RESEARCH ARTICLE

Formulation and Development of β-glucan Hydrogel using Design of Experiments (DOE)

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ABSTRACT

β-Glucan, an endogenous carbohydrate, is a key functional ingredient found in barley and oats and a major component of microbiological and plant cell walls. With no severe adverse effects, it has anti-tumor, diabetic lowering, wound healing, anti-aging and anticholesteremic effects. Twenty formulations were prepared using different polymers like carbapol 934, hydroxypropyl methylcellulose (HPMC) K100M, HPMC K15M, guar gum and xantham gum. F1 formulation exhibited satisfactory results with respect to *in-vitro* drug release, spreadability, extrudability viscosity and drug content. In order to optimize the concentration of polymers used in F1 formulation, design 13 was opted. All the above 8 runs were subjected to evaluation tests, out of which F28 exhibited maximum drug release with optimum viscosity, spreadability and extrudability. The results correlated with the design with less percentage relative error. F28 formulation was observed to have positive correlation for *ex-vivo* drug release. Comparable wound healing activity was observed when performed on HaCaT cell lines.

Keywords: β-Glucan, Extrudability, HaCaT cell lines, Spreadability, Wound healing.

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INTRODUCTION

The name "hydrogel" refers to a three-dimensional cross-linked polymeric network made of synthetic or native polymers that can hold water in its porous structure and do not solubilise in water at physiological temperature.[1,2] β-Glucan, an endogenous carbohydrate, is a key functional ingredient found in barley and oats, as well as a major component of microbiological and plant cell walls. With no severe adverse effects, it has anti-tumor, diabetic lowering, wound healing, anti-aging and anticholesteremic effects.[1] Having a plethora of therapeutic properties, it can be used as an active pharmaceutical agent for formulating topical preparations like gels, films and patches. With the suitability characteristics of β-glucan in formulating a hydrogel and as scanty studies are available using the preparation, it is an attempt to formulate β-glucan hydrogel preparations for wound healing using the Design of experiments.

MATERIALS AND METHODOLOGY

All the chemicals used in the study were of analytical grade. Standard curve of β-glucan was prepared by dissolving known amount of drug in distilled water. The absorbance was read using phosphate buffer as blank at λ_{max} 510 nm, using UV-visible spectrophotometer.

Preparation of Beta-Glucan Hydrogel

Twenty formulations were prepared taking gelling agents guar gum and xanthum gum, hydrophilic polymers like HPMC K15M, HPMC K100M, Carbopol 934, penetration enhancer like menthol and 0.1N NaOH solution as cross linking agent[2] (Tables 1 and 2).

Evaluation of Hydrogels

After being settled in the container, all of the formulated hydrogels were visually inspected for color, presence of identifiable gritty particles, homogeneity, and phase separation both immediately and on a regular basis for a period of 30 days.

Measurement of pH

The pH of all the formulations was evaluated in triplicate and average value is noted.[3]

Spreading Coefficient

Spreading coefficient was measured with a ground glass slide fixed on the wooden block. The hydrogel was layered between this slide and a second glass slide with the same dimensions as the fixed ground slide. The weight was measured and deposited in the pan, which was then hooked to the pulley. The top slide's time (in s) to cover a distance of 5 cm was recorded. A shorter interval indicates a higher coefficient of spreading.[4]

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Table 1: Composition of formulation (F1 to F10)

Ingredients	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*	*F10*
βeta-glucan	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Carbopol 934	0.25 g	0.5 g	-	-	-	-	-	-	-	-
HPMC K 100M	-	-	0.25 g	0.5 g	0.75 g	1 g	-	-	-	-
HPMC K 15M	-	-	-	-	-	-	0.25 g	0.5 g	0.75 g	1 g
Guar gum	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g
Triethanolamine	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Menthol	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Propylparaben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
NaOH solution	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Dist.water	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Total weight	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g

Table 2: Composition of formulation F11 to F20

Ingredients	*F11*	*F12*	*F13*	*F14*	*F15*	*F16*	*F17*	*F18*	*F19*	*F20*
βeta-glucan	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Carbopol 934	0.25 g	0.5 g	-	-	-	-	-	-	-	-
HPMC K 100M	-	-	0.25 g	0.5 g	0.75 g	1 g	-	-	-	-
HPMC K 15M	-	-	-	-	-	-	0.25 g	0.5 g	0.75 g	1 g
Xanthan gum	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g
Triethanolamine	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Menthol	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Propylparaben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
NaOH solution	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Dist.water	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Total weight	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g

