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Original Article

Optimization, Synthesis, and Characterization of Nanoemulgel Containing *Wrightia tinctoria* and Evaluation of Its *In Vitro* Anti-Inflammatory Activity

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ABSTRACT

Wrightia tinctoria is a medicinal plant widely recognized for its anti-inflammatory and wound-healing properties. Incorporation of herbal extracts into nanoemulgel systems can enhance drug stability, skin penetration, and therapeutic efficacy for topical applications. The present study aimed to optimize, formulate, and characterize a nanoemulgel containing *Wrightia tinctoria* extract and evaluate its in vitro anti-inflammatory activity. Nanoemulsion formulations were prepared using red sandalwood oil, Tween 80, PEG 400/Transcutol P, and *Wrightia tinctoria* extract through high-energy emulsification techniques. Optimization was carried out using Central Composite Design (CCD) with Design Expert® software by varying oil, Smix, and Carbopol concentrations. The optimized nanoemulsion was incorporated into a Carbopol gel base to prepare the nanoemulgel. Physicochemical characterization included particle size, polydispersity index (PDI),

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zeta potential, viscosity, pH, spreadability, drug content, and in vitro drug release studies. Morphological analysis was performed using FE-SEM and HR-TEM techniques. The optimized formulation exhibited nanosized droplets ranging from 120–150 nm with a low PDI (0.22 ± 0.03), indicating uniform distribution. The nanoemulgel showed suitable viscosity (1800 cP), pH (6.2 ± 0.1), good spreadability, and satisfactory stability under different storage conditions. FE-SEM and HR-TEM analyses confirmed spherical and uniformly dispersed droplets without aggregation. ANOVA results demonstrated that Carbopol significantly influenced viscosity and formulation stability. The optimized formulation exhibited sustained drug release and promising in vitro anti-inflammatory activity. The developed *Wrightia tinctoria* nanoemulgel demonstrated favorable physicochemical characteristics, stability, and effective anti-inflammatory potential, suggesting its suitability as a promising herbal topical drug delivery system for inflammatory conditions.

Keywords: Wrightia tinctoria, Nanoemulgel, Anti-inflammatory activity, Herbal drug delivery, Topical formulation

INTRODUCTION

Inflammation is a protective biological response that occurs when the body is exposed to injury, infection, or other harmful stimuli. This process helps eliminate damaging agents and initiates tissue repair [1]. Although inflammation is essential for maintaining health, prolonged or excessive inflammatory responses can contribute to the development of chronic diseases, including arthritis, psoriasis, dermatitis, cardiovascular disorders, and autoimmune conditions [2]. Conventional anti-inflammatory medications such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment; however, their prolonged use is often associated with adverse effects, including gastrointestinal irritation, renal complications, and skin-related problems [3,4]. These limitations have encouraged the search for safer and more effective therapeutic alternatives, particularly those derived from natural sources.

Medicinal plants have gained considerable attention because of their long history of use and the presence of biologically active compounds [5]. Among them, Wrightia tinctoria has emerged as an important medicinal plant with significant therapeutic value [6]. It has been traditionally used in various systems of medicine for the treatment of inflammatory skin disorders, wounds, and psoriasis [7]. The plant contains several phytoconstituents, including flavonoids, alkaloids, phenolic compounds, triterpenoids, and steroids, which are reported to possess antioxidant and

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anti-inflammatory properties. Previous studies have demonstrated the ability of Wrightia tinctoria extracts to reduce inflammatory responses, making it a promising candidate for topical drug delivery.

Recent advances in nanotechnology have led to the development of nanoemulsion-based systems for improving the delivery of herbal medicines [8]. Nanoemulsions are dispersions with droplet sizes in the nanometer range, offering advantages such as enhanced solubility, improved stability, greater skin penetration, and increased bioavailability of active compounds [9]. Their low viscosity may limit retention at the site of application. To overcome this drawback, nanoemulsions can be incorporated into gel bases to form nanoemulgels. These systems combine the penetration-enhancing properties of nanoemulsions with the convenience, viscosity, and prolonged residence time of gels.

The present study focuses on the formulation and optimization of a Wrightia tinctoria-loaded nanoemulgel for topical anti-inflammatory application. Various formulation parameters, including particle size, zeta potential, viscosity, pH, spreadability, and drug release, were evaluated to obtain a stable and effective product. The anti-inflammatory potential of the developed formulation was also investigated to assess its suitability as an herbal topical delivery system for the management of inflammatory conditions.

METHODOLOGY

Procedure for Optimization of Nanoemulgel Containing *Wrightia tinctoria*

A nanoemulgel containing Wrightia tinctoria leaf extract was formulated using a Central Composite Design (CCD) to optimize key formulation parameters. The study evaluated the effects of extract concentration, oil phase, and surfactant–co-surfactant mixture on particle size, entrapment efficiency, and drug release. Twenty experimental formulations were prepared by combining the aqueous drug solution, oil phase, and Smix, followed by homogenization and sonication to obtain nanoemulsions. The optimized nanoemulsion was incorporated into a Carbopol gel base to produce the nanoemulgel. All formulations were assessed for physicochemical properties and drug release behavior, and statistical analysis identified the most effective formulation [10, 11].

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Table 1: Experimental design matrix showing composition of oil, Smix, water and corresponding responses (zeta potential and viscosity).

Block	Run	Component 1	Component 2	Component 3	Response 1	Response 2
		A: Red sandalwood oil %	B: Smix (Tween 80 + PEG) %	C: Water %	Zeta potential (Mv)	Viscosity (cP)
Batch 1	1	20	70	10	30	2000
Batch 1	2	12.6077	70	17.3923	25	1500
Batch 1	3	12	47	41	28	1900
Batch 1	4	12	47	41	25	2000
Batch 1	5	20	38.3882	41.6118	20	1590
Batch 1	6	5	35	60	30	1700
Batch 1	7	12	47	41	28	2000
Batch 1	8	5	35	60	30	1800
Batch 1	9	12.6077	70	17.3923	26	1500
Batch 1	10	20	50.2703	29.7297	28	1600
Batch 1	11	5	68.9	26.1	20	1400
Batch 2	12	11.6427	52.1738	36.1835	25	2000

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Batch 2	13	5	43.6108	51.3892	28	1900
Batch 2	14	11.8534	30	58.1466	16	1800
Batch 2	15	20	61.2933	18.7067	20	1500
Batch 2	16	11.8534	30	58.1466	15	1700
Batch 2	17	5	61.2562	33.7438	24	2000
Batch 2	18	20	30	50	30	1800

Preparation of optimised nano-emulsion containing *Wrightia tinctoria* extract and red sandal wood oil.

