



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

A Quality by Design Approach: Development and Evaluation of Herbal Hydrogel

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Received: 25-Jul-2022, Manuscript No. JOCPR-22-28612; **Editor assigned:** 27-Jul-2022, PreQC No. JOCPR-22-28612(PQ); **Reviewed:** 11-Aug-2022, QC No. JOCPR-22-28612; **Revised:** 18-Aug-2022, Manuscript No. JOCPR-22-28612(R); **Published:** 25-Aug-2022, DOI:10.37532/09757384.2022.14(7).037

ABSTRACT

The current study was undertaken to formulate and evaluate herbal hydrogel containing ethanolic coix seed extract. Hydrogel was prepared by ethanolic seed extract, carbapol 934p, Aloe vera gel extract, Triethanolamine and methylparaben. The effects of critical parameters (concentration of Carbapol 934p and Aloe vera gel extract) were investigated by executing design of experimentation using 32 factorial designs by Design Expert 12 version software. All the formulations were developed and evaluated for visual inspection, pH, viscosity, spread-ability, drug release and drug content. Optimized formulation was subjected to *in vitro* antifungal activity against *Candida albicans*. It was observed that formulation variables X_1 : Carbapol 934p and X_2 : Aloe vera gel extract showed significant effect on the response Y_1 : Viscosity (cp) and Y_2 : Drug release (% CADD/cm²). Stability studies conducted under accelerated condition and at room temperature were shown acceptable results. It was concluded that hydrogel containing coix ethanolic seed extract showed good consistency, spread-ability, homogeneity as well as stability. This study confirmed that quality by design is an effective approach for understanding the quality parameters for optimizing Hydrogel formulation.

Keywords: Herbal hydrogel; *Candida albicans*; Homogeneity; Phytoconstituents

Abbreviations: CADD: Cumulative Amount of Diffused Drug; QbD: Quality by Design; QTPP: Quality Target Product Profile; CQA: Critical Quality Attributes

INTRODUCTION

Herbal medicinal system is old and is practiced from the beginning of mankind [1]. According to an estimate of the World Health Organization (WHO), about 80% of the world population uses herbs and other traditional medicines. They are known for their safety, efficacy, cultural acceptability and lesser side effects [2].

Coix lacryma jobi L. is belonging to *Poaceae* or *Gramineae* family. Coix plant is a grass crop that is used in traditional Chinese medicine. Adlay seeds are major medicinal part that contains a range of phytoconstituents such as Polysaccharide, Coixol, Coixenolide, Protein, Lipid, Phytosterol and Polyphenols (Figures 1 and 2). Several studies have demonstrated that coix seeds have antimicrobial, anti-diabetic, anti-inflammatory, anti-obesity, anti-cancer activity and other beneficial effect on humans [3-5].



Figure 1: Coix plant



Figure 2: Coix seed

Topical drug delivery system widely used for skin disease like bacterial infection, fungal infection, eczema etc. Topical application of drug has advantage of directly delivered of drug to the site of action. The advantages of topic formulations are avoidance of first pass metabolism, avoid inter-intra patient variation, convenient and easy to apply etc. [6]. Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. It is a semi occlusive film over the skin and release the drug in controlled manner. Hydrogel possess degree of flexibility very similar to natural tissue, due to their significant water content, good transport properties. Hydrogel are biocompatible, easy to modify and time release growth factor [7,8].

There was no report on the formulation containing extract of *Coix* seed. Hence, in the present study to design, formulate and evaluate hydrogel containing ethanolic extract of adlay seed.

To document product development [ICH guideline (Q9)] report by “Quality by Design” approach for Hydrogel formulation. The objective of the product development report is to present the quality by design aspect for gel based formulations. The elements of quality by design are examined and a consistent nomenclature is proposed with the help of Quality Target Product Profile (QTPP), Critical Quality Attribute (CQA), Critical Process Parameter, Critical Material Attribute (CMA), risk assessment, and control strategy [9,10].

Quality by Design is a structured, organised method for determination the relation between factor affecting a process and the output of that process based on quality risk management, ICH guideline (ICH Q8 (R²)).

MATERIALS AND METHODS

Ethanolic extract of *Coix* seed was purchase from Shannxi Green Bio-Engineering Co. Ltd., China. Aloe vera gel extract obtained from Arogya Jyoti Pharmacy, New V.V. Nagar as a gift sample. Carbapol 934p (gel base), Triethanolamine (pH adjust) and Methyl paraben (Preservative) were used in preparation of Hydrogel.

Quality Target Product Profile (QTPP)

The Quality Target Product Profile as described in International Council for Harmonization (ICH) Q8 is an essential element of a QbD approach. The QTPP includes all product attributes that are needed to ensure equivalent safety and efficacy. QTPP for herbal hydrogel has been developed by taking into account the important drug product quality attributes elements (Table 1).

