NANOEMULGEL-BASED DELIVERY SYSTEM FOR BOSWELLIC ACID: FORMULATION, CHARACTERIZATION, AND EVALUATION

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Abstract

3-O-acetyl -11-keto-β- Boswellic acid (AKBA), pentacyclic triterpenoid boswellic acid, isolated from the plant species Boswellia serrata, possesses excellent anti-inflammatory and anti-arthritic properties and has been regarded as a potential natural constituent for the treatment of rheumatoid arthritis (RA). But the use of boswellic acid in the treatment process is hindered by its insolubility in water, low oral availability, and high first-pass effect, which limit its effectiveness. Development of a nanoemulgel dosage form provides a possible solution to circumvent these drawbacks as it enhances skin permeability, improves drug targeting to site of inflammation and enables sustained drug delivery.

In this study, a boswellic acid loaded nanoemulgel was developed through optimization technique for efficient management of rheumatoid arthritis. A full 2^3 factorial experimental design was used to study the effects of variables such as oil concentration (A), Smix concentration (B), and homogenization speed (C). F3 was mixed with Carbopol 940 gel base to formulate nanoemulgel and evaluated physicochemical parameters, viscosity, spreadability, extrudability, pH value, drug content, *in-vitro* drug release and kinetics.

Nanoemulgel was homogeneous, with appropriate pH, adequate viscosity, good spreadability, and high drug content, which makes it suitable for topical application. It showed 99.64% drug release in 24 hours, which is significantly higher than that of Boswellic acid gel (49.80%), indicating enhanced diffusion and release of the drug. Increased skin permeation was due to the nanosized droplets and permeation enhancement effect of the surfactant system. Release kinetic studies showed controlled drug release behaviour, overcoming the limitations associated with conventional Boswellic acid delivery.

Keywords: Boswellic acid, Nanoemulgel, Factorial design, Rheumatoid arthritis.

How to cite this article: Boyapati S, Pashikanthi S, Varma MM. Nanoemulgel-Based Delivery System for Boswellic Acid: Formulation, Characterization, and Evaluation. Int J Drug Deliv Technol. 2026;16(6): 481-491. DOI: 10.25258/ijddt.16.6.173

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by synovial inflammation, joint deformity, pain, disability and systemic complications[1]. There have been significant developments in medications used for management of RA but yet its treatment is still a difficult issue, since the traditional therapy includes NSAIDs and steroids, both of which have undesirable gastrointestinal, cardiovascular and renal side effects when used long-term[2,3]. Thereby, new methods of treatment are needed, which are more effective and at the same time less toxic. One of the natural compounds that is receiving much attention nowadays is 3-O-acetyl -11-keto-β-Boswellic acid (AKBA)[4], the main active ingredient of the resin of Boswellia serrata. The mechanism of action of boswellic acid is mainly based on its selective inhibition of 5-lipoxygenase enzyme leading to inhibition of production of leukotrienes, mediators of chronic inflammation. Besides, boswellic acid also influences several other inflammatory cascades including NF-κB, TNF-α, IL-1β[5,6] and matrix metalloproteinases. Its use in treatment of RA is hampered by the poor aqueous solubility, poor absorption, extensive first pass effect and, thus, low oral bioavailability[1].

The use of nanoemulsions as lipid-based nanovehicles for delivering drugs through the skin has been of great interest due to their nanodroplet size, high solubility power, high surface area of the interface and kinetic stability[8]. The above-mentioned properties allow better dissolution of the drug, partition into the stratum corneum and

transdermal permeation of the drug. In addition, the presence of surfactants and co-surfactants lowers the interfacial tension and transiently changes the organization of the lipids in the stratum corneum, thereby aiding in drug transport through the skin[7].

While nanoemulsions show superior permeation properties, their poor viscosity leads to reduced residence time at the site of application[5]. Encapsulation of the nanoemulsion in a hydrogel results in the formation of the nanoemulgel. Nanoemulgels combine the permeation ability of the nanoemulsions with the advantageous rheology of hydrogels[6]. They have higher physical stability, easy applicability, long skin residence, sustained release and good acceptability by patients[9].