The preparation of an optimized nano-emulsion containing *Wrightia tinctoria* extract and red sandalwood oil begins with drying and powdering the *Wrightia tinctoria* leaves, followed by extraction using ethanol in a Soxhlet apparatus (e.g., 100 mg powder with 700 mL ethanol for 18 hours), and solvent evaporation at 30–35°C to yield a concentrated extract [11]. For the oil phase, the obtained extract is mixed with red sandalwood oil and an oil-soluble surfactant such as Span 80. Separately, a hydrophilic surfactant Tween 80 is dissolved in distilled water to form the aqueous phase. The oil phase is then added dropwise to the aqueous phase under continuous stirring, creating a coarse pre-emulsion. This pre-emulsion is subjected to high-speed homogenization (10,000–15,000 rpm for 10–15 minutes) to reduce droplet size, forming a transparent slightly opalescent nanoemulsion indicative of nanometer-sized droplets. Optimization involves adjusting the concentrations of oil, surfactants, and homogenization parameters, often using statistical tools to achieve minimal droplet size and polydispersity. The final nanoemulsion is characterized by particle size, zeta potential, stability, and encapsulation efficiency to ensure its suitability for topical or pharmaceutical use [12, 13].

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Table 2: Composition of Nanoemulsion Formulations (F1–F3)

Ingredients	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
Red sandalwood oil + *Wrightia tinctoria* extract.	5	7	10
Tween 80 (Surfactant)	30	28	25
PEG 400 / Transcutol P (Co-surfactant)	20	25	30
Distilled Water.	45	40	35
Total	100	100	100

Particle Size Analysis

The particle size and polydispersity index (PDI) of the nanoemulsion were measured using dynamic light scattering (DLS). The sample was diluted with distilled water and analyzed under controlled conditions using a particle size analyzer. The technique measured droplet movement in the dispersion and calculated the average size and PDI. Particle size confirmed the nanoscale nature of the formulation, while PDI reflected size uniformity. Measurements were performed three times, and mean values were recorded.

Zeta Potential Measurement

The zeta potential of the nanoemulsion was measured to evaluate the surface charge and stability of the formulation. A small quantity of the nanoemulsion was diluted with distilled water to achieve suitable conductivity and prevent aggregation during measurement. The diluted sample was introduced into a specialized zeta potential cell, ensuring that no air bubbles were present as they could interfere with the readings. The measurement was carried out using a zeta potential analyzer based on electrophoretic mobility principles. When an electric field was applied, charged particles moved toward the electrode of opposite charge, and their velocity was measured to calculate the zeta potential. The analysis was conducted at room temperature to maintain consistency. The obtained values indicated the degree of electrostatic repulsion between particles; higher absolute values suggested better stability due to reduced aggregation tendency. Each sample was analyzed multiple times, and the average zeta potential value was reported.

pH Determination

The pH of the nanoemulgel formulations was determined to ensure their compatibility with skin and to minimize the risk of irritation. A calibrated digital pH meter was used for accurate

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measurement. Before analysis, the pH meter was standardized using buffer solutions of known pH values to ensure precision. Approximately 1 g of the nanoemulgel was weighed and dispersed in a small volume of distilled water to form a uniform mixture. The electrode of the pH meter was then immersed into the sample, ensuring complete contact with the formulation. The reading was allowed to stabilize before recording the pH value. Measurements were performed at room temperature, and each sample was analyzed in triplicate to ensure consistency. The obtained pH values were compared with the physiological pH of the skin to confirm suitability for topical application.

Viscosity Measurement

The viscosity of the nanoemulgel formulations was measured using a Brookfield viscometer to determine their flow behavior and consistency. The instrument was equipped with an appropriate spindle based on the viscosity range of the sample. Before measurement, the viscometer was calibrated, and the temperature was maintained at a constant level to avoid variations in readings. The nanoemulgel sample was carefully transferred into the sample holder, ensuring the absence of air bubbles. The spindle was immersed into the sample, and viscosity readings were recorded at different rotational speeds to evaluate the rheological behavior. The instrument measured the resistance offered by the formulation to the rotating spindle, which was then converted into viscosity values. All measurements were carried out in triplicate, and the average values were calculated. The results helped in assessing the suitability of the formulation for topical application in terms of spreadability and stability.

Spreadability

The spreadability of the nanoemulgel was evaluated using the parallel plate method, which is a simple and effective technique to assess ease of application. A known quantity of the nanoemulgel was placed on a clean glass slide, and another slide was carefully placed over it to form a uniform layer. A specific weight was then placed on the upper slide to apply uniform pressure. The time required for the upper slide to move a predetermined distance was recorded using a stopwatch. The spreadability was calculated using the formula $S = (M \times L) / T$, where M represents the applied weight, L is the length moved by the slide, and T is the time taken. This test was repeated multiple times to ensure accuracy and reproducibility. Higher spreadability values indicated better ease of application and uniform distribution over the skin surface.

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Drug Content Determination

The drug content of the nanoemulgel was determined to ensure uniform distribution of the active ingredient within the formulation. An accurately weighed quantity of the nanoemulgel was taken and dissolved in a suitable solvent to extract the drug completely. The mixture was sonicated for a specific duration to enhance dissolution and ensure complete drug release from the formulation matrix. The resulting solution was then filtered to remove any undissolved particles. The filtrate was appropriately diluted and analyzed using a UV-visible spectrophotometer at a predetermined wavelength corresponding to the drug. A calibration curve was prepared using standard drug solutions, and the drug content in the sample was calculated based on absorbance values. The analysis was performed in triplicate, and the average value was reported.

***In-Vitro* Drug Release Study**

The in vitro drug release study was conducted using a Franz diffusion cell to evaluate the release behavior of the nanoemulgel. A suitable membrane, such as a dialysis membrane or excised animal skin, was mounted between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 7.4) and maintained at a constant temperature of 37 ± 0.5°C with continuous stirring to simulate physiological conditions. A measured quantity of the nanoemulgel was placed in the donor compartment. At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with fresh buffer to maintain sink conditions. The collected samples were analyzed using a UV-visible spectrophotometer, and the cumulative percentage drug release was calculated. The experiment was conducted in triplicate to ensure reliability.

Skin Irritation Study

The skin irritation study was carried out to evaluate the safety of the nanoemulgel for topical application. The study was performed on suitable animal models following ethical guidelines. The dorsal surface of the animal was carefully shaved to remove hair and expose the skin. A specific quantity of the nanoemulgel was applied to the test site, while a control site was maintained without treatment or with a placebo. The application area was observed at regular intervals of 24, 48, and 72 hours for any signs of irritation such as redness, swelling, or inflammation. The severity of irritation, if any, was recorded and compared with the control. The absence of significant irritation indicated that the formulation was safe and suitable for topical use.

