Ind. J. Pharm. Edu. Res., 2026; 60(2s):s815-s825.
https://www.ijper.org

Original Article

Formulation and Evaluation of a Bromelain-Loaded Nanoemulgel for Effective Acne Treatment

Archana S Patil*, Pratik Ugare, Arpana Patil, Sheetal Patil, Rajashree Masareddy

KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.

ABSTRACT

Aim: This study aimed to design, optimize, and evaluate a bromelain-infused nanoemulgel to enhance its transdermal delivery and improve therapeutic outcomes in the management of *Acne vulgaris*. **Background:** *Acne vulgaris* remains a prevalent dermatological condition that necessitates more effective treatment strategies. Bromelain, a proteolytic enzyme, offers significant potential for acne management but is hindered by challenges such as poor aqueous solubility, limited skin penetration, and extensive first-pass metabolism. **Materials and Methods:** Nanoemulsions were formulated utilizing pseudo-ternary phase diagrams and optimized through a 3^2 factorial design. The most promising formulation, characterized by high drug content (87.55%) and a minimal globule size (105.8 nm), was incorporated into a xanthan gum-based gel matrix. Penetration enhancers, including propylene glycol, were integrated to facilitate enhanced skin absorption. The bromelain-loaded nanoemulgel was evaluated for homogeneity, pH, viscosity, extrudability, *in vitro* drug release and *ex vivo* skin permeation using rat abdominal skin. **Results:** The nanoemulgel demonstrated superior drug release and skin permeation compared to both emulgel and plain gel formulations. The synergistic effects of the surfactant and co-surfactant blend in the nanoemulsion significantly enhanced the delivery and penetration of bromelain, thus improving its therapeutic efficacy for *Acne vulgaris*. **Conclusion:** The bromelain-loaded nanoemulgel exhibited promising results in terms of both drug release and transdermal penetration, indicating its feasibility as an effective topical formulation for acne management. Further clinical trials are warranted to assess its efficacy and safety for broader clinical application.

Keywords: Anti-microbial study, Bromelain, Nanoemulsion, *Propionibacterium acnes*.

Correspondence:
Dr. Archana S Patil
KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi-590010, Karnataka, INDIA.
Email: archanapatil@klepharm.edu

Received: 15-12-2025;
Revised: 05-01-2026;
Accepted: 25-02-2026.

INTRODUCTION

Acne is a chronic inflammatory skin condition primarily affecting the pilosebaceous follicles, with significant prevalence across all ethnicities and age groups.[1,2] It is especially common in adolescence due to increased androgen levels, making it prevalent among teenagers of all genders, nationalities, and socioeconomic backgrounds.[3,4] Although acne affects both men and women, adult females show a higher prevalence, with an estimated 12% affected.[5] In the United States, around fifty million people suffer from acne, and the annual treatment costs exceed three billion USD.[6,7] Though not life-threatening, acne can profoundly impact mental well-being, frequently manifesting as diminished self-assurance, withdrawal from social engagement, and heightened emotional turmoil.[8]

Several factors are implicated in *Acne vulgaris*, including the proliferation of *Propionibacterium acnes*, aberrant follicular hyperkeratinization, increased sebum production, and inflammation.[6] Additionally, genetic predisposition and diet play important roles in acne development.[9] Clinically, acne is distinguished by its presentation of a spectrum of lesions including comedones, papules, pustules, nodules, cysts, and scarring.[10] Given the substantial effects on quality of life and the limitations of current therapies such as drug complexity and inconsistent response-new therapeutic strategies are required to manage acne effectively. Plant-derived compounds have emerged as valuable resources for developing innovative acne treatments.[11-13]

Bromelain, a proteolytic enzyme extracted from pineapple, is noted for its medicinal properties, including anti-inflammatory, antioxidant, and anti-acne activities.[14] Traditionally, various cultures have used pineapple for its therapeutic benefits, and bromelain has been recognized as a medicinal compound since 1876. Bromelain's effects are attributed to sulfhydryl proteolytic enzymes, which come in two primary forms: stem bromelain and fruit bromelain vary in their levels and structural makeup, with differences depending on the specific part of the pineapple and its

DOI: 10.5530/ijper.20261446

species. As a proteolytic enzyme, bromelain has shown benefits in treating skin conditions such as acne, furrows, and xerosis.[15,16]

Nanoemulgel technology, a recent advancement in topical drug delivery, is being explored to treat skin conditions caused by bacterial, viral, and fungal infections, including eczema, herpes simplex, and acne.[17] Nanoemulgels are created by incorporating a nanoemulsion into a hydrogel base, forming a thermally stable, isotropic colloidal system with globules typically ranging from 10 to 100 nm.[18,19] These globules enable therapeutic benefits by enhancing skin hydration, elasticity, and drug delivery across the skin.[20] Nanoemulsions consist of an oil phase and a surfactant/co-surfactant mixture (S_{mix}), and are categorized into oil-in-water and water-in-oil systems.

The nanoemulgel system offers additional benefits, such as sustained release due to the mucoadhesive properties of the gel, and enhanced skin permeation, which increases the therapeutic effect. The mucoadhesive nature also extends the formulation's contact time on the skin, leading to prolonged drug efficacy. Nanoemulgels are also aesthetically appealing, being non-greasy, non-staining, and easy to apply and remove, with good spreadability.[21] Furthermore, hydrophilic compounds can be encapsulated in water-in-oil nanoemulsions by direct entrapment in oil-based nanoglobules, while the careful selection of oils and surfactants can result in non-toxic, non-irritating nanoemulsions that increase drug bioavailability.[22]

Current commercial formulations, such as bromelain facewash and nanogels, are limited by poor skin penetration due to bromelain's larger molecular size. To overcome this, this study aims to develop a bromelain-infused nanoemulgel formulation to improve skin penetration and deliver effective antimicrobial action against *Propionibacterium acnes*. The novelty of this research lies in the use of nanoemulsion technology within the nanoemulgel, enhancing bromelain's skin penetration and bioavailability. This innovative formulation combines the benefits of nanoemulsions and hydrogels, improving drug delivery, skin hydration, and elasticity, while offering sustained release and prolonged therapeutic effects. The mucoadhesive properties of the nanoemulgel further enhance skin permeation, addressing the limitations of current acne treatments and providing an effective solution for acne management.

MATERIALS AND METHODS

Materials

Bromelain 2400Gdu was purchased from Ultreze enzyme Gujarat (India). Oleic acid and Propylene glycol was obtained from Loba Chemie Pvt. Lmt. Tween 80, Methyl and Propylparaben and xanthan gum were obtained from SDFC limited.

Screening of Components

The Bromelain solubility in different oils (Oleic acid, Liquid Paraffin, Castor Oil, Sesame and Clove Oil), surfactant (Span80, Span20, Cremophore RH40, Tween80 and Tween20) and co-surfactant (Propylene glycol, PEG 400 and PEG 300) was estimated by dissolving 500 mg quantity of Bromelain in each of selected phases i.e. oils, surfactants, and co-surfactants were placed in sealed containers and stirred uniformly for 24 hr using a magnetic stirrer, maintaining a temperature of 37±1.0°C. Equal volume samples were then subjected to centrifugation for 15 min at 3000 rpm. The clear liquid above the solid phase was filtered through a 0.45 µm membrane filter and diluted with the mobile phase. The solubility was determined using a UV spectrophotometer (Shimadzu-1700, Japan) at a wavelength of 278 nm.