Materials and Methods

Boswellic acid was obtained as a gift sample. Carbopol and propylene glycol were purchased from Loba Chemie Ltd., Mumbai, India. Chemicals and reagents used in this study were analytical grade.[10]

Formulation of Boswellic acid nanoemulsions

Quality by Design (QbD) is a systematic methodology which starts with a set of defined parameters and objectives derived from product and process understanding to assure product quality[10]. The formulation variables were statistically optimized using a 2^3 factorial design (three factors at two levels) to obtain maximum in vitro drug release with controlled release characteristics and optimum particle size of the formulation. Minitab software, version 22.5.1, was used to design the experiment and analyze the data.[11]

Optimization, formulation and *in-vitro* evaluation of Boswellic acid nanoemulsions

Solubility studies of Boswellic acid

Boswellic acid solubility was studied in different oils like oleic acid, peppermint oil, eucalyptus oil, lemongrass oil, castor oil, coconut oil, arachis oil, and cinnamon oil as well as in various surfactants and co-surfactants like Tween 20, Tween 80, polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400), and propylene glycol.[2,12] The amount of Boswellic acid added was in excess of the amount of oil or excipient used, and after vortex mixing, the mixture was incubated for 24 hours in a bath shaker at 25 ± 2 °C to allow for equilibrium. Solubility was determined by using UV-visible spectrophotometer and the samples were analyzed in triplicate[11,13].

Smix screening

The suitable ratio of Smix (surfactant and cosurfactant) was selected by mixing different ratios of Smix where the concentration of cosurfactant was increased and the concentration of surfactant was decreased until a stable solution was formed[12]. Different weight ratios, such as 1:1, 1:2, 2:1, 3:1 and 1:3 were used to combine the surfactant and cosurfactant and diluted with water until a stable solution was formed[14,15]. This step was done in order to fix the ratio of surfactant. Based on the previous observation of the fixed surfactant, a mixture of the smix in weight ratios of 1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.5, 1:2, 1:25, 1:3, 1:3.5 and 1:4 was made and titrated with water and kept aside for 24 h to observe any separation[16].

Ternary plot construction

The pseudoternary phase diagram was composed aqueous phase, oil phase and Smix (surfactant/co-surfactant ratio) at fixed mass ratios[17]. In the first step, the oil phase and Smix were mixed well and then the distilled water was slowly added to the mixture to form the nanoemulsion[18]. To optimize the Smix composition, different ratios of the surfactant to co-surfactant (1:2 and 1:3) were used. To identify the widest nanoemulsion region, sixteen different oil-to-Smix volume ratios were evaluated: 1:9, 2:8 (1:4), 3:7 (1:2.3), 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2 (1:0.25), 9:1 (1:0.1), 1:2, 1:3, 1:3.5, 1:5, 1:6, 1:7, and 1:8[19]. The formulations were visually assessed for transparency and turbidity at every stage of water titration and observations were used to create the pseudoternary phase diagram[19,20].

Drug–Excipient compatibility studies

These studies were conducted to check the compatibility of Boswellic acid with selected excipients and also to ensure the stability and performance of the transdermal patch formulation[21]. The interactions between the drug and the formulation components were checked by Fourier Transform Infrared spectroscopy[11]. The pure drug and physical mixtures of drug and excipients were subjected to FTIR analysis and the spectra were compared to evaluate any significant chemical interactions[22].

Optimization of Boswellic acid nanoemulsion

For this study, 2^3 factorial design was chosen to optimize the nanoemulsion by using minitab software[23]. The polynomial equation was mention below. Variables employed in full factorial design was presented in table 1.

$$Y=B_0+B_1A+B_2B+B_3C+B_{12}AB+B_{13}AC+B_{23}BC+B_{123}ABC$$

Table 1: Variables employed in full factorial design

Independent variables		
Factor	**(-1)**	**(+1)**
A: Virgin coconut oil (%)	2	6
B: Smix (%)	2	8
C: Homogenizing speed (min)	20	30
Dependent variables		
Globule size (nm)		
Poly dispersity index (PDI)		
Zeta potential (mV)		

Formulation of Boswellic acid nanoemulsions

Nanoemulsions loaded with Boswellic acid were produced via high-energy emulsification[13] (high-speed homogenization) using coconut oil and Smix. Drug was dissolved in required quantity of coconut oil, stirred well and Smix was added to the oil phase to obtain an isotropic mixture[2,21,22]. Then oil phase was gradually added dropwise into the aqueous phase by maintaining constant magnetic stirring to prepare coarse emulsion[24]. This coarse emulsion underwent high-speed homogenization process at predetermined speeds (either 10 min or 30 min according to factorial design method) to get finer nanoemulsions. Formulated nanoemulsions were kept in tightly sealed containers at room temperature for 24 hours prior to their characterization[25].

