RESEARCH ARTICLE

Formulation Development and Optimization of Prolonged-Release Lemon Grass Oil-Loaded Organogel with an Enhanced Antifungal Activity using 3 Factor 2 Level Full Factorial Design Approach

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ABSTRACT

The present research work aimed to develop biocompatible, prolonged-release lemon grass oil (LGO) loaded organogel with an enhanced antifungal activity using 3-factor 2-level full factorial design approach. Viscosity (cP), Spredability (gm. cm/sec), and the time at which 90–100% diffusion occurred (hours) were chosen as dependent variables, while Span 80, Tween 80, and Carbopol 934 concentrations were regarded as independent variables. The developed organogel formulations were characterized for color, texture, pH, assay, viscosity, diffusion, spredability, and antifungal activity. The results of the study showed that each independent variable had a substantial impact on the dependent variables ($p<0.05$). All organogel formulations were found to be pale yellow with smooth to viscous texture. The pH of the gels (5.61 ± 0.05 to 5.82 ± 0.05) was found to be compatible with the skin pH. The assay/drug content (97.10 to 101.81) of organogels was also found within an acceptable range. The spredability and viscosity parameters were also found to be satisfactory. Nearly 9–12 hours were required for 90-100% diffusion of the LGO from organogel formulation. Excellent prolonged release behavior was observed in almost all formulation batches. In comparison to the marketed formulation, the F4-optimized formulation showed significantly prolonged release behavior. The viscosity in all batches varied from 2548 (F6) to 3555 cP (F2) while the spredability ranged between 9.32 (F2) to 13.84 gm. cm/sec. The optimized batch of organogel (F4) showed the highest antifungal activity based on zone of inhibition (32 mm) followed by Clindac A 1% gel and Nystatin as standard. In conclusion, this study demonstrates the successful development of LGO-loaded prolonged-release organogel with enhanced antifungal activity.

Keywords: Lemon grass oil, organogel, antifungal, spredability, viscosity, diffusion

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INTRODUCTION

A tropical perennial plant, lemongrass produces aromatic oil. The characteristic lemon odor of the essential oil found in the shoot gives rise to the name lemongrass. Cymbopogon is a member of the genus Cymbopogon in the family Graminae (Poaceae) [1]. Lemongrass oil (LGO), extracted from the leaves, offers a range of therapeutic benefits attributed to its diverse bioactive compounds. Renowned for its antimicrobial properties, the oil demonstrates efficacy against bacteria and fungi, promoting skin health and preventing infections [2]. Its anti-inflammatory and antioxidant qualities make it valuable in reducing inflammation, potentially aiding conditions like arthritis [3]. Additionally, LGO is utilized in aromatherapy for its calming aroma, providing relaxation and stress relief. With potential analgesic properties, it may offer relief from pain associated with muscle aches or headaches. Furthermore, LGO exhibits antifungal activity, acts as a natural insect repellent, and finds application in skincare products for its antimicrobial and anti-inflammatory attributes [4].

A literature survey reveals that low water solubility of LGO may result in a diminished impact. Furthermore, the volatility of citral, the main active ingredient, may cause volatilization, a reaction with other compounds in the formulation, and ultimately skin irritation. LGO cannot be incorporated into a hydrogel formulation due to the physical and chemical instability of LGO in an aqueous phase [5, 6]. To surmount these problems, there is an urgent need to develop biocompatible, stable, prolonged-release LGO-loaded delivery technology. By developing an easy-to-administer dose form, recent advancements in new drug delivery systems aim to increase patient compliance while simultaneously enhancing the safety and efficacy of therapeutic compounds. Organogels are biocompatible, thermodynamically stable, isotropic gels that, by percutaneous absorption, provide both localised and systemic effects [7]. Compared to conventional topical dosage forms organogel is found to be more advantageous and efficient. The efficacy, feasibility, and shelf life of organogel are found to be superior to those of alternative lipid-based carrier systems. Organogel has three-dimensional scaffolds like a network and plays a vital role in enhancing the delivery of phytochemicals, bioactive compounds derived from plants, due to their unique properties [8]. Formed in organic solvents, organogels

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Table 1: variables and levels

Variable	(-1) Low level	(+1) High level
Independent variables		
X1= Span 80 (%)	2.5	5
X2= Tween 80 (%)	5	7.5
X3= Carbopol 934 (%)	0.4	0.6
Dependent variables		
Response 1 = Time at which 90-100% diffusion occurs (Hrs.)		
Response 2 = Viscosity (cP)		
Response 3 Spredability (gm. cm/sec)		

effectively solubilize hydrophobic phytochemicals, addressing their poor water solubility and enhancing dispersion and bioavailability [9]. The protective matrix provided by organogels improves the stability of phytochemicals, preventing degradation caused by environmental factors such as light, oxygen, and heat. Serving as controlled-release systems, organogels enable sustained and controlled delivery of phytochemicals, contributing to prolonged therapeutic effects and reduced administration frequency [10]. The gel-like structure enhances the penetration of phytochemicals through biological membranes, particularly beneficial for topical applications in improving absorption. The controlled release and targeted delivery provided by organogels contribute to reducing potential toxicity and side effects
associated with some phytochemicals, enhancing the safety profile of phytochemical-based therapies[11, 12]. In summary, organogels offer a versatile and effective platform for addressing challenges related to the solubility, stability, and controlled release of phytochemicals, holding great promise for innovative therapeutic formulations in medicine, cosmetics, and functional foods.
Considering the limitations of the LGO and potential advantages of the organogels in drug delivery applications the current study method is to synthesise, develop, and evaluate LGO-loaded organogels with improved antifungal activity.