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RESULTS AND DISCUSSION

The Central Composite Design (CCD) in Design Expert Software version 13

The nanoemulgel containing Wrightia tinctoria leaf extract and red sandalwood oil was optimized using Central Composite Design (CCD) and Response Surface Methodology (RSM) with Design Expert® software (Version 13.0). Three formulation variables, namely red sandalwood oil (A), Smix (B), and Carbopol (C), were selected as independent factors and studied at different levels. Viscosity and zeta potential were chosen as the response variables because of their importance in determining formulation stability and topical performance. The design generated 20 experimental runs comprising factorial, axial, and center points. The obtained data were analyzed using polynomial models, and ANOVA was applied to assess the significance of the models and formulation variables. Model suitability was evaluated using statistical parameters such as R^2, adjusted R^2, predicted R^2, and lack-of-fit values. Response surface and contour plots were used to examine the interactions among the variables. Numerical optimization and desirability analysis were performed to identify the most suitable formulation, and the optimized batch was validated to verify the reliability of the predicted results.

ANOVA for Linear model (Response 1: Viscosity)

The ANOVA results for the linear viscosity model show that the formulation variables collectively have a significant effect on viscosity, as indicated by an F-value of 3.83 and a p-value of 0.0305. Among the individual factors, only Carbopol (C) is statistically significant, with an F-value of 9.97 and a p-value of 0.0061, demonstrating that it is the primary contributor to the viscosity of the formulation. This finding is consistent with the known role of Carbopol as a gelling agent that markedly increases formulation thickness and structural strength. Red sandalwood oil (A) and Smix (B) do not significantly influence viscosity within the studied concentration range, as evidenced by their high p-values (0.6250 and 0.2772, respectively). The residual variation suggests the presence of unexplained variability, and the significant lack-of-fit result ($F = 5.19$, $p = 0.0409$) indicates that the linear model does not fully describe the system. Therefore, although the model identifies Carbopol as the key determinant of viscosity, future studies should consider nonlinear effects or interactions to improve predictive accuracy.

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Table 13: Analysis of variance (ANOVA) for the linear model of viscosity, showing the effects of red sandalwood oil (A), Smix (B), and Carbopol (C) on the response, including model significance, individual factor contributions, and lack-of-fit assessment.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.137E+05	3	2.379E+05	3.83	0.0305	significant
A-A: Oil	15433.72	1	15433.72	0.2484	0.6250	
B-B: Smix	78644.69	1	78644.69	1.27	0.2772	
C-C: Carbopol	6.197E+05	1	6.197E+05	9.97	0.0061	
Residual	9.943E+05	16	62140.82			
Lack of Fit	9.143E+05	11	83113.92	5.19	0.0409	significant
Pure Error	80000.00	5	16000.00			
Cor Total	1.708E+06	19				

The Model F-value of 3.83 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case C is a significant model term. The Lack of Fit F-value of 5.19 implies the Lack of Fit is significant.

The regression analysis was used to determine how each formulation component affected viscosity. The intercept value of 1940.00 represents the average viscosity of the formulation at the midpoint levels of all variables. Among the factors studied, Carbopol showed the greatest influence on viscosity, with a positive coefficient of 213.01. This indicates that increasing the Carbopol concentration increases the viscosity of the formulation, which is consistent with its function as a gelling agent. The confidence interval for Carbopol did not cross zero, confirming its significant effect. In comparison, red sandalwood oil and Smix had negative coefficients, suggesting a slight reduction in viscosity with increasing concentration. However, these effects were not statistically significant because their confidence intervals included zero. The variance inflation factor values were 1.0000 for all variables, indicating that there was no correlation among the factors and that the regression estimates were reliable.

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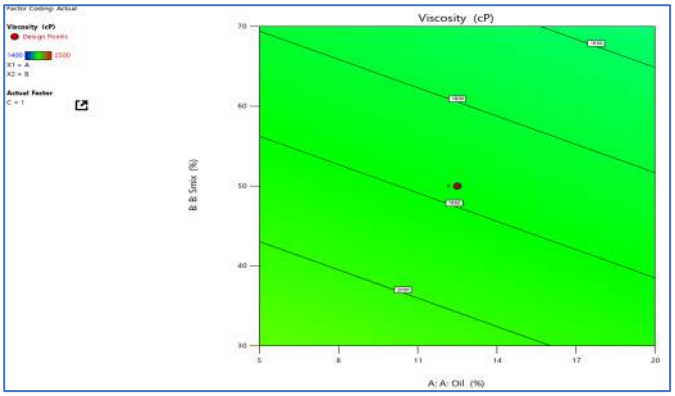


Figure 12. Response surface plot showing the effect of Carbopol concentration (A) and Tween 80 concentration (B) on the spreadability of the nanoemulgel formulation.

The contour plot illustrating the effect of red sandalwood oil (A) and Smix (B) on viscosity, with Carbopol concentration held constant, shows that both variables have only a minor influence on the response. The contour lines are almost parallel and evenly spaced, indicating a gradual and nearly linear change in viscosity across the design space. This suggests that the interaction between oil and Smix is minimal. Only slight variations in viscosity are observed, with a small decrease occurring as the concentrations of Smix and oil increase. This behavior may be related to improved dispersion and reduced internal resistance within the formulation. The central region of the plot corresponds to moderate viscosity values, indicating stable formulation characteristics at intermediate levels of both factors. Overall, the contour plot confirms that red sandalwood oil and Smix have limited effects on viscosity, whereas Carbopol remains the primary factor responsible for controlling the rheological properties of the nanoemulgel.

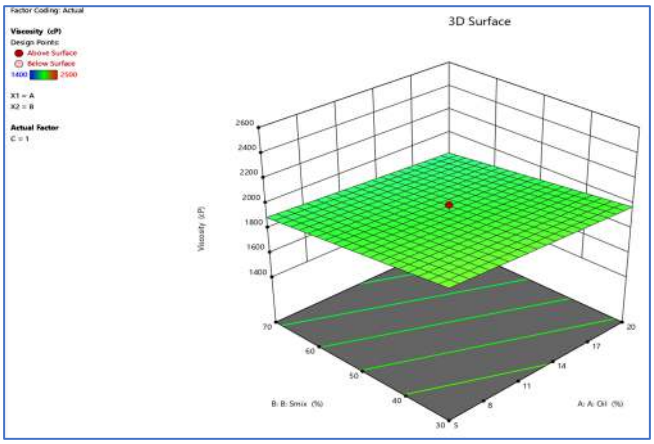


Figure 13. Response surface plot illustrating the influence of Carbopol concentration (A) and Tween 80 concentration (B) on the viscosity of the nanoemulgel formulation.