Table 1: Qtp for hydrogel

Parameters	QTPP element	Justification	Critical quality attributes		
			Low	Medium	High
Dosage form	Hydrogel	Topical application	√		
Dosage design	Controlled release	Better for topic application	√		
Strength	5%	Effective dose			√

Assay	99%	Effect on safety and efficacy			√
pH	6–7	Compatible with skin		√	
Viscosity	Medium	Impact on drug release		√	
Spreadability	Good	Uniformity on skin		√	
Drug release	Permeation	Impact on therapeutic point on view			√

Critical Quality Attributes (CQA)

Critical Quality Attribute (CQA) is “a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The initial CQA were defined from QTPP to identify satisfactory quality of the product. The potential CQA of excipients required for development of herbal hydrogel [11].

Risk Assessment

To identify all the potential high risk factors for further study, risk assessment was conducted. In the risk assessment process, Risk Priority Numbers (RPN) was mapped into three categories (high, medium and low). The initial risk assessment for critical input material and formulation components and their impact on the product quality was determined [12].

Design of Experiment (DoE)

DoE is a structured, organized method for determining the relationship between factors affecting a process and the output of that process. Design expert version 12 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for formulation design. A 3^2 factorial design was employed where the amount of two factors were varied at three levels (-1, 0 and +1) as hypothesized by the design. In this design, two factors, Carbapol 934p and Aloe vera gel extract in each in three levels (Table 2) were evaluated and experimental trials were performed in all 9 possible combinations. (Table 3) Carbapol 934p and Aloe vera gel extract were selected for independent variables and viscosity and drug release were selected for dependent variables.

Table 2: Selected factor combination as per 3^2 full factorial designs

Variables	Level		
	-1	0	1
Independent variable			
Carbapol 934p	1	1.5	2
Aloe vera gel	1	1.5	2

extract			
Dependent variable			
Viscosity	Y ₁ =Viscosity (cp)		
Drug release	Y ₂ =Drug release (%CADD/cm ²)		

Table 3: Formulation trial batches of herbal hydrogel Concentration of each ingredient is expressed in % w/v

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Seed extract	5	5	5	5	5	5	5	5	5
Carbapol 934 p	1.5	1	2	2	2	1	1	1.5	1.5
Aloevera gel extract	2	1.5	1.5	2	1	1	2	1	1.5
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water (ml)	100	100	100	100	100	100	100	100	100

Drug Excipient Compatibility Studies

A Fourier-Transform Infrared (FT-IR) was used to identify if any interaction existed between seed extract and the excipients used. The samples were analyzed *via* potassium bromide pellet method in the region 4000-400 cm⁻¹ [13] (Figures 3-5) (Table 4).

Table 4: Ir spectra-interaction between seed extract and excipient

Band assignment	Std. Range (cm ⁻¹)	Observed (cm ⁻¹)
1. C-N (S)	1350-1550	1024
2. C=C (S)	1600-1475	1500
3. N-H (S)	1640-1550	1639
4. C=O (S)	1725-1705	1712
5. C-H (S)	3130-3050	2926