Evaluation of nanoemulsions

Size of the globules

The size of globules in the prepared nanoemulsions containing boswellic acid was evaluated by the DLS method using a malvern zetasizer nano ZS instrument. By diluting nanoemulsion with distilled water to prevent multiple light scattering and then was analyzed at 25 ± 1°C. The average size of the globules was measured in nanometers (nm)[26].

Polydispersity index (PDI)

PDI of the nanoemulsions was measured by means of the malvern zetasizer nano ZS at the same time when determining the globule size. The properly diluted samples were analyzed under identical conditions, and the average PDI was registered[27]. The PDI value was used to assess the homogeneity of the globule size distribution in the nanoemulsion.

Zeta potential

The zeta potential of the nanoemulsions was determined using a malvern zetasizer nano ZS instrument based on electrophoretic light scattering. The nanoemulsion was appropriately diluted with distilled water and placed into the zeta cell[28]. Then, the analysis was conducted at 25 ± 1°C. The average zeta potential was measured in triplicate and reported in millivolts (mV).

Drug content

The estimation of drug content involved the process of extracting the boswellic acid from the nanoemulsions through the use of pH 7.4 phosphate buffer and sonication to make sure that all the drug is extracted from the sample. The extracted solution was then filtered and subjected to analysis by the use of the UV-visible spectrophotometer at the pre-calculated λmax 248 nm of boswellic acid[29]. The percentage of drug content was thus determined from comparison of the concentration obtained with the theoretical drug content.

Entrapment efficiency

Entrapment efficiency was estimated by the separating the free drug from nanoemulsions by centrifuging at 3,000 rpm for 30 minutes. Spectrophotometric determination of the free drug was done using dilution with pH 7.4 phosphate buffer. The entrapment efficiency (%) was calculated by the difference of the total drug and the free drug.

Viscosity

The measurement of the viscosity of the formulated nanoemulsions was done by use of the Brookfield digital viscometer with the appropriate spindle at 25 ± 1°C. About 20 – 25 mL of the sample was put in the sample chamber and the viscosity was determined after steady reading was achieved[30].

pH

The measurement of the pH of the nanoemulsions was done by utilizing a digital pH meter calibrated to pH buffer solution of 4.0 and 7.0. The pH probe was inserted into the sample directly, and the stabilized pH value was noted. All tests were repeated thrice, and the average pH value was reported[31].

Formulation of Boswellic acid loaded nanoemulgels

The optimized nanoemulsion (F3) was mixed with pre-hydrated carbopol 940 which had been neutralized using triethanolamine, under magnetic stirring. Nanoemulsion was slowly mixed with the gel base to make sure that it would not separate and was evenly distributed. PEG 400 was used as a humectant and plasticizer to enhance the properties of the formulation. Finally, the nanoemulgel was stirred thoroughly to get a homogenous and bubble free formulation[30,31].

Characterization and evaluation of nanoemulgel

Homogeneity

The prepared formulations of nanoemulgel were assessed for homogeneity visually after reaching equilibrium at room temperature. The formulations were assessed for their homogeneity in terms of their appearance, consistency, color, transparency, and freedom from phase separation, grittiness, air bubbles, or particulates. A homogeneous formulation that lacks visible aggregates was accepted for further studies[32].

Viscosity

Viscosity of the nanoemulgel formulations was evaluated using a brookfield digital viscometer. Viscosity readings were noted at 25 ± 1°C with specified rotation speed after allowing the spindle to stabilize in the formulation[10,20,24].