MATERIALS AND METHODS

Lemon grass oil was purchased from local market Span 80 and tween 80 were obtained from Loba chemie Mumbai. Carbopol 934, Propyl paraben and methyl paraben were acquired from Sigma Aldrich. For every other use, analytical grade chemicals and reagents were employed.

Experiments by 3-factor, 2-level (2^3) factorial design

It has been observed in a preliminary study that, diffusion of lemon grass oil, viscosity, and spredability of the organogel was greatly influenced by Tween 80, Span 80, and Carbopol 934 concentrations. The organogel formulation was optimised using a 3-factor, 2-level full factorial design. Dependent and independent variables at different levels are depicted in Table 1.The different batches as per 3-factor, 2-level full factorial design are depicted in Table 2.

Formulation of Lemon grass-loaded organogel

Accurately weighed quantity of span 80 and lemongrass oil were added in a clean and dried beaker and mixed well to form a uniform mixture. In a second beaker tween 80 was added and to this accurately weighed quantity of Carbopol 934 was added. Lemongrass oil and span 80 were combined and added mixture of tween 80 and Carbopol 934 and continuous mixing was done till it formed a cream-like mixture. During the mixing of both phases drop by drop addition of water was done. Methylparaben (0.03%) and propylparaben (0.01%) were added to the above mixture and further mixing was done. Finally, the pH of the formulated organogel was adjusted between 5.4 to 5.9 with the help of 0.1 N hydrochloric acid to obtain a pale yellow creamy organogel. The qualitative and quantitative formula of organogel is presented in Table 3.

Characterization of lemon grass oil-loaded organogel

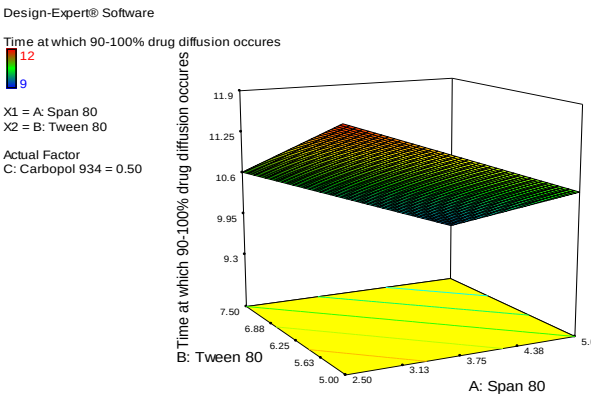

Figure 2: 3D surface responses of Span 80 and Tween 80 at a higher level of Carbopol 934 on diffusion time

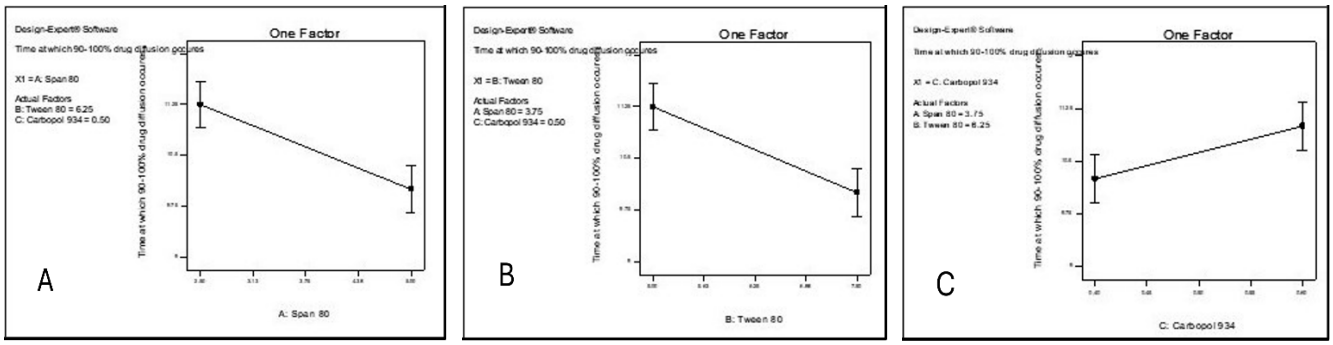

Figure 1: Effect of Span 80 (A), Tween 80 (B), and Carbopol 934 (C) on diffusion time

Table 2: Formulation of organogel using 2^3Factorial Design

Run No.	Factor1 A: % Span 80	Factor 2 B: % Tween 80	Factor 3 C: % Carbopol 934
1	2.5	7.5	0.4
2	2.5	5	0.6
3	5	5	0.6
4	2.5	5	0.4
5	5	5	0.4
6	5	7.5	0.4
7	5	7.5	0.6
8	2.5	7.5	0.6

Color and Texture

All 8 batches of formulated organogel were observed against a white background to check the color. Texture analysis was done by taking a small amount of dosage form in between two figures and texture was determined.

pH

After precisely weighing 0.5 g of organogel, it was added to a 100 ml beaker. There was also the addition of 50 ml of water and mixed vigorously and its pH was measured immediately.

