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Original Article

Quality by Design Driven Development of Topical Gel Encompassing Papain and Bromelain to Elicit Wound Healing

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ABSTRACT

Background: Topical gels are an efficient and targeted therapy for local dermatological problems because they distribute medications effectively and are easy to wipe off the skin. The presented study was aimed to use Quality by Design (QbD) features to develop, optimise, and evaluate a topical gel containing certain plant enzymes for wound healing activity. **Objectives:** The objective of this study was to use contour plots and multiple linear regression analysis to determine the relative relevance of selected independent variables on dependent variables, as well as to identify the desired zone where the product with required qualities produced. **Materials and Methods:** Quality Attributes (CQAs) were assigned to the patient-centric Quality Target Product Profile (QTPP). The quantities of chitosan and sodium carboxymethyl cellulose have been selected as Critical Material Attributes (CMA) based on early observations and were employing a 3^2-full factorial design. CQAS were chosen as viscosity, spreadability, extrudability, and bioadhesive strength, and using multiple linear regression analysis (MLRA) a quantitative association among CQAs and CMAs was observed. **Results:** The full factorial design aids in predicting the optimal chitosan and sodium carboxy methyl cellulose combination. The numerical response analysis aids in comprehending the impact of various factors on various replies. TGF-O showed higher wound healing activity than control and standard formulations in an excision and incision wound healing model. **Conclusion:** The current work successfully underlines the possibility of using a quality by design approach to synthesise and create topical gel formulations for plant enzymes in a simple and cost-effective manner.

Keywords: Quality by design, Plant enzyme, Factorial design, Wound, Topical gel, Spreadability.

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INTRODUCTION

Topical gels are an efficient and targeted therapy for local dermatological problems because they distribute medications effectively and are easy to wipe off the skin. Chitosan (CS) is a polysaccharide derived from the shells of crustaceans like crabs and shrimp. CS is the only biopolymer with cationic properties, making it stand out due its topical use.[1-4] Papain is a proteolytic enzyme derived from papaya plant latex released by specific lactifier cells. It has anti-inflammatory, antibacterial, antioxidant properties and wound-healing synergy with chitosan.[5] Bromelain is a proteinase complex derived from the pineapple plant. It has anti-inflammatory, antioxidant and other therapeutic actions. It's been employed in single-enzyme preparations as well as multi-enzyme preparations.[6,7]

A variety of product and process variables are involved in the development of topical gels. For designing and producing formulations, a Quality by Design (QbD) technique is employed, in which production processes ensure that predefined product specifications are met.[8]

QbD a method for discovering all potential components affecting a product's safety, efficacy, and quality that is systematic, scientific, risk-based, and holistic.[9] The main purpose of QbD is to find out how process and formulation variables influence quality of the product and then use statistical tools to optimise parameters in relation to final specifications.[10,11]

The goal of this study was to employ contour plots and Multiple Linear Regression Analysis (MLRA) to examine the relative importance of selected independent variables on the dependent variable, as well as to identify the desired zone where the product with required qualities produced. To optimize the formulation,

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Table 1: QTPP for topical gel.

Attributes	QTPP	Justification
Drug Delivery type	Polymeric gel system	Improvement in absorption.
Dosage form type	Gel	Improvement in bioavailability.
Route of Administration	Topical	Desired route for skin problem.
Packaging	Collapsible tube	Patient compliance is improved
Impurities	Below safety threshold	There are no contaminants in the formulation for patient safety.
Stability	At room temperature for at least 24 months	To keep the formulation's therapeutic value throughout storage.

a two-factor and three-level (3^2) factorial design was used. The quantities of CS and Sodium Carboxy Methyl Cellulose (NaCMC) were taken into account. The chosen QbD technique facilitates the identification of the best formulation composition and experimental procedures.

MATERIALS AND METHODS

Materials

Sigma Aldrich, Mumbai, India, provided CS (85-95 percent deacetylated) and NaCMC. Advanced Enzymes Technology Ltd., Thane (W), India graciously gave papain (EC 3.4.22.2) as a gift sample, while bromelain (EC 3.4.22.32) were bought from Indore based company Prisha herbals. All of the other chemicals used in this study have been of high analytical quality.

Preparation of Gel

Topical gels were made by slowly stirring CS and NaCMC into half of water containing 1 percent v/v glacial acetic acid. After the swelling had subsided, the remaining water was added and thoroughly blended. By adding triethanolamine and stirring continuously until a homogenous gel was produced, the pH was raised to 6.8-7. By adding distilled water, the final capacity was increased to 100 mL. All of the formulations were allowed to equilibrate at room temperature for 24 hr before being sonicated to remove any air bubbles. The gel containing plant enzymes was prepared using the same procedure.[12,13] The produced gels were kept in cold until they were studied further.

Defining QTPP and CQAs

The initial stage in QbD-based topical gel product development is to define QTPP, which specifies the summary of therapeutic product's quality attributes in order to make a better drug delivery system for topical delivery. Table 1 summarises the various components of QTPP for topical gel formulation, whereas Table 2 provides specific explanations for each CQA.