Construction of pseudo ternary diagram

Based on solubility and drug excipient compatibility assessments oleic acid was selected as the oil phase. Surfactant and cosurfactant were chosen as Tween80 and PG respectively. As an aqueous phase, distilled water was used. Surfactant and cosurfactant (S_{mix}) mixed in a (1:1) ratio and ternary phase diagram was developed. For the ternary phase diagram, oil and S_{mix} were completely mixed in various glass vials at varied quantity ratios ranging from 1:9 to 9:1 Titration of oil, surfactant, and co-surfactant solutions in various weight ratios was accomplished by adding water dropwise while swirling with a moderate magnetic field After equilibration, the mixtures were evaluated visually and transparently. Stable formulations had minimal viscosity, were single phase, and were clear. The titration data was utilized to create a pseudo ternary phase diagram. CHEMIX software was used to develop pseudo ternary phase diagrams.[23]

Optimization of formulations by 3^2 factorial design

The oil and S_{mix} ratio were optimized using a 3^2 factorial design (Design Expert 10.0.7). As independent variables, the formulation variables oil concentration (A) and S_{mix} ratio (B) were chosen. The drug content (Y1) globule size (Y2) and PDI (Y3) were chosen as Dependent response. Nine formulations were created using the factorial design. The formulas were named F1, F2, and F3..... F9. Optimization describes how responses alter when the two independent factors are varied at the same time.

Preparation of Nanoemulsion

The formulation of Nanoemulsion was prepared using Probe Sonication Method. Bromelain-loaded Nanoemulsions were formulated using a 3^2 statistical approach that produced 9 alternative formulations for varied quantities of oil (oleic acid) and S_{mix} (Tween 80: PG) showed in (Table S4). In this method, the drug was dissolved in the aqueous phase which contains Propylene glycol as co-surfactant. Measured quantities of Oleic

acid and Tween80 are combined in a beaker and heated at 70°C by keeping 150 rpm on magnetic stirrer. 2.5% Bromelain was added to this mixture and heated for 10 min, till drug became entirely soluble in aqueous phase. The aqueous phase gently mixed to the oil phase while stirring for 5 min. Probe sonication for 5 min significantly reduced the droplet size in the emulsion.[22]

Characterization of Nanoemulsion

All the optimized formulations are evaluated for Physical appearance, pH, viscosity, particle size, PDI and Zeta Potential.

Physicochemical Evaluation

The colour and clarity of all formulated nanoemulsions were visually assessed. The low viscosity of the nanoemulsions suggests that they consist of discrete spherical droplets, resulting in minimal resistance to flow. The viscosity of the formulations was assessed using a Brookfield digital viscometer (DV III+) with spindle #7, operating at 200 RPM and a torque range of 10-100%. The zeta potential was quantified employing a Malvern particle size analyser. The particle size distribution and morphological characteristics were analyzed using advanced Transmission Electron Microscopy using the JEOL JEM-2100 system. A small volume of the nanoemulsion was placed onto a carbon-coated copper grid with a 200-mesh size was used, followed by staining with a 2% w/v phosphotungstic acid solution for 2 min.

Determination of drug content

For drug content determination, 1 g of Bromelain Nanoemulsion was mixed in distilled water and sonicated for approximately 10 min at a frequency range of 20 kHz to 40 kHz, maintained at room temperature (around 25°C). After that solution was filtered initially and diluted thereafter as needed. The resultant solution absorbance was measured using UV Spectrophotometry at λ_{max} 278 nm, and the drug concentration was estimated. The average of three readings is taken.

Thermodynamic Stability Study

Centrifugation Test

All Nanoemulsion formulations (F1-F9) were centrifuged at 3000rpm for 20 min to determine the separation of phase, creaming, or cracking.[24] The formulation that passed the test will be investigated further, for thermodynamic study.

Heating-Cooling Cycles Test

The formulations (F1-F9) were heated and cooled by storing them at 40°C for duration of 48 hr, and then cooling at 4°C for next 48 hr. This is done in triplicate. This test is performed to determine the influence of racking on formulation stability.[25]

Freez-Thaw Cycles Test

The formulations (F1-F9) were frozen overnight at (-20°C), then melted at 25°C for additional analysis of the thermodynamic stability of the Formulated Nanoemulsions.[26]

Stability Study

In the short-term stability study, the Nanoemulsion formulation placed at 25°C, away from direct light for 30 days, after that Average Drug content, Globule Size and PDI were determined.

Preparation of Emulsion

Concentration of oil phase and S_{mix} in emulsion was kept same as optimized Nanoemulsion. Emulsion was formulated by combining the required quantity of the drug in an aqueous phase with co-surfactant Propylene glycol and surfactant Tween80 in an oil phase, i.e. Oleic acid. At 70°C, both the aqueous and oil phases kept it on magnetic stirrer at 250 rpm. After that, the aqueous phase was mixed into the oil phase while it was kept at room temperature with constant stirring.[27]

Preparation of Three Different Gels

Preparation of Plain gel

Optimized batch was selected for formulation of Nanoemulgel, Emulgel and Plain gel. Hydrogel was prepared by dissolving the 200 mg of Xanthan Gum in sufficient volume of water. Clear and transparent 2% gel of Xanthan gum was prepared.

Incorporation of Formulated Nanoemulsion and Emulsion into a Hydrogel

Formulated With continual stirring, Nanoemulsion and emulsion were integrated into hydrogel (1:1 v/v) with 2% Xanthan gum as gelling agent. The prepared Plain gel and Nanoemulsion, emulsion-based hydro gel was placed in an appropriate container.

Evaluation of Different Gel Formulations

Physicochemical Evaluation

The formulated gel was assessed through observation for its appearance, translucency, transparency, and colour. The pH of the prepared gel was measured using an electronic pH meter, with three readings averaged for three different gel samples. The viscosity of the gels depends on the type and quantity of polymer used, with an optimal range between 2000 and 6000 cps. Viscosity is a critical parameter for assessing the physical properties of gels, as a decrease in viscosity negatively impacts spreadability and extrudability, thereby affecting the gel's stability.

Determination of spreadability

It describes the amount to which a gel spreads when applied to skin or an affected region. The spreadability qualities of the formulation also influence its medicinal efficacy. Glass slides and

a pulley is attached to one end of a wooden block were used to test the spreadability of the gels.

Nanoemulgel (1gm) was placed between two slides and uniformly pushed to form thin layer. The extra gel that had adhered to the slides was scraped. Both slides were positioned and firmly secured to a stand to ensure stability, allowing only the upper slide to move freely under the influence of the attached weight. A 5 g weight was meticulously affixed to the upper slide. The time required for the upper slide to traverse the entire length and detach from the lower slide due to the applied weight was recorded. The procedure was repeated three times, and the mean traversal time was calculated.[28]

Spreadability was calculated by

$$S=m\times l/t$$

Here, S=Spreadability, m=weight attached to upper slide, l=length of slide and t=time (sec) taken to travel the distance.

Determination of extrudability

To measure extruded gel percentage, a sealed squeezable tube containing gel approximately 20 g was pressed from crimped end and a clamp was used to prevent any rollback. After removing the cap, the gel was extruded from the tube. The amount of extruded gel was measured and collected. The extruded gel % was then calculated.[28]

In vitro drug release study for formulated gels

This study was carried out by using a cellulose acetate dialysis membrane and Franz diffusion cells with a diffusion area of 3.204 cm^2. This was set up between the Franz diffusion cells with two chambers. Buffer pH 7.2 was used to fill receiver chamber as diffusion medium and entire setup was kept on a magnetic stirrer

Table 1: Solubility data of Bromelain in various oils.

