

Development, Optimization and Evaluation of Eucalyptus Oil-Based Organogel for Enhanced Topical Antifungal Delivery

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Abstract

Background: Topical delivery of essential oils presents formulation challenges because their volatile constituents may be affected by evaporation, while their distribution and release depend on the properties of the delivery vehicle. Organogel offer a structured lipid-based system for incorporating oil-phase ingredients into semisolid formulations. **Objective:** This study aimed to develop and optimize a eucalyptus oil-based organogel using soy lecithin and beeswax as structuring agents and to evaluate its physicochemical characteristics, in-vitro release behavior and preliminary antifungal activity. **Methods:** Preliminary formulations were prepared using eucalyptus oil, soy lecithin, beeswax, isopropyl myristate (IPM), ethanol and butylated hydroxytoluene (BHT). A (3²) factorial design was employed to investigate the effects of soy lecithin (3–7% w/w) and beeswax (1–3% w/w) across nine formulations (F1–F9). The formulations were evaluated for appearance, homogeneity, phase separation, pH, viscosity, spreadability, eucalyptus oil content and in-vitro diffusion. The optimized formulation was further assessed for preliminary antifungal activity. **Results:** Formulation F5, containing 5% eucalyptus oil, 5% soy lecithin and 2% beeswax, was selected as the optimized batch. It exhibited a pH of 5.56 ± 0.04 , viscosity of $9,416 \pm 157$ cP, spreadability of 16.1 ± 0.3 g·cm/s and eucalyptus oil assay of 98.51%. Cumulative in-vitro diffusion reached 98.06% at 10 h. After six months at 40°C/75% relative humidity, the assay decreased to 94.95%, and cumulative diffusion decreased to 93.94%. Preliminary antifungal testing showed inhibition zones of 29 mm for F5, 28 mm for nystatin and 32 mm for the marketed comparator. **Conclusion:** The developed Organogel demonstrated promising physicochemical characteristics, in-vitro diffusion behaviour. and antifungal efficacy and topical delivery potential.

Keywords: Eucalyptus oil; Organogel; factorial design; formulation ; antifungal activity.

1. Introduction

Topical drug delivery is an important approach for managing localized skin conditions because it allows therapeutic agents to be applied directly to the affected area. The effectiveness of topical formulations depends on the physicochemical properties of the active ingredient, the characteristics of the delivery vehicle, and the release of the incorporated substance. The stratum corneum, the outermost layer of the skin, acts as a major barrier to the penetration of many compounds. Therefore, developing suitable topical formulations is essential for improving formulation performance and achieving the intended local effect [1,2]. Plant-derived essential oils have attracted considerable interest in pharmaceutical research because of their complex chemical composition and reported biological activities. However, their incorporation into pharmaceutical formulations can be challenging because of volatility, susceptibility to oxidation, limited aqueous solubility, and possible variations in chemical composition [3]. These properties may affect formulation quality, storage stability, and reproducibility. Suitable formulation strategies are therefore required to incorporate essential oils into semisolid systems and evaluate their performance under controlled experimental conditions [4]. Eucalyptus essential oil has been investigated for its antimicrobial and antifungal properties. Studies involving different *Eucalyptus* species have reported biological activity against selected microorganisms, including certain fungal pathogens. However, the observed activity may vary according to the botanical species, chemical composition, concentration, and test organism [5,6]. Consequently, the biological activity of eucalyptus oil should be assessed experimentally rather than assumed from findings reported for other species or formulations. Incorporating the oil into a suitable topical vehicle may provide a practical approach for further evaluating its pharmaceutical potential. Organogels are semisolid systems in which an organic liquid phase is immobilized within a three-dimensional

network formed by one or more structuring agents. These systems can provide consistency to liquid ingredients and influence formulation characteristics such as viscosity, spreadability, and release behavior. Lecithin-based organogels have received attention as potential topical delivery systems because phospholipids can form structured assemblies in suitable liquid environments [7]. Recent reviews have also discussed the potential applications of organogels for delivering lipophilic compounds and their relevance to skin-related conditions [8]. Nevertheless, the performance of an individual formulation depends on its composition, preparation method, and evaluation conditions. Enhanced skin penetration cannot be established without appropriate skin permeation experiments [9]. In the present investigation, soy lecithin and beeswax were selected as structuring agents for developing a eucalyptus oil-based organogel. Soy lecithin provides phospholipids that may contribute to the organization of the formulation, whereas beeswax can contribute to the consistency of lipid-based semisolid systems [10]. Isopropyl myristate (IPM) was used as the oil-phase vehicle, while ethanol was included as a formulation component and butylated hydroxytoluene (BHT) as an antioxidant. The combined effects of these ingredients may influence the physical properties and performance of the final product. However, their individual contributions must be interpreted in the context of the complete formulation [11]. Design of experiments (DoE) offers a systematic approach to investigating formulation variables and selecting promising compositions. Factorial designs allow multiple factors to be examined within a defined experimental space and can support the interpretation of their effects on selected responses [12]. The reliability of optimization depends on the experimental design, the statistical model, and the quality of the underlying data [13]. In this study, a (3²) factorial design was employed to investigate soy lecithin concentrations of 3%, 5%, and 7% w/w and beeswax concentrations of 1%, 2%, and 3% w/w. Nine formulations, designated F1–F9, were prepared and evaluated for their physicochemical properties and in-vitro diffusion behavior. Preliminary antifungal assessment was also undertaken to explore the biological potential of the selected formulation. Although published studies have investigated the antifungal activity of eucalyptus essential oils, comparative conclusions require a clearly identified fungal strain, suitable antifungal reference treatment, appropriate controls, and replicated measurements [15]. Inhibition-zone measurements alone are insufficient to establish clinical efficacy or superiority over established treatments. Accordingly, the present study aimed to develop and optimize a eucalyptus oil-based Organogel using soy lecithin and beeswax, characterize its physicochemical properties, evaluate its in-vitro diffusion behavior, and investigate its preliminary antifungal activity. The findings are intended to establish the feasibility of the formulation and identify the additional analytical, stability, antifungal, and skin-permeation studies needed for further development.