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The cube plot illustrating predicted viscosity values provides a comprehensive understanding of how red sandalwood oil (A), Smix (B), and Carbopol (C) interact to influence the rheological behavior of the formulation across their respective ranges. The variation in viscosity values at different vertices of the cube clearly indicates that Carbopol concentration has the most pronounced effect on the response. Higher viscosity values are consistently observed at elevated levels of Carbopol, confirming its dominant role as a thickening agent that enhances the structural integrity of the system. In contrast, changes in oil and Smix concentrations produce comparatively smaller variations in viscosity, suggesting their secondary influence. The plot also demonstrates that the combination of high Carbopol with moderate levels of oil and Smix results in the highest predicted viscosity, indicating a synergistic effect under specific conditions. Conversely, lower Carbopol levels correspond to reduced viscosity values regardless of the proportions of the other two components, reinforcing its primary contribution. The distribution of predicted values across the cube appears systematic and gradual, reflecting a predictable response pattern and supporting the adequacy of the model in capturing factor effects. Overall, the cube plot emphasizes that careful control of Carbopol concentration is essential for achieving the desired viscosity, while oil and Smix play supporting roles in fine-tuning the formulation properties.

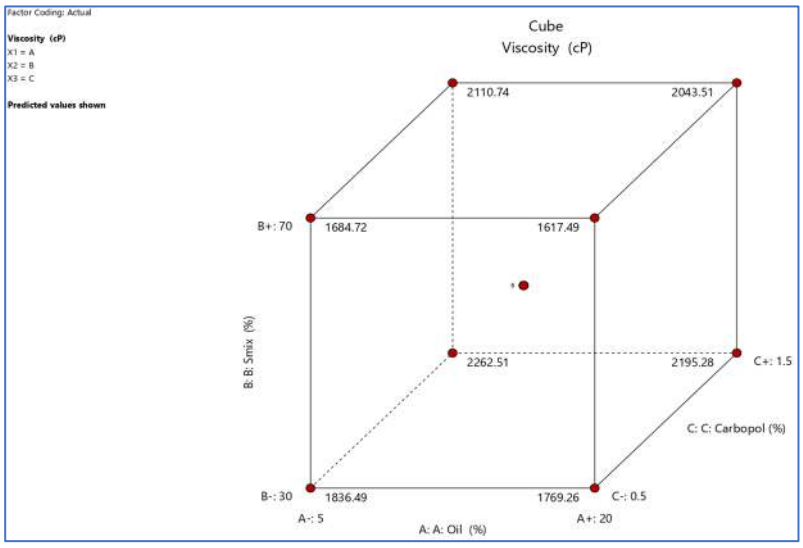


Figure 14. Response surface plot depicting the effect of Carbopol concentration (A) and Tween 80 concentration (B) on the drug release (%) from the nanoemulgel formulation.

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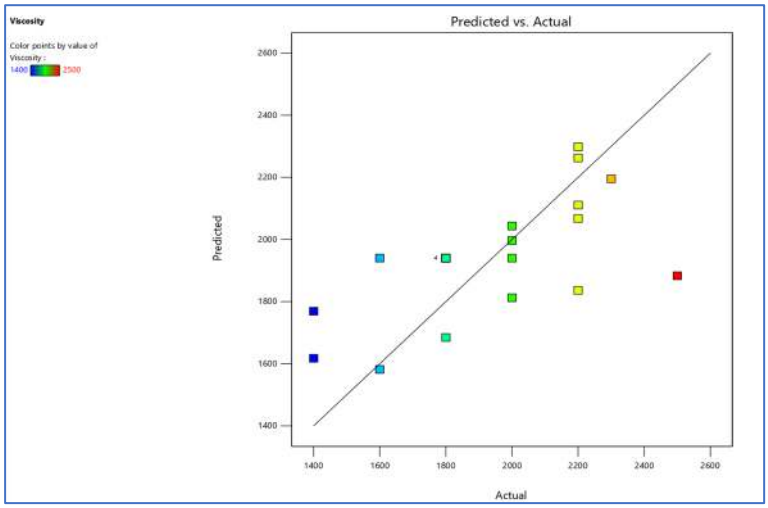


Figure 15. Overlay plot showing the optimized region for nanoemulgel formulation with desired spreadability, viscosity, and drug release characteristics.

ANOVA for 2FI model (Response 2: Zeta potential)

The ANOVA results indicate that the developed model is statistically significant, with an F-value of 4.00 and a p-value of 0.0172, showing that the formulation variables collectively influence the response. Among the factors studied, Carbopol (C) had the greatest effect, as evidenced by its significant F-value of 9.37 and p-value of 0.0091. This highlights its important role in determining the response, likely due to its ability to increase viscosity and strengthen the formulation structure. Smix (B) also showed a significant effect ($p = 0.0373$), suggesting that it contributes to the overall system performance. In contrast, red sandalwood oil (A) was not found to have a significant influence, as indicated by its p-value of 0.3806. The interaction terms AB and BC were not significant, while the AC interaction showed a borderline effect ($p = 0.0516$), suggesting a possible interaction between oil and Carbopol. The significant lack-of-fit value ($p = 0.0029$) indicates that the model does not completely explain the observed variation and may require additional terms or a higher-order model for improved prediction. Overall, Carbopol and Smix were identified as the main factors affecting the response, while further model refinement may enhance its accuracy.

Table 14: Analysis of variance (ANOVA) showing the effects of red sandalwood oil (A), Smix (B), and Carbopol (C), along with their interaction terms, on the response, including model significance, factor contributions, and lack-of-fit evaluation.

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Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	188.90	6	31.48	4.00	0.0172	significant
A-A: Oil	6.48	1	6.48	0.8238	0.3806	
B-B: Smix	42.34	1	42.34	5.38	0.0373	
C-C: Carbopol	73.71	1	73.71	9.37	0.0091	
AB	15.12	1	15.12	1.92	0.1889	
AC	36.12	1	36.12	4.59	0.0516	
BC	15.13	1	15.13	1.92	0.1889	
Residual	102.30	13	7.87			
Lack of Fit	98.80	8	12.35	17.64	0.0029	significant
Pure Error	3.50	5	0.7000			
Cor Total	291.20	19				

The Model F-value of 4.00 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. The Lack of Fit F-value of 17.64 implies the Lack of Fit is significant.

Coefficients in Terms of Coded Factors

Regression coefficient analysis was performed to determine the influence of individual formulation variables on the response while keeping the remaining factors constant. The intercept value of 26.20 represents the predicted response at the central levels of all variables. Among the factors studied, Carbopol (C) produced the largest positive coefficient (2.32), indicating that increasing its concentration significantly enhanced the response. The confidence interval for Carbopol did not include zero, confirming its statistical significance. In contrast, Smix (B) showed a negative coefficient (-1.76), suggesting that higher Smix concentrations reduced the response. Its confidence interval also excluded zero, demonstrating a significant effect. Red sandalwood oil (A) exhibited a small positive coefficient (0.6890), but its confidence interval crossed zero, indicating that its influence was not statistically significant. The interaction terms AB, AC, and BC were also not significant, although the AC interaction showed a slight effect that may deserve further study. The variance inflation factor values of 1.0000 confirmed the absence of multicollinearity among the variables. Overall, the results indicate that Carbopol and Smix were the main factors affecting the response, whereas red sandalwood oil and the interaction terms contributed only minimally within the investigated range.