Solubility in oils			Solubility in Surfactant		Solubility in Co- Surfactant	
Sl. No	Solvent	Solubility	Solvent	Solubility	Solvent	Solubility
1	Castor Oil	20 mg/mL	Span80	32 mg/mL	Propylene Glycol	52 mg/mL
2	Oleic acid	48 mg/mL	Span20	40 mg/mL	PEG 400	33 mg/mL
3	Liquid Paraffin	32 mg/mL	CremophoreRH40	16 mg/mL	PEG 300	38 mg/mL
4	Sesame Oil	24 mg/mL	Tween80	54 mg/mL	-	-
5	Clove Oil	43 mg/mL	Tween20	43 mg/mL	-	-

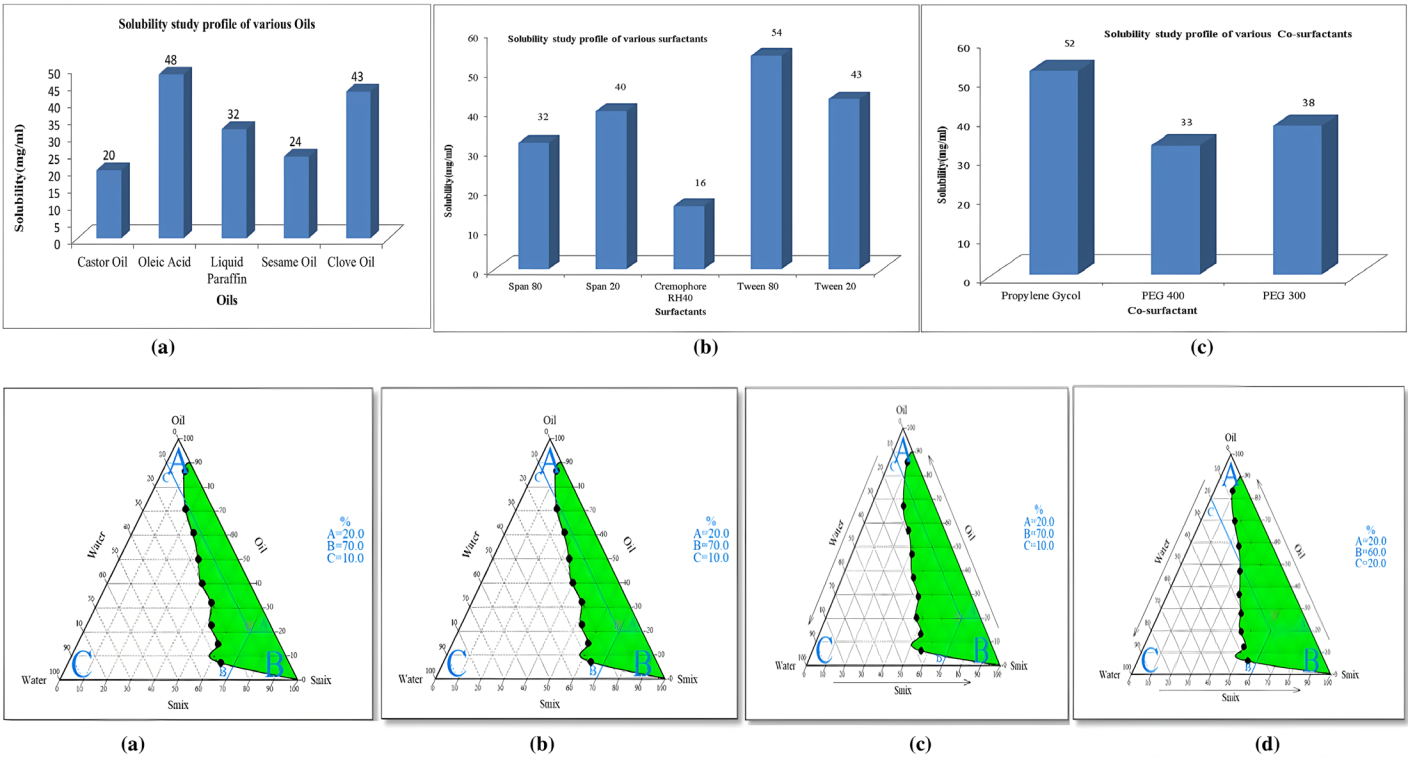

Figure 1: A. Solubility graph of Bromelain in various (a) Oils (b) Surfactant (c) Co-surfactant. B. Pseudo ternary Phase Diagram of Smix a) 1:1, b) 1:2, c) 2:1, and d) 3:1.

Table 2: Experimental Design.

	Factor 1 A: Oil (mL)	Factor 2 B: Smix (mL)	Response 1 Drug Content (%)	Response 2 Globule Size (nm)	Response 3 PDI
F1	1.75	14	45.92	204	0.51
F2	2	14	61.2	168.9	0.27
F3	1.75	16	45.39	160.6	0.3
F4	2.25	14	87.55	105.9	0.32
F5	2	18	78.27	164.6	0.13
F6	2.25	18	80.24	101.2	0.19
F7	2	16	59.06	165.8	0.18
F8	2.25	16	71.3	103.2	0.25
F9	1.75	18	61.4	146.5	0.23

set to 100 rpm. 1 g each of the 3 gels was equally applied on donor membrane. At predefined time intervals of 1/2, 1, 2, 4, 6, and 8 hr, samples were removed from the receiver solution and the cell supplied to their given volumes of fresh buffer. The solution was introduced to the receiver compartment with great care to avoid air trapping. The samples were filtered, and the percentage of drug release was estimated using absorbance at maximum 278 nm.[29]

Ex vivo skin permeation study

Preparation of Rat Abdominal Skin

Abdominal skin from male Wistar rats, sourced from a commercial supplier, was selected for permeation studies due to its suitability for *in vitro* testing, small size, and cost-effectiveness. The abdominal hair was carefully removed using an electric clipper to minimize interference with the permeation process. The excised skin was subsequently stored overnight at 4°C in Phosphate-Buffered Saline (PBS) at pH 7.4, ensuring preservation of its integrity for later use in the experimental setup.[30]

Evaluation of Bromelain Permeation from Various Gel Formulations

Bromelain permeation through the rat skin was assessed using Franz diffusion cells. In this configuration, the skin was mounted between the donor and receptor chambers with the stratum corneum facing the donor side. A 0.5 g aliquot of the gel formulation containing 2.5% bromelain was applied to the skin in the donor compartment. The receptor chamber was filled with 10 mL of phosphate buffer (pH 7.4), maintained at 37±0.5°C, and continuously stirred at 150 rpm to ensure uniform diffusion.[31]

In vitro anti-bacterial study

The anti-bacterial study was carried out with a P. acnes bacterium strain.[32]

Minimum Inhibitory Concentration (MIC)

Antimicrobial agents are classified according to their MICs, which are the lowest doses at which they inhibit or prevent bacterial growth. The most precise way for measuring MIC values is to utilize a 96-well plate using the standard protocol. Each well of a sterile 96-well microtiter plate was filled with 100 µL of sterile Brain Heart Infusion (BHI) broth. After filtering the newly formulated Nanoemulgel formulation via a 0.45 µ filter, 100 µL of formulation was loaded to each first well and serially diluted into the subsequent seven wells. The formulation contained 1000 µg/mL. The concentrations in the first through eight wells were 1000, 500, 250, and 125 µg/mL, respectively it was done in triplicate. 10 µL of bacterial culture was added to each well, which was then covered with foil and incubated at 37°C for 24 hr in a bacterial incubator. The culture was regarded as a beneficial control. After a 24-hr incubation period, 10 µL of resazurin dye was given to each well and the colour was examined for 2 hr for a change. The MIC concentration was determined by the concentration that caused the colour shift.[33,34]

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration required to completely eliminate a particular bacterium. A loopful of inoculum was withdrawn from each well of the incubated 96-well plate and streaked onto the BHI agar plates with varied concentrations of the Nanoemulgel formulation, followed by 24 hr incubation. The visual growth of bacteria on the agar plates was inspected visually.[35,36]

RESULTS

Design and Characterization of Nanoemulsion

Selection of solvents based on solubility studies

Solubility of Bromelain in different oils and surfactant, and co-surfactants was determined. Following a solubilizing assessment, as an oil phase oleic acid was chosen, due to its Greater solubility potential (48 mg/mL). PG was chosen as a Co-surfactant with a solubility potential of 52 mg/mL and tween 80 as a co surfactant with a solubility potential of 54 mg/mL (Figures 1-3) (Tables 1-3).

Development of pseudoternary phase diagram

Based the solubility studies results, oleic acid was selected as the oil phase. Tween80 and PG as surfactants and co-surfactants, respectively. As the aqueous phase, distilled water was used.