Materials and Methods :

Materials

Eucalyptus oil was selected as the antifungal active ingredient for the development of a topical Organogel formulation. Soy lecithin was used as the primary organogelator, while beeswax served as a secondary structuring agent to improve the consistency of the formulation. Isopropyl myristate (IPM) was used as the oil-phase vehicle and penetration enhancer. Ethanol was incorporated as a cosolvent, and butylated hydroxytoluene (BHT) was added as an antioxidant. Methanol was used for analytical sample preparation, and phosphate buffer (pH 5.5) containing ethanol was employed as the receptor medium for the in-vitro diffusion study. The formulation strategy was based on the ability of lipid-based structuring agents to influence gel formation, consistency, and release characteristics [16].

Preliminary Formulation Development

Three preliminary formulations (P1–P3) were prepared to evaluate the influence of soy lecithin and beeswax concentrations on Organogel formation and performance. The concentration of eucalyptus oil was maintained at 5% w/w, whereas soy lecithin was varied between 3% and 7% w/w and beeswax between 1% and 3% w/w. Ethanol (5% w/w) and BHT (0.05% w/w) were maintained at constant concentrations. IPM was adjusted to maintain the total formulation weight at 100%. The preliminary formulations were prepared using a heat-assisted incorporation method. Eucalyptus oil, IPM, and BHT were weighed accurately and mixed to obtain a uniform oil phase. Separately, soy lecithin and beeswax were heated to approximately 70–75°C with continuous stirring until the beeswax melted and a uniform gelator phase was obtained. The oil phase was gradually incorporated into the molten gelator phase under continuous stirring. The mixture was allowed to cool gradually, and ethanol was added during the cooling stage to minimize evaporation. Stirring was continued until a smooth and homogeneous Organogel was obtained. The formulations were transferred into clean, tightly closed containers and allowed to equilibrate at room temperature for 24 h before evaluation. This approach was consistent with established principles of wax-based Organogel preparation [17].

Optimization Using a 3² Factorial Design

A two-factor, three-level factorial design was employed to investigate the influence of formulation variables on the physicochemical and release characteristics of eucalyptus oil organogel. Design-Expert software (version 7.0) was used to construct the experimental design and analyze the responses. Soy lecithin concentration was selected as independent variable A, with levels of 3%, 5%, and 7% w/w. Beeswax concentration was selected as independent variable B, with levels of 1%, 2%, and 3% w/w. The experimental design consisted of nine formulation batches (F1–F9), representing all combinations of the selected factor levels. The concentrations of eucalyptus oil, ethanol, and BHT were maintained at 5%, 5%, and 0.05% w/w, respectively, while IPM was adjusted to maintain the total formulation weight. The formulations were prepared using the procedure described in Section 2.2. The evaluated responses were viscosity (R1), spreadability (R2), and the time required to achieve 90–100% cumulative release (R3). The effects of the independent variables on the measured responses were investigated using analysis of variance (ANOVA). Factorial experimental design is an established approach for evaluating formulation variables and their effects on pharmaceutical product characteristics [18,19].

Evaluation of Organogel Formulations

Appearance and Homogeneity

The prepared Organogel formulations were visually examined for colour, odour, texture, consistency, and uniformity. Approximately 1 g of each formulation was placed on a clean glass slide and inspected under normal illumination for lumps, coarse particles, air bubbles, visible oil droplets, and other physical defects. Homogeneity was assessed by spreading the formulation between two clean glass slides. The samples were considered visually homogeneous when a uniform texture was observed without visible aggregates or phase separation [20].

Phase Separation and Oil Leakage

Approximately 5 g of each formulation was transferred into a clean, dry, transparent container and maintained at room temperature for 24 h. The formulations were subsequently inspected for the formation of a separate oil layer, surface oil droplets, syneresis, or collapse of the gel structure. The absence of visible phase separation and oil leakage was recorded as an indication of satisfactory preliminary physical stability [21].

Determination of Apparent pH

Approximately 1 g of each Organogel was dispersed in 100 mL of distilled water and mixed thoroughly. The dispersion was allowed to equilibrate for approximately 2 h at room temperature. A digital pH meter was calibrated using standard buffer solutions before measurement. The pH of the homogenized dispersion was recorded at $25 \pm 1^\circ\text{C}$. Measurements were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD) [22].