Assay/Drug content

A 100 ml volumetric flask was thoroughly cleaned and dried before 800 mg of the test sample—or 20 mg of lemongrass oil—was precisely weighed. After adding 70 ml of methanol and sonicating for 5 to 10 minutes, the volume was raised to the necessary amount using methanol. An initial 3 to 5 ml of filtrate were discarded before the solution was filtered using a 0.45 µ PVDF syringe filter. 1ml filtered solution was pipet out and diluted with methanol to a maximum of 20 ml. At 238 nm, the sample solution was examined in a single replicate and % assay was calculated against the standard.[13]

Viscosity

The viscosity of every one of the eight prepared batches was measured using a Brookfield Viscometer (Amtech Model Number. LVDVE) at 20 rpm, using spindle number 64. The spindle was rotated at 20 rpm and viscosity (cP) and Torque (%) were measured. The lesser the torque better be viscosity.[14]

Diffusion study sample preparation

To ascertain the drug's release profile from organogel, a Franz diffusion cell-like device developed in a laboratory was utilised. A diffusion membrane (Cellophane membrane) was positioned among the receptor and donor chambers, which made up the cell. The receptor compartment was designed to allow for sampling, and the donor compartment, which had an inner diameter of 24 mm, was open, meaning that one end of it was exposed to the atmosphere. Phosphate buffer solution pH 5.8 (PBS) was the diffusion medium that was employed. A dosage form containing 5 mg of the drug ingredient, weighing about 200 mg, was obtained. It was situated in the donor compartment over the drug release membrane and kept apart from the receptor compartment by a cellophane membrane. Prior to use, the cellophane membrane was immersed in PBS (pH 5.8) for 24 hours. A clamp was used to keep the donor and receptor chambers together. The donor compartment was repositioned until the cellophane membrane barely made contact with the diffusion medium. All of the components were secured onto a magnetic stirrer. A thermostatically controlled magnetic stirrer was used to hold the receptor compartment with 25 ml of PBS. It was kept at 37 ± 0.5^0C and continuously agitated at 100 rpm. At pre-arranged intervals, one ml samples were obtained, diluted with up to twenty ml of methanol, and their drug concentration was determined at λ max (238 nm) using a UV Spectrophotometer in comparison to a blank. Every time a sample was withdrawn, an equivalent volume of phosphate buffer was added back into the receptor phase.

Spredability test

Two slides were sandwiched with 1 gm of the organogel. On the upper slide, there was a 100 g weight. After the sample had been evenly distributed throughout the slide, the weight was taken off, and the excess formulation was discarded. The apparatus's board held the bottom slide in place, while a non-flexible string supported the upper slide and supported a 20gm load. The amount of time the upper slide took to slip off was recorded and the length for which the slide was displaced was measured by a digital vernier caliper.

S=ML/T

Where,

S: Spredability; M: Weight on upper slide; L: Length moved on glass slide and T: Time

Anti-fungal activity for optimized batch and marketed formulation

1 gm accurately weighed quantity of organogel (Optimized batch F4) was taken and 1 ml methanol was added. Vigorous shaking was done and the sample was used for the further process. Similarly, 1 gm accurately weighed quantity of marketed gel (Clindac A 1%) was taken and 1

Table 3: Qualitative and quantitative formula composition of organogel

S.no.	Ingredients	Quantity (mg)							
		F1	F2	F3	F4	F5	F6	F7	F8
1.	Lemongrass oil	250	250	250	250	250	250	250	250
2.	Span 80	250	250	500	250	500	500	500	250
3.	Tween 80	750	500	500	500	500	750	750	750
4.	Carbopol 934	40	60	60	40	40	40	60	60
5.	Methyl paraben	3	3	3	3	3	3	3	3
6.	Propyl paraben	1	1	1	1	1	1	1	1
7.	Water (q. s. to 10 gm)	8706	8936	8686	8956	8706	8456	8436	8686
Total		10 gm.							

ml methanol was added. Vigorous shaking was done and the sample was used for the further process.

RESULTS AND DISCUSSION

Physicochemical characterization of organogels

Several physicochemical properties were assessed for the developed organogel formulations. All organogel formulation was observed to have a smooth to viscous texture and a pale yellow colour. The smoothness and viscous properties of organogels are due to the presence of Span 80, Tween 80, and Carbopol 934 [15]. The pH of the gels (5.61 ± 0.05 to 5.82 ± 0.05) was found to be suitable with the skin pH. Skin pH normally ranges from 4.7 to 5.75 on the pH scale, which indicates that it is mildly acidic. The assay/drug content (97.10 to 101.81) of organogels was also found within an acceptable range of 85 % to 115%. The spredability and viscosity parameters were also found to be satisfactory. The compiled physicochemical properties of the organogels are depicted in Table 4.