Pseudo ternary diagrams were developed by titration of a mixture of oil and S_{mix} in the absence of bromelain with increasing amounts of water. S_{mix} was made using different ratios of Surfactant/ Co-surfactant weight ratios (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, and 4:1). Each S_{mix} and oil were combined in various weight ratios (oil: S_{mix}) of 1:9, 1:7, 1:6, 1:4, 3:7, and 8:2. (Table 4 and Tables S1-S3). Each combination was vortexed and then titrated by aqueous medium until turbidity formation. The concentration changed with addition of water incrementally, the values were utilized to define the limits of the Nanoemulsion. Using the CHEMIX School 7.00 software. The combinations that resulted in clear oil/ water systems were identified and represented on a triangular diagram. The S_{mix} ratio of 1:1 exhibited the largest emulsifying area of all the pseudo ternary phase diagrams (Figure 4 and Table 4 and Tables S1-S3).

Table 3: (a) Compositions of various Nanoemulsion formulations (b) Composition of different gel formulations.

Compositions of various Nanoemulsion formulations									
Ingredients (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Bromelain	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Oleic acid	7	8	7	9	8	9	8	9	7
S_{mix}	56	56	64	56	72	72	64	64	72
Water	34.5	33.5	26.5	32.5	17.5	16.5	25.5	24.5	18.5

Compositions of Different gel formulations			
Formulation	**Bromelain(% w/w)**	**Xanthan Gum(% w/w)**	**Water Up to(mL)**
Gel	2.5	2	q. s
Nanoemulgel	2.5	2	q. s
Emulgel	2.5	2	q. s

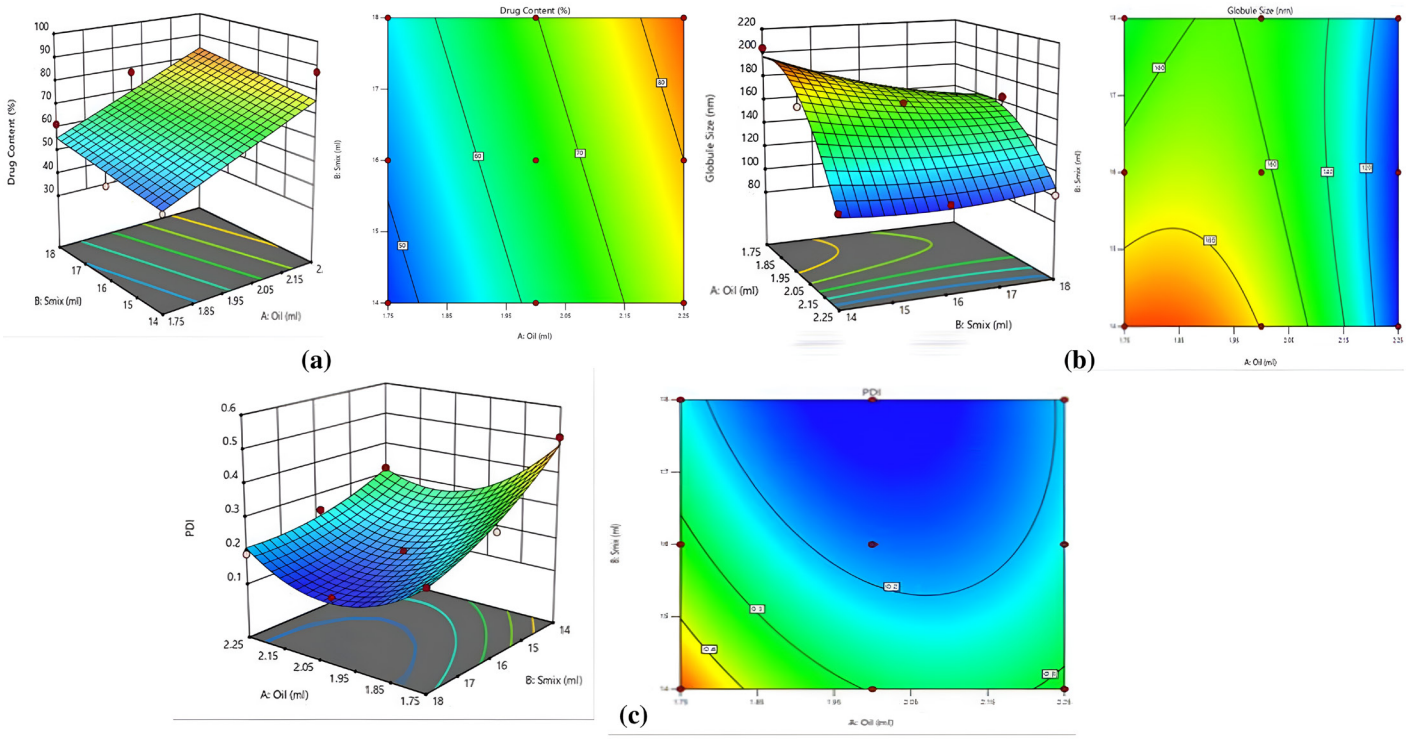

Figure 2: Contour plot and 3D Response plot for (a) Drug Content (b) Globule Size and (c) PDI signifying the effefts of two factors on response.

Pseudoternary Phase Diagram-Based Screening for S_{mix} Ratio Optimization

Based on the pseudo ternary phase diagrams, the S_{mix} ratio of 1:1 shows greater emulsifying region and was considered most satisfactory.

Screening of Oil

S_{mix} ratio based on particle size and PDI

The S_{mix} ratio of 1:1 was kept constant in trial formulations TF1 to TF6 by varying Oil: S_{mix} ratio. According to the results, Oil and S_{mix} concentration in formulations TF3, TF4, and TF5 produced good particle size with good PDI (Table S4).

Formulation optimization by 3^2 factorial designs

The effects of two independent variables, % Oil (A) and % S_{mix} (B), on Drug Content (Y1), Globule Size (Y2), and PDI (Y3) were analyzed using a 3^2 full factorial design (Table S5). The variables were tested at three levels to determine their quadratic influence. Regression analysis and F-statistics helped to create models to identify the most significant terms. A reduced equation, which included only the statistically significant components, was used to generate contour plots, revealing the relationship between the variable levels and the responses.

Generation of polynomial equation

The following equations were derived by the statistical model for each response:

$$Y=\beta0+\beta1A+\beta2B+\beta3AB+\beta4A2+\beta5B2$$

The independent variable is denoted by Y, while β0 represents the arithmetic mean response across the 9 experimental runs. The coefficient of 1 reflects the calculated value for factor A. The primary effects of the amounts of A and B show the average response when these components are varied individually from lower to higher levels. The interaction terms (AB) indicate how the response changes when both factors are adjusted simultaneously. The data obtained from the Design of Experiments (DOE) strongly suggests that the globule size is influenced by this variations.PDI, and Drug content is depending on the independent factors chosen. Conclusions can be derived from the polynomial equations based on the mathematical sign they carry, which is either +ve or -ve, indicating an impact of synergistic or antagonistic

$$Y1\ (Drug\ Content)=65.59+14.40XA+4.21XB$$

$$Y2\ (Globule\ Size)=162.89-33.47XA-11.08XB$$

$$Y3\ (PDI)=0.1722-0.0467XA-0.0917XB$$

Where,

A=Conc.of Oil (Oleic acid)

B=Conc. of S_{mix} (Tween80: PG)

Analysis of Data statistically

To identify components with no significant effect, the Analysis of Variance (ANOVA) method was utilized. The data were analyzed using Design-Expert software (version 10.0.7). The results

Table 4: (a) Physicochemical Evaluation of Nanoemulsions (b) Physicochemical Evaluation of gels.

