RESEARCH ARTICLE

Optimization *In-vitro* Evaluation and Scratch Wound Healing Assay of Hexacosane Topical Gel for Wound Healing

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ABSTRACT

The compounds hexocosane, a sterol hydrocarbon identified in the dichloromethane extract and isolated from marine sponges of the genus *Agelas*. Hexocosane possesses anti-inflammatory and antioxidant activities that reduce inflammation and oxidative stress, they promote cell proliferation and angiogenesis, facilitating tissue regeneration and repair. This multifaceted approach makes this secondary metabolite a promising candidate for developing advanced wound care therapies that effectively address both infection control and wound healing. On the basis of viscosity, *in-vitro* release, and skin retention, optimal gel formulations were developed with hexocosane (1%) to achieve the best results. A response surface methodology Box-Behnken design was applied on three factors and three levels [carbopol 940 (1, 1.25 and 1.5%), hydroxypropyl methylcellulose (HPMC) (1,1.5 and 1.2%), and propylene glycol (0–1–1.5% and 1–2%, respectively] using three factors and three levels. In order to assess the spreadability and viscosity, a glass slide and a Brookfield viscometer were used. An assay was conducted to determine how topical gel affects wound healing. The optimization study of the gel formulation, involving variations in adhesive polymer (Carbopol 940), release retarding polymer (HPMC K4M), and penetration enhancer (Surfactant), has identified three noteworthy formulations (F4, F8 & F14). Each formulation is characterized by specific attributes such as viscosity, *in-vitro* drug release, and skin retention. All samples were found to elicit significant wound healing efficacy as evidenced from the representative photomicrographs. The maximum efficacy was elicited by formulation (F4) with 100 mg drug at the time duration of 36 hours

Keywords: Hexocosane, Box-Behnken design, Topical gel, Scratch wound healing assay.

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INTRODUCTION

In the absence of proper healing, wounds disrupt skin and underlying tissues, leading to chronic injuries. Chronic wounds are often stuck in the inflammatory phase of healing and can persist for months or even years.[1] Common types include diabetic foot, venous leg, and pressure ulcers. Marine sponges have garnered considerable attention for their potential to promote wound healing due to their rich repertoire of bioactive compounds.[2] These compounds, including peptides, polysaccharides, sterols, and alkaloids, exhibit various biological activities that contribute to the wound healing process.

Hexacosane is a colorless crystal with straight chain alkane comprising 26 carbon atoms including in the sterol hydrocarbon category.[3] The studies have indicated that certain polysaccharides and sterol hydrocarbons extracted from sponges can enhance fibroblast proliferation and collagen synthesis, which are essential for tissue repair and strength. Additionally, these compounds can promote the migration of keratinocytes to cover the wound surface, facilitating faster closure.[1, 3]

Hexacosane is also being assessed for its healing effects. A gelling agent, carbopol 940, and HPMC K4M, are present in this mixture. Different factors can affect the properties of gels in addition to physical and chemical parameters.

MATERIALS AND METHODS

Optimization of Gel

The chosen method used for optimization and formulation of gel was response surface methodology (RSM). Optimize the formulation of a gel was based on varying factors (adhesive polymer, release retarding polymer, and penetration enhancer)

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Table 1: Optimization design

Factor	*Name*	*Units*	*Type*	*Subtype*	*Minimum*	*Maximum*	*Coded low*	*Coded high*	*Mean*	*Std. dev.*
A	Adhesive polymer (Carbopol 940)	%	Numeric	Continuous	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7071
B	Release retarding polymer (HPMC K4M)	%	Numeric	Continuous	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7071
C	Penetration enahncer (Surfactant)	%	Numeric	Continuous	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7071

and observing their effects on three responses (Viscosity, *in-vitro* drug release for 12 hours, and skin retention).