A preliminary risk evaluation and screening of factors

The risk evaluations were completed to determine the Critical Material Attributes (CMA) and/or Critical Process Parameters (CPPs) for the formulated topical gel that affect the CQAs of the pharmaceutical drug delivery. To generate a preliminary list of high-risk characteristics, a fishbone diagram was created that had an impact on the quality of the topical gel that had been prepared shown in Figure 1. A preliminary study was designed to discover CMAs/CPPs with a large risk evaluation matrix (REM) for qualitative risk evaluation by allocating low, medium, and high risk levels to every material characteristic and/or process variables explained in Table 3.

A factor screening research was used to assess the impact of every material characteristic and process variable on topical gel CQAs. Preliminary screening batches were made with varying percentages of CS and NaCMC and examined for viscosity, spreadability, extrudability, and bio-adhesive strength as shown in Table 4.

Design of Experiment (DoE)

The effect of selected independent factors on responses was evaluated using a 3^2 complete factorial design in this study. The design was based on preliminary observations, which were carried out to guarantee that the requirements for the creation of topical gel were met. The concentrations of CS (X_1) and NaCMC were chosen as independent variables (factors) that changed at three levels (low, middle and high). Table 2 shows the dependent variables (responses) that were chosen: viscosity (Y_1), spreadability (Y_2), Extrudability (Y_3) and bioadhesive strength (Y_4). Table 3 shows the experiment matrix, as well as the causes and responses that were evaluated.

Design-Expert software version 8.0.0.1 was used to create the quadratic response surface and mathematical model (Stat-Ease Inc, USA). MLRA including independent variables and their interrelation with considered response was performed using the model equation established by the 3^2 factorial designs. The quadratic model equation of the MLRA is as follows:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2+\beta_6X_1X_2^2+\beta_7X_1^2X_2+\beta_8X_1^2X_2^2 \ldots\ldots\ldots(1)$$

Where Y is the dependent variable, 0 is the intercept, 1 to 8 are the regression coefficients, X_1X_2 and Xi^2 (i=1,2,...) are the interaction and polynomial terms, respectively; X_1X_2 and Xi^2 (i=1,2,...) are the interaction and polynomial terms, respectively. ANOVA was used in the software to do statistical validation of polynomials.

Table 2: CQAs for topical gel and their justification.

Quality attributes of topical gel	Target	Is this a CQA?	Justification
Physical attributes		No	Color, odour, texture, and pH were not deemed as important because they are unrelated to patient safety.
Color	Acceptable to patient		
Odor	No unpleasant odor		
Appearance	Acceptable to patient		
pH	Same as skin pH, No skin irritation		
Viscosity	Optimum	Yes	All are critical attributes as they directly affect the therapeutic activity of prepared topical gel.
Spreadability	Optimum	Yes	
Extrudability	Optimum	Yes	
Bioadhesive strength	Optimum	Yes	

Table 3: REM for initial risk estimation of topical gel.

	Risk Estimation Matrix								
Drug product CQAs	Type of polymers	Polymer: viscosity modifier ratio	Amount of chitosan	Amount of Sodium CMC	Amount of enzymes	Solvent ratio	Stirring speed	Temperature	Sonication
Viscosity	High	High	High	High	Med	Med	Med	Med	Low
Spreadability	High	High	High	High	Low	Low	Low	Low	Low
Extrudability	High	High	High	High	Low	Low	Low	Low	Low
Bioadhesive strength	High	High	High	High	Low	Low	Low	Low	Low

Evaluation of the gel

Physical characteristics

The appearance, clarity and pH of all of the produced gels were checked.

Viscosity

Using a Brookfield viscometer RVDV-II+Pro and a spindle T bar (S96), The viscosity of the gel that was developed was calculated. The viscosity measurements were recorded after 10 mL of gels were rotated at 100 rpm.[14]

Spreadability

It denotes the size of a region across which the gel glides easily when applied to the skin or damage part of skin. A formulation's medicinal impact is also influenced by its spreading value. It is measured in seconds and expressed as the time it takes for two slides to fall off the gel. About 1 g of gel has been sandwiched among two slides, and the time it took to separate the slides was recorded. About 1 g gel is applied among the two slides towards the direction of a specified weight, which minimises the time it takes to separate the two slides and promotes spreadability.[15]

It was calculated using the formula below:

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

Where S – Spreadability, L – Length travelled by the glass slide, M – Weight, T – Time (in seconds) it took to separate the slides completely.

Extrudability

It was utilised to determine the gel's ability to be extruded from a collapsible tube. The gel-filled collapsible tube's crimp end was squeezed in. The gel eject out till the force was released when the cap has been separated. In 10 s, the weight in grams necessary for a 0.5 cm gel ribbon was measured. The average extrusion pressure is measured in g and recorded.