(a) Physicochemical Evaluation of Nanoemulsions						
Formulations Code	pH ±SD	Viscosity±SD (cps)	Globule Size (nm)	PDI	Zeta potential (mv)	Drug content (%)
F1	5.2±0.2	744±1.3	201	0.51	-7.632	45.92
F2	4.2±0.11	746±1.5	168.9	0.27	-5.15	61.2
F3	5.8±0.25	744±1.4	160.6	0.3	-5.592	45.39
F4	6.2±0.14	744±1.6	105.9	0.32	-6.875	87.55
F5	5.7±0.09	778±1.4	164.6	0.13	-5.619	78.27
F6	5.8±0.19	991±1.1	101.2	0.19	-4.199	80.24
F7	6.5±0.14	1016±1.5	165.8	0.18	-8.227	59.06
F8	5.1±0.05	911±1.1	103.2	0.25	-5.002	71.3
F9	4.9±0.8	662±1.6	146.5	0.23	-6.064	61.4

(b) Evaluation of gels for Homogeneity, pH, Viscosity and Spreadability					
Formulated Gel	Homogeneity	Viscosity (cps)	Spreadability (cm^2 /g)	pH	Extrudability
Plain Gel	Homogenous	1720	1.47	6.8	+ + +
Emulgel	Homogenous	1280	1.5	6.2	+ +
Nanoemulgel	Homogenous	2770	1.64	6.6	+ + +

indicated that all dependent variables had *p*-values less than 0.05 ($p<0.05$). The F values for Globule Size, PDI, and Drug Content models were 18.28, 19.07, and 9.75, respectively, showing that the models were statistically significant. R-squared, or the coefficient of determination, quantifies how closely the data fit the regression line. In multiple regression contexts, this measure is known as the coefficient of multiple determination, reflecting how much of the variability in the dependent variable can be explained by the independent variables combined (Tables S7-S9).

Generation of 3D response surface plots

The changes in the response surface were determined by constructing three-dimensional (3D) plots for the measured responses, which are useful for studying the effect of two factors simultaneously. The software-generated 3D plots established the relationship between the response and independent variables, aligning with the polynomial term reflecting the effect of oil concentration (A) and S_{mix} concentration (B) on the responses. Figures illustrate the influence of independent variables on dependent variables. The 3D response surface plot for drug content (Y1) in Figure demonstrates an increase in drug content with rising concentrations of oil and S_{mix}. The plot for globule size (Y2) in Figure shows that increasing S_{mix} concentration reduces globule size, while oil concentration has no significant effect. The PDI (Y3) plot in Figure indicates a decrease in PDI with increasing S_{mix} concentration, while PDI initially decreases with rising oil concentration up to a certain level but then increases at higher oil concentrations, possibly due to the combined effect of oil.

Preparation of Nanoemulsion Formulation

Bromelain-loaded nanoemulsions were prepared for 9 different software-generated runs in 3^2 complete factorial design. The prepared nanoemulsions were evaluated for various types of parameters (Table S6).

Evaluation of Nanoemulsions

Physicochemical Evaluation of Nanoemulsion

All of the viscosities and pH of Bromelain-loaded nanoemulsions (F1-F9) were determined using a Brookfield digital viscometer (DV III+) with spindle #7 at 200 RPM and digital pH meter. The Malvern particle size analyser was used to determine the Globule size and PDI for all Formulations (F1-F9). A total of 20 runs were recorded using Zetasizer software, yielding an average of 20 runs. The zeta potential is regarded as a significant measure of nanoparticle stability in the dispersion medium (Table S10).

Based on Drug content, Globule size and PDI F4 Batch selected as Optimized Batch.

Drug content determination

The following equation, derived from a linear regression analysis of the calibration curve, was used to determine drug content. The

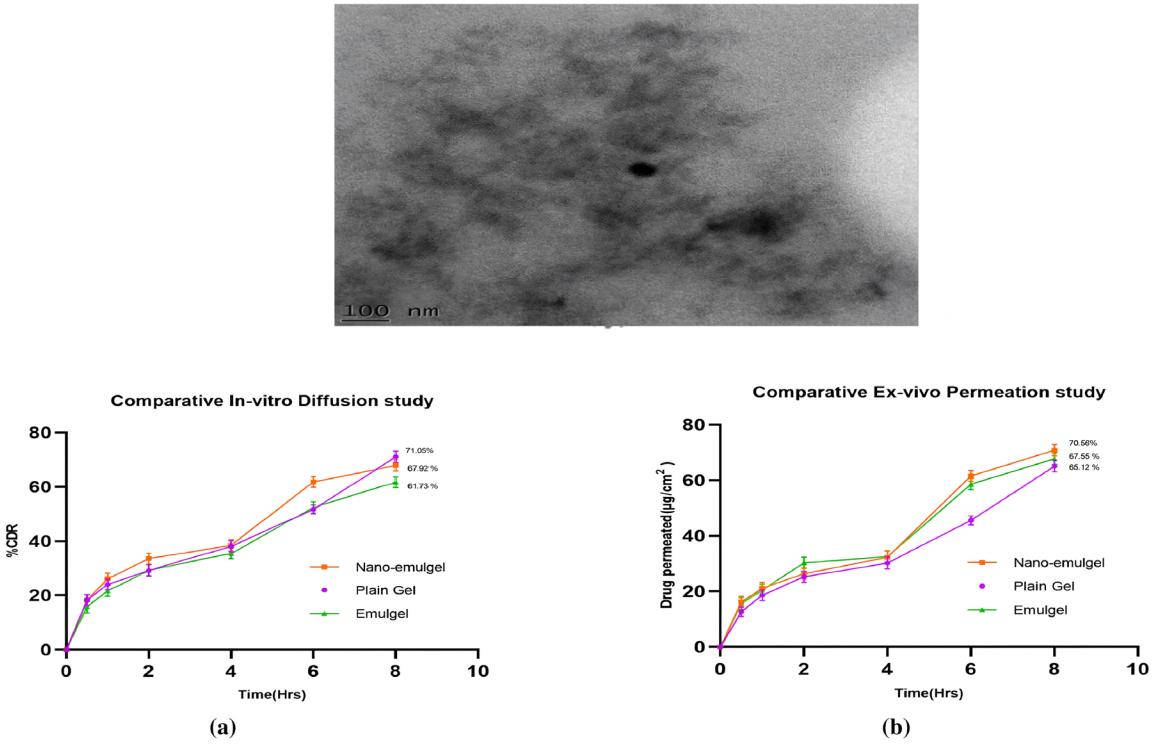

Figure 3: A. Transmission electron microscopy image of optimised formulation F4. B. (a) In vitro Drug Diffusion of Three Different Gels. (b) Ex vivo Skin Permeation study of Three Different gels.

drug content of all nanoemulsion formulations ranged between 45% and 90% (Table S11).

$Y=0.0002x+0.0102$ $R^2=0.9987$

Determination of thermodynamic Stability

The thermodynamic stability determination was intended to detect and eliminate nonstable formulation. As demonstrated in Table, all of the Nanoemulsion (F1-F9) successfully withstood rigorous conditions without exhibiting any signs of phase separation, creaming, cracking, or drug precipitation. This indicates the method's effectiveness in producing a stable nanoemulsion.

Morphological evaluation

The Transmission Electron Microscope (TEM) generated a distinct image, wherein the nanoemulsion droplets appeared dark against a luminous background. The observed average droplet size was below 150 nm which is within the nanoscale range, thereby classifying the emulsion as a nanoemulsion.

Stability Study

To assess stability, the nanoemulsion was subjected to testing at two different temperatures, followed by storage at room temperature for one month. The results, including maximum drug content, globule size, and Polydispersity Index (PDI), are summarized below.

Formulation of Nanoemulgel, Emulgel and Plain gel

Nanoemulgel and emulgel were manufactured by combining 2% Xanthan gum solution with Bromelain Nanoemulsion and Emulsion in a 1:1 ratio. The prepared Nanoemulgel, Emulgel, and plain gel were tested for Different parameters.

Evaluation of different gel formulations

Formulation F4 was chosen for Nanoemulgel, Emulgel formulation based on drug content, globule size, and PDI formulation.

Evaluation of Nanoemulgel, Emulgel and Plain gel

Physicochemical Evaluation of all formulations

The prepared gels were examined visually for clarity, colour and transparency. All formulations were found to be homogenous. The viscosity of formulations plain gel, Emulgel, and Nanoemulgel was observed to be 1,720 cP, 1,280 cP, and 2,770 cP, respectively. The pH of the gels was examined using a digital pH meter. The pH of the formulations plain gel, Emulgel, and Nanoemulgel was found to be 6.8 6.2, and 6.6, respectively.