The experimental design aimed to optimize the formulation of a gel by systematically varying three key factors: adhesive polymer (A), release retarding polymer (B), and penetration enhancer (C). A total of 17 experimental runs were conducted, each representing a unique combination of factor levels. The responses measured were viscosity (cp), *In-vitro* drug release for 12 hours (%), and skin retention (%).[4]

The methodology involved statistical analysis to understand the impact of each factor and their interactions with the responses. The goal was to identify the optimal combination of factor levels that would yield the desired gel characteristics. This optimization methodology encompassed experimental design, data collection, statistical analysis, and iterative refinement to guide the formulation of a gel with specific attributes, including optimal viscosity, drug release, and skin retention. The systematic exploration of the factor space aimed to provide valuable insights into the formulation process and enhance the efficacy of the gel for its intended application.[4,5] Table 1 summarizes the optimization design of three factors.

The gel optimization study, version 13.0.5.0, employed a randomized Box-Behnken design with a quadratic model, aiming to explore the relationship between input variables and response in 17 experimental runs. The absence of blocks indicates a straightforward experimental setup, and the build time was 22 ms. The chosen design type and model suggest a comprehensive approach to understanding and optimizing the gel characteristics.

Factors: Three factors (A, B, and C) represent different components or variables in the gel optimization study. The names of the factors indicate the specific components being considered, such as adhesive polymer (Carbopol 940), release retarding polymer (HPMC K4M), and penetration enhancer (Surfactant).

Type

All factors are of numeric type, implying that they involve numerical values.

Minimum and maximum

The minimum and maximum values define the range of each factor. In this case, all factors range from -1.0000 to 1.0000.

Coded low and coded high

Coded low and coded high values provide a standardized representation of the minimum and maximum, making it easier to compare factors on a standardized scale.

Mean and Std. Dev.

Mean represents the average value and Std. Dev. (standard deviation) gives an indication of the variability of each factor. Table 2 describes about the fit summary.

Source

A linear model, a fractional factorial (2nd order), a quadratic model, or a cubic model is indicated by this parameter.

Sequential p-value

Represents the *p-value* associated with adding the corresponding term to the model. Statistics are significant when *p-value*s are low.

Lack of fit p-value

Evaluates the adequacy of the model by testing whether the model fits the data well. A low *p-value* indicates lack of fit.

Adjusted R^2

Provides a measure of how well the model explains the variability in the data, adjusted for the number of predictors. Higher values indicate a better fit.

Predicted R^2

Indicates the predictive capability of the model. Negative values may suggest poor predictive performance.

Interpretation

In summary, the linear model is statistically significant with a good fit, while the 2FI model is not significant and may not provide a good fit. The Quadratic model is suggested as it has a low *p-value*, high adjusted R^2, and a positive predicted R^2. The Cubic model, although suggested, is aliased, and its interpretation may be influenced by confounding variables or limitations in the experimental design.

Formulation of topical gel

The polymers are dispersed in a glycerol and water solution, with the concentration of the glycerol varying with the concentration of hexacosane. Triethanolamine was used to neutralize and viscousify the dispersion after it was neutralized.[5]

Table 2: Fit summary

Source	*Sequential p-value*	*Lack of Fit p-value*	*Adjusted R²*	*Predicted R²*	
Linear	0.0004	0.0002	0.6882	0.5133	
2FI	0.8807	0.0001	0.6197	-0.0400	
Quadratic	0.0001	0.0119	0.9687	0.7972	Suggested
Cubic	0.0119		0.9956		Aliased

Characterization of Gel [6-8]

Physical evaluation

Color and appearance of the specimen were examined.

Measurement of pH

In order to determine the pH of the gel, a pH meter was used.

Spreadability

Greater spreadability is associated with shorter intervals. Our formula for calculating spreadability is as follows:

$$S = M \times L / T$$

Where, S = spreadability, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Viscosity

Measurements of gel viscosity were performed using a Brookfield viscometer mounted on a spindle [9,10]