Statistical analysis of the time at which 90-100% diffusion occurs (Response 1)

Organogels play a crucial role in drug delivery through controlled release, ensuring a gradual and sustained diffusion of active pharmaceutical ingredients (APIs) [16]. This controlled release enhances bioavailability by maintaining drug concentrations within the therapeutic window for a longer amount of time, particularly beneficial for drugs with narrow therapeutic ranges [17]. By avoiding sharp peaks in drug levels, organized-based systems help minimize side effects, contributing to a more tolerable and safer therapeutic profile. Prolonged release formulations of organogels require less frequent dosing, leading to improved patient compliance. This convenience encourages adherence to treatment regimens involving fewer administrations. Organogels also offer stability, protecting drugs from degradation and maintaining efficacy over time, especially for drugs sensitive to environmental factors [18]. The results of the time at which 90-100% drug diffusion occurs from organogels are presented in Table 5 in addition to the levels of the dependent variables. Nearly 9–12 hours were required for 90 (F6)-100% (F2 and F4) diffusion of the lemon grass oil from organogel formulation. The independent variables had a great impact on the diffusion process. Span 80 and Tween 80 showed an inverse relation with diffusion time i.e., as Span 80 and Tween 80 increased, diffusion time decreased (Figure 1A and 1B). But in the case of Carbopol 934, a direct relation was observed with diffusion time i.e., as Carbopol concentration increased, diffusion time was increased (Figure 1C). A graphic depicts the link and the effect of the independent factors on diffusion time in Figure 2. The diffusion time (R1) in coded factors final polynomial equation is shown below. The

Table 4: Physicochemical properties of the organogels

Batches	Color	Texture	pH	% Assay	Spreadability (gm. cm/sec)	Viscosity (cP)
F1	Pale yellow	Smooth	5.54 ± 0.05	98.48	12.03	3041 ± 11.15
F2	Pale yellow	Viscous	5.82 ± 0.05	101.81	9.32	3555 ± 11.79
F3	Pale yellow	Viscous	5.65 ± 0.05	98.12	9.85	3412 ± 11.15
F4	Pale yellow	Smooth	5.74 ± 0.05	99.60	11.86	3105 ± 13.50
F5	Pale yellow	Smooth	5.79 ± 0.05	97.33	12.67	2845 ± 20.46
F6	Pale yellow	Smooth	5.81 ± 0.05	101.30	13.84	2548 ± 19.50
F7	Pale yellow	Viscous	5.74 ± 0.05	97.10	11.98	2874 ± 11.14
F8	Pale yellow	Smooth	5.61 ± 0.05	99.93	10.86	3225 ± 17.00

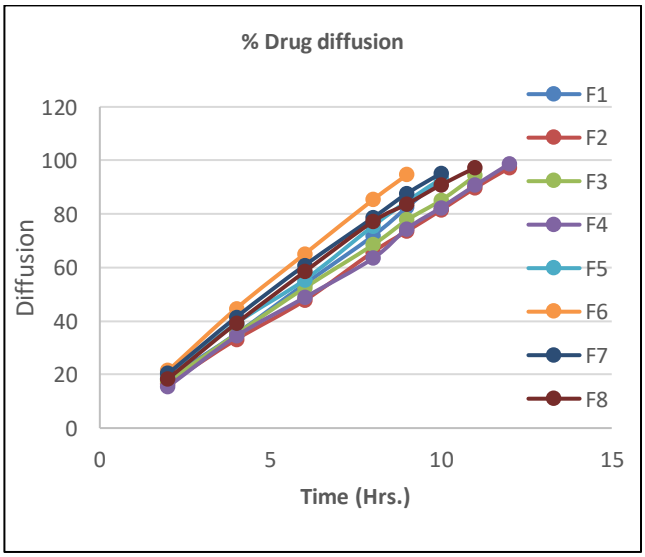

Figure 3: Comparative in vitro drug diffusion of organogel formulations (F1 to F8) in phosphate buffer solution pH 5.8

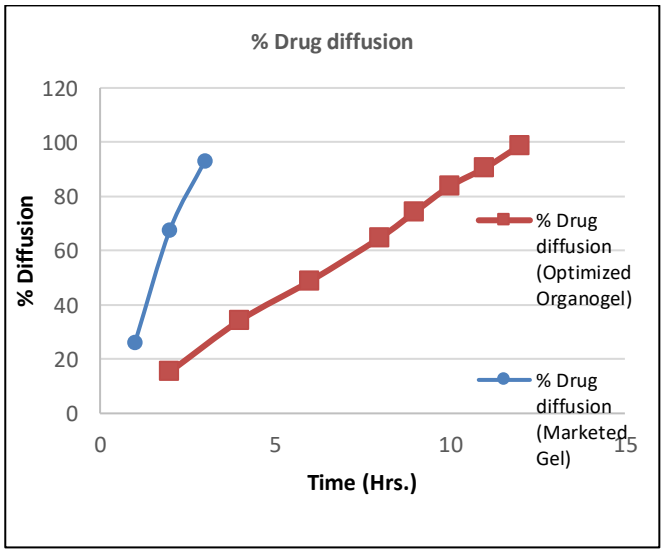

Figure 4: Comparative *in vitro* drug diffusion of marketed gel with optimized organogel formulations (F4) in phosphate buffer solution pH 5.8