Drug content

An accurately weighed equivalent amount of 5mg of nanoemulgel was added to 100 mL of methanol and pH 7.4 phosphate buffer in volumetric flask and stirred for 30 min. Solution was then passed through 0.45 µm filter to separate undissolved polymeric material. Suitable dilutions were made in methanol and analyzed for the drug content using UV-visible spectrophotometer at 248 nm. Concentration of drug was calculated with the help

of calibration curve and expressed as percentage of theoretical drug content. Analysis was done in triplicate.

Extrudability

The extrudability of the nanoemulgel was determined by the use of tube extrusion technique to determine the ease of extruding the sample from a collapsible aluminum tube. Nanoemulgel was loaded into an aluminum tube and the tube appropriately sealed. Force was placed on the tube and the volume of the gel extruded through the nozzle for 10 seconds was recorded. Alternatively, the force needed to extrude a 0.5 cm ribbon of gel was determined and results expressed in grams. Nanoemulgels that could easily be extruded without too much force were said to have good extrudability. This test was done in triplicates[32].

Spreadability

Slip-and-drag method was used to assess the spreadability of the nanoemulgel. The emulgel was put between two glass plates (20 × 20 cm) and one gram of it was placed. Uniform spreading was achieved by placing the 500 g on the upper plate for 5 min and the diameter of the spread was then measured on two axes at right angles to one another and the mean diameter recorded[33]. A weight of 50 gms was fastened to the upper plate and the time taken for the plate to move a fixed distance of 10 cm was recorded for the determination of drag time. The equation used for the calculation of spreadability was S = (M × L)/T with M being the applied weight, L the distance travelled, and T the time taken. The measurements were performed three times at 25 ± 2 °C[33].

***In-vitro* studies of drug release**

In-vitro drug release study was carried out in Franz diffusion cell with dialysis membrane. Phosphate buffer solution (pH 7.4) was put into the receptor compartment and stirred continuously at 600 rpm at 32 ± 0.5 °C. The formulation equivalent of 5 mg Boswellic acid was placed in the donor compartment. Aliquots were removed at regular time intervals (0.5–24 h) and immediately replaced with the same volume of fresh receptor medium to keep the sink conditions. All the samples were subjected to UV-VIS Spectrofluorometric analysis at 248 nm. The release kinetics of the drug was determined by fitting the released data to the zero-order, first-order, Higuchi and Korsmeyer–Peppas kinetic models. The release profile of the optimized nanoemulgel was compared with that of the conventional 1% Carbopol based Boswellic acid gel.

***In-vitro* skin permeation studies**

The *in-vitro* skin permeation study was performed with franz diffusion cells with semipermeable membrane of dermatomed porcine ear skin (thickness: 0.8–1.0 mm). Phosphate buffer (pH 7.4) was used as a receptor compartment, kept at 32 ± 0.5 °C and continuously stirred at 600 rpm during the experiment. Formulation evenly applied on the skin surface in a diffusion area of 1 cm². Samples were taken at fixed time intervals over a 24-hour period, and an equal volume of fresh receptor medium was added to replace each sample that was withdrawn, to ensure constant diffusion conditions. The samples were analyzed spectrophotometrically at 248 nm. The cumulative amount of drug permeated, the flux and the permeability coefficient (Kp) were calculated. Each experiment was repeated three times ([34]).

Anti-Arthritic Activity (*In-vitro*) (MTT Assay)

The in vitro anti-arthritic activity was evaluated by MTT assay. Cells were plated at 1 × 10⁴ cells/well in 96-well plates and cultured overnight for cell attachment. Treatment solutions were made up by serial dilutions of emulgel extracts made by incubating known amounts of the formulation in culture medium normalized to surface area for 24 h. After desired time of treatment (24 or 48 h), the culture medium was replaced by MTT solution (0.5 mg/mL) and the plates were incubated at 37 °C for 3–4 h. The supernatant was gently removed after incubation and the crystals of formazan were dissolved in 100 µL of DMSO/wells. To assess cell viability, absorbance was measured at 570 nm and 630 nm was used as the reference wavelength.

Percent viability = $[(A_{sample} - A_{blank}) \div (A_{negative\ control} - A_{blank})] \times 100$

IC_{50} values were obtained by fitting a four-parameter logistic model to the concentration–response data. All conditions were run in triplicate wells and in three independent experiments.