Scratch Wound Healing Assay

Cell line maintenance

The National Centre for Cell Sciences in Pune provided an Indian cellular line for this study. As an antibiotic cocktail, 1% DMEM-Himedia medium containing 10% heat-inactivated fetal bovine serum (FBS) was used in addition to penicillin 100 units per mL, streptomycin 100 grams per mL, and amphotericin B 2 g/mL. A Galaxy® 170 incubator was used to incubate 24-cm^2 TC flasks with cells (Eppendorf, Germany) at 37°C and 5% CO2. We prepared samples with DMSO (10 mg/mL) and sterilized them with 0.21mm Millipore filters. A 6-well plate with 5% CO2 was used to prepare and sterilize samples. According to previously described procedures, DMEM medium was diluted further with samples and then added to wells containing at least 80% confluent cells at final concentrations of 25, 50, or 100 mg/mL. In the control wells, there was no treatment applied. [11,12]

Scratch wound healing assay procedure

- Cell monolayers are scratched by pipette tip 200 L.

After washing and smoothing the cells, 5 mL of fresh growth medium should be added. All debris should be removed and scratches should be smoothed.

The first step is to make scratches equal in size on both sides of the specimen.

Wider or narrower cells should be used as control cells to minimize variations caused by these differences.

- As a guide when acquiring the image, reference points should be marked near the scratch. A razor blade or ultrafine tip marker can be used to lightly etch the bottom of a well plate, for instance. Place the dish under a phase-contrast microscope and place the reference marks outside the capture area but within easy reach of the eyepiece. As soon as possible, take a photograph of the scratch.
- Well plates should be incubated at 37.5°C when cultured tissues. Photomicrographs were taken after 0, 12, 24 and 36 hours. Cell types require varying times of incubation. As soon as the well plates have been examined outside, they can be placed back in the incubator for re-incubation.

If you want the scratch to heal completely, incubate the cells under the fastest migration conditions for as long as possible.

- Incubate the dish, and then examine it under the phase-contrast microscope

It is recommended to take an additional image once the previously photographed region has been aligned and the reference point has been acquired.

Take photos of the wound as soon as possible to ensure it heals completely.

Cell migration rate and average wound area

Analyzing the images with Image J, a software package from the United States, was done to determine migration rates and percentages of areas that had been closed since 0 hours previously. Increasing closed areas causes migratory cells. This leads to faster wound healing.[13,14]

RESULTS AND DISCUSSION

Gel optimization was done and it is described in Table 3.

Adhesive Polymer (Factor 1 - A)

Significant effect

Among Run 3 (A=1) shows a substantial increase in viscosity (980 cp). This suggests that higher levels of Adhesive Polymer (Carbopol 940) positively impact viscosity.

Trade-off consideration

While increased adhesive polymer enhances viscosity, it's crucial to assess the potential trade-offs with other responses, such as drug release and skin retention.

Release Retarding Polymer (Factor 2 - B)

Significant effect

Run 8 (B = 0) exhibits the highest *in-vitro* drug release (56.4%), indicating that the absence of release retarding polymer (HPMC K4M) leads to a significant increase in drug release.

Table 3: Optimization of gel by varying the factor and determining its effect on response

	Factor 1	*Factor 2*	*Factor 3*	*Response 1*	*Response 2*	*Response 3*
Run	*A:Adhesive polymer (Carbopol 940)*	*B:Release retarding polymer (HPMC K4M)*	*C:Penetration enahncer (Surfactant)*	*Viscosity*	*In-vitro drug release for 12 hours*	*Skin retention*
	%	%	%	cp	%	%
1	0	0	0	456	66.8	33.2
2	0	1	1	488	86.6	13.4
3	1	-1	0	980	68.8	31.2
4	1	0	1	1398	88.4	11.6
5	1	0	-1	1124	68.9	31.1
6	1	1	0	1345	86.2	13.8
7	0	0	0	494	68.4	31.6
8	-1	0	1	324	56.4	43.6
9	0	-1	-1	388	24.8	75.2
10	0	1	-1	446	33.6	66.4
11	0	0	0	464	67.4	32.6
12	0	0	0	426	62.8	37.2
13	-1	1	0	428	54.2	45.8
14	0	-1	1	348	78.4	21.6
15	-1	0	-1	356	24.6	75.4
16	0	0	0	466	64.8	35.2
17	-1	-1	0	247	37.2	62.8

Trade-off consideration

Higher drug release may be advantageous for certain applications, but it's essential to balance this with other factors like viscosity and skin retention.