Table 3: Evaluation tests of formulated hydrogel

Formulation code	*Non homogeneity*	*pH*	*Drug content*	*Spreadability gms.cms/min*	*Extrudability*	*Viscosity(Cps)*
F1	-ve	6.66 ± 0.03	100.9 ± 0.3	1.723 ± 0.02	6.12 ± 0.09	17,828
F2	-ve	6.15 ± 0.04	91.56 ± 1.01	5.67 ± 0.014	2.66 ± 0.17	23,730
F7	-ve	7.11 ± 0.04	90.93 ± 1.8	0.221 ± 0.05	13.08 ± 0.03	6,529
F8	-ve	7.36 ± 0.03	97.18 ± 0.91	0.74 ± 0.03	10.87 ± 0.16	12,730
F9	-ve	6.64 ± 0.01	99.06 ± 0.5	1.26 ± 0.021	6.54 ± 0.02	17,320
F10	-ve	6.56 ± 0.03	90.31 ± 0.58	3.38 ± 0.05	3.35 ± 0.14	20,236
F11	-ve	6.52 ± 0.03	99.06 ± 1.07	1.76 ± 0.03	4.84 ± 0.02	17,770
F12	-ve	7.03 ± 0.08	94.06 ± 1.5	5.14 ± 0.06	2.36 ± 0.10	20,569
F17	-ve	7.1 ± 0.08	95.93 ± 0.35	1.32 ± 0.03	10.27 ± 0.07	15,480
F18	-ve	7.30 ± 0.013	99.06 ± 0.37	1.76 ± 0.01	4.77 ± 0.05	17,100
F19	-ve	7.41 ± 0.02	96.56 ± 1.6	4.15 ± 0.04	5.67 ± 0.03	17,547
F20	-ve	6.91 ± 0.04	89 ± 1.2	10.9 ± 0.04	7.10 ± 0.01	22,298

Standard deviation(n=3)

Extrudability

The amount of gel extruded from a lacquered aluminum collapsible tube when weight in grams is applied to extrude at least 0.5 cm ribbon of gel in 10 seconds determines extrudability.[5]

Extrudability was computed using the formula:

%Extrudability = [Iw-Fw]×100/ Iw

Where,

Iw= initial product weight in the collapsible tube

Fw = ultimate weight in collapsible tube upon extrusion.

Viscosity

A brook field viscometer with spindle no 64 was used to determine the viscosity of the prepared formulations. (Brookfield Engineering Laboratories). The reading was noted at maximum 30 rpm.[6]

Drug Content

1 g of the resulting formulation in a phosphate buffer and stirred for 30 minutes, filtered and the absorbance was measured at 510 nm at 6.8 pH. The percent drug content was computed using the absorbance reading.

In-vitro release studies

Modified Franz diffusion (FD) cell was used for determining *in-vitro* drug release studies. The formulation was smeared on the dialysis membrane in between donor and receptor compartment of the FD cell with Phosphate buffer pH 6.8 as a dissolution media. A circulating water jacket was kept at 37°C and stirred continuously while the entire assembly was kept on a magnetic stirrer. At appropriate time intervals, the sample (l-mL) was removed and replaced with equal volumes of new dissolving media. The cumulative percent drug release was estimated by spectrophotometric analysis at 510 nm.[7]

EXPERIMENTAL DESIGN

Design of experiments (DoE), a statistical strategy that allows the formulator to identify the most important factors that influence the experimental response and establish their best values.

A 2^2 factorial design was opted in which the amount of polymer carbopol 934 (*A*) and guar gum (*B*) were selected as factors and studied at two levels. *In-vitro* release (*Y*1), spreadability (*Y*2), and viscosity (*Y*3) were chosen as the response variables.

Ex-vivo Drug Release Study

An *ex-vivo* drug release test of a selected formulation was done in a modified Franz diffusion cell with goat skin. A section of goat skin was cut and cleaned appropriately. With the dorsal side of the skin facing up, the adipose tissue was removed and placed between the donor and receptor compartments. Phosphate buffer pH 6.8 was used as the dissolving media, just as it had been in the *in-vitro* studies. A thermostatically controlled water jacket was used to maintain the cell's temperature at 32°C. A magnetic bead was used to constantly swirl the solution, and the complete assembly was kept on a magnetic stirrer. The samples were removed and replaced with equal quantities of fresh dissolving liquid at regular intervals. The sample was spectrophotometrically analyzed at 510 nm[8].

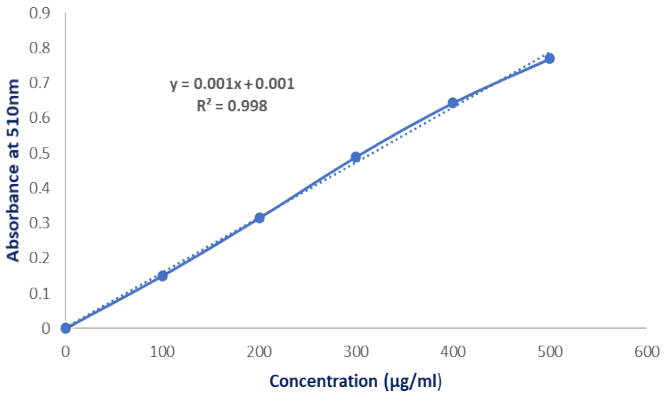

Figure 1: Standard plot of Beta-glucan at λmax-510 nm

Skin Irritation Study

The hydrogel (1-gm) formulations were applied to the skin in order to conduct skin irritation tests. Visual observations were made after cleaning remaining hydrogel to track the progression of erythema/edema for 24 hours.