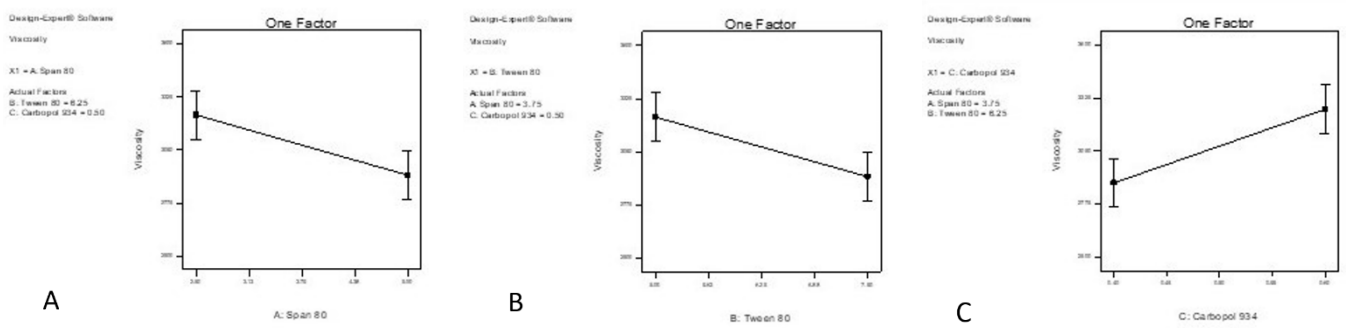

Figure 5: Effect of Span 80 (A), Tween 80 (B), and Carbopol 934 (C) on viscosity

response for specific levels of each element can be predicted using the equation expressed in terms of coded factors.

R1 = +10.63 -0.63 A-0.63 B+0.37C

P-values less than 0.0500 indicate significant model terms**.** Here, the model terms A, B, and C are significant. Values higher than 0.0500 signify the lack of significance for the model terms (Table 6). The model's F-value of 19.67 suggests that it is significant (Table 6). Only 0.74% of cases might have an F-value this high due to noise. The adjusted R^2 of 0.889 and the predicted R^2 of 0.7460 correspond reasonably well (Table 6). Signal-to-noise ratio is determined using the Adeq precision. A ratio greater than four is ideal. A ratio of 13.000 in our model suggested a sufficient signal. This model can be used to navigate the design area.

Table 5: Effect of independent variables on diffusion time

Runs	Factor 1	Factor 2	Factor 3	Response 1
	A: % Span 80	B: % Tween 80	C: % Carbopol 934	Time at which 90-100% diffusion occurs (Hrs.)
F1	2.5	7.5	0.4	10
F2	2.5	5	0.6	12
F3	5	5	0.6	11
F4	2.5	5	0.4	12
F5	5	5	0.4	10
F6	5	7.5	0.4	9
F7	5	7.5	0.6	10
F8	2.5	7.5	0.6	11

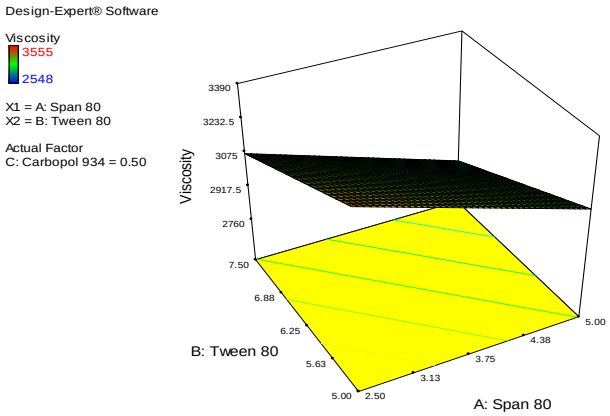

Figure 6: 3D surface responses of Span 80 and Tween 80 at a higher level of Carbopol 934 on viscosity

Comparative drug diffusion profile

The comparative drug diffusion of all organogel formulations (F1 to F8) in phosphate buffer solution pH 5.8 is presented in Table 7 and Figure 3. Excellent prolonged re lease behavior was observed in almost all formulation batches. Span 80 and Tween 80 contribute to emulsification and improved drug dispersion, while Carbopol 934 plays a crucial role in forming a gel-like matrix, increasing viscosity, and influencing the hydration and swelling behavior [19]. The combination of these components in an organogel formulation can result in a system with