Results and discussion

Solubility studies

Coconut oil was chosen for the oil phase owing to its superb skin affinity, and ensures the delivery of the drug into the skin without causing irritation. Despite the moderate solubility of Boswellic acid in coconut oil, the addition of tween 80 and propylene glycol (Smix) helps to improve its solubilization and ensures even distribution of the drug in the hydrogel base. Solubility of boswellic acid in different oils, surfactants and co-surfactants was Fig. 1

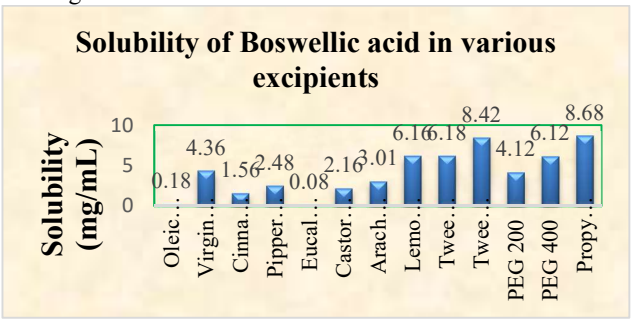


Figure 1: Solubility of boswellic acid in different oils, surfactants and co-surfactants

Smix selection

In the solubility study, it was noted that the ratio between tween 80 and propylene glycol greatly affected the stability of the Smix. The more the amount of propylene glycol in the ratio, the better the clarity and physical stability of the Smix on aqueous dilution. Of all the combinations tested, the Smix ratios between 1:2 and 1:3 had clarity, did not show any signs of phase separation within 24 h, and showed homogeneity.

Pseudoternary phase diagram

Pseudo-ternary phase diagrams Fig. 2 for the Boswellic acid nanoemulsion system, depicting the nanoemulsion zones using different Smix ratios of (A) 1:1 and (B) 1:3. The blue-colored zone represents the isotropic nanoemulsion zone, while the non-blue zone denotes unstable or biphasic zone. An increase in the Smix ratio from 1:1 to 1:3 led to an increase in the nanoemulsion zone[34].

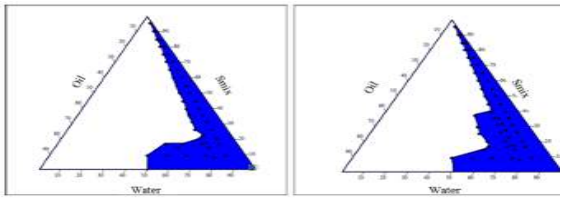


Figure 2: Pseudo-ternary phase diagram

FTIR analysis

FTIR analysis was performed for boswellic acid, propylene glycol, tween 80, coconut oil, and the optimized boswellic acid nanoemulsion to determine drug excipient compatibility Fig. 3 & 4. FTIR spectra of the pure Boswellic acid and optimized nanoemulgel formulation (F3) was compared to assess drug–excipient compatibility and to look for any possible chemical interactions. The O–H stretching was observed at 3505.51 cm^{-1}, aliphatic C–H stretching (CH_2/CH_3) at 2971.05 cm^{-1}, C=O stretching at 1626.38 cm^{-1}, C=C stretching at 1508.70 cm^{-1}, CH_2 bending at 1427.67 cm^{-1}, CH_3 bending at 1377.51 cm^{-1}, and C–O stretching at 1026.38 cm^{-1}. In the optimized formulation (F3), the corresponding peaks were observed at 3339.59, 2947.94, 1641.82, 1493.26, 1331.20, 1097.76, and 1028.31 cm^{-1}, respectively.

The broad O–H stretching band was preserved with slight shift, whereas aliphatic C–H stretching and carbonyl (C=O) stretching bands were still visible, suggesting the chemical structure of the drug is preserved. Minor changes in the carbonyl and hydroxyl absorption bands could be due to intermolecular hydrogen bonding or physical interaction between Boswellic acid and excipients instead of the creation of new chemical bonds. The C–O stretching band at 1097.76 cm^{-1} and the ester C–O–C band at 1028.31 cm^{-1} verified the presence of Tween 80, propylene glycol and coconut oil in the formulation. Overall, no new peaks were observed, the characteristic functional group bands were not disturbed and there was no significant peak distortion, thus it can be concluded that there was no chemical incompatibility between Boswellic acid and the selected excipients and hence the drug was successfully incorporated into the nanoemulgel.