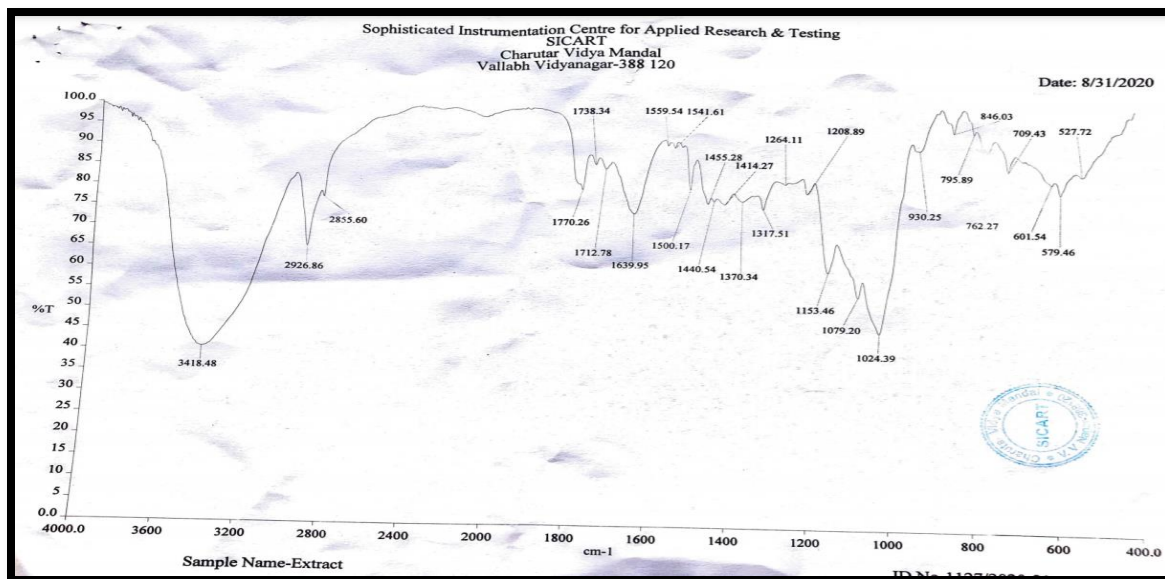


Figure 3: FT-IR Spectra for ethanolic seed extract

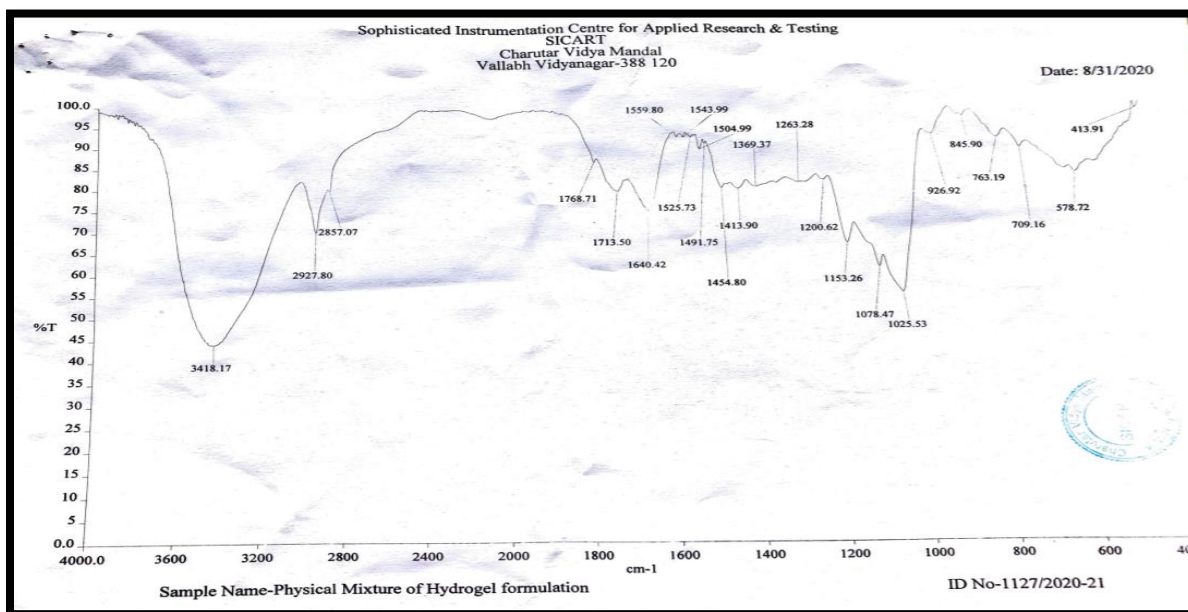


Figure 4: FT-IR spectra of physical mixture of hydrogel formulation

Formulation of Hydrogel

Weighed accurately amount of Carbapol 934p and dissolved in water using magnetic stirrer. Coix seed extract, Aloe vera gel extract and Methylparaben were added to the gel base. Triethanolamine was added to adjust the pH of hydrogel between 6.0 to 7.0 with constant stirring.

Evaluation of Hydrogel Formulation

The formulated gel formulations were evaluated for physical examination. The characterization and response in the design of the experiments conducted on the gel preparations include pH, spreadability, homogeneity, viscosity, drug release and drug content [14-16] (Table 5).

Table 5: Variables and response as per design expert

Run	Spreadability	pH	Viscosity (cp)	% CADD/cm ² (after 5 hrs.)	Drug content (%)
F1	Good	6.82	32645	52.26	98.86
F2	Good	6.99	28923	62.12	97.16
F3	Average	6.93	33249	52.9	97.12
F4	Average	6.92	34920	50.12	97.27
F5	Average	6.55	33712	51.98	98.26
F6	Good	6.92	28092	62.53	97.78
F7	Good	6.89	32590	52.68	98.51

Spreadability: The gel was weighed to be as high as 0.5 g and then placed on glass plate. Then, put another other glass plate above the gel mass. The gel was spread over the slides. Then gel was spread uniformly over the glass plate.

Homogeneity: After the gels have been set in container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

Viscosity: A maximum of 100 g of gel was put into a container, and then placed on a viscometer with spindle no. 64 installed. Then, the spindle was lowered onto the gel with 10 rpm speed.

Drug content: 1 g of Hydrogel was accurately weighed dissolved in 80 ml Phosphate buffer (pH 6.8). Sonicated for 10 min. and made up volume up to 100 ml with Phosphate buffer. From this 1 ml was pipetted out and diluted to 10 ml same solvent. The absorbance was measured by UV spectroscopy at 286 nm against blank.

In vitro Diffusion study

The diffusion studies of the prepared gels were carried out in Franz diffusion cell for studying the release of gels through a cellophane membrane. Place 1 gm hydrogel was taken on cellophane membrane and the diffusion studies carried out at $37 \pm 1^\circ\text{C}$ using Phosphate buffer (pH 6.8) as dissolution medium. Three ml of each sample was withdrawn periodically at 1, 2, 3, 4 and 5 hr. and each sample was replaced with equal volume of dissolution

medium. The samples were analyzed for the drug content by using Phosphate buffer as blank. The absorbance was analyzed by UV spectroscopy at 286 nm.