Bioadhesive strength

This relates to how well the gel adheres to the skin. To assess bioadhesive strength, using double-sided adhesive tape, different specimen of topical gel have been adhered to the bottom of a glass vial that had been inverted. The distance between these two vials

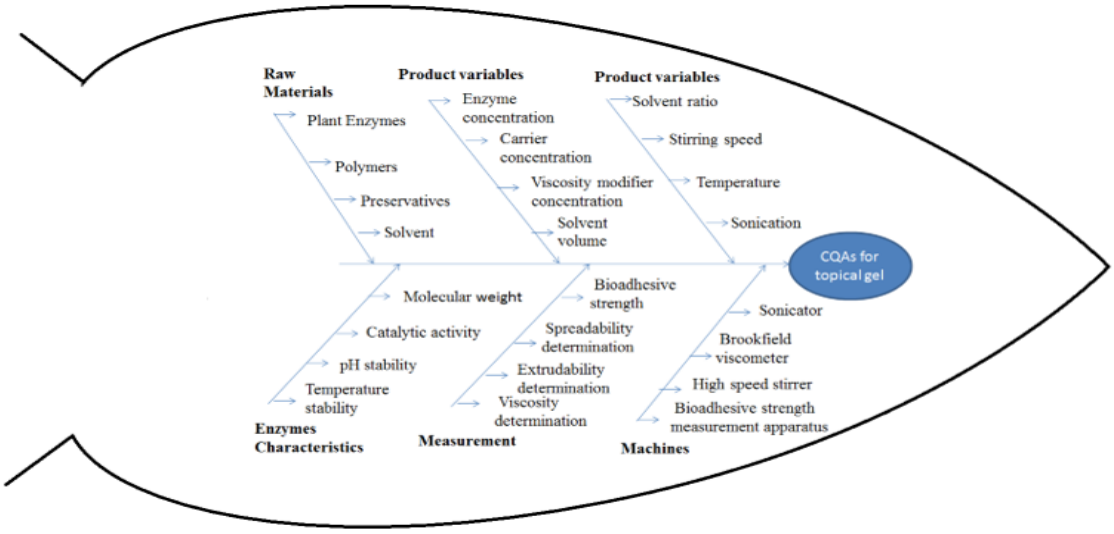

Figure 1: Modified Ishikawa diagram.

Table 4: The factors and responses.

Independent variables (factors)	Dependent variables (response)
X_1= CS (1, 2, 3 g)	Y_1= Viscosity (cp)
X_2= NaCMC (0.5, 1, 1.5 g)	Y_2 = Spreadability (g.cm/sec)
	Y_3 = Extrudability (g /cm^2)
	Y_4 = Bioadhesive strength (g /cm^2)

was selected so that the gel sample stayed attached to the goat skin's mucosal barrier.

To facilitate effective attachment of the gel to the mucosa, for 10sec, adequate pressure was applied to both sides of the vial. A continuous weight is attached to the other arm of the new balance in the pan, pushing the vial away from the other vial. It was noted how much force was necessary to separate the two vials. The mucoadhesive force, which was reported as the detachment stress in dynes/cm^2, was computed using the lowest weight which detaches the mucous membrane tissue from the surface of every formulation.

It was calculated using the following formula:

$$Mg / A = \text{Bioadhesive strength (dynes/cm}^2\text{)}$$

Where M-Weight necessary to separate in grams, g-Gravitational acceleration (980 cm/sec^2), A – Area of exposed mucosa.

Differential Scanning Calorimetry (DSC)

Using a DSC, the physical properties of plant enzymes in topical gel were studied. Thermograms for pure enzymes and a physical mixture of polymer and enzymes have been taken in a nitrogen environment at a heating rate of 10°C/min with a heat flow from 0 to 300°C.

Evaluation of wound healing activity of optimized formulation

Animal experiment design

After getting approval from the Institutional Animal Ethical Committee proposal no. KNCOP/R&D/AN-PROT/14-15/05, all animal research was carried out in accordance with CPCSEA principles (Committee for the Purpose of Control and Supervision of Experiments on Animal). In this study, albino wistar rats weighing 150-200 g were used. The rats were kept in separate cages made of hygienic plastic with unrestricted access to food and fresh water the cages were housed in an animal house with ample ventilation. The animals were sorted in the manner shown in Table 5.

Excision wound formation

Rats were anaesthetized locally with diethyl-ether and depilated using a sterile blade to remove hairs from the dorsal upper part of the body prior to excision. Under aseptic settings, spherical wounds with a diameter of around 2.5 cm were produced and monitored throughout the experiment. The wound area (sq. mm.) was calculated by marking the wound area on a transparent polyethene graph paper placed over the wound. It was assumed that this was the location of the first wound. All topical gels were applied on a daily basis beginning on the first day of the injury. The wound regions were measured three days apart. Using the reported wound area, the wound contraction % was calculated. The amount of days it took for the scar to fade and no raw wound to remain was used to calculate the epithelization period.[16,17]

Incision wound formation

The animals within every group being anaesthetized with diethyl-ether and depilated by eliminating hairs with a sterilized blade prior to the incision. A 6 cm long paravertebral incision was made on the backs of the rats. With non-absorbable suture

materials (monofilament polyamide black USP) and a 26 mm 3/8 circle reversed piercing needle, the divided skin was held together again and sewn uniformly and tightly at around 0.5 cm intervals following the incision. All of the groups were treated as if they were suffering from an excision wound. After the wounds had entirely healed, the sutures was extracted on day 9 and the repaired wounds' tensile strengths were determined on day 10.

Statistical analysis

The arithmetic mean and standard deviation were used to express all experimental data. The data was statistically analysed using the Instate Graph Pad Prism software. The significant differences among several preparations were compared using one way analysis of variance– ANOVA with post hoc test. Significant was defined as a probability (p) value of less than 0.05.