Scratch Wound Healing Activity

A "wound gap" in human fibroblast cell monolayer was generated by scratching with a new 200 uL pipette tip across the center of the well , and the "healing" of this gap by cell migration and growth towards the centre of the gap was watched and quantified. Images of cells were taken at various time intervals 0, 24, 48 hours. Factors that affect cell motility and/or development might cause the rate of "healing" of the gap to increase or decrease.[9]

% of Wound Healing Scored= (Initial area-Final area)/ Initial area *100

RESULTS AND DISCUSSION

Development of Calibration Curve for βeta-glucan

The λ_{max} of βeta-glucan was determined using UV-visible spectrophotometer and was found to be 510 nm. The calibration curve of β-glucan revealed that there exists a linear relationship between various concentrations of β-glucan obeying Beer law in a concentration range of 100-500 µg/mL.

Evaluation Parameters

Physical Appearance

Various formulations of the prepared hydrogel were inspected visually for their color, homogeneity, consistency and phase separation. All the formulations Fl, F2, F7 to F12 and F17 to F20 appeared homogenous, white with smooth consistency while formulation F3 to F6 and F13 to F16 observed to be non-homogenous after for 1 and 1/2 months.

Measurement of pH

The pH values hydrogels of βeta-glucan exhibited using calibrated pH meter were in the range of 6– 8 indicating the normal pH range of the skin with no skin irritation.

Table 4: *In-vitro* drug release profiles of hydrogels formulations

Timein hours	*F1% drug release*	*F9% drug release*	*F11% drug release*	*F18% drug release*	*F19% drug release*
0.5	8.33 ± 1.7	2.86 ± 1.7	4.68 ± 2.4	18.4 ± 2.4	6.3 ± 1.5
1	17.63 ± 1.7	11.20 ± 0.8	7.92 ± 0.9	21.61 ± 2.3	14.63 ± 2.4
2	30.93 ± 0.8	30.23 ± 0.4	16.47 ± 0.7	31.10 ± 1.2	21.83 ± 3.7
3	41.31 ± 0.9	40.70 ± 0.9	21.80 ± 1.5	39.83 ± 0.9	21.87 ± 1.6
4	53.72 ± 1.4	42.44 ± 1.5	35.67 ± 2.6	47.1 ± 3.7	22.93 ± 2.1
5	65.01 ± 2	45.15 ± 2.6	44.62 ± 0.7	50.50 ± 1	42.84 ± 1.8
6	72.2 ± 2.4	46.94 ± 3.9	59.28 ± 1.9	63.07 ± 1.8	50.84 ± 0.7

Standard deviation(n=3)

Table 5: Formulation table (Runs)of two factorial design

Formulation code	*Carbapol 934*	*Guar gum*
F21	0.125g	0.01g
F22	0.5g	0.01g
F23	0.125g	0.07g
F24	0.5g	0.07g
F25	0.125g	0.045g
F26	0.25g	0.045g
F27	0.1875g	0.0475g
F28	0.125g	0.05g

Drug content estimation

Percentage drug content of various hydrogel formulations was found to be in the range of 89% w/v to 100.9% w/v, indicating the uniformity of drug content.

Spreadability and Extrudability studies

Spreadability, an essential criterion for a hydrogel, denotes the extent of the area on which the gel readily spreads on application to the skin. Among all the above formulations, F1, F9, F11, F18, F19 were showing satisfactory spreadability properties in terms of applicability on the skin. Formulations F1, F9, F11, F18, F19 were showing satisfactory extrudability with values. The extrudability values were ranging (2.36% E - 13.08% E) (Table 4).

Viscosity Determination

Formulated hydrogels were evaluated for viscosity using Brook field Viscometer using spindle no 64 at 30 rpm. The viscosity values were ranging 6529 Cps-23730 Cps (Table 3).

The results revealed that formulations F1, F9, F11, F18 and F19 exhibited satisfactory values in terms of pH, percentage drug content, spreadability, extrudability and viscosity. The above formulations were observed to have suitable properties of hydrogel. Thus, they were selected and further tests were performed

In-vitro Drug release profile of βeta-glucan

The kind and concentration of the polymer determine the rate of drug release from a formulation. The *in-vitro* drug release order and the drug release values observed the following decreasing order F1>F18>F11>F19>F9 that is 72.2>63.07>59.28>50.84>46.94% w/v, respectively.