Statistical Optimization

The evaluation of the quality of fit of the model was performed by using analysis of variance (ANOVA) technique. Based on comparison of several statistical parameters the best fit model was selected, the statistical parameters included the R²-coefficient of determination, Adjusted R², Predicted R², p value provided by DoE software. The relationship between the dependent and independent variables was demonstrated using response surface plots i.e. Contour and 3D surface plots, these plots were used to study the effect of various factors on the response (Tables 6 and 7). Optimized formulations were run for triplicate and evaluate for pH, viscosity, drug content and drug release. (Table 8)

Table 6: Statistical parameters–Analysis Of Variance (ANOVA)

Response	p-Value	R ²	Adjusted R ²	Predicted R ²
Y ₁ -Viscosity	0.0091	0.9932	0.9819	0.9316
Y ₂ -Drug release	0.0085	0.9813	0.9501	0.7823
Note: p-values were less than 0.0500 indicate model terms are significant				

Table 7: Polynomial equation

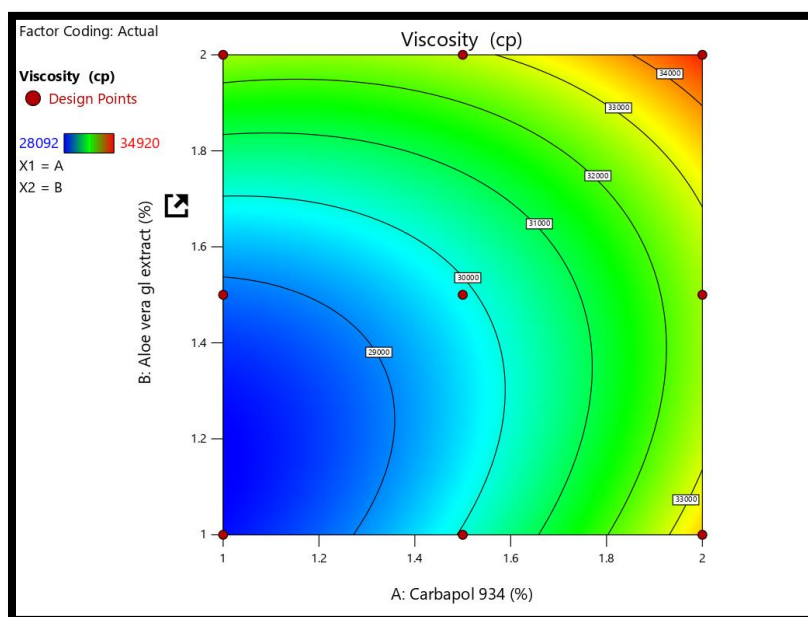
Response	Model	Polynomial equation
Viscosity	Quadratic model	$29887.4 + 1879.33 \times A + 1388.33 \times B \pm 822.5 \times AB + 817.333 \times A^2 + 1564.33 \times B^2$
Drug release	Quadratic model	$58.5389 + -3.72167 \times A + -3.15833 \times B + 1.9975 \times AB \pm 1.23833 \times A^2 \pm 2.86833 \times B^2$
Note: “×” Multiplication		

Table 8: Evaluation parameters of optimized formulation

Formulation	pH \pm SD	Viscosity \pm SD	Drug content (%)	% CADD/cm ²
F9	6.92 \pm 0.02121	30123 \pm 0.707107	98.06 \pm 0.02121	58.25 \pm 0.3606

Table 9: Antimicrobial study data

Culture	Blank	Control	Reference	Hydrogel-excluding Seed extract	Hydrogel
Candida albicans	NZ	10 mm	25 mm	12 mm	20 mm

**Figure 5: Counter plot for Response Y₁ Viscosity****Anti-Bacterial Activity**

Optimized formulation was subjected to perform *in vitro* anti-fungal activity. Facility provided by Vasu Research Center, Baroda (Table 9).

Stability Study

Stability studies were carried out for statistically optimized formulation according to the International Council for Harmonization (ICH) Q1A (R2) guidelines. The samples were stored in closed high density polyethylene (HDPE) bottle which was maintained at room temperature $27^{\circ} \pm 2^{\circ}$ /RH $65 \pm 5\%$ and accelerated conditions $40^{\circ} \pm 2^{\circ}$ /RH $75 \pm 5\%$ for 45 days. The hydrogel formulation was withdrawn after a period of 30 days and 45 days and evaluated for physical appearance, pH, viscosity and drug content [17] (Table 10).

