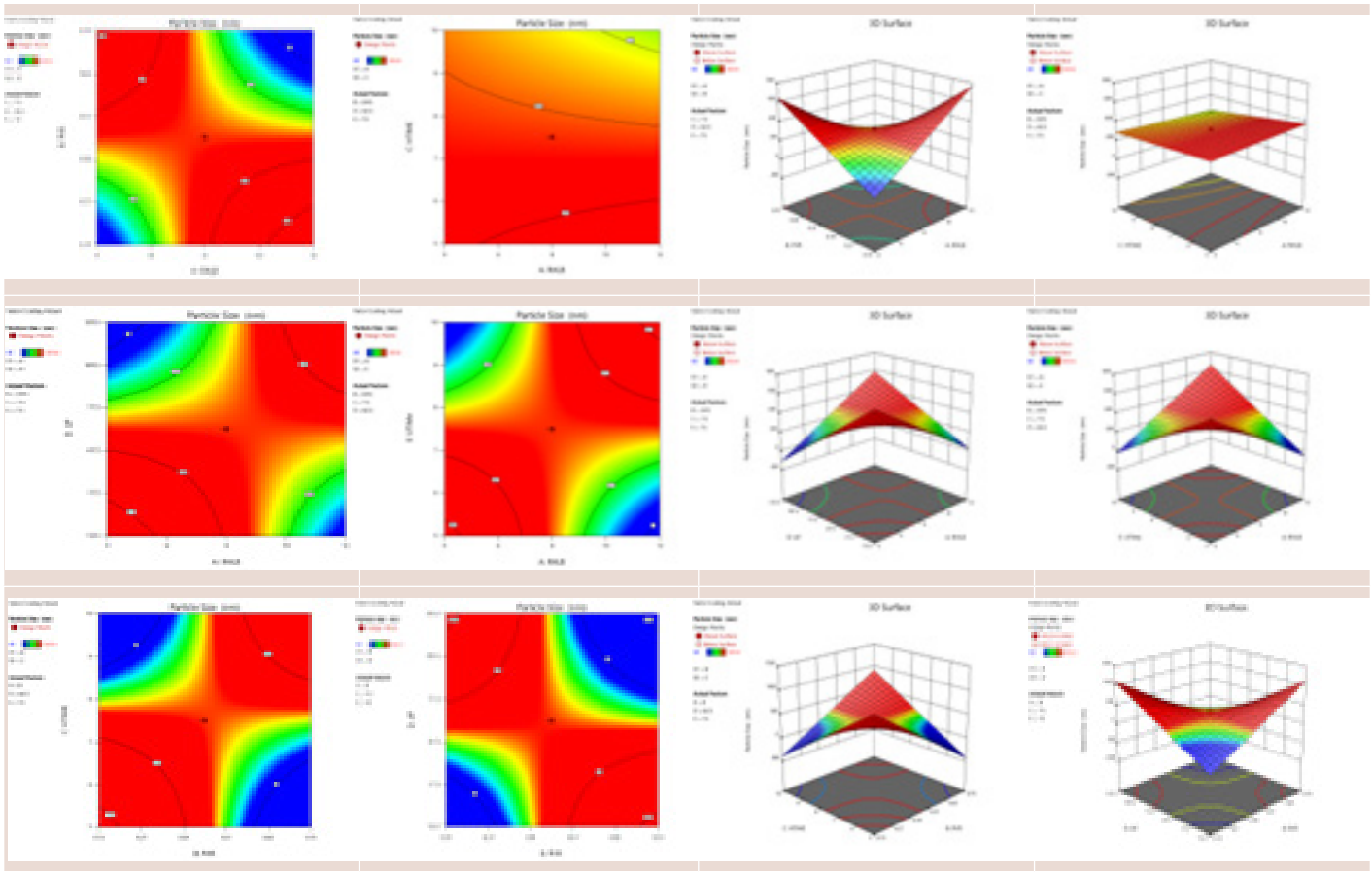


Figure 2: Contour plot and response surface plots for all factors on the response particle size(R1).