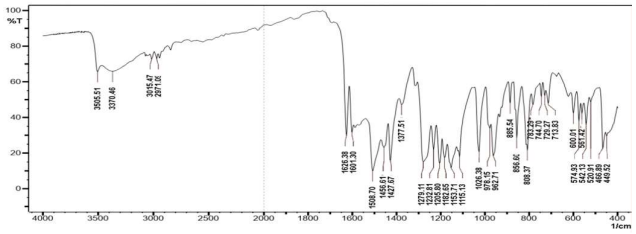


Figure 3: FTIR spectrum of Boswellic acid

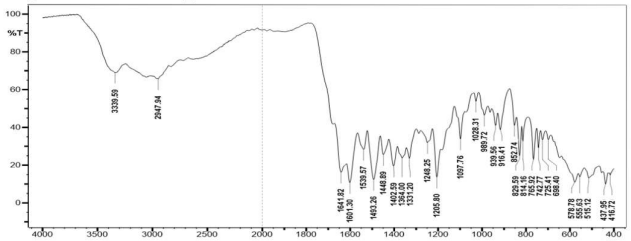


Figure 4: FTIR spectrum of optimized boswellic acid Nanoemulsion composite

Boswellic acid peaks characteristic of functional groups present were found in the optimized formulation, but with minor shifts and broadening in some bands, which implies that the interaction between boswellic acid and the components in the optimized formulation could be intermolecular hydrogen bonding. No new peaks or any loss of the characteristic absorption bands in the optimized formulation implies that there are no chemical interactions between boswellic acid and other components[35].

Optimization of nanoemulsions

The concentrations of coconut oil (A), Smix (B) and homogenization time (C) were optimized to minimize globule size (Y_1), polydispersity index (PDI, Y_2) and zeta potential (Y_3) of the Boswellic acid nanoemulsion by conducting 2^3 factorial design. The experimental results of the eight formulations (F1–F8) are summarized in (Table 2 & Fig, 5).

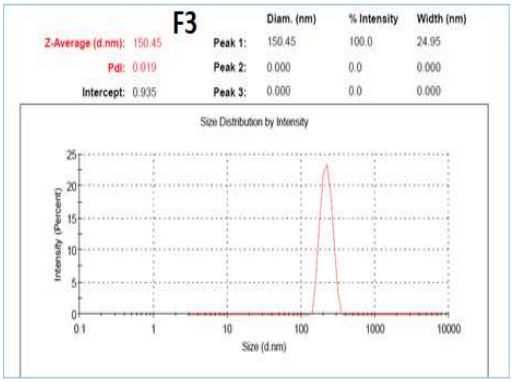
The globule size of the prepared nanoemulsions varied from 150.45 to 226.01 nm, which shows that formulation variables have a significant effect. F3 had the smallest globule size (150.45 nm) while F7 had the largest globule size (226.01 nm) among all the formulations. This decrease in globule size in F3 was attributed to the higher Smix concentration (8%) and longer homogenization time (30 min) which facilitated efficient emulsification and reduced the interfacial tension, thereby fine droplets were formed. Formulations with higher oil concentrations (6%) on the other hand, tended to yield larger droplets as a result of the higher viscosity and higher resistance to droplet disruption during homogenization.

The polydispersity index (PDI) of the formulations ranged from 0.019 to 0.30, reflecting different

particle size distribution of the formulations. The optimized formulation F3 showed the lowest PDI (0.019) indicating the most uniform and monodisperse nanoemulsion. On the other hand, formulations with more oil content showed larger particle size distributions, which means they were less homogeneous, especially for F7 (PDI = 0.30). The optimized surfactant system has successfully produced the PDI of 0.45 for F3 which is quite low. The zeta potential of the various formulations ranged between −13.4 and −16.6 mV, which means that all the formulations had negative surface charge. Even though the absolute zeta potential values were less than ±30 mV, the formulations did not destabilize physically, mostly because of steric stabilization which was provided by the non-ionic surfactant (Tween 80), rather than electrostatic repulsion. The optimized formulation F3 had a negative zeta potential of −13.4 mV, which was adequate for providing a colloidal stability in addition to steric stabilization effect.