Table 10: Stability study data of optimized formulation

Evaluation parameters	Initial	30 days		45 days	
		RT	40°/75% RH	RT	40°/75% RH
pH	6.87	6.87	6.85	6.87	6.85
Viscosity (cp)	30130	30129	30127	30126	30125
Drug content (%)	98.75%	98.73%	98.70%	98.70%	98.65%

RESULTS AND DISCUSSION

QTPP serves as a summary of quality attributes of a drug product that need to be achieved to ensure its safety and efficacy. The QTPP for Herbal formulation consist of information regarding dosage form, dosage design and route of administration, dosage strength, and drug product quality attributes, and based on prior scientific knowledge and data an Initial Risk Assessment of drug substance (Herbal Extracts) and formulation variables (Excipients) was carried out to meet the Quality Target Product Profile (QTPP). Concentration of Carbapol 934p (%) (X_1) and concentration of Aloe vera gel extract (%) (X_2) were considered as the major formulation parameters for design of experimentation. A 3^2 full factorial design was employed for development and optimization of Hydrogel. The experimental runs, with independent variables and measured responses for the developed hydrogel formulation are shown in (Table 2).

The all prepared formulations were inspected visually for their color, homogeneity, and grittiness. It observed that all the formulations were showed good homogeneity with no any grittiness and brown in color. The formulation batches F1-F9 were subjected for physical characterization, pH, spreadability, viscosity, drug release and drug content.

The pH of the hydrogel was found to be within 6.55-6.99. The viscosity of hydrogel prepared was in the range of 28092-34920 cp. The drug release (%CADD/cm²) of the hydrogel was found to be 50.12–62.53% and Coixol content was within the range of 97.12%-98.86%.

Statistical optimization of Herbal hydrogel was performed by comparison of several statistical parameters, provided by Design-Expert® Software, Version 12. The values of the coefficients X_1 , X_2 , their interaction and

quadratic-terms are related to the effect of these variables on the response. To demonstrate graphically the influence of each factor on responses of viscosity the Contour plots, 3D surface plot, residual verses predicted and normal verses residual were generated (Figures 6-10).