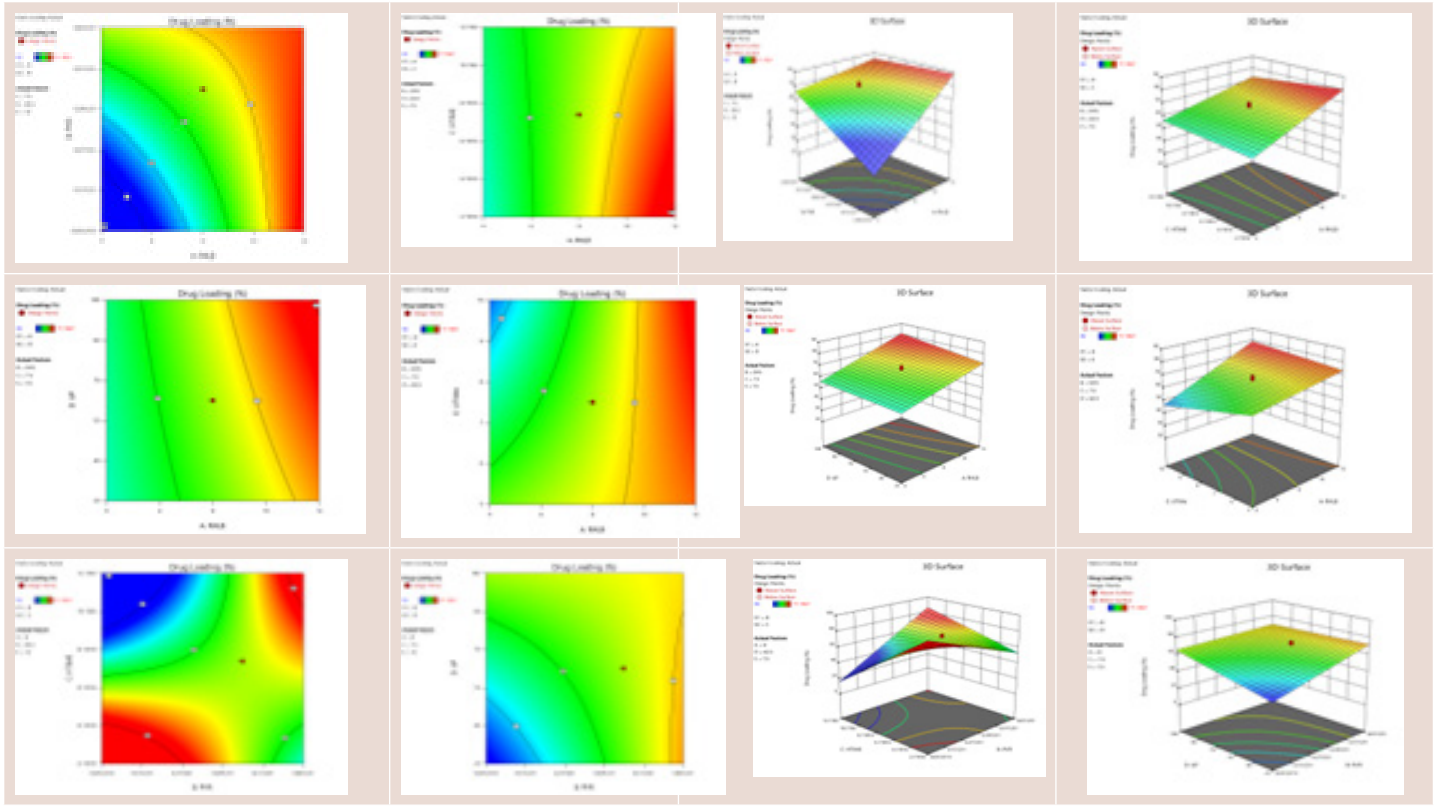


Figure 3: Contour plot and response surface plots for all factors on the response % drug loading(R2).

Table 2: Formulation table.