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Table 15: Regression coefficient estimates showing the effects of red sandalwood oil (A), Smix (B), and Carbopol (C), along with their interaction terms, on the response, including confidence intervals and multicollinearity assessment (VIF).

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	26.20	1	0.6273	24.84	27.56	
A-A: Oil	0.6890	1	0.7591	-0.9509	2.33	1.0000
B-B: Smix	-1.76	1	0.7591	-3.40	-0.1208	1.0000
C-C: Carbopol	2.32	1	0.7591	0.6833	3.96	1.0000
AB	-1.37	1	0.9918	-3.52	0.7676	1.0000
AC	2.12	1	0.9918	-0.0176	4.27	1.0000
BC	-1.38	1	0.9918	-3.52	0.7676	1.0000

Regression coefficient analysis and response surface plots were used to understand the influence of formulation variables on viscosity and drug release of the nanoemulgel. The coefficient values indicated the direction and magnitude of the effect of each factor on the studied responses. Carbopol (X_1) was found to have the greatest effect on viscosity, with higher concentrations producing a marked increase due to the formation of a stronger and more compact gel network. In comparison, Wrightia tinctoria extract (X_2) showed only a minor effect on viscosity, suggesting that its primary role was related to therapeutic activity rather than modification of rheological properties. The response surface plots for drug release revealed significant interactions among the formulation variables. Increasing the concentration of Wrightia tinctoria extract enhanced drug release, whereas higher levels of red sandalwood oil (X_3) tended to reduce release by creating a more hydrophobic environment. Similarly, increasing Carbopol concentration decreased drug release by limiting drug diffusion through the viscous gel matrix. The interaction between Carbopol and the extract indicated that appropriate extract levels could improve release, although excessive Carbopol reduced this effect. The curved response surfaces suggested the presence of nonlinear relationships among the variables. Overall, Carbopol was identified as the main factor controlling viscosity and drug release, while Wrightia tinctoria extract and red sandalwood oil primarily influenced the release profile of the formulation.

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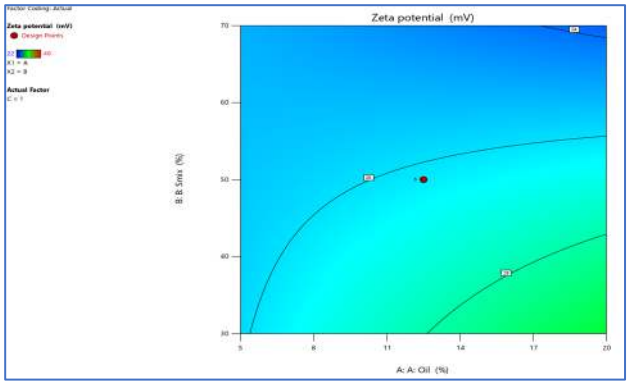


Figure 16: Response surface plot showing the effect of Carbopol (X_1) and Wrightia tinctoria extract (X_2) on the viscosity of the nanoemulgel.

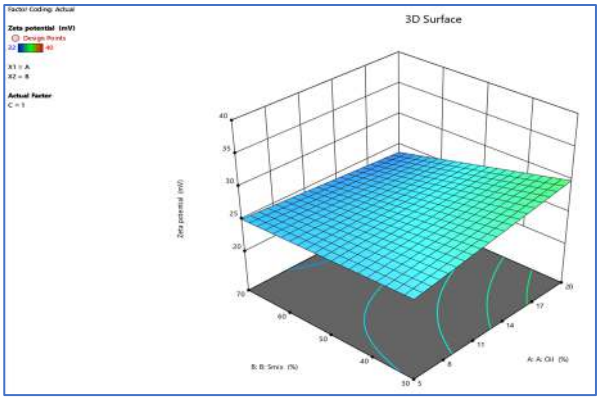


Figure 17: Response surface plot showing the effect of Wrightia tinctoria extract (X_2) and red sandalwood oil (X_3) on the drug release (%) of the nanoemulgel.

The response surface plot illustrating the combined effect of Wrightia tinctoria extract (X_2) and red sandalwood oil (X_3) on drug release (%) provides valuable insight into how these formulation components interact to influence the release behavior of the nanoemulgel. The surface profile clearly indicates that drug release is not governed by a single factor but is the result of a complex interplay between the extract and oil concentrations. An increase in Wrightia tinctoria extract (X_2) is associated with an enhancement in drug release, which may be attributed to improved solubilization of the active constituents and increased diffusion from the formulation matrix. Higher extract concentration likely promotes a more favorable partitioning of the drug into the external phase, thereby facilitating its release.

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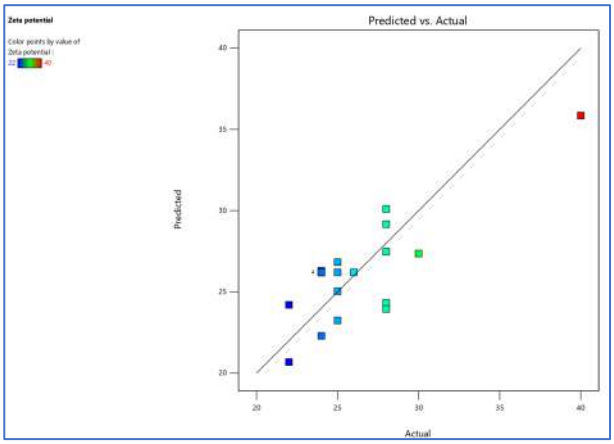


Figure 18: Response surface plot showing the effect of red sandalwood oil (X_3) and Carbopol (X_1) on the drug release (%) of the nanoemulgel.

An increase in red sandalwood oil (X_3) concentration was associated with a reduction in drug release, particularly at higher levels. This may be due to the hydrophobic nature of the oil, which creates a diffusion barrier and limits the movement of the drug from the formulation. The response surface showed a curved pattern, indicating that the relationship between extract concentration and oil content is nonlinear and that their combined influence on drug release is more complex than a simple additive effect. Maximum drug release was observed at lower oil concentrations and moderate to higher levels of Wrightia tinctoria extract, suggesting the existence of an optimal formulation region. In contrast, formulations containing high oil content and low extract concentration exhibited lower drug release, indicating restricted drug mobility within the nanoemulgel matrix. The gradual changes across the response surface further suggest that small variations in component concentrations can significantly affect the release behavior.