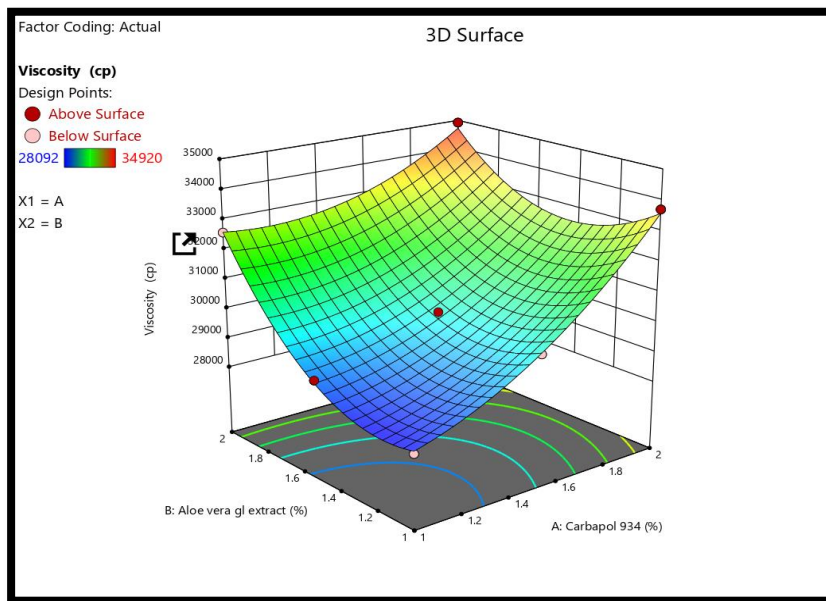


Figure 6: 3D surface response for Y_1 Viscosity

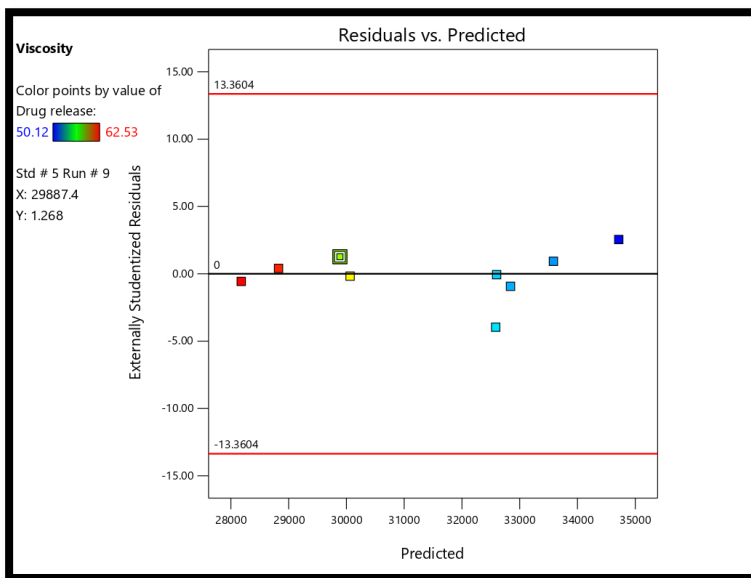


Figure 7: Residual verses Predicted for response Y_1 viscosity

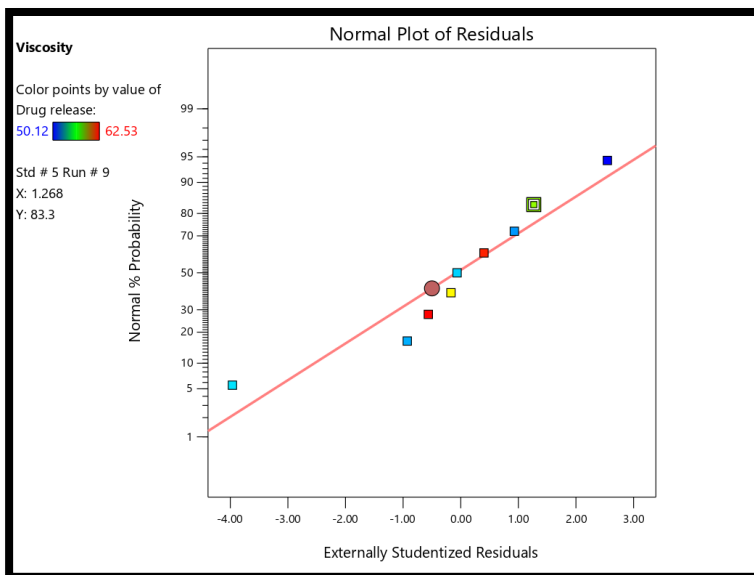


Figure 8: Normal plot versus residual for response Y_1 Viscosity

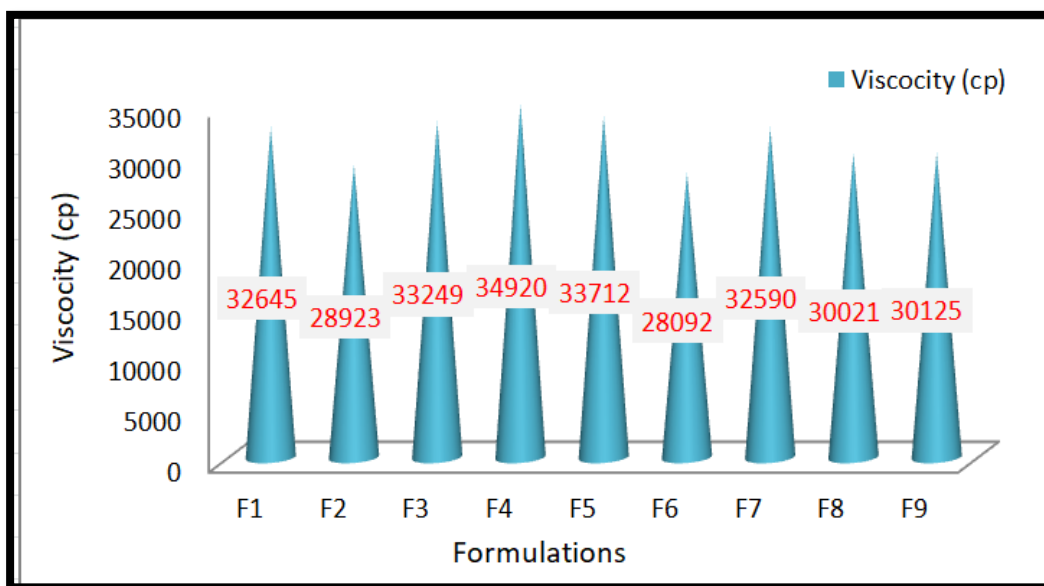


Figure 9: Graphical presentation of all formulation (F1-F9)