Stability studies

Three months of stability testing at a temperature of 20-40°C were performed on all of the selected formulations, as per International Council for Harmonization (ICH) recommendations, and were evaluated for changes in appearance, odour and pH.[18]

RESULTS AND DISCUSSION

Studies on risk evaluation and screening of factors

The resulting relationship between the elements affecting CQAs of the therapeutic product is depicted by the Ishikawa fish bone diagram in Figure 1 for manufactured topical gel. The REM for qualitative risks associated with each material characteristic and process parameter is shown in Table 1. According to REM, high risk parameters included polymer type, polymer: viscosity modifier ratio, amount of CS and NaCMC, whereas low and medium risk factors were enzyme amount, solvent ratio, stirring speed, temperature, and sonication. DoE only optimised high-risk factors because they were crucial to the responses.

Table 5: Animal groups for experimental design.

Group	Treatment
Group - I	Negative control received no treatment
Group - II	Test group received TGF-O
Group - III	Positive control received standard drug (Framycetin Sulphate IP)

Optimization of formulation variables

Pharmaceutical formulations are created by modifying one parameter at a time, with little regard for the overall effect of factors and responses. In order to get at the best solution for the formulation, DoE is important for understanding the complexities of the relationships between inputs and responses. The current study employs a simple design that includes two variable studies with three degrees of testing. As a result, for the formulation of the needed topical gel, a 3^2 complete factorial design was adopted. Viscosity, Spreadability, Extrudability and Bioadhesive Strength were tested in a total of nine formulation batches.

Effect of factor on viscosity

The viscosity of a topical gel composition can be used to estimate its potential effect. As it is directly related to the other evaluation parameters, viscosity plays a vital function. The right viscosity is crucial; a formulation with too much or too little viscosity will not produce good results. Table 6 shows the viscosity data for the developed formulation.

The following is the viscosity model equation

$$\text{Viscosity (cp)} = 82.34 + 0.84\ X_1 + 2.21X_2$$

$$[R^2 = 0.9939,\ F \text{ value} = 492.32,\ P < 0.0001]$$

Table 6: Full Factorial Design using Observed Response values.

Batch Code	Levels of independent variables (factors) employed		Responses			
	CS (%) X_1	NaCMC (%)X_2	Viscosity	Spreadability	Extrudability	Bioadhesive strength
TGF1	1(-1)	0.5(-1)	79.12	15.65	17.00	1.10
TGF2	2(0)	0.5(-1)	80.25	15.10	16.65	1.13
TGF3	3(1)	0.5(-1)	80.95	14.90	16.35	1.18
TGF4	1(-1)	1.0(0)	81.35	14.30	15.90	1.21
TGF5	2(0)	1.0(0)	82.65	13.45	15.50	1.25
TGF6	3(1)	1.0(0)	83.15	13.10	15.25	1.27
TGF7	1(-1)	1.5(1)	83.85	12.90	15.00	1.28
TGF8	2(0)	1.5(1)	84.50	12.55	14.75	1.30
TGF9	3(1)	1.5(1)	85.25	12.36	14.53	1.32

When the concentration of X_1 and X_2 was increased at different levels (-1, 0, 1), the viscosity of the topical gel formulation was observed to increase at each level. Topical Gel Formulation (TGF1) with a low concentration has a low viscosity (79.12 cps), while TGF9 with a high concentration has a high viscosity (85.25 cps). This could be due to the fact that polymer concentrations have a direct impact on viscosity.

In polynomial equations, the positive coefficient X_2 was bigger than X_1, implying that the concentration of X_2 has a more direct effect on viscosity than the concentration of X_1, which has less effect on the response.

Effect of factor on Spreadability

Spreadability refers to the amount of gel that spreads easily when applied to the skin or affected area. A formulation's medicinal impact is also influenced by its spreading value. The spreadability model equation is shown below.

$$\text{Spreadability (g.cm/sec)} = 13.81\text{-}0.42\ X_1\text{-}1.31X_2$$

$$[R^2 = 0.9697,\ F = 96.08,\ P< 0.0001]$$

When the concentration of X_1 and X_2 was increased at different levels (-1, 0, 1) the spreadability of topical gel formulation was shown to be reduced at each level. The low-concentration TGF1 formulation has a high spreadability (15.65), while the high-concentration TGF9 formulation has a low spreadability (12.36). This could be due to the fact that polymer concentrations have an indirect effect on spreadability.

In polynomial equations, the value of negative coefficientX_2 was bigger than X_1, implying that the concentration of X_2 has a greater impact on the spreadability than the concentration of X_1, which has a minor impact on the response.

Effect of factor on extrudability of gel

It was used to test the topical gel formulation's extrudability from the collapsible tube. The following is the extrudability model equation:

$$\text{extrudability (g/cm}^2) = +15.52\text{-}0.29\ X_1 - 0.95\ X_2 + 0.045\ X_1X_2 + 0.38\ X_{12} + 0.16\ X_{22}$$

$$[R^2 = 0.9993,\ F \text{ value} = 888.63,\ P<0.0001]$$

The negative values of X_1 and X_2 indicate that as their concentrations were increased, the extrudability of topical gel formulation was observed to diminish at each level. TGF1 formulations with low concentrations have a high extrudability (17.00) while TGF9 formulations with high concentrations have a low extrudability (14.53).