Penetration Enhancer (Factor 3 - C)

Significant effect

Run 9 (C = -1) demonstrates the highest skin retention (75.2%) at a lower level of penetration enhancer (Surfactant), suggesting a significant effect on skin retention.

Trade-off Consideration

While increased skin retention is beneficial, it's important to assess potential impacts on drug release and viscosity.

Interaction Effects

Significant effect

Run 14 (A=0, B=-1, C=1) displays high skin retention (78.4%) and moderate drug release (21.6%). This suggests a significant interaction effect, highlighting the trade-offs between different factors.

Overall optimization considerations

Achieving an optimal gel formulation involves balancing the trade-offs between viscosity, drug release, and skin retention.

Run 14 represents a compromise with relatively high skin retention and drug release. However, further analysis is needed to find the optimal combination that meets specific requirements.

The effects of each variable on the responses

A: Adhesive Polymer (Carbopol 940)

- Viscosity: Runs 3, 4, 5, and 6, where A is positive, show an increase in viscosity. Run 8, where A is negative, has lower viscosity.
- *In-vitro* Drug Release: Higher levels of A generally correspond to higher drug release, with Run 4 (A=1) having the highest.
- Skin Retention: The effect of A on skin retention is less clear, as there is no consistent pattern across the runs.

B: Release Retarding Polymer (HPMC K4M)

- Viscosity: There is no consistent trend in viscosity with changes in B.
- *In-vitro* Drug Release: Run 2 (B=1) has the highest drug release, suggesting that higher levels of B may lead to increased drug release.
- Skin Retention: The effect of B on skin retention is less clear, similar to A.

C: Penetration Enhancer (Surfactant)

- Viscosity: There is no consistent trend in viscosity with changes in C.
- *In-vitro* Drug Release: Runs 9 and 10, where C is negative,

Table 4: Fit summary Response 1: viscosity

Source	*Sequential p-value*	*Lack of Fit p-value*	*Adjusted R²*	*Predicted R²*	
Linear	0.0004	0.0002	0.6882	0.5133	
2FI	0.8807	0.0001	0.6197	-0.0400	
Quadratic	0.0001	0.0119	0.9687	0.7972	Suggested
Cubic	0.0119		0.9956		Aliased

show lower drug release compared to other runs.

- Skin Retention: Higher levels of C generally correspond to higher skin retention, with Run 14 (C=1) having the highest.

Fit summary is presented in Table 4.

Effects of the variables on Response 1 (Viscosity)

Significant Variables

- Adhesive Polymer (A): Highly significant with a large F-value (363.92) and extremely low *p-value* (< 0.0001), indicating a substantial impact on viscosity.
- Release Retarding Polymer (B): Also significant with a significant F-value (16.52) and low *p-value* (0.0048), suggesting a notable effect on viscosity.
- A^2 (Adhesive Polymer Squared): The squared term of Adhesive Polymer is highly significant, emphasizing a non-linear relationship with viscosity.

Marginally significant variable

- AC Interaction: This shows a marginally significant impact on viscosity, with a F-value of 5.59 and a *p-value* of 0.0500.

Not significant variables

- Penetration Enhancer (C): Not significant as the *p-value* is relatively high (0.2243).
- AB Interaction, BC Interaction, B^2, C^2: None of these variables or interactions are significant.

Lack of Fit Analysis:

- The lack of fit is significant (*p-value* of 0.0119), indicating that the model may not fit the data well. It suggests the need for further investigation or refinement of the model. (Table 4)

The above Figure 1 showing on increase the variables (adhesive polymer and release retarding polymer) its shows a significant increase in viscosity

Polynomial equation R1 (Viscosity) = 461.2+ 436.5 A (Adhesive Polymer (Carbopol 940) + 93 B (Release Retarding polymer (HPMC K4M) + 335.9 A^2 (Adhesive Polymer (Carbopol 940).