Formulation code	Factors					Responses	
	RHLB	PVR	HTIME (min)	UPOWER (Hz)	U TIME (min)	Particle Size (nm)±SD (*n*=3)	Drug Loading (%) ±SD (*n*=3)
F1	4	0.6	5	25	10	218.0±24.56	50±1.04
F2	4	0.3	10	25	5	121.6±19.05	43±1.06
F3	12	0.3	10	100	5	100.2±17.21	73±2.04
F4	4	0.3	5	100	10	201.1±31.66	48±1.08
F5	4	0.3	10	100	5	76.3±22.52	52±1.09
F6	4	0.6	10	100	10	68±20.22	62±1.18
F7	12	0.3	10	25	10	112.5±23.4	63±2.23
F8	8	0.45	7.5	62.5	7.5	322.6±37.06	60.8±1.22
F9	4	0.6	5	100	5	71±19.16	62.01±1.24
F10	12	0.6	10	25	5	70.6±18.02	77.19±1.36
F11	12	0.6	5	25	10	221.9±87.22	72±1.35
F12	12	0.6	5	100	10	72.8±16.38	74±2.36
F13	12	0.3	5	25	5	269.5±22.19	76±2.03
F14	8	0.45	7.5	62.5	7.5	302±17.09	67±1.06
F15	8	0.45	7.5	62.5	7.5	312±20.03	69±1.06

cumulative permeation curve back to the time axis (x-axis). The permeation constant was calculated by dividing the Jss by the concentration of drug in the donor compartment. To assess the enhancement effect, the enhancement ratio (E) was calculated by dividing the Jss value of each test formulation by the Jss value of the control formulation.

In vivo study for antiarthritic activity

A total of 24 female albino Wistar rats, each weighing between 200-250 g, were employed in the study. The animals were procured from the Central Animal House of Krupanidhi College of Pharmacy, with ethical approval obtained under Approval No. KCP/IAEC/PCOL/PCEU/145/2024. Environmental conditions were strictly regulated, maintaining a 12-hr light/dark cycle, at a temperature of approximately 24°C and 50% relative humidity. All animals had free access to water and were fed with a standard pellet diet. The animals were divided into four groups: a negative control group (I), a positive control group (II), a standard group treated with oral tofacitinib citrate solution (III), and a test group (IV).[22, 23]

Arthritis was induced in groups II, III, and IV by administering 0.1 mL of Complete Freund's Adjuvant (CFA) into the surface of the left hind paw two days prior the treatment. This induced localized inflammation on the experimental animals. After induction of arthritis, the treatment protocol was initiated and continued for 28 days.

Group II animals were functioned as the positive control group, receiving no therapeutic intervention. Group III animals were given tofacitinib citrate dispersed in water at an oral administration dose of 30 mcg per day. The drug was prepared as a suspension in 0.2% w/v carboxymethylcellulose in distilled water. The formulation was adjusted to a concentration of 0.03 mg/mL, and a fixed volume of 1 mL was administered once daily via oral gavage. Group IV animals underwent topical application of nanoemulgel once daily for 28 days. To ensure a smooth application process, these animals were sedated using mild ether anesthesia before applying the gel and fitted with Elizabethan Collar.

X-ray image Analysis

At the end of the treatment period, rats were weighed, and X-ray images of the affected paws were taken from each group.

Histopathological examination

At the end of the 28-day study, all rats were euthanized using an excess of carbon dioxide. Following euthanasia, tibiotarsal joints were carefully excised from each experimental group and immediately preserved in 10% buffered neutral formalin solution. The collected samples were subsequently processed and were sent for histopathological study at VEMSA BIO-TECH Pvt Ltd, Bengaluru, utilizing an LB-239 digital microscope equipped with camera to facilitate imaging.[24]

RESULTS

Screening of lipid

During preformulation, the solubility of tofacitinib citrate in various oils, surfactants, and co-surfactants was evaluated to optimize nanoemulsion components and ensure stability and efficacy.

The solubility of tofacitinib citrate in different oils was assessed, revealing the highest solubility in eucalyptus oil (0.5531±0.02 mg/mL), followed by castor oil (0.198±0.03 mg/mL) and olive oil (0.094±0.2 mg/mL).

Among surfactants, tween 20 (2.59±0.04 mg/mL) exhibited the highest solubility, followed by Tween 80 (2.3115±0.04 mg/mL), Transcutol HP (2.23±0.01 mg/mL), and Span 80 (1.345±0.02mg/mL).

For cosurfactants, Labrafil M2125 (0.888±0.0005 mg/mL) showed the highest solubility, followed by Propylene glycol (0.463±0.017 mg/mL), Ethanol (0.241±0.003 mg/mL), and Glycerol (0.15±0.003 mg/mL).