Effect of factor on bioadhesive strength

The capacity of the gel to adhere to the skin layer is determined by its bioadhesive strength. It's also vital for the topical gel formulation's medicinal impact. The following is the model equation for bioadhesive strength:

$$\text{Bioadhesive strength} = +1.24+0.030\ X_1 + 0.082\ X_2 - 1.000E-002\ X_1X_2 + 0.000\ X_1^2 - 0.025\ X_2^2$$

$$[R^2 = 0.9972,\ F \text{ value} = 211.80,\ P = 0.0005]$$

The positive values of X_1 and X_2 indicate that increasing their concentration increased the bioadhesive strength of topical gel formulation at each level, correspondingly. The low-concentration formulation TGF1 has a high bioadhesive strength (1.10), while the high-concentration formulation TGF9 has a low bioadhesive strength (1.32).

According to the ANOVA results, all models were significant (p 0.001) for all response parameters examined shown in Table 7. The Design Expert 8.0.0.1 software also produces counter and 3D response surface plots for viscosity, spreadability, extrudability and bioadhesive strength shown in Figure 2.

Validation of optimization model

To create a new optimised formulation with optimal response, a desirability-based numerical optimization technique was utilised to optimise all of the replies with varied objectives. Using the Design expert software, based on the acceptability criterion, numerical analysis was used to determine the desired values of responses. The Overlay plot and counter plot shown in Figure 3 depict the desirability zone. By making a topical gel formulation with the required mix of the components proposed by the model, the accuracy of the optimised model was tested. The viscosity, spreadability, extrudability, and bioadhesive strength of the Optimized topical gel formulation (TGF-O) were all tested.

Table 8 shows the results of tests with expected and observed reactions using a mathematical model. With modest error values, the optimised topical gel formulation (TGF-O) demonstrated viscosity (84.24±0.395), spreadability (13.75±0.360), extrudability (15.78±0.175), and bioadhesive strength (1.50±0.260). The resulting optimised model for 3^2 factorial designs was well suited, according to the results.

Differential Scanning Calorimetry (DSC)

To assess the physical state of the medication in the formulation, DSC experiments were carried out. DSC thermograms of pure enzymes (Papain and Bromelain) revealed an extremely sharp endothermic peak at (216.38°C and 154.96°C), respectively, with peak onset at (139.54°C and 149.87°C), which corresponds to their melting point. Both enzymes have a strong endothermic peak, indicating that they are crystalline in form. The DSC

Table 7: ANOVA summary for the response parameters.

Sources	Sum of Squares	D.F.	Mean Square	*F*- Value	*p*-value prob>*F*
a) For Viscosity (cps)					
Model	33.61	2	16.80	492.32	0.0001(S)
X_1	4.22	1	4.22	123.54	0.0001(S)
X_2	29.39	1	29.39	861.10	0.0001(S)
b) For Spreadability (g.cm/sec)					
Model	11.28	2	5.64	96.08	0.0001(S)
X_1	1.03	1	1.03	17.61	0.0057(S)
X_2	10.24	1	10.24	174.55	0.0001(S)
c) For Extrudability (g /cm^2)					
Model	6.04	5	1.21	888.66	0.0001(S)
X_1	0.52	1	0.52	384.14	0.0003(S)
X_2	5.45	1	5.45	4011.79	0.0001(S)
$X_1 . X_2$	8.100E-003	1	8.100E-003	5.96	0.0924(NS)
X_1^2	2.939E-003	1	2.939E-003	2.16	0.2378 (NS)
X_2^2	0.053	1	0.053	39.25	0.0082(S)
d) For Bioadhesive strength (g /cm^2)					
Model	0.047	5	9.413	211.80	0.0005(S)
X_1	5.400E-003	1	5.400E-003	121.50	0.0016(S)
X_2	0.040	1	0.040	900.37	0.0001(S)
$X_1 . X_2$	4.000E-004	1	4.000E-004	9.00	0.0577(NS)
X_1^2	0.000	1	0.000	0.000	1.0000(NS)
X_2^2	1.250E-003	1	1.250E-003	28.12	0.0131(S)

Thermogram of the formulation, which shows that CS has a broad endothermic peak (89.79°C) spanning the full scanning range of 0°C to 300°C, indicated that the physical state has changed into an amorphous state. However, no identifiable peak of enzymes in formulation was detected at the tested temperature, implying that after manufacturing and interacting with the polymer, the drug was in an amorphous or high-energy form, meaning that it was in an amorphous or high-energy state. Because the amorphous state is regarded as a condition of severe disorder, the solid medicines in the formulation remain widely distributed inside the polymer matrix shown in Figure 4.

Evaluation of wound healing activity of optimized formulation

Rate of wound contraction

It is a crucial metric for assessing wound healing activity, and it's quantified using the excision wound model. In comparison to the untreated group and the group treated with conventional medication, the group treated with TGF-O showed considerable wound contraction explained in Table 9 and shown in Figure 5.

Epithelization time

It indicates how long it will take to completely remove the scar from the injured tissue. Wound healing is improved when the epithelization time is reduced, and vice versa. In this study, the time required to completely remove a scar was shown to be shorter in the group treated with TGF-O than in the other groups, indicating that the group treated with an optimised topical gel formulation has better wound healing activity than the other groups shown in Table 9.