The response surface plot illustrating the interaction between red sandalwood oil (X_3) and Carbopol (X_1) demonstrated their combined effect on drug release from the nanoemulgel. Carbopol had a major influence on the release profile due to its ability to increase viscosity and form a stronger gel structure. As the concentration of Carbopol increased, drug release decreased because the denser polymer network restricted drug diffusion. Red sandalwood oil also affected drug release by altering the hydrophobic character of the formulation. Lower oil concentrations favored greater drug release, whereas higher concentrations reduced release by retaining the drug within the internal oil phase. The reduction in release became more evident when high oil concentrations were combined with elevated Carbopol levels, where both increased viscosity and hydrophobicity

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acted together to slow drug diffusion. The curved nature of the response surface indicated an interaction between these variables, suggesting that their combined influence was not purely linear. An optimum region was observed at moderate concentrations of both Carbopol and red sandalwood oil, where formulation stability was maintained while allowing adequate drug release. Such a balance is desirable for achieving a controlled and sustained release profile in topical nanoemulgel formulations.

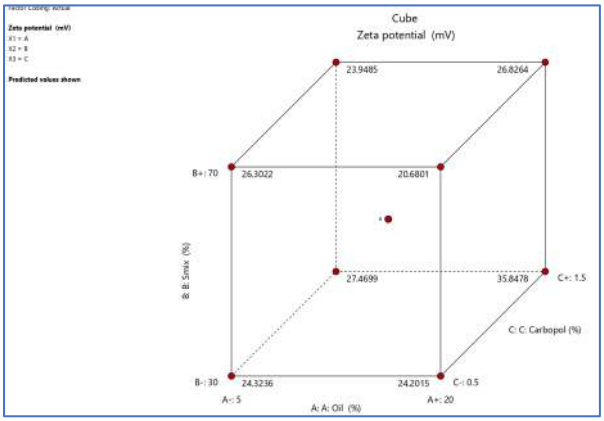


Figure 19: Response surface plot showing the effect of Carbopol (X_1) and Wrightia tinctoria extract (X_2) on the drug release (%) of the nanoemulgel.

The response surface plot illustrates the combined effect of Carbopol (X_1) and Wrightia tinctoria extract (X_2) on drug release from the nanoemulgel. Carbopol significantly influences drug release by increasing the viscosity of the formulation. Higher Carbopol concentrations form a denser gel network, which slows drug diffusion and reduces release. In contrast, increasing the concentration of Wrightia tinctoria extract improves drug release, possibly due to enhanced solubility and diffusion of the active constituents. The curved response surface indicates a nonlinear interaction between the two variables. Maximum drug release was observed at moderate Carbopol levels and higher extract concentrations, providing an optimal formulation balance.

Factors

Optimization of the nanoemulgel was performed using Central Composite Design (CCD) and Design Expert® software. The effects of oil concentration, Smix, and Carbopol on viscosity and zeta potential were evaluated through 20 experimental runs. Viscosity values ranged from 1400 to 2500 cP, while zeta potential values varied between 22 and 40 mV. Carbopol had the greatest influence on viscosity, whereas higher Smix levels improved formulation stability by increasing

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zeta potential. The optimized formulation contained 12.5% oil, 50% Smix, and 1% Carbopol, producing a viscosity of 1800 cP and zeta potential of 24 mV with a desirability value of 0.99.

Table 16: Composition and levels of formulation components used in the experimental design, including red sandalwood oil (A), Smix (B), and Carbopol (C), with their respective low and high levels and coding in actual values.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	A: Oil	12.50	5.00	20.00	0.0000	Actual
B	B: Smix	50.00	30.00	70.00	0.0000	Actual
C	C: Carbopol	1.0000	0.5000	1.50	0.0000	Actual

Two-sided Confidence = 95% Population = 99%

Table 17: Point prediction statistics for the optimized formulation showing predicted mean, median, standard deviation, standard error, confidence intervals, and tolerance intervals for viscosity and zeta potential at 95% confidence and 99% population levels.

Analysis	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Viscosity	1940	1940	249.281	55.7408	1821.83	2058.17	932.426	2947.57
Zeta potential	26.2	26.2	2.8052	0.627261	24.8449	27.5551	14.356	38.044

Table 18: Confirmation composition of the optimized formulation indicating the selected levels of oil (A), Smix (B), and Carbopol (C).

A: Oil	B: Smix	C: Carbopol
12.5	50	1

Table 19: Experimental design matrix with observed responses for viscosity and zeta potential along with desirability values, highlighting the optimized and selected formulation run.

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Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Desirability
		A: Oil %	B: Smix %	C: Carbopol %	Viscosity cP	Zeta potential mV	
10	1	25.1134	50	1	2500	30	0.85
14	2	12.5	50	1.8409	2200	28	0.90
1	3	5	30	0.5	2200	28	0.90
15	4	12.5	50	1	1600	26	0.91
4	5	20	70	0.5	1400	22	0.92
20	6	12.5	50	1	1800	24	0.99 selected
5	7	5	30	1.5	2200	28	0.95
12	8	12.5	83.6359	1	2000	25	0.89
11	9	12.5	16.3641	1	2200	28	0.85
2	10	20	30	0.5	1400	22	0.95
6	11	20	30	1.5	2300	40	0.85
3	12	5	70	0.5	1800	24	0.90
7	13	5	70	1.5	2200	28	0.90
19	14	12.5	50	1	1800	24	0.91
18	15	12.5	50	1	1800	24	0.92
9	16	-0.113446	50	1	2000	25	0.85
16	17	12.5	50	1	1800	24	0.90
8	18	20	70	1.5	2000	25	0.92
17	19	12.5	50	1	2000	25	0.85
13	20	12.5	50	0.159104	1600	24	0.90

The nanoemulgel formulation was optimized using Central Composite Design (CCD) with Design Expert® software to evaluate the influence of oil concentration (A), Smix (B), and Carbopol (C) on viscosity and zeta potential. Twenty experimental formulations were prepared according to the design, and the responses were analyzed using a quadratic model. The viscosity of the formulations ranged from 1400 to 2500 cP, while zeta potential values varied between 22 and 40 mV. An increase in Carbopol concentration resulted in a noticeable rise in viscosity due to the formation of a stronger gel network. Higher oil levels also contributed to increased viscosity, whereas increasing Smix concentration tended to lower viscosity by improving droplet dispersion. Zeta potential measurements confirmed good formulation stability, with higher Smix concentrations producing better electrostatic stabilization. Among all formulations, Run 6, containing 12.5% oil, 50% Smix, and 1% Carbopol, was identified as the optimum formulation. It exhibited a viscosity of 1800 cP, a zeta potential of 24 mV, and the highest desirability value of 0.99. Statistical evaluation confirmed the suitability of the quadratic model, and the optimized formulation met the desired criteria for stability and topical application.