ANOVA for selected factorial model

Response 1: in-vitro drug release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1093.77	3	364.59	63.46	< 0.0001	significant
A-carbapol	937.47	1	937.47	163.17	< 0.0001	
B-guar gum	217.15	1	217.15	37.80	< 0.0001	
AB	276.73	1	276.73	48.17	< 0.0001	
Residual	63.20	11	5.75			
Lack of Fit	42.80	4	10.70	3.67	0.0644	not significant
Pure Error	20.40	7	2.91			
Cor Total	1156.96	14				

Fig.2: ANOVA performed by software for response 1 (Y1:% *in-vitro* drug release)

Among the selected formulations F1 showed maximum drug release (Table 4).

Experimental Design

From the above evaluation tests and *in-vitro* drug release it was found that F1 formulation formulated using 0.250 g of carbapol and 0.05 gm of guar gum was showing best results when compared to the remaining formulations. In order to optimize the concentration of polymers used in the F1 formulation, two factorial design was opted. Concentrations of carbapol 934 and guar gum are independent variables. *In-vitro* drug release, viscosity, spreadability are dependent variables. A 2^2 factorial design was employed where the amounts of two carriers (factors) were varied at two levels as hypothesized by the Design Expert 13 (32-bit) software (Stat- Ease Inc., Minneapolis, USA). The amount of polymer carbapol 934 (*A*) and guar gum (*B*) were selected as factors and studied at two levels. *In-vitro* release (*Y*1), viscosity (*Y*2), and spreadability (*Y*3) were taken as the response variables.

All formulations in Table 5 were prepared in laboratory and evaluation tests were conducted. The results were tabulated in Table 6

Two Factorial Design for Response 1(Y1)

The obtained *in-vitro* dissolution values (Table 4) were given as responses in the Design expert Software 13. These significance of responses were evaluated by analysis of variance (ANOVA) (Figure 2) and linear regression. 3D response surface graphs and contour graphs are generated by the software. The Model F-value of 63.46 implies the model is significant. p<0.0500 indicate model terms are significant. The Lack of Fit *F-value* of 3.67 implies there is a 6.44% chance that a Lack of Fit *F-value*

Table 6: Evaluation results of runs generated by Design expert 13, Minneapolis

Formulation code	*Non homogeneity*	*pH*	*Percentage drug content*	*Spreadibility g.cm/min*	*Viscosity cps*	*In-vitro drug release*
F21	-ve	6.62 ± 0.02	98.4 ± 1.07	1.56 ± 0.04	13,465	63.2 ± 1.1
F22	-ve	6.19 ± 0.03	93.09 ± 1.19	2.61 ± 0.06	21,547	58 ± 1.4
F23	-ve	6.72 ± 0.03	99.6 ± 2.04	1.85 ± 0.08	18,903	77.2 ± 0.8
F24	-ve	6.15 ± 0.04	91.56 ± 1.5	2.78 ± 0.05	22,800	56 ± 0.5
F25	-ve	6.95 ± 0.04	99.6 ± 0.35	1.703 ± 0.04	17,487	75.2 ± 0.6
F26	-ve	6.69 ± 0.04	99.2 ± 0.5	1.89 ± 0.02	17,995	71.2 ± 1.2
F27	-ve	6.64 ± 0.02	99.8 ± 0.57	1.9 ± 0.07	17,985	70.3 ± 1.9
F28	-ve	6.90 ± 0.03	99.91 ± 1.6	1.715 ± 0.03	1.715 ± 0.03	76.4 ± 1.6

Standard deviation (n=3)

this large could occur due to noise. The predicted R^2 is in reasonable agreement with adjusted R^2 for all the responses, *i.e.,* the difference is less than 0.2 (Table 7) indicating the suitability of the model.

A mathematical relationship between factors and responses were generated using multiple linear regression analysis in the form of equations. These equations represent the quantitative effect of variables (X1 and X2) and their effects on the response Y1. A positive sign represents a synergistic effect while a negative sign indicates an antagonistic effect. Equation no.1 indicates that variable X1 i.e., concentration of carbapol was showing negative influence and concetration of guar gum was showing positive influence.

Equation for Y1 (% *in-vitro* drug release) for the formulation is given as:

$$Y1 = 61.07 - 1.82X1 + 381.55X2 - 946.32X1X2 \quad \text{Eq. (1)}$$

Figure 3 depicts that as the level of X1 (carbapol 934) increases from 0.125 to 0.25 the percent drug release was found to decrease from 74.009% to 68%.