Determination of Spreadability

Spreadability was calculated by

$$S=m\times l/t$$

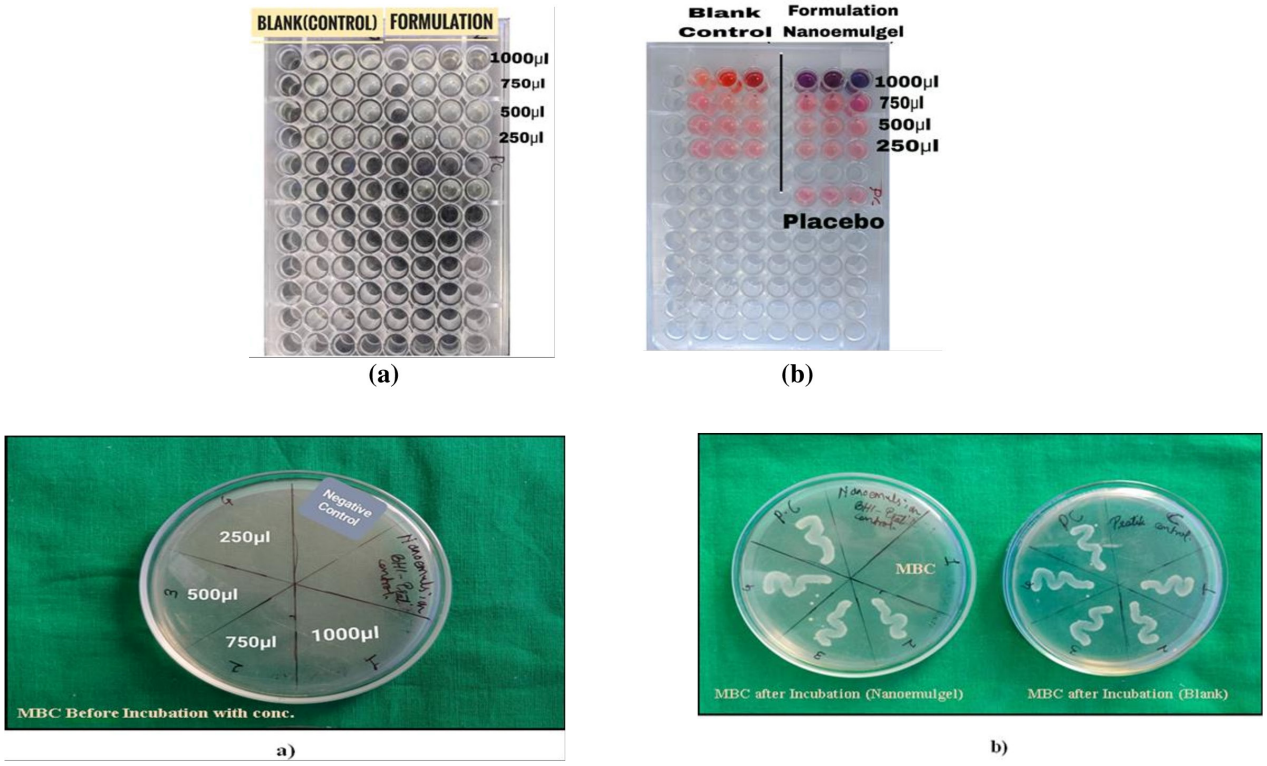

Figure 4: A. MIC using resazurin broth microdilution assay in 96-well plate, a) Before incubation without Dye, b) After incubation with Dye. B. MBC using Agar plate Diffusion Method a) MBC before Incubation b) MBC after Incubation.

In this context, the variables are defined as follows

S represents spreadability, m denotes the weight attached to the top slides, l indicates the length of the slides, and t represents the time (in sec) required to travel the distance.

Determination of extrudability

Collected extruded gel was weighed from the closed collapsible tube. The extruded gel % was measured. All formulations showed good (+ +) to excellent (+ + +) extrudability.

Evaluation of gels for *in vitro* drug release study

After 8 hr, release of drug was 71.05±0.65%, 61.73±0.64% and 67.91±0.73% for Plain gel, Emulgel, and Nanoemulgel respectively.

As a result, the various gel formulations are ranked in order of maximum drug release: plain gel>Bromelain Nanoemulgel>Bromelain Emulgel. The excess water level of the gel formulation enhances the release of drug, result in plain gel.

Compared to gel formulations, the notably lower release percentage of Bromelain from the formulated emulgel and nanoemulgel could be attributed to the higher viscosity of these formulations, which may hinder the efficient diffusion of the encapsulated drug. Additionally, the reduced water content and the inclusion of oleic acid in these formulations also contribute to this effect. Interestingly, the nanoemulgel demonstrated a significantly higher ($i<0.05$) drug release compared to the emulgel. This improvement is likely due to the smaller droplet size in the nanoemulgel formulation.

From all gel formulations release kinetics of Bromelain were analyzed using the Higuchi diffusion model. As shown in Table. As a result, diffusion is the preferred method for Bromelain release via these gels.

Ex vivo skin penetration study

The Comparative study for penetration through abdominal skin of rat of plain gel, Emulgel and Nanoemulgel was carried out for 8 hrs. The amount of drug permeation from Plain gel, Emulgel and Nanoemulgel showed 65.12%, 67.55% and 70.56% respectively. The cumulative % of Bromelain that penetrated through rat skin (μg/cm^2) from the nanocarrier gel was considerably higher compared to its penetration from the emulsion-based gel, the Bromelain-loaded gel, or the Bromelain solution.

Actually, the incorporation of a surfactant and a permeation enhancer increase the flux of Bromelain from Emulgel and Nanoemulgel. Furthermore, the outer aqueous phase has the potential to moisturize the *stratum corneum*, causing cell swelling and facilitating drug transport.

Also, the increased permeation of Nanoemulgel incorporated with Bromelain could be attributed to the Nanosized particles, which provide a larger contact area for drug penetration and facilitate the delivery of high drug concentrations to the affected area.

In vitro anti-bacterial study

Minimum inhibitory concentration (MIC)

Visually calculating the MIC values involved contrasting the colour with a control well. Violet turned pink, indicating the lowest inhibitory concentration. The MIC value was determined to be 750 μg/mL, which was the lowest concentration that did not cause a colour change.

Minimum Bactericidal Concentration (MBC)

By streaking these MIC wells on BHI agar plates, the minimum bactericidal concentration was assessed. The concentration that had no colonies was classified as MBC. At 1000 μg/mL, the MBC concentration was seen. Bromelain has been found to be antibacterial at concentrations of 1000 μg/mL and higher.

CONCLUSION

The formulation of the bromelain-loaded nanoemulgel successfully overcame the inherent limitations of the enzyme, including its poor aqueous solubility and suboptimal skin penetration. The optimized formulation exhibited excellent physicochemical properties, with a remarkable penetration efficiency of 70.56% over an 8-hr period, significantly enhancing the bioavailability of bromelain. Moreover, the nanoemulgel demonstrated substantial antibacterial activity against *Propionibacterium acnes*, further substantiating its therapeutic potential. These findings validate the study's hypothesis, positioning the nanoemulsion-based system as a promising strategy to enhances the effective delivery of bromelain for targeted topical acne treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study was carried out without financial support from any public, commercial, or non-profit funding agencies.

ABBREVIATIONS

BR: Bromelain; **%:** Percentage; **°C:** Degree Centigrade; **nm:** Nanometre; **μg:** Microgram; **μL:** Microliter; **CDR:** Cumulative Drug Release; **DSC:** Differential Scanning Calorimetry; **FTIR:** Fourier transmission infrared; **g:** Gram; **Hr:** Hours; **mg:** Milligram; **mL:** Millilitre **rpm:** Rotation per minute; **UV** Ultraviolet; **w/v:** Weight by volume; **λ_{max}:** Maximum Absorbance **MIC:** Minimum Inhibitory Concentration; **MBC:** Minimum Bactericidal Concentration; **P. Acnes**: Propionibacterium acnes; **PDI:** Polydispersity Index.