Evaluation of nanoemulsion using DoE

The experimental design resulted in 15 trial runs, the observations showed that particle size ranged from 68±10.22 nm to 312±10.03, depending on variations in process intensity and formulation factors as shown in Table 2. Similarly, the percentage of drug loading exhibited a range, fluctuating from as low as 43±0.06% to as high as 77.19±0.06%. The model was analyzed at a significance level $p<0.05$.

The analysis of factors on the responses is listed in Table 3.

Contour graphs and response surface graphs in Figures 2 and 3, depict the relationships between critical factors and responses in the experimental design.

Based on the influence of these critical factors on the response variables, optimization was performed at a significance level of $p<0.05$. The design model predicted optimal conditions at an RHLB value of 8, a Phase Volume Ratio (PVR) of 0.4, a Homogenization Time (HTIME) of 10 min, and sonication power (UP) of 99.83 Hz for 5 min, as shown in Figure 4A. The optimized formulation was formulated, and the bias between the predicted and experimental conditions was found to be less than 5%, indicating good agreement and model reliability of the model as shown in Table 3.

The optimized nanoemulsion exhibited particle size of 180±40.2 nm, zeta potential -25±1.05 mV, % drug loading of 71.28±2.03, viscosity of 35±1 cP at room temperature and a pH of 6.2.

The zeta potential of the optimized product was found to be -29 mV.

Table 3: ANOVA analysis of the responses and evaluation of optimized product.

Re	Particle Size (nm)		Statistical Significance	Drug loading (%)		Statistical Significance
Source	**F-value**	***p*-value**		**F-value**	***p*-value**	
Model	33.96	0.0072	*	12.00	0.0323	*
A-RHLB	0.6528	0.4782		101.35	0.0021	*
B-PVR	30.52	0.0117	*	15.04	0.0304	*
C-HTIME	69.05	0.0037	*	0.1909	0.6917	
D-UP	40.47	0.0079	*	6.57	0.0830	
E-UTIME	2.70	0.1990		6.31	0.0868	
AB	182.34	0.0009	*	3.59	0.1545	
AC	11.31	0.0436	*	1.42	0.3185	
AD	187.40	0.0008	*	0.4429	0.5533	
AE	179.26	0.0009	*	1.31	0.3361	
BC	210.61	0.0007	*	2.47	0.2140	
BD	193.91	0.0008	*	1.55	0.3011	
Optimized product evaluation						
Solution Response	Predicted Mean		Observed ±SD (***n***=3)		Percentage biased	
Particle Size (nm)	173.764		180±20.2		3.58	
Drug Loading (%)	67.9014		71.288±2.03		4.54	

Table 4: Permeation parameters.

Permeability Parameters	Nano gel	Pure drug Gel
Time lag (h) ±SD (*n*=3)	3±0.50	15±0.69
Drug flux (Jss) (mg/sqcm/h)	0.061	0.012
Permeation constant (Kp) (cm/hr)	0.307	0.06
Permeation enhancement	5.12	

SD=Standard Deviation.

Transmission Electron Microscopy (TEM) images (Figure 4B) confirmed the formation of nanosized vesicles, with clear segregation observed, indicating formulation stability.

Evaluation of nanoemulgel

The formulated nanoemulgel exhibited an off-white appearance, moderate stiffness, and accompanied by a faint eucalyptus odour. pH of the formulation was found to be 6.6 and supported topical compatibility.

The viscosity of the nanoemulgel varied at different angular velocities, and the rheogram is shown in Figure 4C. The consistency index of the formulation was calculated to be 39.81Pa-S.

The formulation showed excellent spreadability (6.45±0.6 g cm/s).

The nanogel system demonstrated sustained drug diffusion, while the pure drug gel exhibited comparatively slower diffusion, as shown in Figure 4D.

Ex vivo skin permeation studies were conducted to evaluate and compare the drug permeation potential of a nanoemulgel formulation versus a conventional gel containing the pure drug. Key permeation parameters such as flux, permeability coefficient, lag time, and enhancement ratio were calculated for both formulations. The *ex vivo* permeation graph is shown in Figure 4E.

The permeation parameters are listed in Table 4. The lag time was markedly reduced, and the flux and permeation enhancement was improved by 5 times compared to the gel of pure drug.