Table. 2: Factorial design showing the levels of formulation variables and the corresponding responses of nanoemulsions

Formulation	A: Coconut oil (%)	B: Smix (%)	C: Homogeniztion speed (min)	Y1 Globule size (min)	Y2 PDI	Y3 Zeta potential (mV)
F1	2	8	10	167.02	0.19	-14.7
F2	2	2	10	176.49	0.22	-16.6
F3	2	8	30	150.45	0.019	-13.4
F4	6	2	10	192.11	0.25	-15.2
F5	6	8	10	206.15	0.27	-15.6
F6	6	2	30	212.44	0.28	-13.6
F7	6	8	30	226.01	0.30	-14.8
F8	2	2	30	192.71	0.27	-16.0



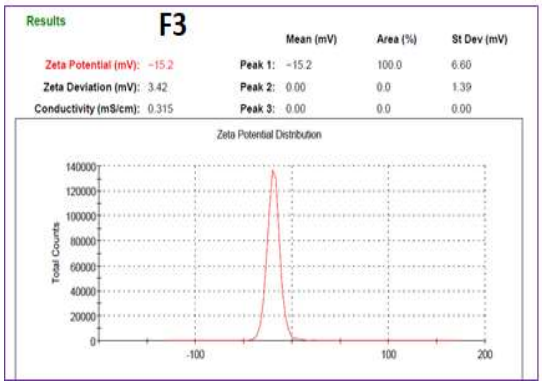


Figure 5: Globule size and zeta potential of F3

Physicochemical evaluation of Boswellic acid nanoemulsion formulations (F1–F8)

The drug content, pH, entrapment efficiency and viscosity of the prepared Boswellic acid nanoemulsions (F1–F8) were evaluated to determine their physicochemical properties and suitability for further development. The results are presented in Table 3.

The drug content of the formulations was found to be in the range of 93.8 ± 0.28% to 102.1 ± 0.16% which showed an efficient incorporation of the drug and good content uniformity of the developed nanoemulsions. The optimized formulation F3 showed the maximum drug content (102.1 ± 0.16%), indicating that there was no significant drug loss during the formulation process. The drug content in all the formulations was found to be near to the theoretical value which indicates the reproducibility of the formulation method.

All the formulations were found to have the pH range between 5.64 ± 0.02 to 5.96 ± 0.01 which is acceptable for the normal physiological pH of the skin. The pH values demonstrate that the formulations are not likely to irritate the skin and are suitable for transdermal use. This optimized formulation F3 showed the optimum pH value of 5.64 ± 0.02 which is within the tolerable limit for topical preparations.

The entrapment efficiency ranged from 86.32 ± 0.26% to 99.42 ± 0.01% which suggests that the Boswellic acid was effectively encapsulated within the nanoemulsion droplets. The formulation F3

exhibited the maximum entrapment efficiency (99.42 ± 0.01%) which could be explained by the optimization of the ratio of oil to the surfactant and the better emulsification process which led to improvement of the solubilization of the drug in the internal oil phase. The reduced entrapment efficiency of formulations like F6 (86.32 ± 0.26%) and F8 (89.34 ± 0.41%) may be attributed to insufficient amount of surfactant used or the insufficient solubilization ability of the drug, which leads to some drug leakage during formulation.

Influence of formulation composition on flow characteristics, the viscosity of the nanoemulsions was found to be 48.1 ± 0.27 to 80.1 ± 0.18 cP. Formulation F3 had a viscosity of 53.5 ± 0.18 cP, which is low enough to allow easy incorporation into a Carbopol gel matrix and has good flow properties during the process. The higher the concentration of oil, the higher was the viscosity of the formulations, due to the higher volume of internal phase[35].