Overall, the equation suggests that as the values of adhesive polymer (A) and release retarding polymer (B) increase, there is a positive impact on viscosity. The squared term for Adhesive Polymer introduces a nonlinearity, implying that the relationship may exhibit curvature or have a more complex shape. *The in-vitro* drug release fit summary is presented in Table 5.

The linear model appears to be the most promising for explaining *In-vitro* Drug Release, as it is statistically significant, has good adjusted and predicted R^2 values, and is suggested by the lack of fit analysis. The 2FI and quadratic models are not recommended due to their lack of significance and poor predictive performance. The cubic model is statistically significant but may have confounding factors, and further investigation is needed to assess its practical significance. The higher-order models (2FI, quadratic, and cubic) do not significantly improve the model's explanatory power, with the cubic model being complicated by aliasing. Therefore, the linear model is suggested for further consideration in understanding the relationship between the variables and *in-vitro* drug release (Table 5).

Figure 2 shows on increase in the variables (adhesive polymer, release retarding polymer and surfactant) it shows a significant control of drug release.

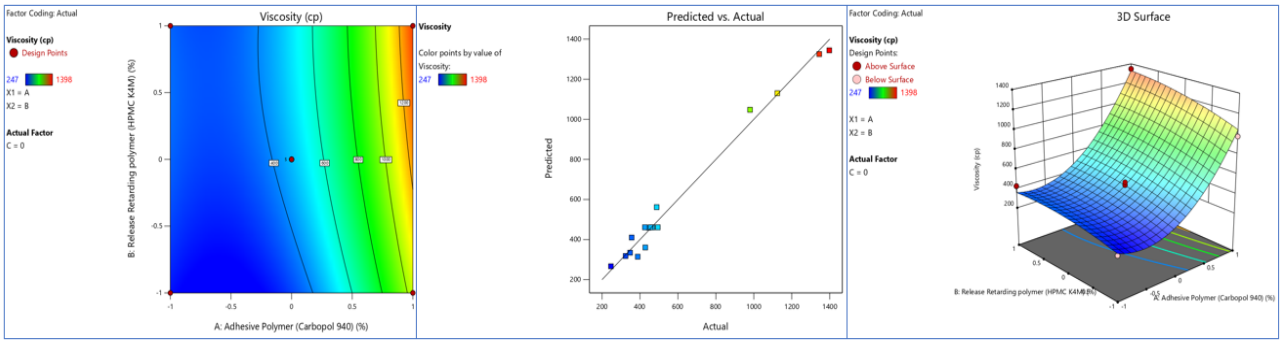

Figure 1: Effect of variable on viscosity showing the contour plots; predicted Vs observed value relation; and 3D surface design.

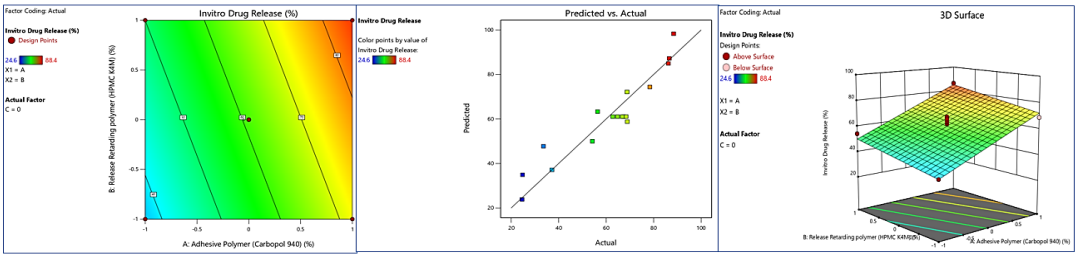

Figure 2: Effect of variable on drug release showing the contour plots; predicted Vs observed value relation; and 3D surface design

Table 5: Response 2: *In-vitro* drug release fit summary

Source	*Sequential p-value*	*Lack of fit p-value*	*Adjusted R²*	*Predicted R²*	
Linear	< 0.0001	0.0085	0.8626	0.7871	Suggested
2FI	0.9075	0.0047	0.8305	0.5422	
Quadratic	0.3683	0.0035	0.8414	-0.0665	
Cubic	0.0035		0.9879		Aliased