Viscosity Determination

The viscosity of the Organogel formulations was measured using a Brookfield rotational viscometer fitted with spindle No. 64. Approximately 50 g of formulation was transferred into a suitable container, avoiding the incorporation of air bubbles. The sample was equilibrated at $25 \pm 1^\circ\text{C}$ before measurement. The spindle was immersed in the centre of the sample without touching the container walls or bottom. Viscosity was measured at a rotational speed of 20 rpm after the reading had stabilized and was expressed in centipoise (cP). The same spindle, speed, temperature, and measurement conditions were maintained for all batches. Each formulation was evaluated in triplicate, and results were expressed as mean $\pm$ SD [23].

Determination of Spreadability

Spreadability was determined using the slide-pull method. Approximately 1.0 g of Organogel was placed at the centre of a clean glass slide, and a second glass slide was placed over the sample. The upper slide was connected to a 20 g load using a lightweight thread passing over a pulley. The time required for the upper slide to travel a fixed distance of 7.5 cm was recorded using a stopwatch.

Spreadability was calculated using the following equation:

$$S = \frac{M \times L}{T}$$

where (S) represents spreadability, (M) is the applied mass, (L) is the distance travelled by the slide, and (T) is the time required for the movement. Results were expressed in g·cm/s. Measurements were performed in triplicate and reported as mean $\pm$ SD [24].

Determination of Eucalyptus Oil Content

Preparation of Standard Solution

The eucalyptus oil content of the Organogel formulations was determined using a UV–visible spectrophotometer at 210 nm, with methanol as the blank [25]. Accurately weighed eucalyptus oil (10 mg) was transferred into a 20 mL volumetric flask. Approximately 15 mL of methanol was added, and the solution was sonicated for 2–3 min before making up the volume to 20 mL with methanol to obtain a nominal concentration of 500 µg/mL. A 1.4 mL aliquot of this stock solution was diluted to 20 mL with methanol to obtain a working concentration of 35 µg/mL. The working standard was analyzed at 210 nm. Five replicate absorbance measurements were recorded, and the mean, SD, and percentage relative standard deviation (%RSD) were calculated [26].

Preparation of Sample Solution

Approximately 1,000 mg of Organogel, equivalent to 50 mg of eucalyptus oil based on the nominal formulation composition, was weighed accurately and transferred into a 100 mL volumetric flask. Approximately 70 mL of methanol was added, and the mixture was sonicated for 5–10 min before making up the volume to 100 mL with methanol. The solution was filtered through a 0.45 µm PVDF syringe filter, discarding the initial 3–5 mL of filtrate. A 0.7 mL aliquot of the filtrate was diluted to 10 mL with methanol and analyzed at 210 nm. The eucalyptus oil content was calculated by comparison with the standard absorbance and the corresponding dilution and sample-weight factors. The analytical procedure should be validated for its intended quantitative use, including appropriate assessment of specificity, linearity, accuracy, and precision [27].

In-Vitro Diffusion Study

In-vitro diffusion of eucalyptus oil from the Organogel formulations was evaluated using a Franz diffusion cell fitted with a suitable dialysis membrane. The membrane was hydrated and equilibrated with the receptor medium before the experiment. The receptor compartment was filled with phosphate buffer (pH 5.5) containing 10% v/v ethanol and maintained at $32 \pm 0.5^\circ\text{C}$ with continuous stirring at approximately 100 rpm. An accurately weighed quantity of 0.5 g of Organogel, corresponding to a nominal 25 mg of eucalyptus oil, was uniformly applied over the membrane in the donor compartment. The donor chamber was covered to minimize evaporation of volatile constituents. At predetermined time intervals, 0.35 mL aliquots were withdrawn from the receptor compartment and diluted to 10 mL with methanol. The samples were analyzed using a UV–visible spectrophotometer at 210 nm against an appropriate blank. After each withdrawal, the receptor compartment was replenished with an equal volume of fresh receptor medium to maintain the original volume. Cumulative release was calculated from the measured concentrations, accounting for the dilution and replacement of previously withdrawn samples and expressed as a percentage of the nominal eucalyptus oil content. The receptor medium was selected to provide a mildly acidic aqueous environment with ethanol to assist the solubilization of the hydrophobic oil constituents. Receptor-fluid composition, temperature control, membrane suitability, and adequate solubility of the test substance are important considerations in diffusion testing [28,29]. The procedure described here represents an in-vitro release assessment across a dialysis membrane; it should not be interpreted as a measurement of skin permeation.

Stability Study

The physical stability of the prepared eucalyptus oil Organogel formulations was initially assessed by examining their appearance, homogeneity, phase separation, and oil leakage after 24 h of equilibration at room temperature. Approximately 5 g of each formulation was transferred into a clean, dry, transparent container and stored at room temperature. The samples were examined for changes in colour, odour, texture, consistency, formation of a separate oil layer, surface oil droplets, syneresis, and collapse of the gel structure. The absence of visible changes was recorded as evidence of preliminary physical stability [30]. For a formal stability study, the optimized formulation should be stored in its intended container under defined long-term and accelerated storage conditions. Samples should be examined at predetermined intervals for appearance, homogeneity, pH, viscosity, eucalyptus oil content, and in-vitro release characteristics, as appropriate. The storage conditions, sampling intervals, acceptance criteria, and analytical procedures should be established before initiating the study, following the principles described in ICH Q1A(R2) [31]. The duration and conditions of any stability study must be reported according to the experiments performed.