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Formulation of Nano emulsion

The nanoemulsion containing Wrightia tinctoria extract and red sandalwood oil was prepared using a high-energy emulsification technique to obtain a stable nanosized dispersion. The formulation components were selected and optimized to achieve uniform droplet distribution and good physical stability. The oil phase, comprising Wrightia tinctoria extract and red sandalwood oil, was incorporated at 10% w/w and served as the source of the active constituents. Tween 80 was used as the surfactant at 25% w/w to reduce interfacial tension and stabilize the dispersed droplets, thereby preventing coalescence. A co-surfactant, either PEG 400 or Transcutol P, was included at 30% w/w to improve the flexibility of the interfacial layer and assist in the formation of smaller droplets. Distilled water constituted the remaining 35% w/w of the formulation and acted as the continuous phase. The optimized nanoemulsion appeared clear to slightly opalescent, indicating successful formation of nanosized droplets with uniform dispersion. The combination of surfactant and co-surfactant contributed to enhanced stability and reduced droplet size, resulting in a homogeneous system suitable for topical delivery of the incorporated bioactive compounds.

Formulation of Nanoemulsion

The three samples labeled F1, F2, and F3 show noticeable differences in stability and phase behavior. F1 and F2 appear relatively uniform, with only slight foam formation at the top, suggesting better dispersion and stability of the nano emulsion. In contrast, F3 shows visible phase separation and aggregation, indicating reduced stability. This difference may be due to variations in formulation, such as surfactant concentration or mixing conditions. The presence of foam in all samples suggests air incorporation during preparation. Overall, F1 and F2 demonstrate more promising characteristics, while F3 requires further optimization to improve homogeneity and prevent separation over time.

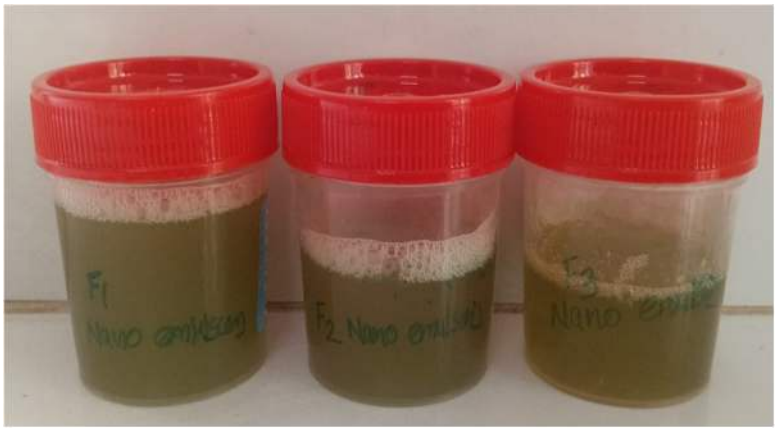


Figure 20: Visual comparison of nano emulsion formulations F1, F2, and F3, showing differences in appearance, foam formation, and phase stability after preparation.

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Characterization of Nanoemulsion Formulation (F)

Particle Size and Polydispersity Index (PDI)

The developed nanoemulsion exhibited an average droplet size of 120–150 nm, confirming the successful formation of a nanosized dispersion. The small droplet size provides a larger surface area, which may enhance the delivery and absorption of the incorporated active compounds. The formulation showed a polydispersity index (PDI) of 0.22 ± 0.03, indicating a narrow and uniform droplet size distribution. Such a low PDI value reflects good homogeneity and suggests that the droplets are evenly dispersed throughout the system. These characteristics contribute to improved physical stability, minimize the risk of aggregation, and support the suitability of the nanoemulsion for topical pharmaceutical applications.

Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM analysis of the developed nanoemulsion revealed important information about its morphology and structural properties. The micrograph showed that the droplets were predominantly spherical with smooth surfaces, indicating efficient emulsification and good formulation stability. The particles were uniformly distributed throughout the field and showed no evidence of aggregation or flocculation, suggesting a well-dispersed system. The absence of large clusters supports the particle size and polydispersity results, confirming a homogeneous formulation with a narrow size distribution. The nanosized droplets and intact structure demonstrate the effectiveness of the preparation process and indicate suitability for pharmaceutical and cosmeceutical applications.

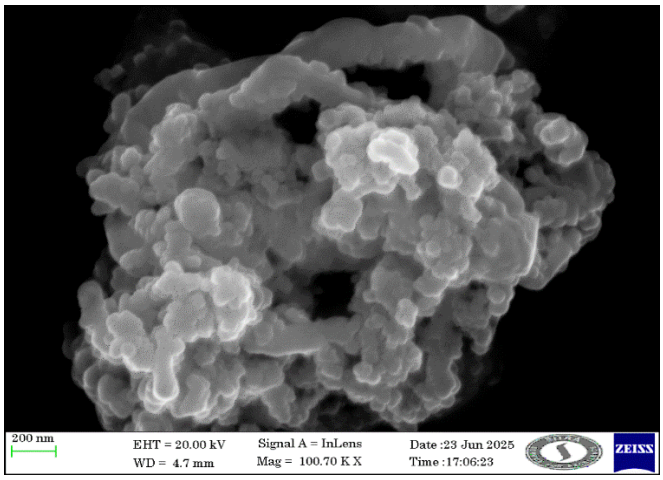


Figure 21: FE-SEM analysis of Nanoemulsion.

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High Resolution-Transmission Electron Microscopy (HR-TEM) analysis of Nanoemulsion.

HR-TEM analysis was performed to examine the size, morphology, and internal structure of the developed nanoemulsion. The images showed predominantly spherical droplets with well-defined boundaries, confirming successful emulsification and stabilization of the oil phase. The droplet size was found to be within the nanoscale range (100–150 nm), which agreed with the particle size measurements obtained by dynamic light scattering. The droplets were uniformly distributed and showed no signs of aggregation or coalescence, indicating good formulation stability. The smooth morphology and homogeneous dispersion suggest effective surfactant stabilization, making the nanoemulsion suitable for efficient drug delivery and pharmaceutical applications.

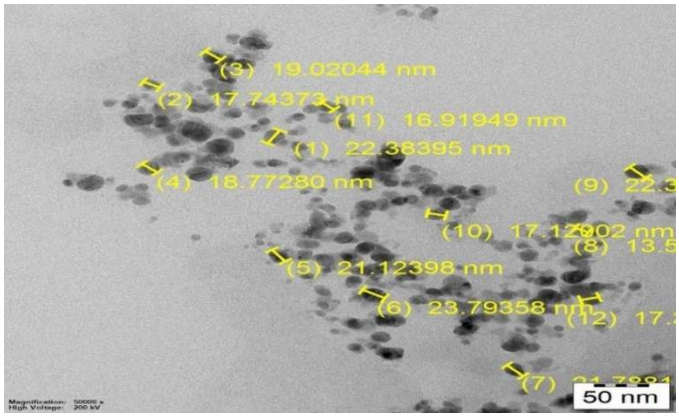


Figure 22: HR-TEM analysis of Nanoemulsion.