In vivo study for antiarthritic activity

The photo, Xray and histopathology images of the different groups of animals are shown in (Figure 5). The images were taken after the treatment period depicting comparative efficacy of the nanogel formulation. This was further supported by histopathological studies. Histopathological evaluation revealed distinct differences among the groups. The negative control group (group -I) displayed normal joint architecture with intact chondrocytes and well-preserved joint space, confirming healthy tissue morphology. The positive control group (Group -II) showed marked pathological alterations, including narrowed joint space, reduced chondrocyte proliferation, and cartilage erosion, indicating severe arthritic damage. The group -III animals demonstrated partial protection, with moderate preservation of chondrocytes and joint space, and degenerative changes were still evident. The group -IV animals exhibited near-normal histology, with prominent chondrocyte proliferation, better maintenance of joint space, and minimal cartilage erosion, highlighting its better protective and restorative effects compared to the oral solution.

No skin irritations were observed in the group IV animals on application of the nanogel.

DISCUSSION

Screening of lipid

The results of the solubility study provide a rationale for the selection of eucalyptus oil, tween 20, and labrafil as key components in the formulation of nanoemulsion.

Eucalyptus oil, although an effective natural penetration enhancer, was incorporated at a low level (<5%) which generally regarded as safe for topical use.[26] Tween 20 (polysorbate 20) and Labrafil, a PEGylated lipid excipient manufactured by Gattefossé are widely used in dermatological and cosmetic preparations with good biocompatibility and minimal dermal irritation profile. Hence these three components were selected for the preparation of nanoemulsion.

Evaluation of nanoemulsion using DoE

The evaluation of the design and the model sensitivity was carried out by ANOVA at a significance level of $p<0.05$. The F-value for the model was 33.96 with a p-value of 0.0072, indicating the statistical significance of the model in evaluating the effect of factors on particle size (Table 3). The goal was to minimize the particle size and maximize drug loading. The statistical analysis revealed that factors such as RHLB (Required Hydrophilic-Lipophilic Balance), PVR (Phase Volume Ratio), Homogenization Time (HTIME), and Ultrasonication Power (UPOWER) significantly influenced particle size as main factor and interaction effects.

The model was also found to be statistically robust for the evaluation of factors affecting drug loading. The statistical analysis (Table 3) highlighted that RHLB and PVR were the most significant factors, with F-values of 101.35 and 15.04 respectively, and p-values below 0.05. RHLB and PVR were the critical factors for drug loading[25]. The analysis confirmed that a balance between process intensity and formulation parameters is critical for achieving optimal nanoemulsion characteristics.

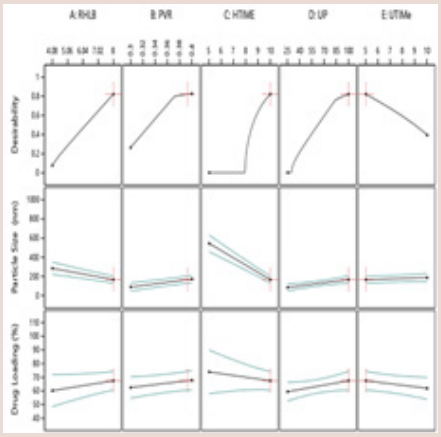
Closer contour lines in the particle size (Figure 2) graph and drug loading (Figure 3) highlighted the regions where the particle size can be minimized and drug loading can be maximized respectively.

The response surface graphs (Figure 2) for particle size resembled a valley. The curvature and slopes on the response surface graphs

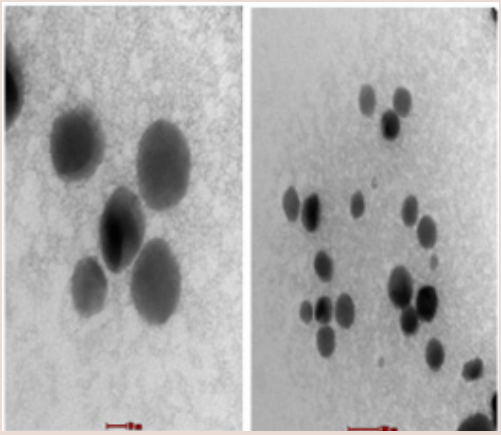
of particle size provided insights into the sensitivity of particle size with the changes in the factor levels. Steeper slopes indicated regions where small changes in the factors have significant impact on particle size.