Accelerated Stability Study

The accelerated stability of the optimized eucalyptus oil-based Organogel formulation (F5) was evaluated under elevated temperature and humidity conditions. According to the progress report, the formulation was stored at

40°C/75% relative humidity (RH) for six months. Stability assessments were documented at the end of the third and sixth months, and the results were compared with the initial formulation characteristics. The evaluated parameters included physical appearance, pH, viscosity, spreadability, eucalyptus oil content (assay), and cumulative in-vitro diffusion at 10 h. Changes in these parameters were used to assess the physical and performance-related stability of the formulation during storage. The initial values were used as the reference for comparison with the measurements obtained after three and six months [32].

Antifungal Activity

The preliminary antifungal activity of the optimized eucalyptus oil-based Organogel (F5) was assessed by measuring the inhibition zone produced by the formulation and comparing it with the reported reference preparations. The optimized formulation contained 5% w/w eucalyptus oil, 5% w/w soy lecithin, and 2% w/w beeswax. The reported inhibition-zone diameters were 29 mm for F5, 28 mm for the nystatin reference, and 32 mm for the marketed comparator. The inhibition-zone diameter was recorded in millimetres and used as the endpoint for preliminary comparison of antifungal activity. The results were interpreted descriptively because the available experimental record did not provide sufficient information regarding the fungal test organism, inoculum preparation, culture medium, sample quantity, incubation conditions, number of replicates, or statistical analysis. These experimental details should be included only after verification from the original laboratory records [33].

Statistical Analysis

All experimental measurements were expressed as mean $\pm$ standard deviation (SD), where replicate measurements were available. The influence of soy lecithin and beeswax concentrations on viscosity, spreadability, and cumulative-release characteristics was evaluated using analysis of variance (ANOVA) in Design-Expert software, version 7.0. Model adequacy was assessed using the F-value, p-value, coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and residual analysis. A p-value below 0.05 was considered statistically significant [34,35]. Results obtained from single measurements were not treated as replicate data.

Results and Discussion:

Preliminary formulation development and physical evaluation

Three preliminary eucalyptus oil-based Organogel formulations (P1–P3) were evaluated for appearance, homogeneity, pH, viscosity, spreadability, eucalyptus oil content, and in-vitro diffusion. All formulations were reported to have a smooth, uniform, pale-yellow appearance with a characteristic eucalyptus oil odour. No visible lumps, coarse particles, phase separation, or oil leakage were observed after 24 h of equilibration. The pH values ranged from 5.42 ± 0.05 to 5.58 ± 0.04 . Viscosity increased from 8050 ± 125 cP for P1 to $12,100 \pm 205$ cP for P3, whereas spreadability decreased from 18.8 ± 0.3 to 12.6 ± 0.4 g·cm/s. The reported times required to achieve 90–100% cumulative diffusion were 8, 10, and 11 h for P1, P2, and P3, respectively. These observations indicated that changes in the concentrations of the structuring agents were associated with differences in the physical properties and release behaviour of the formulations.

Table 1. Preliminary Evaluation of Eucalyptus Oil-Based Organogel Formulations (P1–P3)

Parameter	P1	P2	P3
Soy lecithin (% w/w)	3	5	7
Beeswax (% w/w)	1	2	3
Appearance	Pale yellow, smooth	Pale yellow, smooth	Pale yellow, smooth
pH (mean $\pm$ SD)	5.42 ± 0.05	5.51 ± 0.06	5.58 ± 0.04
Viscosity (cP, mean $\pm$ SD)	8050 ± 125	9550 ± 169	$12,100 \pm 205$
Spreadability (g·cm/s, mean $\pm$ SD)	18.8 ± 0.3	16.0 ± 0.2	12.6 ± 0.4
Time to achieve 90–100% cumulative diffusion (h)	8	10	11
Eucalyptus oil assay (%)	97.60	98.39	98.50

Optimization Using a 3² Factorial Design

Nine formulations (F1–F9) were prepared using a 3² factorial design with soy lecithin concentrations of 3%, 5%, and 7%

w/w and beeswax concentrations of 1%, 2%, and 3% w/w. Eucalyptus oil (5% w/w), ethanol (5% w/w), and BHT (0.05% w/w) were maintained at constant concentrations, while isopropyl myristate was adjusted to maintain the total formulation weight.

Table 2. Composition of Factorial Formulations

Ingredient (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eucalyptus oil	5	5	5	5	5	5	5	5	5
Soy lecithin	3	3	3	5	5	5	7	7	7
Beeswax	1	2	3	1	2	3	1	2	3
Ethanol	5	5	5	5	5	5	5	5	5
BHT	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
IPM	85.95	84.95	83.95	83.95	82.95	81.95	81.95	80.95	79.95

Physical appearance and homogeneity of factorial formulations

The formulations were reported to be smooth, homogeneous, and pale yellow, with the characteristic odour of eucalyptus oil. No visible phase separation or oil leakage was observed after 24 h. These findings supported satisfactory initial physical appearance and homogeneity across the prepared batches.