Wound breaking strength

It is a crucial metric that measures the tensile strength of a fully healed wound and is assessed using the wound incision model.

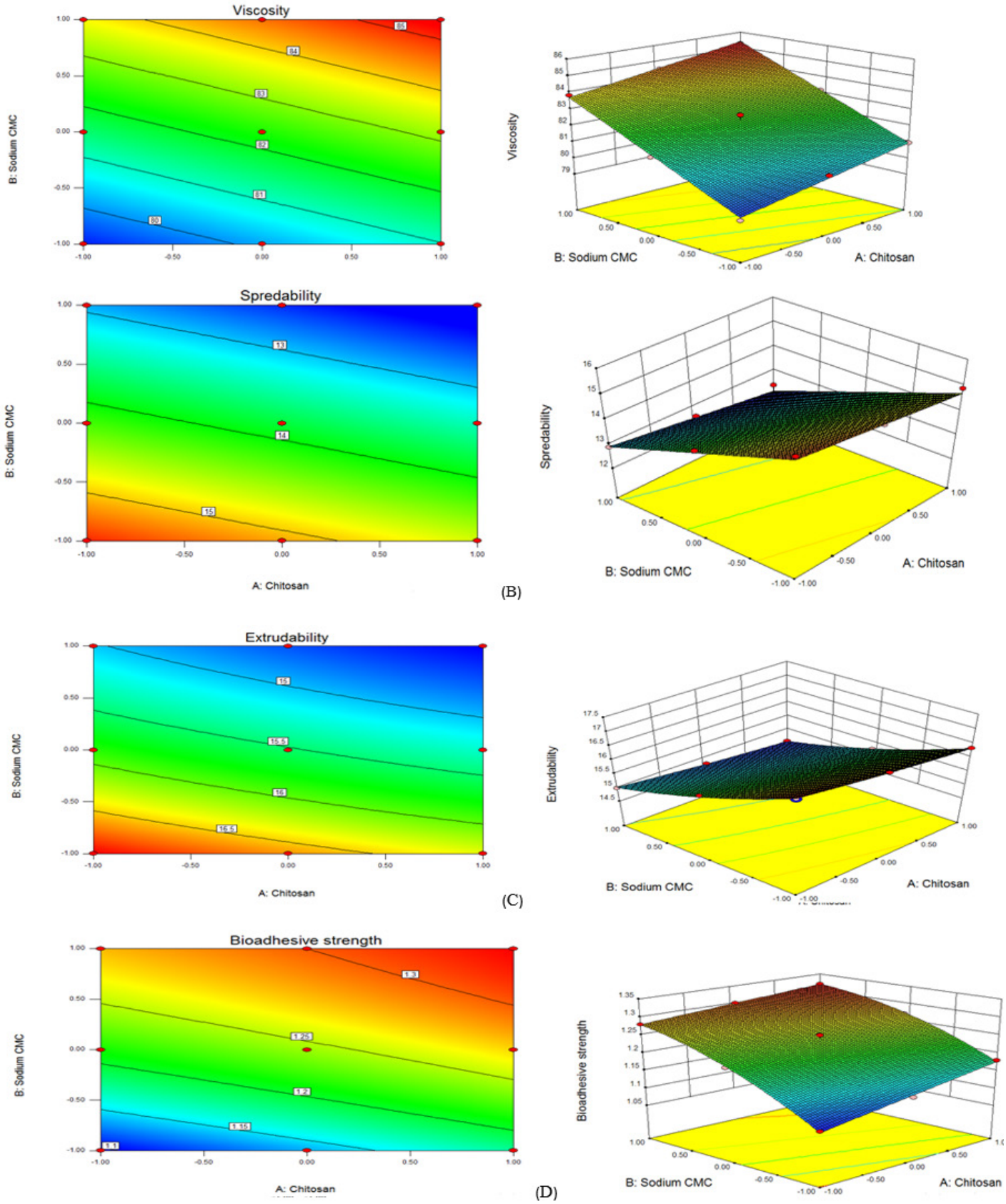

Figure 2: The combined effect of CS and NaCMC concentrations on A) Viscosity, B) Spreadability, C) Extrudability, and D) Bioadhesive strength is depicted in a response surface plot and a counter plot.