3D response plots in the figure no 4.14 indicates that drug release increases with decrease in carbapol concentration and increase in concentration of guar gum which positively correlates with the actual equation (Eq. 1). The above results indicate the model is suitable and the concentration of carbapol 934 is having negative influence and guar gum is having positive influence (Eqn 1) the responses reveal the same as the concentration of carbapol 934 increases, the drug release was found to decrease (Figure 4 and 5). The actual experimental values were compared to predicted values generated by the software. It was observed to have closed correlation with minimum variation between actual value and predicted value with respect to F28 (Table 8)

Two Factorial Design for Response 2(Y2)

The obtained viscosity (Table 9) was given as responses in the Design expert Software 13. These significances of responses were evaluated by analysis of variance (ANOVA) (Figure 6) and linear regression. 3D response surface graphs and contour graphs are generated by the software. The Model *F-value* of 251.59 implies the model is significant. There is only a 0.01% chance that an *F-value* this large could occur due to noise.

p-values less than 0.0500 indicate model terms are significant. In this case A, B and AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit *F-value* of 2.89 implies the lack of fit is not significant relative to the

Table 7: Fit Statistics

Std. dev	*2.40*	*R2*	*0.9454*
Mean	65.28	Adjusted R2	0.9305
C.V%	3.67	Predicted R2	0.8987
		Adequate Precision	20.6210

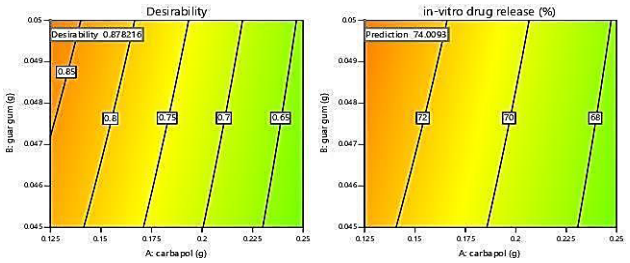

Figure 3: Contour plot for the effect of Carbapol 934 and guar gum on response Y1.

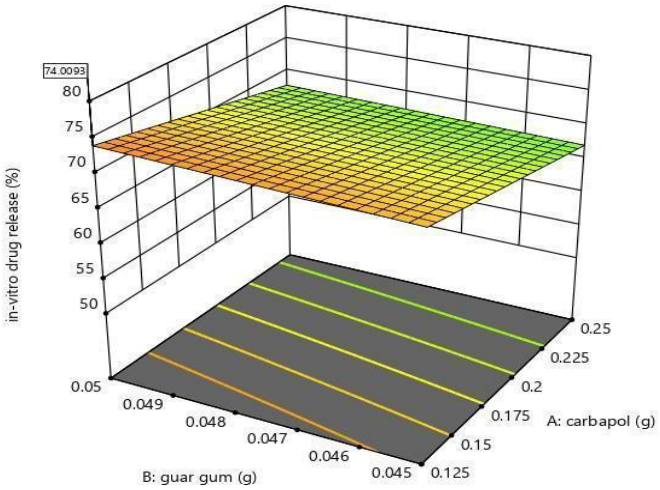

Figure 4: 3D Response surface graph for Y1 response

Table 8: Comparison of predicted and actual values for response Y1 (% *in-vitro* drug release)

Formulation code	*Response 1: %Drug release*	
	Actual value	*Predicted value*
F21	63.20	63.48
F22	58.00	59.25
F23	77.20	79.27
F24	56.50	53.75
F25	75.20	72.69
F26	71.20	67.14
F27	70.30	70.43
F28	76.40	74.01

Table 9: Comparison of predicted and actual values for response Y2 (Viscosity)

Formulation code	*Response 2: Viscosity*	
	Actual value	*Predicted value*
F21	13465	13819.08
F22	21547	21511.72
F23	18903	18949.45
F24	22800	22927.78
F25	17487	16811.80
F26	17995	18653.78
F27	17985	17920.76
F28	17598	17239.33

Table 10: Comparison of predicted and actual values for response Y3 (Spreadability)

Formulation code	*Response 3: Spreadability*	
	Actual value	*Predicted value*
F21	1.56	1.52
F22	2.61	2.61
F23	1.85	1.81
F24	2.78	2.88
F25	1.70	1.69
F26	1.89	1.88
F27	1.90	1.88
F28	1.72	1.71

Table 11: Percentage relative error calculation

Response	*% Relative error*
In-vitro drug release	0.83
Viscosity	0.01
Spreadability	0.35

pure error. There is a 10.48% chance that a lack of fit *F-value* this large could occur due to noise. Non-significant lack of fit is good, we want the model to fit.

The predicted R^2 is in reasonable agreement with adjusted R^2 for all the responses, *i.e.,* the difference is less than 0.2.

A mathematical relationship between factors and responses were generated using multiple linear regression analysis in the form of equations. These equations represent the quantitative effect of variables (X1 and X2) and their effects on the response Y2. A positive sign represents synergistic effect while a negative sign indicates an antagonistic effect.

Equation for Y2 (Viscosity) for the formulation is given as:

$$Y2 = 17920.76 + 895.20X1 + 187.97X2 - 25.79X1X2$$

Eq. (2).