Table 3. Physical Appearance and Homogeneity

Parameter	Reported result
Colour	Pale yellow
Appearance and texture	Smooth, uniform and homogeneous
Odour	Characteristic eucalyptus oil odour
Visible lumps or coarse particles	Not observed
Separated oil droplets	Not observed
Phase separation after 24 h	Not observed
Oil leakage after 24 h	Not observed

Determination of apparent pH

The pH values of the factorial formulations ranged from 5.48 ± 0.05 to 5.64 ± 0.04 . Formulation F1 exhibited the lowest reported pH, whereas F9 showed the highest. The optimized formulation F5 had a pH of 5.56 ± 0.04 . The pH readings showed relatively small differences among the batches. The report therefore documented a narrow range of apparent pH values across the formulations.

Table 4. Apparent Ph of Factorial Formulations

Formulation	Soy lecithin (% w/w)	Beeswax (% w/w)	pH (mean $\pm$ SD)
F1	3	1	5.48 ± 0.05
F2	3	2	5.51 ± 0.04
F3	3	3	5.50 ± 0.05
F4	5	1	5.54 ± 0.04
F5	5	2	5.56 ± 0.04
F6	5	3	5.58 ± 0.05
F7	7	1	5.56 ± 0.04
F8	7	2	5.61 ± 0.05
F9	7	3	5.64 ± 0.04

Viscosity determination

Table 5. Viscosity of Factorial Formulations

Formulation	Soy lecithin (% w/w)	Beeswax (% w/w)	Viscosity (cP, mean $\pm$ SD)
F1	3	1	7215 ± 115
F2	3	2	8022 ± 132

F3	3	3	8799 ± 147
F4	5	1	8644 ± 130
F5	5	2	9416 ± 157
F6	5	3	9945 ± 165
F7	7	1	9864 ± 155
F8	7	2	10,698 ± 167
F9	7	3	11,573 ± 149

The observed trend was consistent with an association between higher structuring-agent concentrations and increased resistance to flow. F5 exhibited an intermediate viscosity among the factorial formulations.

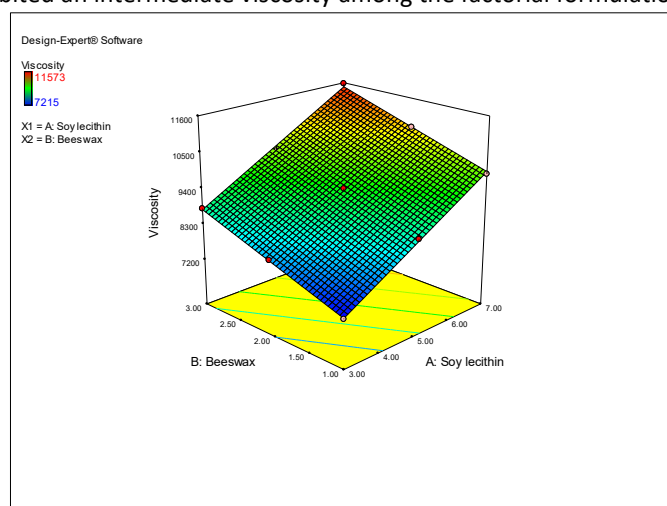


Figure 1: 3D Graph for Viscosity

Determination of spreadability

Spreadability decreased from 18.2 ± 0.2 g·cm/s for F1 to 12.5 ± 0.4 g·cm/s for F9. The spreadability of F5 was 16.1 ± 0.3 g·cm/s. Overall, formulations with higher viscosity tended to exhibit lower spreadability. The findings showed an inverse relationship between viscosity and spreadability across the factorial batches. F5 displayed an intermediate spreadability value alongside its intermediate viscosity.

Table 6. Spreadability of Factorial Formulations

Formulation	Applied mass (g)	Distance travelled (cm)	Time (s)	Spreadability (g·cm/s, mean ± SD)
F1	20	7.5	8.2	18.2 ± 0.2
F2	20	7.5	8.6	17.4 ± 0.3
F3	20	7.5	9.0	16.6 ± 0.3
F4	20	7.5	8.8	17.1 ± 0.2
F5	20	7.5	9.3	16.1 ± 0.3
F6	20	7.5	9.7	15.4 ± 0.2
F7	20	7.5	10.6	14.2 ± 0.4
F8	20	7.5	11.3	13.3 ± 0.3
F9	20	7.5	12.0	12.5 ± 0.4