The response surface diagrams of drug loading (Figure 3) showed a gradual elevation in drug content with increasing RHLB and optimal PVR, forming a peak in the graph, indicating a favorable zone for drug loading. The optimized product evaluation suggested validated the statistical model and % bias less than 5% between the predicted and experimented results indicated the reproducibility of the optimized condition. Zeta potential of the optimized nanoemulsion indicated stability of the dispersion.

Transmission electron microscopy confirmed the presence of mono-dispersed nanosized stable vesicles further validated the design efficiency and findings of zeta potential.



A



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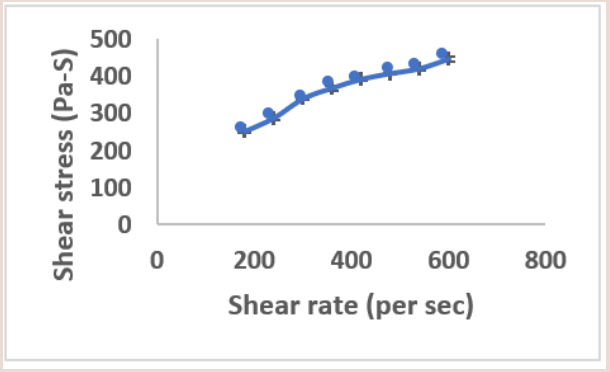

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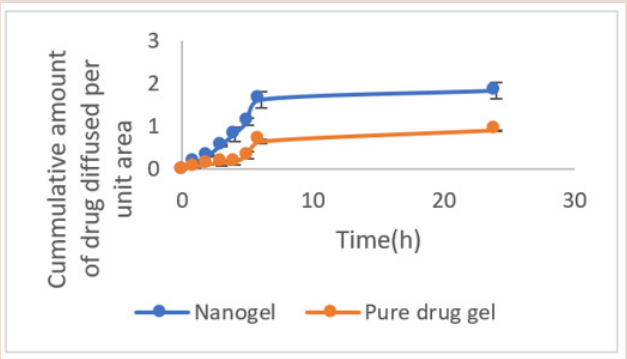

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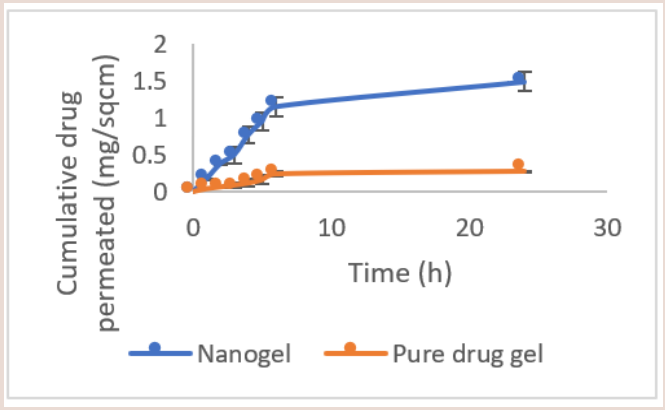

E

Figure 4: Prediction profiler- All factors optimization graph (A), Surface morphology study (TEM) of the optimized nanoemulsion (B) Rheogram of the nanoemulgel (C), Cumulative drug diffusion study *in vitro* (D) and Cumulative drug permeation study *ex vivo* (E).