Table 12: In-vitro and ex-vivo drug release profile for formulation F28

Time in hours	*In-vitro drug release of F28*	*Ex-vivo drug release of F28*
0.5	10.6 ± 1.5	12.9 ± 0.5
1	21.5 ± 0.8	24.5 ± 0.9
2	31.9 ± 2.4	39.8 ± 0.8
3	45 ± 1.08	47.7 ± 1.2
4	51.5 ± 1.29	54.6 ± 2.4
5	62.9 ± 0.6	60.5 ± 0.9
6	76.4 ± 0.7	76.4 ± 0.7

Table 13: Skin irritation studies

Group	*Erythma*		*Edema*	
	12 hours	*24 hours*	*12 hours*	*24 hours*
Control	0	0	0	0
Standard	0	0	0	0
F28	0	0	0	0

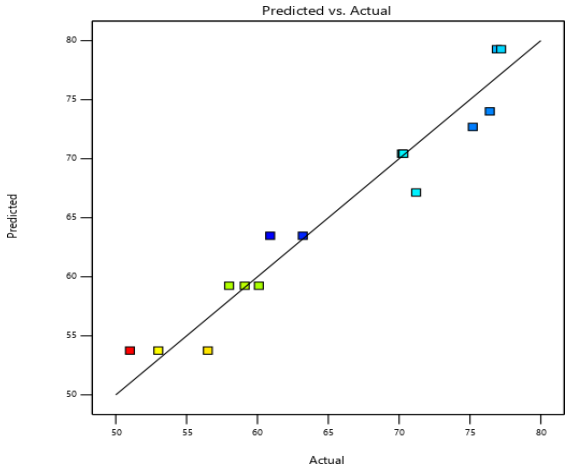

Figure 5: Comparison of predicted and actual values for the response Y1

ANOVA for selected factorial model

Response 2: viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.289E+08	3	4.295E+07	251.59	< 0.0001	significant
A-carbapol	8.792E+07	1	8.792E+07	514.99	< 0.0001	
B-guar gum	2.946E+07	1	2.946E+07	172.60	< 0.0001	
AB	8.421E+06	1	8.421E+06	49.33	< 0.0001	
Residual	1.878E+06	11	1.707E+05			
Lack of Fit	1.170E+06	4	2.924E+05	2.89	0.1048	not significant
Pure Error	7.083E+05	7	1.012E+05			
Cor Total	1.307E+08	14				

Figure 6: ANOVA performed by software for response 2 (viscosity)

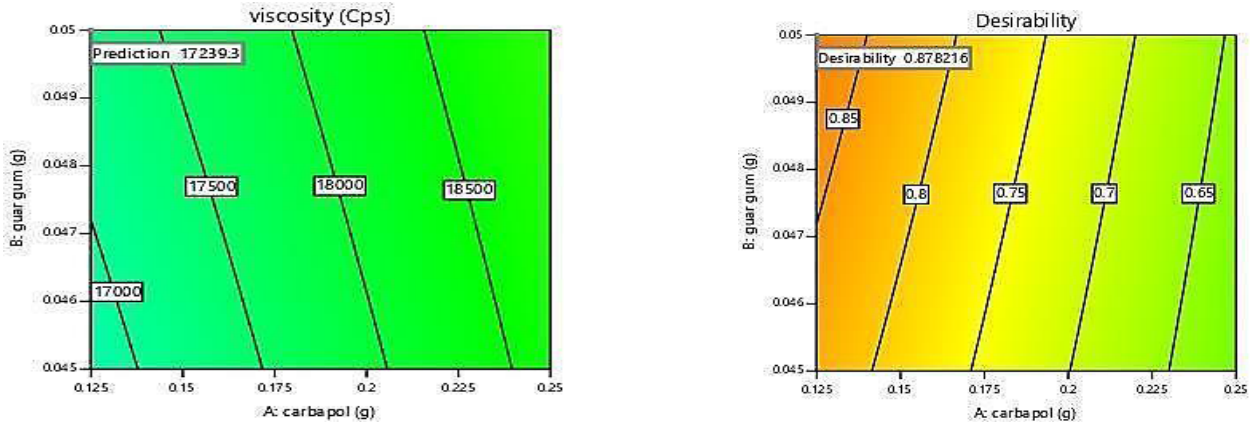

Figure 7: Contour plot for response Y2 (Viscosity)

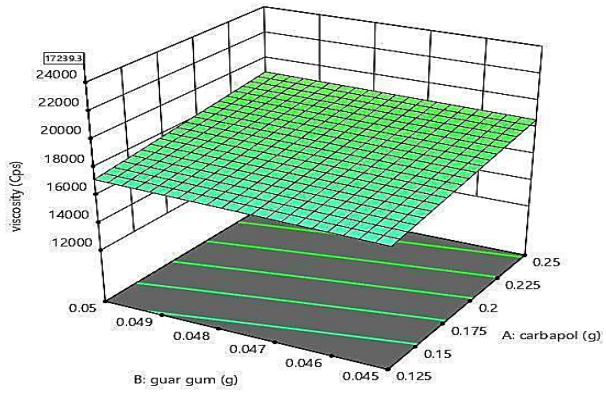

Figure 8: 3D response plot for response Y2 (Viscosity)