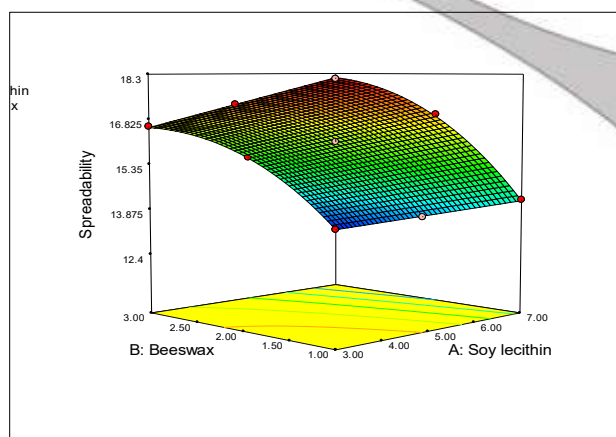


Figure 2: 3D Graph for Spreadability

Factorial design and statistical analysis

The factorial design was used to investigate the effects of soy lecithin and beeswax on viscosity, spreadability, and the time required to achieve 90–100% cumulative diffusion. For the diffusion-time response, the reported model was significant ($F = 52.20$, $p = 0.0002$). Soy lecithin was a significant model term ($F = 88.2$, $p < 0.0001$), as was beeswax ($F = 16.2$, $p = 0.0069$).

Table 7. ANOVA Results for Diffusion-Time Response

Source	Sum of squares	df	Mean square	F-value	p-value
Model	9.666667	2	4.833333	52.20	0.0002
Soy lecithin (A)	8.166667	1	8.166667	88.20	<0.0001
Beeswax (B)	1.500000	1	1.500000	16.20	0.0069
Residual	0.555556	6	0.092593	—	—
Cor total	10.22222	8	—	—	—

For viscosity, the model was significant ($F = 782.3649$, $p < 0.0001$). Both soy lecithin and beeswax were reported as significant terms ($p < 0.0001$). The model showed $R^2 = 0.9962$, adjusted $R^2 = 0.9949$, and predicted $R^2 = 0.9912$.

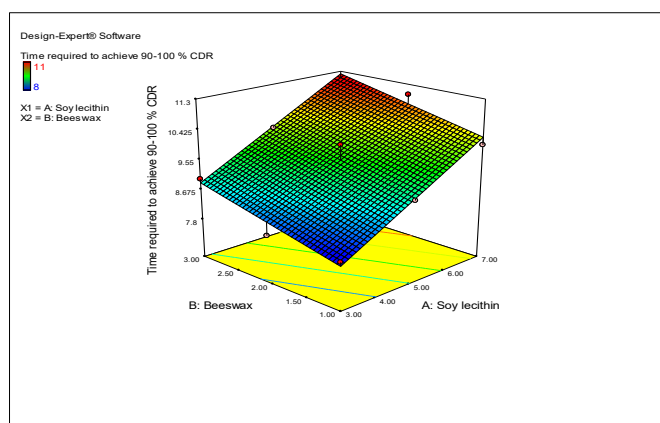


Figure 3: 3D Graph for Diffusion (Time Required to Achieve 90-100% CDR)

Table 8. ANOVA Results for Viscosity

Source	Sum of squares	df	Mean square	F-value	p-value
Model	14,449,773	2	7,224,886	782.3649	<0.0001
Soy lecithin (A)	10,932,300	1	10,932,300	1183.831	<0.0001
Beeswax (B)	3,517,473	1	3,517,473	380.8983	<0.0001
Residual	55,408.06	6	9234.676	—	—
Cor total	14,505,181	8	—	—	—

For spreadability, the reported model was significant ($F = 2530.185$, $p < 0.0001$). Soy lecithin, beeswax, and the quadratic term A^2 were significant terms in the reported model.

Table 9. ANOVA Results for Spreadability

Source	Sum of squares	df	Mean square	F-value	p-value
Model	30.36222	3	10.12074	2530.185	<0.0001
Soy lecithin (A)	24.80667	1	24.80667	6201.667	<0.0001
Beeswax (B)	4.166667	1	4.166667	1041.667	<0.0001
A^2	1.388889	1	1.388889	347.2222	<0.0001
Residual	0.020000	5	0.004000	—	—
Cor total	30.38222	8	—	—	—

Table 10. Fit Statistics for the Factorial Models

Statistical parameter	Diffusion time	Viscosity	Spreadability
Standard deviation	0.30	96.10	0.063
Mean	9.56	9352.89	15.64
Coefficient of variation (%)	3.18	1.03	0.40
R^2	0.9457	0.9962	0.9993
Adjusted R^2	0.9275	0.9949	0.9989
Predicted R^2	0.8924	0.9912	0.9980
PRESS	1.10	127,043.45	0.061
Adequate precision	18.974	76.259	135.978

Determination of eucalyptus oil content

Table 11. Eucalyptus Oil Content in Factorial Formulations

Formulation	Sample weight (mg)	Absorbance	Assay (%)
F1	1000.2	0.6608	97.91
F2	999.8	0.6633	98.32
F3	999.9	0.6607	97.92
F4	1000.2	0.6623	98.13
F5	999.9	0.6647	98.51