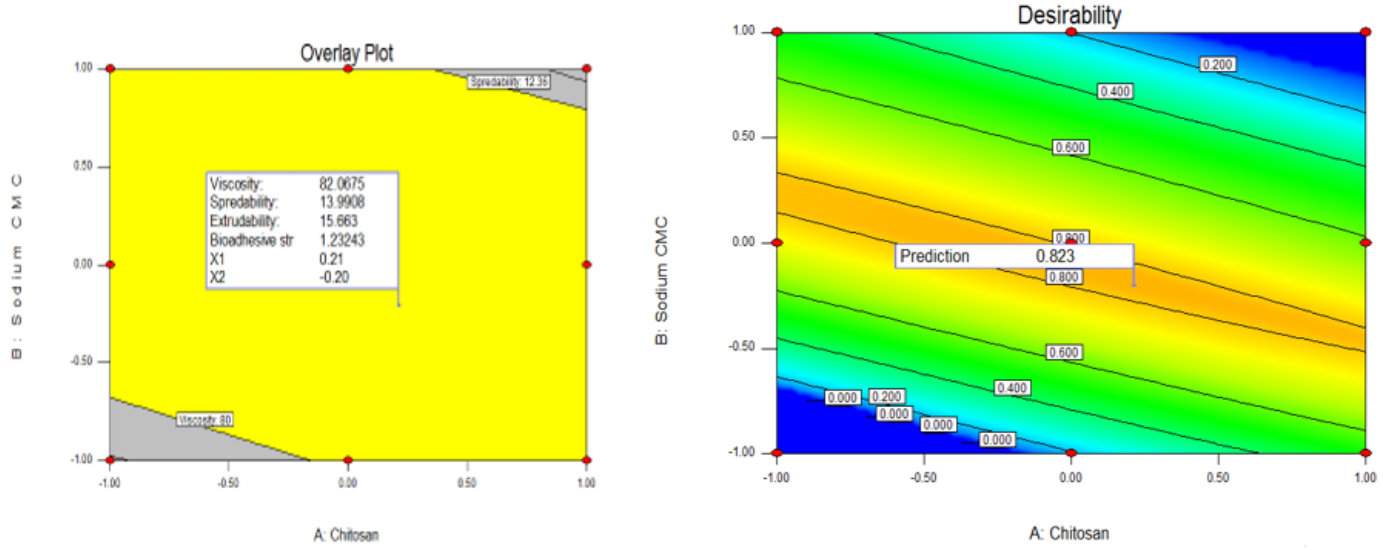

Figure 3: The desirability region is shown by an overlay plot and a counter plot.

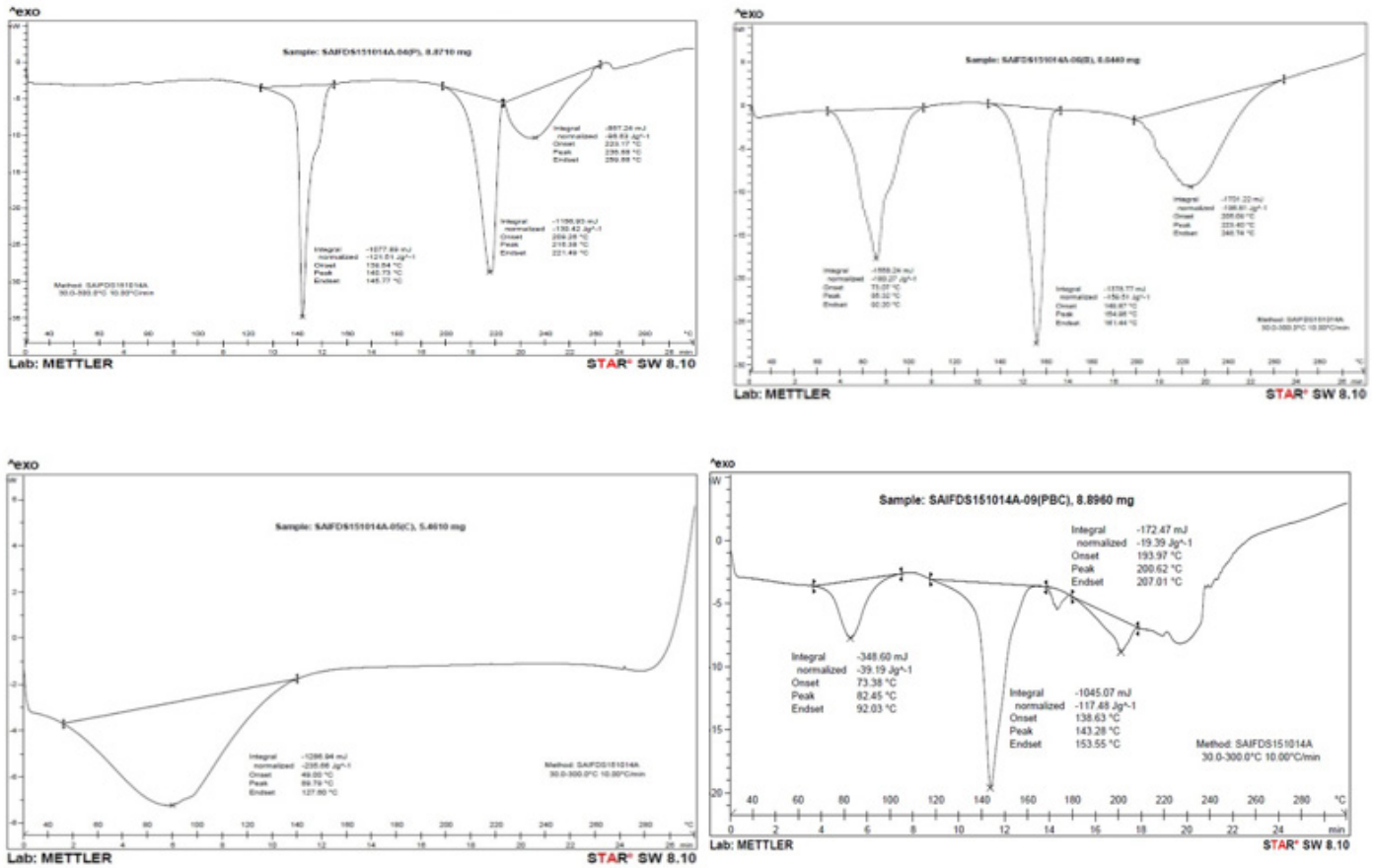

Figure 4: DSC thermogram for a) papain b) Bromelain c) CS d) formulation.