Table 6: Fit summary response 3: Skin Retention

Source	*Sequential p-value*	*Lack of Fit p-value*	*Adjusted R²*	*Predicted R²*	
Linear	< 0.0001	0.0085	0.8626	0.7871	Suggested
2fi	0.9075	0.0047	0.8305	0.5422	
Quadratic	0.3683	0.0035	0.8414	-0.0665	
Cubic	0.0035		0.9879		Aliased

The polynomial equation relating the R² value (representing drug release) to two independent variables, adhesive polymer (Carbopol 940) denoted as A, and release retarding polymer (HPMC K4M) denoted as B. The equation is as follows:

Polynomial Equation R2 (Drug release) = 17.4875 + 6.425 Adhesive Polymer (Carbopol 940) A + 19.7375 Release Retarding polymer (HPMC K4M) B

Intercept 17.4875: The intercept or constant term. It represents the expected R² value when both A and B are zero.

Coefficient of A 6.425: The coefficient for the adhesive polymer (Carbopol 940) A. This indicates the impact of A on the R² value. The positive sign suggests that an increase in A is associated with an increase in R².

Coefficient of B 19.7375: The coefficient for the release retarding polymer (HPMC K4M) B. This represents the impact of B on the R² value. The positive sign suggests that an increase in B is associated with an increase in R².

Table 6 shows the fit summary response 3: Skin Retention

Linear

Recommended due to low sequential *p-value*, good adjusted R², and predicted R².

2fi

Not statistically significant, potential lack of fit.

Table 7: Fit statistics response 3: Skin retention

Std. Dev.	*7.55*	*R²*	*0.8883*
Mean	38.92	Adjusted R²	0.8626
C.V. %	19.39	Predicted R²	0.7871
		Adeq Precision	20.3318

Quadratic

Not statistically significant, potential lack of fit, and negative predicted R².

Cubic

Statistically significant, high adjusted R², but marked as "Aliased," indicating a potential issue.

The overall model is statistically significant (*p-value* < 0.0001), indicating that at least one variable has a significant effect on skin retention. (Table 6)

A-Adhesive Polymer (Carbopol 940)

This variable is highly significant (*p-value* < 0.0001), suggesting that the Adhesive Polymer (Carbopol 940) has a substantial effect on skin retention.

B-Release Retarding Polymer (HPMC K4M)

This variable is statistically significant (*p-value* = 0.0316), indicating that the Release Retarding Polymer (HPMC K4M) has a noticeable effect on skin retention, although it is less significant than the Adhesive Polymer.

C-Penetration Enhancer (Surfactant)

This variable is highly significant (*p-value* < 0.0001), suggesting that the Penetration Enhancer (Surfactant) has a substantial effect on skin retention.

The fit statistics collectively suggest that the skin retention model has a good fit, with a strong correlation (high R²) and reasonably good predictive ability. The adjusted R² accounts for the model's complexity, and the adequacy precision indicates a favorable signal-to-noise ratio. However, the variability, as indicated by the coefficient of variation (C.V. %), is moderately high. Overall, the model appears to be effective in explaining and predicting skin retention. (Table 7)

The above Figure 3 showing the skin retention over predicted Vs observed value relation; and 3D surface design

Polynomial equation R3 (Skin retention) = 38.9235 -17.4875A(AdhesivePolymer (Carbopol 940) -6.425 B (Release Retarding polymer (HPMC K4M)-19.7375 C (Surfactant concentration)