Table 3: Physicochemical evaluation of Boswellic acid nanoemulsion formulations (F1–F8)

Formulation code	Drug content (%)	pH	Entrapment efficiency (%)	Viscosity (CPS)
F1	100.1±0.46	5.70 ± 0.02	98.46±0.24	68.6±0.16
F2	93.8±0.28	5.94 ± 0.01	96.32±0.22	72.6±0.24
F3	102.1±0.16	5.64 ± 0.02	99.42±0.01	53.5±0.18
F4	95.2±0.34	5.90 ± 0.02	92.16±0.24	48.1±0.27
F5	100.4±0.26	5.71 ± 0.01	96.12±0.18	52.2±0.24
F6	99.7±0.18	5.96 ± 0.01	86.32±0.26	50.4±0.14
F7	99.2±0.16	5.66 ± 0.01	94.65±0.18	80.1±0.18
F8	99.7±0.22	5.91 ± 0.01	89.34±0.41	50.4±0.32

***In-vitro* diffusion and permeation studies of nanoemulgel**

In-vitro drug release (Fig. 6) characteristics of the nanoemulgel containing Boswellic acid was compared with pure Boswellic acid using Franz diffusion cell. It was found that the nanoemulgel showed significantly higher and more sustained release rate than the normal gel. Higher release rate from nanoemulgel is due to the smaller size of droplets that increases the surface area for diffusion as well as maintains Boswellic acid in a dissolved form. Moreover, the use of surfactants and co-surfactants enhances the wettability of the drug as well as minimizes the diffusion barrier effect and thus, enables sustained drug release from the carbopol gel matrix. However, the insoluble nature of the pure Boswellic acid in aqueous phase prevents its dissolution and diffusion through the membrane. The model with the highest coefficient of determination (R^2) was taken into account as the most adequate one. The sustained release indicates that the main mechanism of drug release is drug diffusion from the hydrated polymeric matrix.

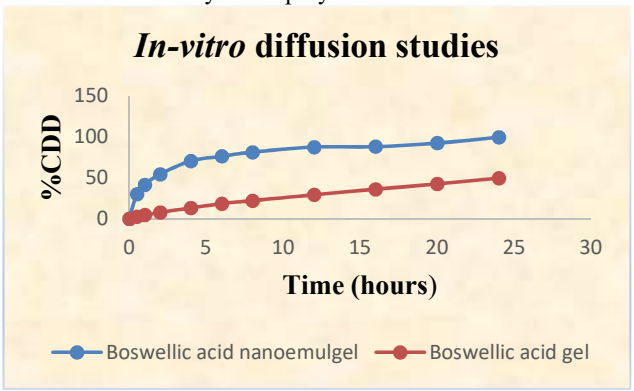


***Figure. 6: In-vitro* diffusion and permeation studies of nanoemulgel**

***In-vitro* cytotoxic activity**

The IC_{50} values were calculated to determine the cytotoxic and anti-inflammatory potency of the optimized formulation. The cytotoxicity IC_{50} value was found to be greater than 600 µg/mL, indicating that the formulation is non-toxic to macrophage cells. In contrast, the nitric oxide inhibition assay showed an IC_{50} value of 64.2 µg/mL, suggesting strong anti-inflammatory activity. These results confirm that the optimised hydrogel film demonstrates excellent cytocompatibility along with significant anti-inflammatory potential, making it suitable for transdermal therapeutic applications.

Conclusion

Development and optimization of Boswellic acid loaded nanoemulgel for improved transdermal drug delivery was successfully achieved through 2^3 full factorial design approach. The optimized nanoemulsion (F3) had favorable physicochemical properties, good stability, high drug loading along with desirable rheological properties. The *in-vitro*

drug release from the nanoemulgel was significantly improved (99.64% at 24 h) in addition to improved skin permeation capacity when compared to Boswellic acid gel, due to the presence of nanodroplets and surfactant system synergy. Kinetic study revealed a sustained and controlled release pattern of drug, thereby indicating prolonged drug release from the system. Effective utilization of nanoemulsion with carbopol gel system efficiently resolved the issues regarding poor solubility and permeation of Boswellic acid, which could be used as a promising transdermal delivery system for the management of RA.

ACKNOWLEDGEMENTS:

The authors wish to thank the Department of Pharmaceutical Technology, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam and Shri Vishnu college of pharmacy for their constant support to complete this work.

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest

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