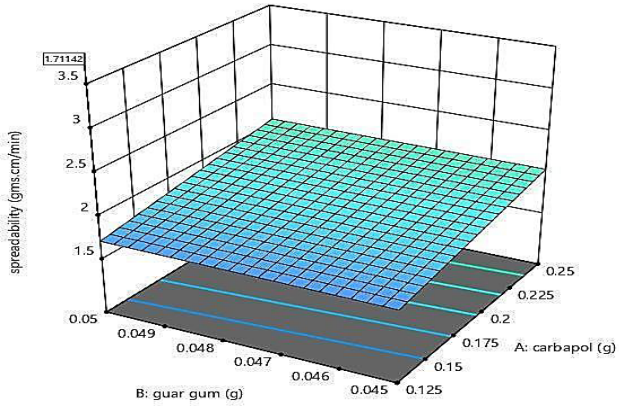

Figure 10: 3D response plot for response Y3 (Spreadability)

Figure 7 depicts that as the level of X1 (carbapol 934) and X2 (Guar gum) increases from -1 to +1 the viscosity was found to increase from 17239 Cps to 18500 cps

3 D response plots shown in the Figure 8 shows inclining trend in viscosity with increase in X1 (carbapol 934) and X2 (guar gum) which positively correlates with the actual equation (Eq. 2).

ANOVA for selected factorial model

Response 3: spreadability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.83	3	1.28	105.95	< 0.0001	significant
A-carbapol	3.55	1	3.55	294.61	< 0.0001	
B-guar gum	0.1206	1	0.1206	10.02	0.0090	
AB	0.0002	1	0.0002	0.0152	0.9041	
Residual	0.1324	11	0.0120			
Lack of Fit	0.0267	4	0.0067	0.4412	0.7761	not significant
Pure Error	0.1058	7	0.0151			
Cor Total	3.96	14				

Figure 9: ANOVA performed by software for response Y3 (Spreadability)

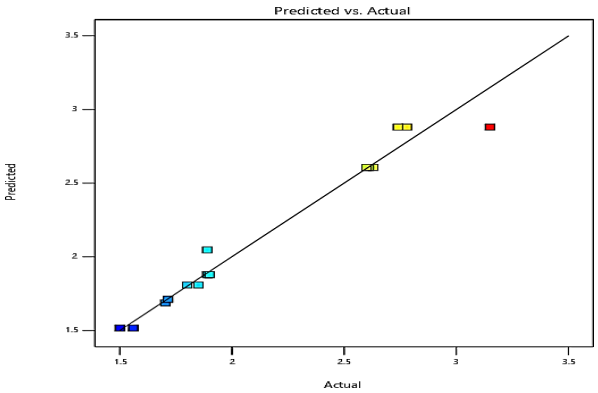

Figure 11: Comparison between predicted and actual values for response Y3 (Spreadability)

The above results indicate that the model is suitable and that the concentration of carbapol and guar gum has a positive influence on viscosity as indicated by (Eqn. 2) and Figure 7 and 8.

The actual experimental values were compared to predicted values generated by the software. It was observed to have closed correlation with minimum variation between actual value and predicted value with respect to F28 (Table 9).

Two Factorial Designs for Response 3

The obtained spreadibility values were given as responses in the design expert Software 13. These significance of responses were evaluated by analysis of variance (ANOVA)

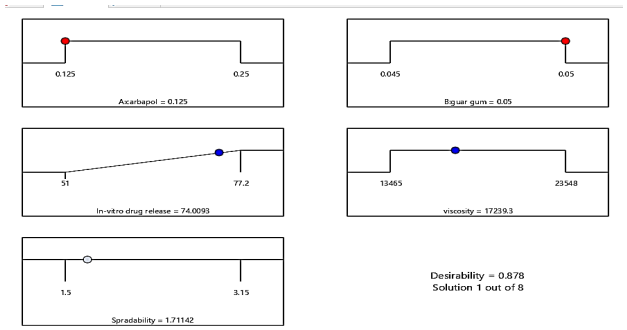

Figure 12: Numerical optimization for hydrogel formulation using carbapol 934 and guar gum