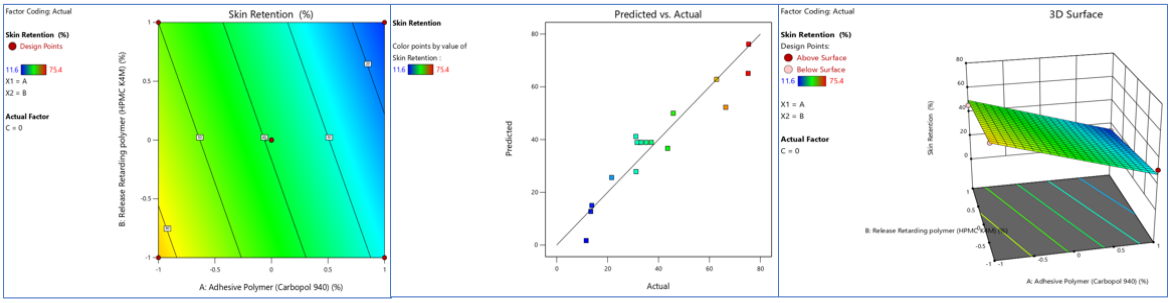

Figure 3: Effect of variable on skin retention showing the contour plots; predicted Vs observed value relation; and 3D surface design

Discussion About the Equation and its Coefficients

Intercept (38.9235)

This is the baseline value of skin retention when all predictors (A, B, and C) are zero.

Coefficient for A (Adhesive Polymer - Carbopol 940)

The coefficient -17.4875 suggests that an increase in the concentration of Adhesive Polymer (Carbopol 940) is associated with a decrease in skin retention.

Coefficient for B (Release Retarding Polymer - HPMC K4M)

The coefficient -6.425 implies that an increase in the concentration of release retarding polymer (HPMC K4M) is associated with a decrease in skin retention.

Coefficient for C (Surfactant concentration)

The coefficient -19.7375 indicates that an increase in Surfactant concentration is associated with a decrease in skin retention.

The negative coefficients for A, B, and C suggest an inverse relationship between each of these factors and skin retention. As the concentration of adhesive polymer, release retarding polymer, or surfactant increases, skin retention is predicted to decrease based on this model.

It's essential to interpret the coefficients in the context of your specific study and data. Additionally, consider the statistical significance of each coefficient and the overall model fit to make robust conclusions about the relationships between the variables and skin retention.

Three well-optimized formulations

Run 4

Factor 1: 1 (Adhesive Polymer)
Factor 2: 0 (Release Retarding polymer)
Factor 3: 1 (Penetration enhancer)

This formulation shows high viscosity (1398 cp), excellent *in-vitro* drug release (88.4%), and relatively low skin retention (11.6%). It could be suitable for scenarios where sustained drug release is desired.

Run 14

Factor 1: 0 (No change in Adhesive Polymer)
Factor 2: -1 (Reduced Release Retarding polymer)
Factor 3: 1 (Penetration enhancer)

This formulation has a moderate viscosity (348 cp), high *in-vitro* drug release (78.4%), and lower skin retention (21.6%). It may be a good balance for achieving sustained release with a moderate skin retention rate.

Run 8

Factor 1: -1 (Reduced Adhesive Polymer)
Factor 2: 0 (No change in Release Retarding polymer)
Factor 3: 1 (Penetration enhancer)

This formulation has a lower viscosity (324 cp), moderate *in-vitro* drug release (56.4%), and higher skin retention (43.6%). It might be suitable for scenarios where sustained release with higher skin retention is desired.

Topical gel composition and its characterization was described in Tables 8 and 9

Table 8: Formulation of topical gel

S. No	*Ingredients*	*F4*	*F8*	*F14*
1	Drug (hexacosane) (%)	1 (100 MG)	1	1
2	Carbopol 940 (%)	1	1.25	1.5
3	Hpmc k4m (%)	1	1.5	2
4	Propylene glycol (%)	20	30	40
5	Glycerin (%)	10	10	10
6	Methylparaben (%)	0.03	0.03	0.03
7	Propylparaben (%)	0.01	0.01	0.01
8	Triethanolamine	Q.S	Q.S	Q.S
9	Purified water	Q.S to 10	Q.S to 10	Q.S TO 10

Table 9: Physiochemical Characterisation of Topical Gel

Formulation code	*Appearance*	*pH*	*Viscosity (cps) spindle – s 64*	*Spreadability (g.cm/sec)*
F4	Yellowish Clear Translucent	6.8	25620	6.5
F8	Yellowish Clear Translucent	6.4	21742	6.8
F14	Yellowish Clear Translucent	6.9	24460	6.5