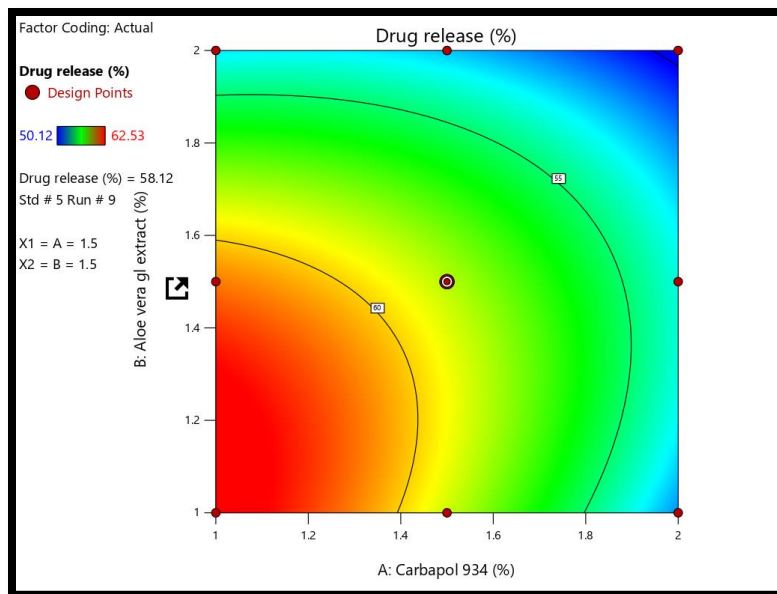


Figure 10: Counter plot for response Y_2 Drug release

The ANOVA result of response Y_1 : Coefficient of determination $R^2=0.9932$, adjusted $R^2=0.9819$, Predicted $R^2=0.9316$ and $p\text{-value}=0.0091$ ($p<0.05$) and for response Y_2 : Coefficient of determination $R^2=0.9813$, adjusted $R^2=0.9501$, Predicted $R^2=0.7823$ and $p\text{-value}=0.00085$ ($p<0.05$) (Tables 6 and 7). From the counter plot presentation, formulation no. 9—was within this appropriate area having viscosity 30125 cp. Based on 3D surface plot concentration of Aloe vera extract was increased the pH of hydrogel also increase, so to maintain pH requirement of Triethanolamine was more and increased viscosity. To increase concentration of Aloe vera gel extract in formulation decrease pH and to maintain pH need to add triethanolamine. So, it increased the viscosity of hydrogel. For responses of drug release the Contour plots, 3D surface plot, residual verses predicted and normal verses residual were generated (Figures 11-14).