Viscosity

The developed nanoemulsion exhibited a viscosity of 45 ± 3 cP, indicating suitable flow properties for pharmaceutical and topical applications. This viscosity allows easy spreading and uniform application while maintaining the physical stability of the formulation. A balanced viscosity is important because very low values may lead to phase separation, whereas excessively high values can reduce ease of application. The observed result suggests effective stabilization of the oil droplets within the aqueous phase by the surfactant system. In addition, the viscosity is adequate to support uniform dispersion and controlled release of the incorporated bioactive compounds during application.

pH Measurement

The pH of the developed nanoemulsion was measured as 6.2 ± 0.1, indicating good compatibility with the natural pH of human skin. This slightly acidic pH is suitable for topical application and helps minimize the risk of skin irritation or damage to the skin barrier. The result suggests that the

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formulation is mild and safe for prolonged use. The consistent pH value also indicates good formulation stability and the absence of unfavorable interactions among the ingredients. In addition, maintaining an appropriate pH supports the stability of bioactive compounds and contributes to the overall effectiveness of the nanoemulsion.

Stability Studies

The stability studies confirmed that the developed nanoemulsion possessed excellent physical stability under different stress conditions. No phase separation, creaming, or cracking was observed after centrifugation at 5000 rpm for 30 minutes, indicating good resistance to droplet aggregation and coalescence. Storage studies conducted at 4°C, 25°C, and 40°C for 30 days showed no significant changes in appearance, particle size, or pH. These results demonstrate that the formulation remained stable under both refrigerated and elevated temperature conditions. The observed stability can be attributed to the optimized composition and uniform nanosized droplets, ensuring prolonged shelf life and effectiveness.

Nanoemulgel formulation

Three nanoemulgel formulations, namely F1, F2, and F3, were prepared by incorporating optimized nanoemulsions into a Carbopol-based gel matrix to improve topical application, stability, and drug delivery performance. The nanoemulsion content was varied at 80%, 82.5%, and 85% w/w in F1, F2, and F3, respectively, to study its effect on the physicochemical characteristics of the formulations. Carbopol 940 was maintained at 1% w/w in all batches to provide the required gel structure and viscosity. Glycerin was added as a moisturizing agent at concentrations of 3%, 4%, and 5% w/w in F1, F2, and F3, respectively, to enhance hydration, texture, and spreadability. Methyl paraben (0.1% w/w) and propyl paraben (0.02% w/w) were incorporated as preservatives to ensure microbial stability during storage. Triethanolamine was added in sufficient quantity to neutralize Carbopol and obtain a smooth gel consistency with an appropriate pH. Distilled water was used to make up the final weight of each formulation to 100%. The gradual increase in nanoemulsion and glycerin levels enabled comparison of formulation performance. F1 was expected to exhibit greater firmness, while F3 was anticipated to provide superior spreadability, hydration, and drug release. F2 offered a balanced combination of these properties, making it a suitable intermediate formulation.

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Figure 23: Physical appearance of nano emulgel formulations F1, F2, and F3, illustrating variations in color, consistency, and phase uniformity after preparation.

CONCLUSION

The present study successfully developed and optimized a nanoemulgel containing Wrightia tinctoria extract and red sandalwood oil for topical anti-inflammatory application. Optimization using Central Composite Design identified the ideal formulation composition, which exhibited desirable viscosity, zeta potential, and overall stability. The optimized nanoemulsion showed nanosized droplets with a narrow size distribution, confirming the formation of a uniform and stable system. The pH of the formulation was compatible with skin, making it suitable for topical use. Morphological studies revealed spherical and well-dispersed droplets, while stability testing confirmed the absence of phase separation or significant changes during storage. The nanoemulgel demonstrated good spreadability, acceptable rheological properties, and sustained drug release characteristics. Carbopol played a major role in improving viscosity and controlling drug release. Overall, the developed nanoemulgel represents a stable and effective herbal delivery system with potential for managing inflammatory skin conditions and improving therapeutic outcomes.

CONFLICT OF INTEREST: Nil

REFERENCE

1. Karmakar E, Ghosh S, Mandal SC. Seedpod extracts of *Wrightia tinctoria* shows significant anti-inflammatory effects in HepG2 and RAW-264.7 cell lines. *Nat Prod Res.* 2023;37(15):2574-2579. doi:10.1080/14786419.2022.2141932.
2. Ojha M, Sharma P, Verma R. Phytotherapeutic analysis of chloroform-based fractions of *Wrightia tinctoria* for anti-psoriatic activity. *Biomed Pharmacother.* 2025;182:117465.

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3. Jurel P, Singh R. Formulation and evaluation of *Wrightia tinctoria* emulgel for the treatment of psoriasis. *Int J Pharm Sci Res.* 2024;15(12):4550-4561.
4. Meenachisundaram S, Murugan KS, Munusamy H. Phytochemical and therapeutic potential of *Wrightia tinctoria*: A comprehensive review. *J Ethnopharmacol.* 2025;338:118902.
5. Yamuna K, Dwarampudi LP, Shanmugam R. A review on phytoconstituents for the management of psoriasis. *Nat Prod Commun.* 2025;20(5):1-15.
6. Subalakshmi S, Devi RS. Wound healing potential of methanolic extract of *Wrightia tinctoria* leaves in experimental models. *J Herb Med.* 2026;42:100735.
7. Khan N, Rahman M, Alam M. GC-MS analysis and antioxidant activity of *Wrightia tinctoria* leaf extract. *Pharmacogn J.* 2021;13(2):412-419.
8. Srivastava R, Ahmed H, Dixit RK. A review on phytochemical, pharmacological, and ethnomedicinal uses of *Wrightia tinctoria*. *J Nat Remedies.* 2014;14(1):1-9.
9. Dal Mas J, Bittencourt CM, de Souza P. Nanoemulsion as a carrier to improve topical anti-inflammatory activity of herbal formulations. *AAPS PharmSciTech.* 2016;17(5):1230-1238.
10. Patel MR, Patel RB, Parikh JR. Recent advances in nanoemulgel formulations for topical drug delivery systems. *Drug Dev Ind Pharm.* 2023;49(4):611-623.
11. Sharma D, Kaur G, Singh I. Optimization and characterization of herbal nanoemulgel for enhanced anti-inflammatory activity. *Colloids Surf B Biointerfaces.* 2024;236:113845.
12. Ahmed TA, Ibrahim HM. Nanoemulgel-based topical delivery systems: formulation strategies and therapeutic applications. *Pharmaceutics.* 2023;15(8):2034.
13. Verma S, Gupta A, Kumar R. Recent trends in nanoemulgel formulations for herbal drug delivery and skin disorders. *Int J Biol Macromol.* 2024; 254:127821.