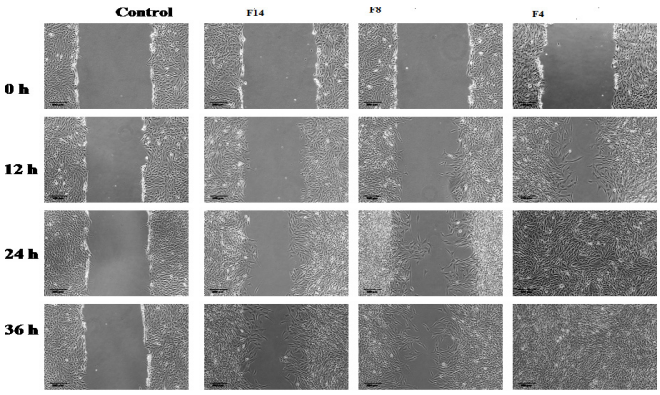

Figure 4: Scratch wound healing assay –cell images

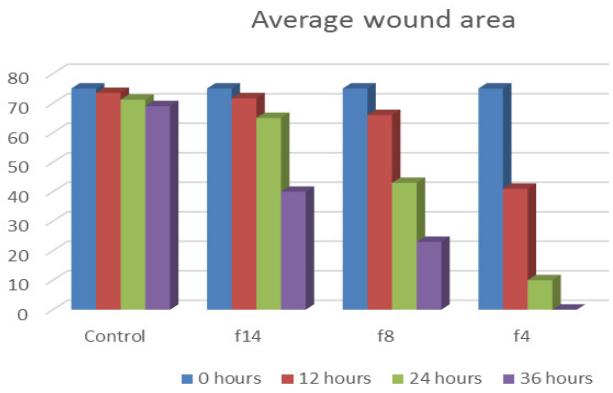

Figure 5: Determination of average wound area

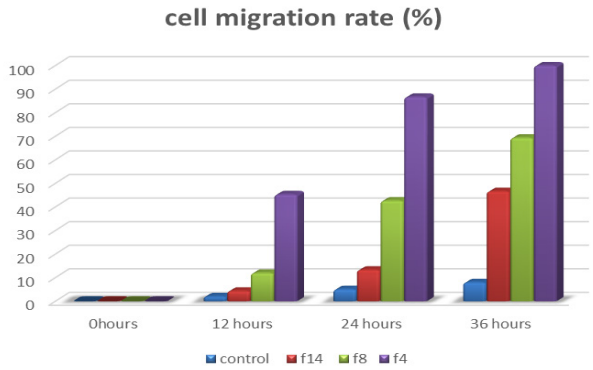

Figure 6: Determination of cell migration rate

Scratch Wound Healing Assay

Figure 4, 5, 6 shows the details of scratch wound healing assay

All samples were found to elicit significant wound healing efficacy as evidenced from the representative photomicrographs. The maximum efficacy was elicited by formulation (F4) at the time duration of 36 hours. This is an indication that the sample can be effective In addition to its therapeutic applications, it is also capable of healing scratches and wounds on human cells. There was a concentration-dependent decrease in wound area over time. F4 of the sample displayed the maximum efficacy. Enhanced cell migration rate, the indicator of enhanced wound healing, was observed in a concentration and time-dependent manner. The maximum efficacy was displayed by formulation 4(F4).

CONCLUSION

In this study, 3 optimized carbopol940/HPMC gels were designed and formulated using response surface methodology by Box-Behnken design. Each formulation is characterized by specific attributes such as viscosity, *in-vitro* drug release, and skin retention. The physicochemical properties of all three formulations were comparable with gel properties.

The scratch wound healing assay of the three optimized formulations was promising. The maximum efficacy was elicited by formulation (F4) at the time duration of 36 hours. This is an indication that the sample can be effectively Wounds caused by scratching or wounding can also be treated with it.

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