Table 6: ANOVA for a selected factorial model of diffusion time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.375	3	2.458333	19.66667	0.0074	Significant
A-Span 80	3.125	1	3.125	25	0.0075	
B-Tween 80	3.125	1	3.125	25	0.0075	
C-Carbopol 934	1.125	1	1.125	9	0.0399	
Residual	0.5	4	0.125			
Cor Total	7.875	7				
Fit statistics						
Std. Dev.	0.35		R^2	0.9365		
Mean	10.63		Adjusted R^2	0.8889		
C.V. %	3.33		Predicted R^2	0.7460		
PRESS	2.00		Adeq Precision	13.000		

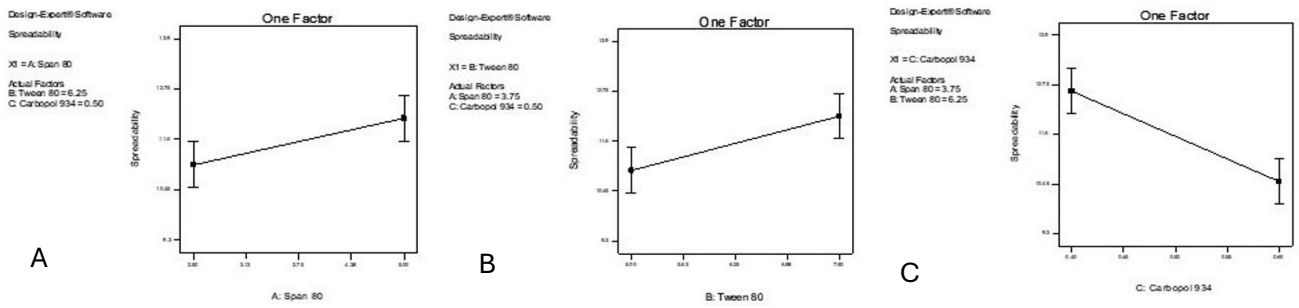

Figure 7: Effect of Span 80 (A), Tween 80 (B), and Carbopol 934 (C) on spredability

Table 7: % Cumulative Drug Release from organogel formulations

Time (Hrs.)	Batches	% Cumulative Drug Release							
		F1	F2	F3	F4	F5	F6	F7	F8
2		18.46	16.85	17.48	15.37	19.59	21.3	20.21	18.32
4		35.57	34.06	36.06	34.29	39.89	44.64	41.55	39.03
6		53.25	47.69	51.81	48.73	55.17	64.94	60.47	58.52
8		71.52	65.54	68.05	64.76	74.79	84.19	78.22	77.68
9		82.64	73.68	77.35	74.27	84.74	94.22	87.83	84.03
10		93.74	82.52	85.21	83.74	93.8	-	95.59	91.02
11		-	89.39	94.29	90.49	-	-	-	97.63
12		-	97.67	-	98.73	-	-	-	-

prolonged and controlled drug release characteristics. The specific ratios and interactions among these components need careful consideration during the formulation process to achieve the desired release profile for the targeted therapeutic effect. The diffusion behavior of marketed Clindac A 1% gel was also studied and compared with the optimized F4 organogel formulation. The marketed formulation showed nearly 93% of drug release within 4 hours as presented in Figure 4. In comparison to the marketed formulation, the F4-optimized formulation showed significantly prolonged release behavior.

Statistical analysis of viscosity (Response 2)

Viscosity is a critical factor in the formation and properties of organogels. It profoundly influences the gelation process by regulating the interaction of molecules or particles, fostering the development of a three-dimensional network typical of gels [20]. Higher viscosity enhances this process by slowing molecular motion and promoting interactions among gelators, leading to more robust and stable gels. The structural properties of organogels, including strength and stability, are determined by viscosity, and they influence rheological properties such as shear-thinning behavior [21]. In summary, viscosity is a multifaceted determinant in organogels, shaping their formation, structure, texture, and applicability across diverse industries. Researchers and industries carefully manipulate viscosity to achieve desired organogel properties. The observations of viscosity with different levels of independent variables are presented in Table 8.

The viscosity in all batches varied from 2548 (F6) to 3555 cP (F2) showing great impact of independent variables. Span 80 and Tween 80 showed inverse relation with viscosity i.e., as Span 80 and Tween 80 increased, viscosity decreased (Figure 5A and 5B). But in the case of Carbopol 934, a direct relation was observed with viscosity i.e., as Carbopol concentration increased, viscosity was increased

Table 8: Effect of independent variables on viscosity

Runs	Factor1	Factor 2	Factor 3	Response 2
	A: % Span 80	B: % Tween 80	C: % Carbopol 934	Viscosity (cP)
F1	2.5	7.5	0.4	3041
F2	2.5	5	0.6	3555
F3	5	5	0.6	3412
F4	2.5	5	0.4	3105
F5	5	5	0.4	2845
F6	5	7.5	0.4	2548
F7	5	7.5	0.6	2874
F8	2.5	7.5	0.6	3225

(Figure 5C). Figure 6 visually depicts the relationship and the effect of the independent factors on diffusion time. The viscosity (R2) final polynomial equation in coded factors is shown below. The equation defined in terms of coded factors can be used to predict the
response for individual amounts of each element.