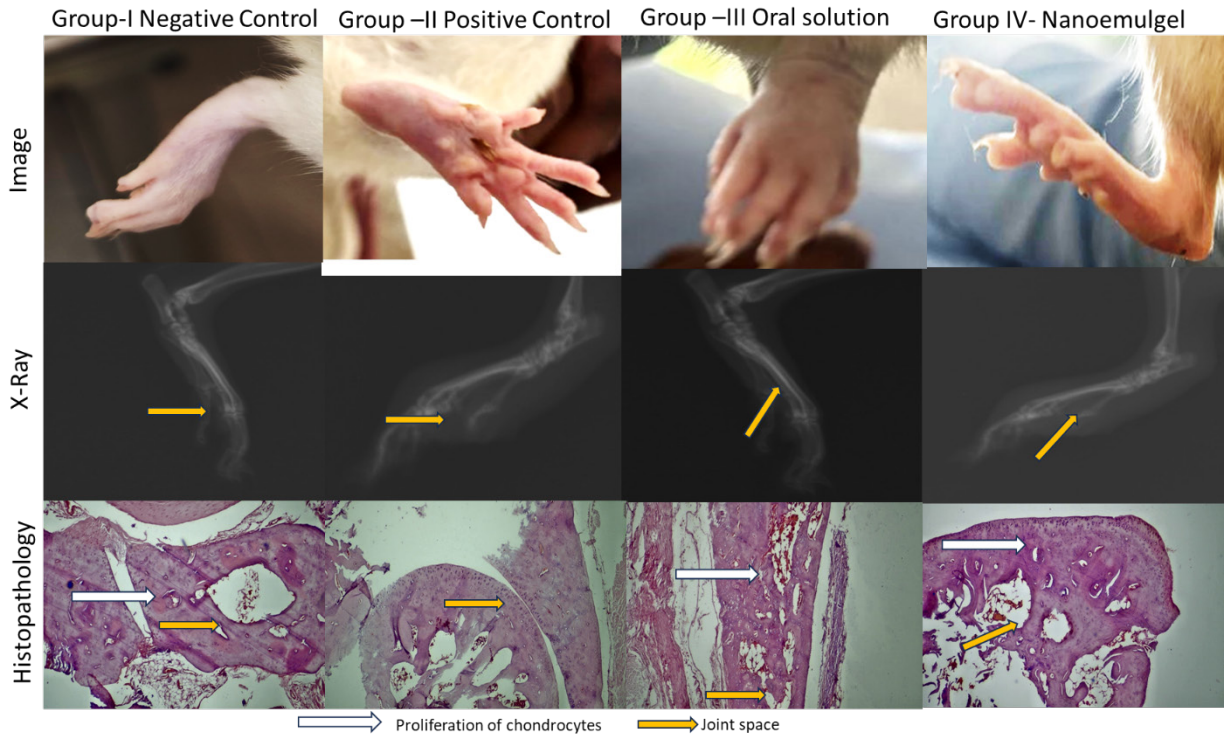

Figure 5: Radiographic analysis and histopathology study on rodents.

Evaluation of nanoemulgel

Carbopol 934 is widely selected as a gelling agent over other synthetic and natural agents because it can generate high viscosity even at low concentrations while producing clear and aesthetically acceptable gels. The nanoemulsion droplets were entrapped in the three-dimensional polymeric network of the gel, thereby enhancing the physical stability of the formulation.

It exhibited pseudoplastic (shear-thinning) behavior which allowed the gel to spread easily upon application and also helped to maintain sufficient consistency afterward for long residence on the skin.

The flow index (n) describes how much the system deviates from Newtonian behavior (where n=1). A flow index less than 1 indicates pseudoplastic or shear-thinning behavior, while a value greater than 1 suggests dilatant or shear-thickening behavior. The nanoemulgel exhibited a flow index of 0.37, indicating strong pseudoplastic characteristics.

The spreadability of the nanogel further supported optimal physicochemical properties for topical use.

The objective of the *in vitro* diffusion study was to compare the drug release profile of the developed nanoemulgel with that of pure drug gel, independent of skin barrier complexity, in order to evaluate the intrinsic release characteristics of the formulation. The nanogel exhibited a faster onset and achieved a higher cumulative release over the 24-hr compared to the pure drug gel. The results underscored the potential of the nanogel for an effective controlled delivery system.

The *ex vivo* study of the nanoemulgel demonstrated a remarkable higher rate of drug penetration through the skin compared to the pure drug gel.

Topical delivery to periarticular tissues is constrained by the stratum corneum and deeper barriers limiting drug access to the synovium.

The nano size droplets promoted follicular deposition and potentially enhanced deformability for intercellular penetration. Presence of Tween 20 in the formulation facilitated to fluidize the structured lipids of the stratum corneum, thereby reducing barrier resistance.[17] Eucalyptus oil, containing terpenes might have promoted penetration by extracting and fluidizing stratum corneum lipids, leading to the formation of transient permeation pathways.[26] Labrafil M2125, a PEGylated lipid excipient, contributed by improving drug solubilization within the formulation and enhancing partitioning into the skin layers. Hence, the combination of these excipients exhibited a synergistic effect to disrupt the skin barrier and facilitate the partitioning of drug-loaded nano-droplets into deeper tissues, including the synovium.[21]

In vivo study for antiarthritic activity

The dose of the gel and frequency of application was calculated from the flux value (J):

$$\text{Dermal Dose} = J \times A \times T$$

Where J=Flux, A - Area of application and T is the exposure time:

$$T = T\ \text{total} - T\ \text{lag}$$

Dermal dose was calculated from the experimented data of drug loading, flux and time lag.[27] The required dose was calculated to be 1.342 mg, based on the amount of drug permeated per unit area (cm^2) over 24 hr, taking into account the lag time. This estimation was supported by *ex vivo* permeation data. Accordingly, a dose corresponding to the application of 0.2-0.25 g of nanoemulgel per cm^2 was selected for once-daily administration.