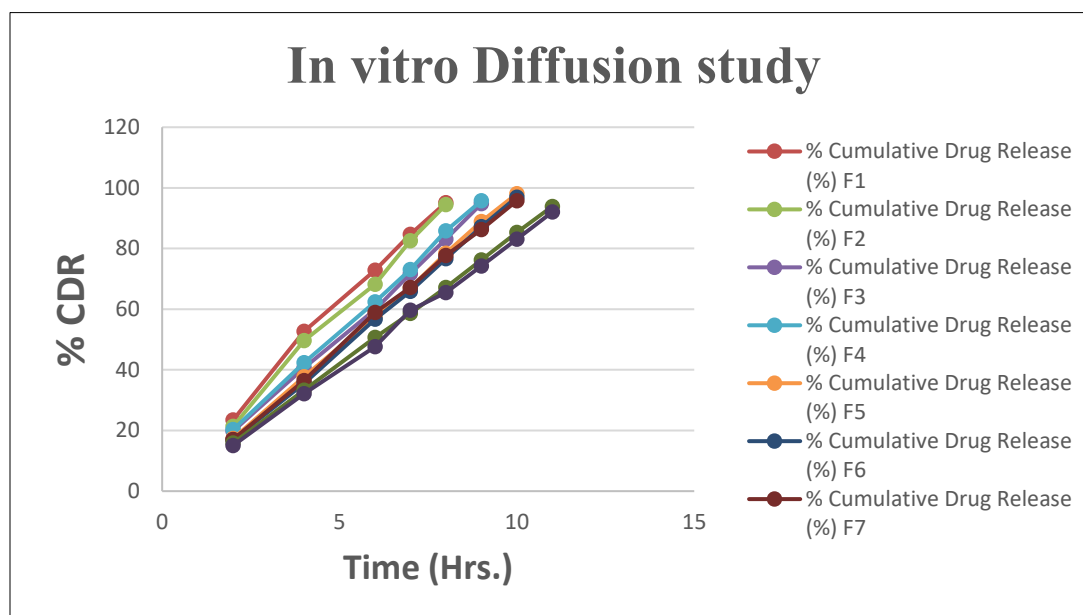
F6	1000.1	0.6603	97.84
F7	1000.2	0.6621	98.10
F8	1000.3	0.6661	98.68
F9	1000.2	0.6643	98.42

In-Vitro Diffusion Study

Table 12. Time Required to Achieve 90–100% Cumulative Diffusion

Formulation	Time (h)
F1	8
F2	8
F3	9
F4	9
F5	10
F6	10
F7	10
F8	11
F9	11

The factorial analysis indicated that soy lecithin and beeswax significantly influenced the diffusion-time response ($p < 0.05$). The model showed an R^2 value of 0.9457, an adjusted R^2 of 0.9275, and a predicted R^2 of 0.8924. These findings describe diffusion across a dialysis membrane under the specified experimental conditions and do not establish skin permeation or transdermal delivery.



Graph 1. In-Vitro Diffusion Profile of Optimized Eucalyptus Oil-Loaded Organogel

Comparison with Marketed Clindac A 1% Gel

The reported in-vitro diffusion results for marketed Clindac A 1% gel showed diffusion values of 26.48%, 68.72%, and 93.82% at 1, 2, and 3 h, respectively. At 4 h, the reported value decreased to 91.51%.

Table 13. Reported In-Vitro Diffusion of Clindac A 1% Gel

Time (h)	Absorbance	Reported diffusion (%)
1	0.0689	26.48
2	0.1788	68.72
3	0.2441	93.82
4	0.2381	91.51

Stability Study

he preliminary physical stability of the eucalyptus oil-based organogel formulations was evaluated after 24 h of equilibration at room temperature. All nine formulations (F1–F9) maintained a smooth, uniform, pale-yellow appearance with the characteristic odour of eucalyptus oil. No visible phase separation, oil leakage, separate oil layer, or major textural defects were observed. These findings indicated satisfactory preliminary physical stability during the 24-h observation period.

Table 14. Preliminary Physical Stability of Organogel Formulations

Parameter	Observation after 24 h
Colour	Pale yellow
Appearance and texture	Smooth and uniform
Homogeneity	Homogeneous
Characteristic odour	Eucalyptus oil odour
Phase separation	Not observed
Oil leakage	Not observed
Separate oil layer	Not observed
Overall observation	No visible physical instability

Accelerated Stability Study

The optimized formulation F5 was evaluated during storage at 40°C/75% relative humidity for 6 months. The assessed parameters included appearance, pH, viscosity, spreadability, eucalyptus oil content, and cumulative diffusion at 10 h. The formulation retained its reported pale-yellow, smooth appearance throughout the assessment period. The pH decreased from 5.56 ± 0.04 initially to 5.48 ± 0.05 after 3 months and 5.43 ± 0.04 after 6 months. Viscosity decreased from 9416 ± 157 cP to 9128 ± 129 cP, while spreadability decreased from 16.1 to 15.4 g·cm/s over 6 months. The eucalyptus oil assay decreased from 98.51% initially to 96.40% at 3 months and 94.95% at 6 months. Similarly, the reported cumulative diffusion at 10 h decreased from 98.06% to 95.98% and 93.94% at the respective assessment points.