R2 = +3075.63 -155.88A-153.63B+190.88 C

P-values less than 0.0500 denote significant terms in the model. In this case, A, B, and C are significant model terms. Values higher than 0.0500 signify the lack of significance for the model terms (Table 9). The model's 13.86 F-value suggests that it is significant (Table 9). Only 1.40 percent of cases with an F-value this high could be the consequence of noise. The adjusted R^2 of 0.9122 and the predicted R^2 of 0.6490 correspond reasonably well (Table 9). Signal-to-noise ratio is determined using the Adeq precision. A ratio greater than 4 is ideal. A ratio of 11.110 in our model suggested a sufficient signal. This model helps in navigating the design area.

Statistical analysis of spredability (Response 3)

The spreadability of organogels holds significant importance across diverse industries, influencing their ease

Table 9: ANOVA for a selected factorial model of diffusion time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	674647.4	3	224882.5	13.85928	0.0140	Significant
A-Span 80	194376.1	1	194376.1	11.97921	0.0258	
B-Tween 80	188805.1	1	188805.1	11.63587	0.0270	
C-Carbopol 934	291466.1	1	291466.1	17.96277	0.0133	
Residual	64904.5	4	16226.13			
Cor Total	739551.9	7				
Fit statistics						
Std. Dev.	127.38		R^2	0.9122		
Mean	3075.63		Adjusted R^2	0.8464		
C.V. %	4.14		Predicted R^2	0.6490		
PRESS	259618		Adeq Precision	11.110		

of application and user acceptance [22]. In cosmetic and skin care applications, the ability of organogels to spread smoothly on the skin is crucial for providing a pleasant user experience and enhancing product performance.

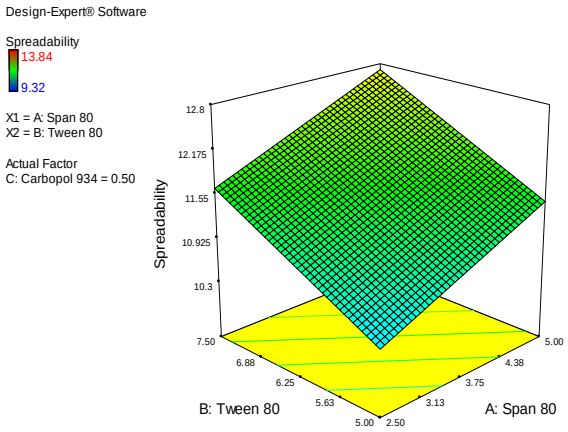

Figure 8: 3D surface responses of Span 80 and Tween 80 at a higher level of Carbopol 934 on spredability

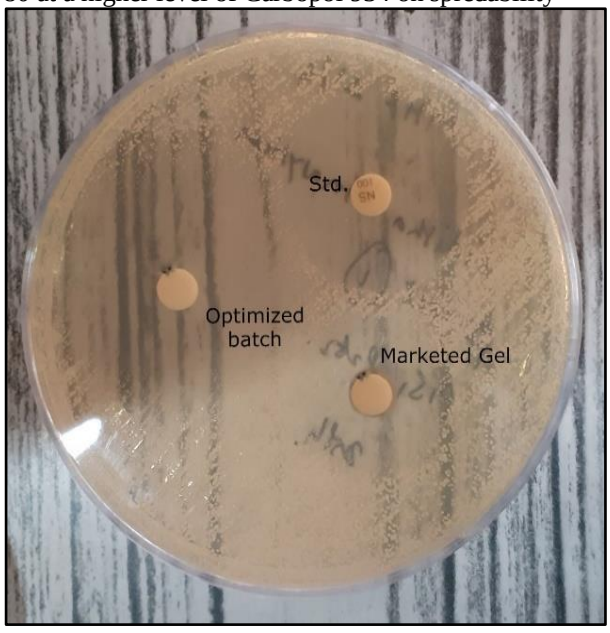

Figure 9: Zone of inhibition of optimized batch, marketed gel, and standard

In pharmaceuticals, especially in topical drug delivery, the spreadability of organogels ensures uniform drug distribution and improved absorption, contributing to the efficacy of drugs [23]. In industrial settings, organogels serve as coatings or lubricants, and their spreadability is integral for uniform coverage, enhancing protective and lubricating properties. Additionally, spreadability plays a crucial role in the formulation process, contributing to the uniformity of the final product, and has aesthetic implications in cosmetic products [24]. Overall, the spreadability of organogels significantly contributes to a positive user experience, making products that are easy to apply and spread more likely to be preferred by consumers. The observations of spredability with different levels of independent variables are presented in Table 10. The spredability in all batches varied from 9.32 (F2) to 13.84 gm. cm/sec (F6) showing a great impact of independent variables. Span 80 and Tween 80 showed direct relation with spredability i.e., as Span 80 and Tween 80 increased, spredability was also increased (Figure 7A and 7B). But in the case of Carbopol 934, an inverse relation was observed with spredability i.e., as Carbopol concentration increased, spredability was found to be decreased (Figure 7C).