The photographic and X-ray images of the animal paws clearly defined the progression and restoration of the disease in different groups of animals. This was further supported by the histopathology studies. The histopathological examination provided clear evidence on the effectiveness of the developed nanogel formulation in the treatment of arthritic conditions.

The enhanced therapeutic outcome observed with the nanogel formulation might be associated with improved localized drug delivery and prolonged retention of the drug at the inflamed site. The nanosized carrier system and the excipient in the nanoemulsion might have promoted better penetration and controlled release of the drug, resulted in the improvement of inflammatory conditions and regeneration of the joint tissues. The nanogel was found to be non-irritating on the skin of group IV animals. These findings are indicating the suitability and superior anti-arthritic activity of the nanogel formulation over the conventional oral treatment.

CONCLUSION

The present study successfully developed and optimized a tofacitinib citrate-loaded nanoemulgel for topical delivery in rheumatoid arthritis. Optimization of experimental design ensured favourable particle size, high drug loading, and stability of nanoemulsion of tofacitinib. The optimized nanoemulgel demonstrated desirable viscosity, pH, and spreadability. *Ex vivo* studies revealed notably improved skin permeation and reduced lag time compared to conventional gel. The nanoemulgel remarkably reduced paw edema, preserved joint architecture, and promoted chondrocyte regeneration, as confirmed by histopathological analysis. The developed nanoemulgel represents a promising topical alternative that demonstrated encouraging preclinical performance and further clinical studies are required to establish its safety and therapeutic efficacy in humans.

ACKNOWLEDGEMENT

The authors are highly grateful for the management of Krupanidhi college of Pharmacy, Bengaluru for providing the infrastructure to conduct the study. Authors extend their gratitude to Viatris, Hyderabad for their generous supply of the drug as gift sample.

ABBREVIATIONS

RA: Rheumatoid arthritis; **WHO:** World Health Organization; **FDA:** Food and Drug Administration; **BCS:** Biopharmaceutics Classification System; **CYP:** Cytochrome P; **NEs:** Nanoemulsions; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **HLB:** Hydrophilic Lipophilic Balance; **PVR:** Phase volume ratio; **HTIME:** Homogenization time; **UPOWER:** Ultrasonication power; **UTIME:** Ultrasonication time; **RHLB:** Required Hydrophilic-Lipophilic Balance; **o/w:** oil-in-water; **2FI:** Two-factor interaction; **DLS:** Dynamic light scattering; **TEM:** Transmission Electron Microscopy; **MWCO:** Molecular weight cut-off; **CFA:** Complete Freund's Adjuvant; **DoE:** Design of Experiments; **PEG:** Polyethylene glycol; **SD:** Standard deviation; **Jss:** Steady-state flux; **Tlag:** Lag time; **Kp:** Permeation constant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study successfully formulates and optimizes a novel tofacitinib citrate-loaded dermal nanoemulgel for the targeted treatment of rheumatoid arthritis. Utilizing eucalyptus oil, Tween 20, and Labrafil M2125, the optimized nanoemulgel overcame the low bioavailability and systemic side effects of conventional oral tofacitinib. The formulation demonstrated high drug loading, sustained release, and significantly enhanced *ex vivo* transdermal permeation. *In vivo* rodent studies confirmed that the topical nanoemulgel effectively reduces paw edema and preserves joint architecture with near-normal histology, providing a highly promising, site-specific alternative to oral rheumatoid arthritis therapies.

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Cite this article: Bhattacharyya S, Dang R, Muheeb NF, Ramanna S, Sivakumar K. Optimization and Characterization of a Tofacitinib Citrate-Loaded Dermal Nanoemulgel for Rheumatoid Arthritis. Indian J of Pharmaceutical Education and Research. 2026;60(4s):s1795-s1806.