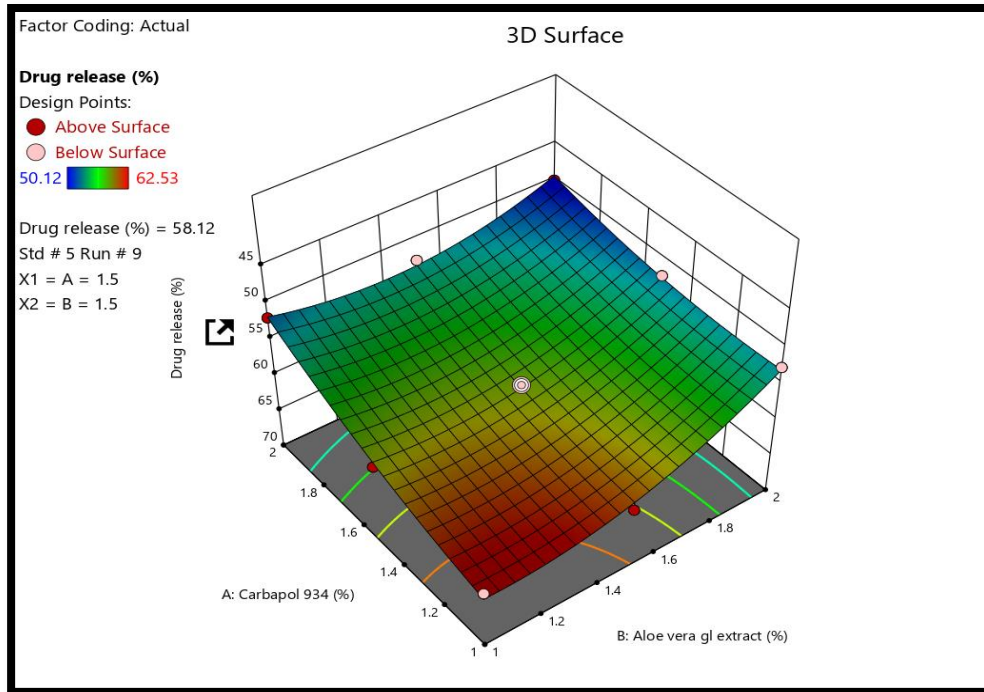


Figure 11: 3D surface response for response Y₂ Drug release

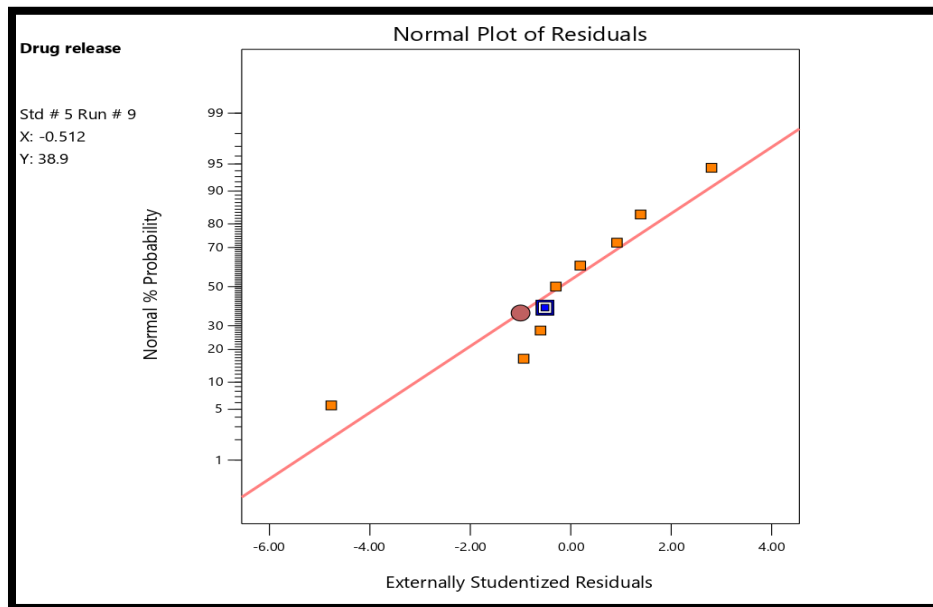


Figure 12: Normal plot verses residual for response Y₂ Drug release

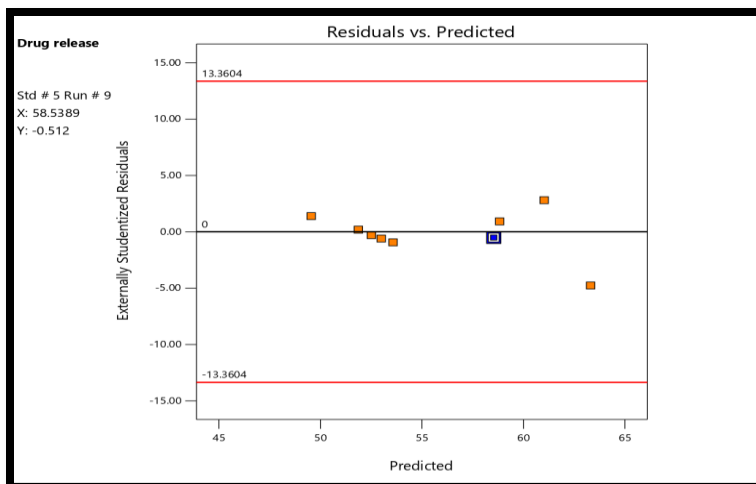


Figure 13: Residual verses predicted for response Y_2 Drug release

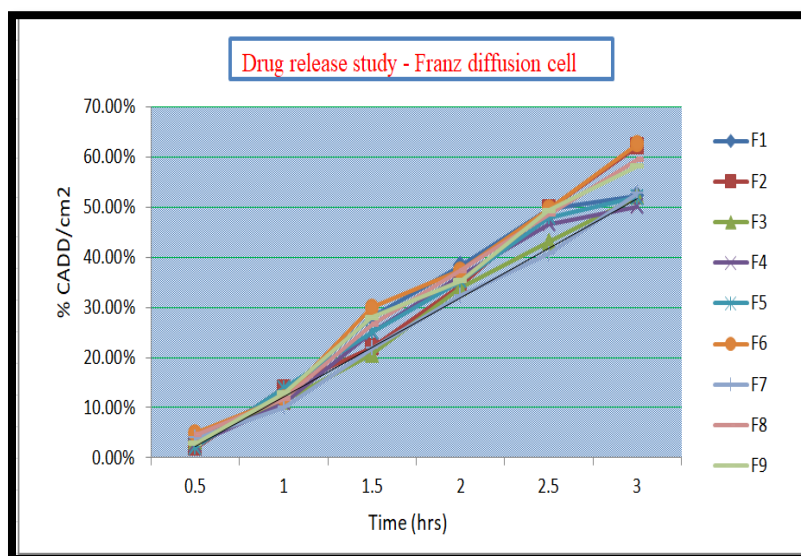


Figure 14: Graphical representation of drug release

From the graphical presentation formulation 9 was given adequate drug release which show in effective area of counter plot. The normal probability plot for both responses, the residuals is normally distributed.

From the Design Space, formulation batch F9 falls under the region of successful operating ranges. Hence formulation F9 (Carbapol 934p-1.5% and Aloe vera gel extract-1.5%) fulfills the criteria of QTPP and CQA for hydrogel formulation. Therefore F9 formulation was selected as optimized formulation. The optimized formulation as evaluated for physical appearance, spreadability, viscosity, drug release and drug content (n=3) (Table 8).

In vitro antifungal activity performed by cylindrical plate method. *Candida albicans* (ATCC 10231) was used for antifungal activity. Sabroud dextrose agar with Chloramphenicol media was used to prepared agar plate. Four Derm Cream was used as a reference sample. The formulation showed antifungal activity having zone inhibition 20 mm (Figures 15 and 16, Table 9).



Figure 15: Zone of inhibition of Reference sample



Figure 16: Zone of inhibition of Hydrogel formulation

The results obtained demonstrated that Hydrogel formulation has good anti-fungal activity. The present research work has demonstrated the successful implementation of QbD approach for the development of hydrogel formulation. The desired QTPP and CQA were predefined in order to obtain the final product with desired quality.

Further it can also be concluded that formulation prepared within the design space will be able to accomplish CQAs in the drug product which further results into the product with desired QTPP

ACKNOWLEDGMENT

The authors are thankful to the Indukaka Ipcowala College of Pharmacy, New V. V. Nagar, Vasu Research Center, Baroda, and Arogya Jyoti Pharma, New V.V. Nagar, Sardar Patel College of Pharmacy, Bakrol and authors also thanks to Dr. Bhavna Patel for their continues support and encouragement.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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