SUMMARY

This study successfully developed a bromelain-loaded nanoemulgel to address the challenges of poor solubility and limited skin penetration associated with the enzyme. The nanoemulsion was optimized using a 3^2 factorial design, yielding a formulation with a high drug content of 87.55% and a small globule size of 105.8 nm. The optimized nanoemulsion was incorporated into a xanthan gum-based gel matrix, augmented with penetration enhancers. *In vitro* and *ex vivo* evaluations demonstrated that the nanoemulgel exhibited superior drug release and skin penetration compared to conventional emulgel and plain gel formulations. These results suggest the potential of the bromelain-infused nanoemulgel as an effective and targeted topical treatment for acne, warranting further clinical investigation.

REFERENCES

1. Song YH, Cai H, Gu N, Qian CF, Cao SP, Zhao ZM. Icariin attenuates cardiac remodelling through down-regulating myocardial apoptosis and matrix metalloproteinase activity in rats with congestive heart failure. *J Pharm Pharmacol*. 2011;63(4):541-549.
2. Liu CH, Huang HY. *In vitro* anti-propionibacterium activity by curcumin-containing vesicle system. *Chem Pharm Bull*. 2013;61(4):419-425.
3. Zouboulis C, Eady A, Philpott M, *et al.* What is the pathogenesis of acne? *Exp Dermatol*. 2005;14:143-152.
4. Wu TQ, Mei SQ, Zhang JX, *et al.* Prevalence and risk factors of facial *Acne vulgaris* among Chinese adolescents. *Int J Adolesc Med Health*. 2007;19(4):407-412.
5. Perkins AC, Maglione J, Hillebrand GG, Miyamoto K, Kimball AB. *Acne vulgaris* in women: prevalence across the lifespan. *J Womens Health*. 2012;21(2):223-230.
6. Zaenglein AL, Pathy AL, Schlosser BJ, *et al.* Guidelines of care for the management of *Acne vulgaris*. *J Am Acad Dermatol*. 2016;74(5):945-973.33.
7. Bhate K, Williams HC. Epidemiology of *Acne vulgaris*. *Br J Dermatol*. 2013;168(3):474-485.
8. Rapp SR, Feldman SR, Graham G, Fleischer AB, Brenes G, Dailey M. The acne quality of life index (Acne-QOLI): development and validation of a brief instrument. *Am J Clin Dermatol*. 2006;7(3):185-192.
9. Kapoor S, Saraf S. Topical herbal therapies: an alternative and complementary choice to combat acne. *Res J Med Plant*. 2011;5(6):650-669.
10. Tahir I, Khan MR, Shah NA, Aftab M. Evaluation of phytochemicals, antioxidant activity, and amelioration of pulmonary fibrosis with *Phyllanthus emblica* leaves. *BMC Complement Altern Med*. 2016;16(1):1-12.
11. Williams HC, Dellavalle RP, Garner S. *Acne vulgaris*. *Lancet*. 2012;379(9813):361-372.
12. Bahramsoltani R, Farzaei MH, Rahimi R. Medicinal plants and their natural components as future drugs for the treatment of burn wounds: an integrative review. *Arch Dermatol Res*. 2014;306(7):601-617.
13. Soleymani S, Farzaei MH, Zargaran A, Niknam S, Rahimi R. Promising plant-derived secondary metabolites for the treatment of *Acne vulgaris*: a mechanistic review. *Arch Dermatol Res*. 2020;312(1):5-23.
14. Hidayat Y, Hermawati E, Setiasih S, Hudiyono S, Saepudin E. Antibacterial activity test of partially purified bromelain from pineapple core extract (*Ananas comosus* [L.] Merr) by fractionation using ammonium sulfate acetone. *AIP Conf Proc*. 2018; 2023.
15. Ozlen SN, inventor; Longevity Network Ltd, assignee. Cosmetic composition containing alpha hydroxy acids, salicylic acid, and enzyme mixture of bromelain and papain. United States patent US 5,441,740. 1995 Aug 15.
16. Loon YK, Satari MH, Dewi W. Antibacterial effect of pineapple (*Ananas comosus*) extract towards *Staphylococcus aureus*. *Padjadjaran J Dent*. 2018;30(1):1.
17. Azimi H, Fallah-Tafti M, Khakshur AA, Abdollahi M. A review of phytotherapy of *Acne vulgaris*: perspective of new pharmacological treatments. *Fitoterapia*. 2012;83(8):1306-1317.
18. Ajazuddin, Alexander A, Khichariya A, *et al.* Recent expansions in an emergent novel drug delivery technology: emulgel. *J Control Release*. 2013;171(2):122-132.
19. Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization, and anti-inflammatory activity of *Swietenia macrophylla* nanoemulgel. *J Nanomedicine Nanotechnol*. 2014;5(2).
20. Ahmad J, Gautam A, Komath S, Bano M, Garg A, Jain K. Topical nano-emulgel for skin disorders: formulation approach and characterization. *Recent Pat Antiinfect Drug Discov*. 2018;14(1):36-48.
21. Ahmad J, Kohli K, Mir S, Amin S. Self-emulsifying nano carriers for improved oral bioavailability of lipophilic drugs. *Rev Adv Sci Eng*. 2012;1(2):134-147.
22. Akram S, Anton N, Omran Z, Vandamme T. Water-in-oil nano-emulsions prepared by spontaneous emulsification: new insights on the formulation process. *Pharmaceutics*. 2021;13(7).
23. Chang M, Guo Y, Jiang Z, *et al.* Sea buckthorn pulp oil nanoemulsions fabricated by ultra-high pressure homogenization process: a promising carrier for nutraceutical. *J Food Eng*. 2020; 287(May):110129.
24. Estanqueiro M, Conceição J, Amaral MH, Santos D, Silva JB, Lobo JMS. Characterization and stability studies of emulsion systems containing pumice. *Braz J Pharm Sci*. 2014;50(2):361-369.
25. Al-Sabagh AM, Khalil SA, Abdelrahman A, *et al.* Investigation of oil and emulsion stability of locally prepared metalworking fluids. *Ind Lubr Tribol*. 2012;64(6):346-358.
26. Mekhilef SF, Hussein A. Novel combination for self-nanoemulsifying drug delivery system of candesartan cilexetil. *Iraqi J Pharm Sci*. 2018;27(2):123-134.
27. Morsy MA, Abdel-Latif RG, Nair AB, *et al.* Preparation and evaluation of atorvastatin-loaded nanoemulgel on wound-healing efficacy. *Pharmaceutics*. 2019;11(11):1-15.
28. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. *Braz J Pharm Sci*. 2016;52(3):493-507.
29. Mulye SP, Wadkar KA, Kondawar MS. Formulation development and evaluation of indomethacin emulgel. *Der Pharm Sin*. 2013;4(5):31-45.
30. Ibrahim MM, Shehata TM. The enhancement of transdermal permeability of water-soluble drug by niosome-emulgel combination. *J Drug Deliv Sci Technol*. 2012;22(4):353-359.
31. Abdallah MH, Abu Lila AS, Unissa R, *et al.* Preparation, characterization, and evaluation of anti-inflammatory and anti-nociceptive effects of brucine-loaded nanoemulgel. *Colloids Surf B Biointerfaces*. 2021;205:111868.
32. Apriani E, Rosana Y, Iskandarsyah I. Formulation, characterization, and *in vitro* testing of azelaic acid ethosome-based cream against *Propionibacterium acnes* for the treatment of acne. *J Adv Pharm Technol Res*. 2019;10(2):75-80.
33. Huang WC, Tsai TH, Chuang L-T, Li YY, Zouboulis CC, Tsai PJ. Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: a comparative study with lauric acid. *J Dermatol Sci*. 2014;73(3):232-240.
34. Rajaram SK, Ahmad P, Sathya Keerthana SS, *et al.* Extraction and purification of an antimicrobial bioactive element from lichen-associated *Streptomyces olivaceus* LEP7 against wound-inhabiting microbial pathogens. *J King Saud Univ Sci*. 2020;32(3):2009-2015.
35. Gao C, Guo N, Li N, *et al.* Investigation of antibacterial activity of aspirin BB against *Propionibacterium acnes*. *Arch Dermatol Res*. 2016;308:79-86.
36. Murbach Teles Andrade BF, Nunes Barbosa L, Bérgamo Alves FC, *et al.* The impact of *Cymbopogon martini* essential oil on *Cutibacterium acnes* strains and its interaction with keratinocytes. *J Pharm Pharmacol*. 2018;70(12):1688-1699.