Table 15. Accelerated Stability of Optimized Formulation F5

Parameter	Initial	3 months	6 months
Appearance	Pale yellow, smooth	Pale yellow, smooth	Pale yellow, smooth
pH (mean $\pm$ SD)	5.56 ± 0.04	5.48 ± 0.05	5.43 ± 0.04
Viscosity (cP, mean $\pm$ SD)	9416 ± 157	9257 ± 146	9128 ± 129
Spreadability (g·cm/s)	16.1	15.8	15.4
Eucalyptus oil assay (%)	98.51	96.40	94.95
Cumulative diffusion at 10 h (%)	98.06	95.98	93.94

Antifungal Activity

The antifungal assessment reported inhibition zones of 29 mm for the optimized eucalyptus oil-based Organogel F5, 28 mm for nystatin, and 32 mm for marketed Clindac A 1% gel.

Table 16. Antifungal Activity

Sample	Zone of inhibition (mm)
Nystatin (standard)	28
Optimized eucalyptus oil-based organogel (F5)	29
Marketed Clindac A 1% gel	32



Discussion:

The present study focused on the development, optimization, and evaluation of a eucalyptus oil-based Organogel using soy lecithin and beeswax as structuring agents. The preliminary formulations exhibited a smooth, homogeneous appearance without visible phase separation or oil leakage after 24 h. The apparent pH values of the factorial formulations ranged from 5.48 ± 0.05 to 5.64 ± 0.04 , with F5 showing a pH of 5.56 ± 0.04 . These findings indicate acceptable initial physicochemical characteristics under the experimental conditions. Lecithin-based Organogel have been investigated as potential carriers for topical delivery because of their ability to incorporate lipophilic substances within structured semisolid systems [16]. The viscosity of the formulations increased from $7,215 \pm 115$ cP for F1 to $11,573 \pm 149$ cP for F9 as the concentrations of soy lecithin and beeswax increased. Conversely, spreadability decreased from 18.2 ± 0.2 to 12.5 ± 0.4 g·cm/s. The optimized formulation F5 exhibited a viscosity of $9,416 \pm 157$ cP and spreadability of 16.1 ± 0.3 g·cm/s, suggesting a balance between consistency and ease of application. The viscosity model was statistically significant ($F = 782.3649$, $p < 0.0001$), with an R^2 of 0.9962, while the spreadability model also demonstrated statistical significance ($p < 0.0001$). These findings support the application of factorial experimental design for systematically evaluating formulation variables and optimizing pharmaceutical preparations [17]. However, confirmation experiments are necessary to establish the predictive reliability of the models. The eucalyptus oil assay ranged from 97.84% to 98.68%, with F5 showing a reported value of 98.51%. The in-vitro diffusion study demonstrated differences in release behaviour among the formulations, with F5 reaching the reported 90–100% cumulative diffusion endpoint in approximately 10 h. Its cumulative diffusion at 10 h was reported as 98.06%. These results indicate substantial release of the measured material across the dialysis membrane under the specified conditions. Nevertheless, because eucalyptus oil contains multiple volatile constituents, UV absorbance at 210 nm may not provide sufficient analytical specificity without appropriate validation. Assessment of specificity, linearity, accuracy and precision is therefore necessary before interpreting the assay as a definitive quantitative measurement [18]. Furthermore, dialysis-membrane diffusion does not establish skin permeation or enhanced topical delivery. During accelerated storage at 40°C and 75% relative humidity, the eucalyptus oil assay decreased from 98.51% initially to 94.95% after six months, while cumulative diffusion at 10 h decreased from 98.06% to 93.94%. The pH and viscosity also showed modest reductions. These changes suggest alterations in the measured physicochemical and performance characteristics during storage. Loss of volatile constituents or changes in chemical composition may contribute, but these possibilities require experimental confirmation. Formal stability evaluation should follow established stability-testing principles and include validated analytical methods, predefined acceptance criteria and appropriate storage conditions [19,20]. The preliminary antifungal assessment reported inhibition zones of 29 mm for F5, 28 mm for nystatin and 32 mm for the marketed comparator. Although these results indicate measurable inhibition under the reported conditions, definitive comparative conclusions cannot be drawn because the fungal strain, experimental controls, replicate measurements and statistical analysis require verification. The suitability of the marketed comparator also needs confirmation. Overall, the findings support the feasibility of developing a eucalyptus oil-based Organogel with measurable physicochemical properties and in-vitro release characteristics. Further research involving validated antifungal testing, chemical characterization, reproducibility studies, dermal safety assessment and ex-vivo skin permeation is required to establish its therapeutic potential.

Conclusion:

The present study demonstrated the feasibility of developing a eucalyptus oil-based organogel using soy lecithin and beeswax as structuring agents. Among the nine formulations prepared using a 3² factorial design, F5, containing 5% eucalyptus oil, 5% soy lecithin and 2% beeswax, was selected as the optimized formulation. It exhibited a pH of 5.56 ± 0.04, viscosity of 9,416 ± 157 cP, spreadability of 16.1 ± 0.3 g·cm/s and a reported eucalyptus oil assay of 98.51%. The formulation also demonstrated 98.06% cumulative in-vitro diffusion at 10 h. During six months of accelerated storage, the formulation retained its smooth appearance, although reductions in eucalyptus oil content and diffusion were observed. Preliminary antifungal testing showed an inhibition zone of 29 mm; however, further validation using an identified fungal strain, appropriate reference treatments and statistical analysis is required. Overall, the findings support further investigation of eucalyptus oil-based Organogel as a potential topical delivery system.

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