Figure 8 visually illustrates the relationship and the effect of the independent factors on diffusion time.

The following is the final spredability (R3) polynomial equation in coded factors. Using the equation indicated in terms of coded factors, the response for individual levels of each element may be predicted.

R3 = +11.55 +0.53A+0.63B-1.05C

P-values less than 0.0500 denote the presence of significant model terms. In this case, key model terms are A, B, and C. Values higher than 0.0500 signify the lack of significance for the model terms (Table 11). The F-value of 16.73 for the model indicates that it is significant (Table 11). Only 1.00% of the cases with an F-value this high might be the consequence of noise. The adjusted R^2 of 0.8708 and the predicted R^2 of 0.7047 correspond reasonably well (Table 11). Signal-to-noise ratio is determined using the Adeq precision. A ratio greater than 4 is ideal. A ratio of 11.737 in our model suggested an adequate signal. This model helps in navigating the design area.

Anti-fungal activity of optimized organogel batch and marketed formulation

Anti-fungal study for the optimized batch of organogel (F4) was performed with respective to marketed gel formulation (Clindac A 1%). Nystatin was used as a standard antifungal agent. The results of antifungal activity are presented in Table 12. The optimized batch of organogel (F4) showed the highest antifungal activity based on the zone of inhibition (32 mm) followed by Clindac A 1% gel and Nystatin as standard (Figure 9). Lemongrass oil, renowned for its antimicrobial properties, exhibited enhanced antifungal activity [25] when incorporated into an organogel, a gel-like structure formed in an organic solvent. This enhanced efficacy results may be due to multiple factors.

The organogel matrix improved lemongrass oil's solubility and dispersion, facilitating better interaction with fungal cells. Acting as a controlled-release system, the gel structure ensured sustained exposure to antifungal compounds, prolonging their effectiveness [26]. The organogel's enhanced adhesion to surfaces, particularly in topical applications, promotes better contact with fungal cells. The synergistic effects of lemongrass oil's bioactive compounds, such as citral and limonene, are amplified by the organogel matrix. The gel structure aids in the penetration of lemongrass oil into fungal structures, disrupting growth [27]. Additionally, organogels offer stability and protection, preventing oil degradation and preserving bioactivity. Designed for targeted delivery, organogels concentrate lemongrass oil at the infection site, further improving antifungal efficacy [28]. In summary, the lemongrass oil-loaded organogel synergistically maximizes antifungal potential through improved solubility, sustained release, enhanced adhesion, synergistic effects, penetration, stability, and targeted delivery.

Table 10: Effect of independent variables on spredability

Runs	Factor1 A: % Span 80	Factor 2 B: % Tween 80	Factor 3 C: % Carbopol 934	Response 3 Spreadability (gm. cm/sec)
F1	2.5	7.5	0.4	12.03
F2	2.5	5	0.6	9.32
F3	5	5	0.6	9.85
F4	2.5	5	0.4	11.86
F5	5	5	0.4	12.67
F6	5	7.5	0.4	13.84
F7	5	7.5	0.6	11.98
F8	2.5	7.5	0.6	10.86

Table 12: The comparative antifungal activity results

S.no	Sample	Zone of inhibition (mm)
1.	Nystatin (Standard)	27 mm
2.	Optimized batch of organogel (F4)	32 mm
3.	Marketed Gel (Clindac A 1%)	24 mm

CONCLUSION

In conclusion, our research has successfully addressed the objective of developing a biocompatible, prolonged-release organogel loaded with LGO that demonstrates enhanced antifungal activity. Employing a systematic 3-factor, 2-level full factorial design approach, we systematically investigated the influence of independent variables on critical dependent variables. This study not only successfully formulated a prolonged-release organogel with LGO but also demonstrated its heightened antifungal activity. The systematic approach employed in the design and optimization process ensures the reproducibility and reliability of the developed formulation. These findings hold promise for the application of our organogel in dermatological interventions, particularly those requiring sustained and effective antifungal therapy. The insights gained contribute to the advancement of pharmaceutical formulations with controlled-release properties, offering a novel and valuable contribution to the field.

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Table 11: ANOVA for a selected factorial model of diffusion time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	14.21564	3	4.738546	16.72551	0.0100	Significant
A-Span 80	2.279113	1	2.279113	8.044518	0.0470	
B-Tween 80	3.137513	1	3.137513	11.07439	0.0292	
C-Carbopol 934	8.799013	1	8.799013	31.05762	0.0051	
Residual	1.13325	4	0.283313			
Cor Total	15.34889	7				
Fit statistics						
Std. Dev.	0.53		R^2	0.9262		
Mean	11.55		Adjusted R^2	0.8708		
C.V. %	4.61		Predicted R^2	0.7047		
PRESS	4.53		Adeq Precision	11.737		

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