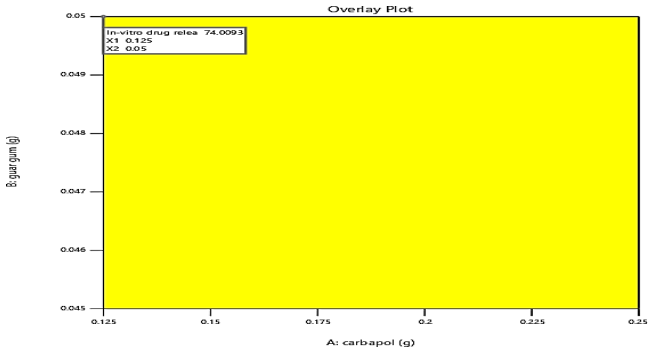

Figure 13: Overlay plot for optimized formulation

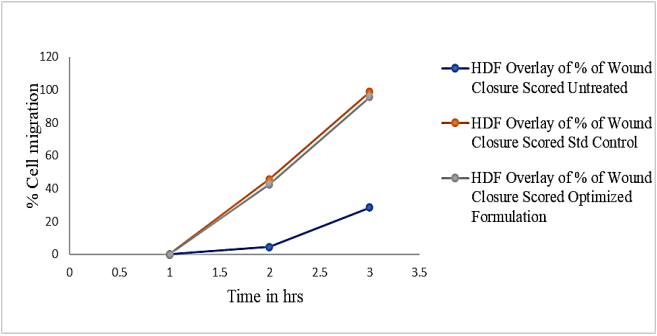

Figure 14: Percentage cell migration with time

The model *F-value* of 105.95 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

p <0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The lack of fit *F-value* of 0.44 implies the lack of fit is not significant relative to the pure error. There is a 77.61% chance that a lack of fit *F-value* this large could occur due to noise. Non-significant lack of fit is good, we want the model to fit.

The predicted R^2 is in reasonable agreement with adjusted R^2 for all the responses, *i.e.,* the difference is less than 0.2 (Figure 9).

A mathematical relationship between factors and responses were generated using multiple linear regression analysis in the form of equations. These equations represent the quantitative effect of variables (X1 and X2) and their effects on the response Y3. A positive sign represents synergistic effect while a negative sign indicates an antagonistic effect.

Equation for Y3 (Spreadability) for the formulation is given as:

Y3= 1.88 +0.1798X1 + 0.0120X2 – 0.0001X1X2

Figure 10 depicts that as the level of X1 (carbapol 934) and X2 (guar gum) increases from -1 to +1 the spredability decreased from 1.711 to 2.

3D response plots shown in the figures below shows declining trend in spreadability with increase in X1 (carbapol 934) nad X2 (guar gum).

The above results indicate the model is suitable and as the concentration of carbapol and guar gum is having a positive effect and combine showing negative influence (Eqn. 3), the above properties correlate experimentally (Figure 10 and 11).

The actual experimental values were compared to predicted values generated by the software. It was observed to have closed correlation with minimum variation between actual value and predicted value with respect to F28 (Table 10).

Optimization

Optimization was performed by the design based on the obtained responses by numerical and graphical representation

Based on responses, minimum and maximum for drug release are given and solutions are obtained. The solution with maximum desirability is considered. This is graphically represented in overlay plot. Overlay plot highlights the point where the response criteria can be met. In a overlay plot the region where the specifications are not met is shaded out. Flag planted is a representation of optimum. The yellow regions refer to the space where factors can be set to satisfy requirements for all the responses (Figure 12 and 13).

Percent relative error was calculated between the predicted mean and the observed mean.

Calculation of Percentage Relative Error

The variations between the experimental results and resulte obtained by the design expert 13 were calculated by calculating % relative error (Table 11). The percent relative error of optimized formulation was found to be 0.83%, 0.01% and 0.35% for Response 1, Response 2 and Response 3, respectively. Since the percent relative error is within the limits *i.e.,* < 1% the software generated data is found to be in close agreement with the practical data.

Ex-vivo Drug Release Profile

Ex-vivo release for the formulation F28 was studied using franz diffusion cell and goat skin and compared with *in-vitro* drug release (Tables 12 and 13). There is no significant variation in the release rate at 6 hours time period.

Skin Irritation Studies

Skin irritation studies were conducted by applying the hydrogel (1-gm) formulations on to the skin. Sensitivity or reaction if any were recorded and tabulated as in the Table 14. All the three groups showed '0' indicating no skin irritation. By applying optimized formulation F28 all the three groups showed no erythematous and edematous reaction Table 13.

Wound Healing Activity

The optimized formulation F28 was subjected to wound healing activity using HaCaT cell lines. The formulation at the concentration of 200 µg/mL showed significant mobilization of keratinocytes and closed the gap compared to the control without adding formulation. The Figure 14 indicates % keratinocyte cell migration at specific time interval and comparison between control, 50 µg/mL standard (allantoin), 200 µg/mL of optimized formulation (Figure 14).

CONCLUSION

The results correlated with the design with less percentage relative error. F28 formulation was observed to have positive correlation for *ex-vivo* drug release. Comparable wound healing activity was observed when performed on HaCaT cell lines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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