Cite this article: Patil AS, Ugare P, Patil A, Patil S, Masareddy R. Formulation and Evaluation of a Bromelain-Loaded Nanoemulgel for Effective Acne Treatment. Indian J of Pharmaceutical Education and Research. 2026;60(2s):s815-s825.

Supplementary Tables

Table S1: Visual observations during water titration for blank Oil: S_{mix} mixtures (S_{mix} ratio: 1:1).

Oil: S_{mix}	Observations made during titration after each incremental addition of water (S_{mix} ratio: 1:1)					
1:9	0 NE	9.1 NE	16.66 NE	23.08 NE	28.57 C	-
1:7	0 NE	11.11 NE	20 NE	27.27 G	-	-
1:6	0 NE	12.5 E	22.22 G	-	-	-
1:4	0 NE	16.66 C	28.57 E	37.5 G	-	-
3:7	0 NE	9.09 C	16.66 E	23.07 E	-	-
8:2	0 NE	9.09 C	16.66 E	-	-	-

Where, NE-Nanoemulsion, C- Cloudy emulsion, E- Milky emulsion, G-Transparent gel, PS- Phase Separation.

Table S2: Visual observations during water titration for blank Oil: S_{mix} mixtures (S_{mix} ratio: 1:2).

Oil: S_{mix}	Observations made during titration after each incremental addition of water (S_{mix} ratio: 1:2)					
1:9	0 NE	9.09 C	16.66 C	23.07 C	28.57 C	-
1:7	0 NE	11.11 C	20 C	27.27 C	-	-
1:6	0 NE	12.5 C	22.22 C	30 C	-	-
1:4	0 NE	16.66 C	28.57 C	37.5 C	-	-
3:7	0 NE	9.09 C	16.66 C	23.07 C	-	-
8:2	0 NE	9.09 C	-	-	-	-

Where, NE-Nanoemulsion, C- Cloudy emulsion, E- Milky emulsion, G- Transparent gel, PS- Phase Separation.

Table S3: Visual observations during water titration for blank Oil: S_{mix} mixtures (S_{mix} ratio: 2:1).

Oil: S_{mix}	Observations made during titration after each incremental addition of water (S_{mix} ratio: 2:1)					
1:9	0 NE	18.18 NE	16.66 NE	23.07 G	-	-
1:7	0 NE	11.11 NE	20 C	27.27 G	-	-
1:6	0 NE	12.5 NE	22.22 C	30 G	-	-
1:4	0 NE	16.66 NE	28.57 C	37.5 G	-	-
3:7	0 NE	9.09 C	16.66 E	-	-	-
8:2	0 NE	9.09 C	16.66 E	-	-	-

Where, NE-Nanoemulsion, C- Cloudy emulsion, E- Milky emulsion, G- Transparent gel, PS- Phase Separation.

Table S4: Visual observations during water titration for blank Oil: Smix mixtures (S_{mix} ratio: 3:1).

Oil: S_{mix}	Observations made during titration after each incremental addition of water (S_{mix} ratio: 3:1)					
1:9	0 NE	9.09 C	16.66 C	23.07 E	28.57 PS	-
1:7	0 NE	11.11 C	20 E	27.27 PS	33.33 PS	-
1:6	0 NE	12.5 C	22.22 C	30 E	36.36 PS	-
1:4	0 NE	16.66 C	28.57 C	37.5 PS	44.55 PS	-
3:7	0 NE	9.09 E	16.66 PS	23.07 PS	28.57 PS	-
8:2	0 NE	9.09 E	18.36 E	24.52 E	-	-

Where, NE-Nanoemulsion, C- Cloudy emulsion, E- Milky emulsion, G- Transparent gel, PS- Phase Separation.

Table S5: Screening of Oil: S_{mix}.

	Oil: Smix: Water	Oil: Smix	Particle Size (nm)	PDI
TF1	5:75:20	1:15	383.3	0.13
TF2	6:72:22	1:12	305.3	0.22
TF3	8:72:20	1:9	281.9	0.18
TF4	7:56:37	1:8	241	0.21
TF5	9:63:28	1:7	275	0.25
TF6	12:72:16	1:6	347	0.025

Table S6: ANOVA for quadratic Drug Content (Y1) models.

Source	Sum of Squares	d_f	Mean Square	F-value	*p*-value	
Model	1349.76	2	674.88	9.76	0.0130	Significant
A-Oil	1243.58	1	1243.58	17.98	0.0054	
B-Smix	106.18	1	106.13	1.54	0.2616	
Residual	414.91	6	69.15			
Cor Total	1764.67	8				

Std. Dev.	8.32	R^2	0.7649
Mean	65.59	Adjusted R^2	0.6865
C.V. %	12.68	Predicted R^2	0.4216
press	1020.63	Adeq Precision	7.7496

Table S7: ANOVA for quadratic Particle Size (Y2) models.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9955.08	5	1991.02	18.29	0.0187	Significant
A-Oil	6720.11	1	6720.11	61.72	0.0043	
B-Smix	737.04	1	737.04	6.77	0.0803	
AB	696.96	1	696.96	6.40	0.0854	
A^2	1744.44	1	1744.44	16.02	0.0280	
B^2	56.53	1	56.53	0.5192	0.5232	
Residual	326.64	3	108.88			
Cor Total	10281.72	8				

Std. Dev.	10.43	R^2	0.9682
Mean	146.74	Adjusted R^2	0.9153
C.V. %	7.11	Predicted R^2	0.6215
press	3891.61	Adeq Precision	11.3304

Table S8: ANOVA for quadratic PDI (Y3) models.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0939	5	0.0188	19.07	0.0176	Significant
A-Oil	0.0131	1	0.0131	13.28	0.0357	
B-Smix	0.0504	1	0.0504	51.22	0.0056	
AB	0.0056	1	0.0056	5.71	0.0967	
A^2	0.0228	1	0.0228	23.12	0.0171	
B^2	0.0020	1	0.0020	2.04	0.2487	
Residual	0.0030	3	0.0010			
Cor Total	0.0968	8				

Std. Dev.	0.0314	R^2	0.9695
Mean	0.2644	Adjusted R^2	0.9187
C.V. %	11.86	Predicted R^2	0.6349
press	0.0354	Adeq Precision	14.6068

Table S9: Thermodynamic study for Nanoemulsions.

Formulation Code	Centrifugation test	Heating -cooling cycle test	Freeze-thawing cycle test
F1	Pass	Pass	Pass
F2	Pass	Pass	Pass
F3	Pass	Pass	Pass
F4	Pass	Pass	Pass
F5	Pass	Pass	Pass
F6	Pass	Pass	Pass
F7	Pass	Pass	Pass
F8	Pass	Pass	Pass
F9	Pass	Pass	Pass

Table S10: Result of stability study.

F4 Formulation	Drug Content (%)	Globule Size (nm)	PDI
0 month	87.55%	105.9	0.32
1 month (4)	85.65%	106.5	0.30
1 month (25)	84.13%	108.2	0.29

Table S11: Release Kinetics of Different gel formulations.

Mathematical Models	Plain gel	Emulgel	Nanoemulgel
Zero Order	0.953	0.979	0.988
First Order	0.886	0.898	0.915
Higuchi	0.973	0.995	0.996
Korsmeyer Peppas	0.969	0.991	0.992