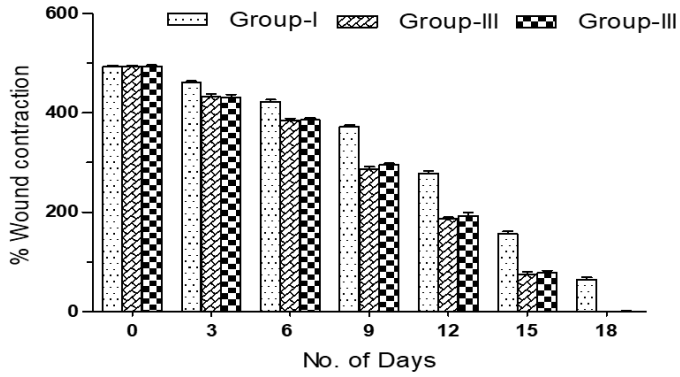

Figure 5: The area of wound contraction as a result of topical administration of prepared gels to an excision wound. The values are in mean ± SEM, with a significance level of $p<0.01$ when compared to the control.

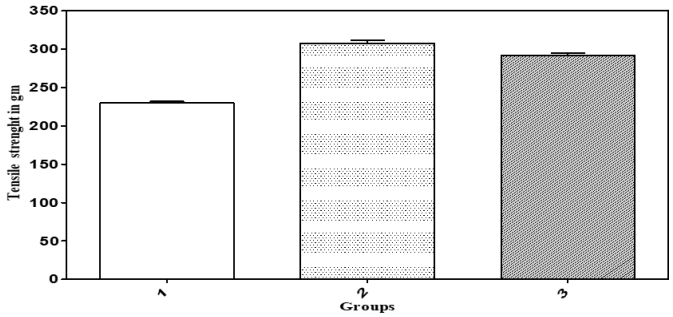

Figure 6: On the 10th post-wounding day the effect of topical administration of prepared gels on the skin's tensile strength in an incision wound.

Table 8: Experiment results for proving optimization capabilities.

Batch	CS (X_1) coded	NaCMC (X_2) coded	Response	Predicted values	Actual values	Error² (%)
TGF-O	0.21	-0.20	Viscosity	82.0673	84.24±0.395	2.64
			Spreadability	13.991	13.75±0.360	-1.72
			Extrudability	15.663	15.78±0.175	0.74
			Bioadhesive strength	1.232	1.50±0.260	-1.78

Table 9: Effect of optimized gels on wound contraction and epithelization time.

Days Groups	0 day	3rd day	6th day	9th day	12th day	15th day	18th day	Epithelization time
Group-I	493.41±2.31	461.75±2.37	422.58±4.12	371.58±4.23	278.08±4.76	157.41±4.41	64.91±4.23	24.83±0.81
Group-II	493.70±2.08	432.63±5.51	384.58±4.30	286.66±5.26	186.91±2.92	74.83±5.31	0.00	16.91±0.66
Group-III	494.03±2.11	431.88±5.18	386.16±3.23	295.08±3.64	193.08±5.92	78.08±4.77	0.50±0.83	17.83±0.75

The tensile strength of totally healed wounds treated with TGF-O was shown in Figure 6 is higher than in the other groups.

CONCLUSION

The current work successfully underlines the possibility of using a quality by design strategy to synthesise and create topical gel formulations for plant enzymes in a simple and cost-effective manner. The full factorial design aids in predicting the optimal CS and NaCMC combination. The numerical response analysis helps to understand the impact of various factors on different responses. The viscosity, spreadability, extrudability, and bioadhesive strength of the improved topical gel formulation (TGF-O) were tested. TGF-O showed higher wound healing activity than control and standard formulations in an excision and incision wound healing model. The encouraging results of this study can also be extended for successfully enhancing the usage of plant enzymes in topical gel formulation for wound healing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

QbD: Quality by Design; **CS:** Chitosan; **NaCMC:** Sodium Carboxy Methyl Cellulose; **MLRA:** Multiple Linear Regression Analysis; **QTPP:** Quality Target Product Profile; **CQA:** Critical Quality Attributes; **CPP:** Critical Process Parameters; **TGF-O:** Optimized Topical Gel Formulation; **DoE:** Design of Experiment; **REM:** Risk Estimation Matrix; **CMA:** Critical Material Attributes.

SUMMARY

The main goal of this study is to develop a topical gel of papain and bromelaine by using contour plots and multiple linear regression analysis to determine the relative importance of selected independent variables on dependent variables and to pinpoint the desired zone where the product with the desired qualities can be produced. Quality Attributes (CQAs) were assigned to the patient-centric Quality Target Product Profile (QTPP). Based on early findings, the quantities of chitosan and sodium carboxymethyl cellulose were chosen as critical material attributes (CMA) in a 32-factorial design. Viscosity, spreadability, extrudability, and bioadhesive strength were chosen as CQAS, and a quantifiable relationship between CQAs and CMAs was discovered using Multiple Linear Regression Analysis (MLRA). TGF-O showed higher wound healing activity than control and standard formulations in an excision and incision wound healing model.

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