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DEVELOPMENT OF A CURCUMIN OINTMENT FORMULATION WITH ANTIFUNGAL POTENTIAL USING A FACTORIAL DESIGN APPROACH

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Abstract

This study aims to develop an ointment formulation of curcumin with antifungal activity, utilizing a factorial design approach. Curcumin, known for its broad-spectrum antimicrobial properties, was selected as the active ingredient. Various formulation parameters were systematically optimized using factorial design to achieve maximum efficacy and stability. The formulated ointments were evaluated for their physicochemical properties, stability, and antifungal effectiveness against common fungal pathogens. Results demonstrated that the optimized curcumin ointment exhibited significant antifungal activity, suggesting its potential as an effective topical treatment for fungal infections. This research highlights the application of factorial design in the development of potent antifungal formulations.

Keywords: Curcumin, antimicrobial, Antifungal activity, Phytochemical analysis, Pharmacological evaluation

Introduction

Curcumin, a natural compound derived from turmeric, has demonstrated potent antifungal activity against a wide range of fungal pathogens, including Candida species, dermatophytes, and Aspergillus species. The use of curcumin in topical formulations offers several advantages, including its low toxicity, broad-spectrum activity, and potential to overcome drug resistance. Incorporating curcumin into an ointment base provides a convenient and targeted delivery system for localized fungal infections, promoting enhanced efficacy and patient compliance[1].

Numerous studies have explored different strategies to enhance the bioavailability and therapeutic efficacy of curcumin, including nanoparticle formulations, liposomal delivery

systems, and combination therapies.Research on curcumin's antimicrobial properties has focused on its potential as an alternative or adjunctive treatment for microbial infections, including fungal infections[2,3].

Given the rising incidence of fungal infections and the emergence of drug-resistant fungal strains, there is a need for new and effective antifungal agents. Curcumin's broad-spectrum antimicrobial activity and favorable safety profile make it an attractive candidate for the development of novel antifungal formulations. Formulating curcumin into ointments for topical application offers the potential for targeted delivery and localized therapy for fungal skin infections[4,5].

In the present studies research, we utilized a factorial design approach to optimize the formulation of curcumin ointment. Factorial design is a powerful statistical tool that allows for the systematic investigation of the effects of multiple formulation variables and their interactions on the desired characteristics of the final product. This method not only enhances the efficiency of the formulation development process but also ensures a comprehensive understanding of the factors influencing the ointment's performance. Our study focuses on the preparation, optimization, and evaluation of a curcumin ointment using a factorial design[6]. We examined various formulation variables, such as the type and concentration of the ointment base, emulsifiers, and penetration enhancers, to determine their impact on the physicochemical properties, stability, and therapeutic efficacy of the ointment[7-8].

Experimental protocols

Pre-formulation studies

Melting Point: The melting point of Curcumin was determined using the open capillary method. The drug powder sample was packed in a capillary and the melting point was determined in Thies's tube.

Solubility: Drug saturation solubility was determined in distilled water by shake flask method. Excess quantity of Curcumin was added to 10ml Phosphate buffer, it was shaken at a speed of 200 rpm with a mechanical shaker for 24hrs, at room temperature. The solution was centrifuged and absorbance was recorded at 425nm using a UV-visible spectrophotometer (Schimadzu UV 2600), solubility was calculated based on the observations.

UV spectroscopy: Accurately weighed 10 mg of curcumin was accurately weighed and transferred into a 100 mL volumetric flask. Approximately 50 mL of ethanol was added to dissolve the curcumin, and the solution was then made up to volume with ethanol. The resulting solution had a concentration of 100 mcg/mL. Subsequently, serial dilutions of curcumin were prepared at concentrations of 2, 4, 6, 8, and 10 mcg/mL by diluting the standard curcumin solution with ethanol. UV absorbance measurements of all standard solutions were taken at the absorbance maximum of 425 nm, using ethanol as a blank. A calibration curve was constructed using the obtained absorbances and the concentrations of the different standard solutions of curcumin.

FT IR spectrum: Fourier Transform Infrared Spectroscopy, commonly referred to as FTIR Analysis or FTIR Spectroscopy, is an analytical method utilized to identify organic, polymeric, and occasionally inorganic substances. This technique employs infrared radiation to examine test samples and observe their chemical characteristics. FTIR spectra of the pure drug and its formulations with other excipients were acquired by directly placing the drug into the spectrophotometer's cavity and analyzing them within the wavenumber range of 4000-400 cm.

Development of ointment formulation of curcumin: The fusion method is a conventional approach for formulating ointments, which entails measuring the required ingredients, melting the base using a heat source, integrating the Curcumin (API), and including any additional excipients. Stirring is employed to ensure uniform blending and dispersion. Subsequently, the ointment is allowed to gradually cool at room temperature until solidification occurs, preventing crystallization or texture inconsistencies. Once completed, the finalized product is poured or

dispensed into appropriate containers, labeled, and stored under appropriate conditions to preserve stability and effectiveness. This method proves practical for developing ointments with desired components and concentrations, offering versatility for both pharmaceutical and cosmetic applications[9].

The formula used for preparation of ointment is as follow

Table1 .Ingredients of curcumin ointment

Sr.	Ingredients	Quantity
1	Curcumin	75 mg
2	Bess Wax	05 gm
3	Cetostearyl Alcohol	1.5 gm
4	Cetyl Alcohol	1.5 gm
5	Liquid Paraffin	02 gm
Total = 10 gm		

Optimization of curcumin ointment

Factorial design is a methodical experimental technique used to assess the factors involved in a study and determine their relative significance. In a 2^3 full factorial design, for instance, three factors are evaluated at two levels each, and experimental trials are conducted at all possible combinations. This allows researchers to comprehensively examine the impact of different factors on the outcome of interest. In the context of formulation studies, two independent variables are often evaluated using factorial design. These variables may encompass factors such as the type and concentration of ingredients, processing conditions, or other formulation parameters. By systematically varying these variables at different levels and observing the resulting responses, researchers can gain insights into the optimal formulation conditions and the relative importance of each factor in influencing the desired outcome[10].

2^3 Factorial Design-

Table2 Possible Combinations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Curcumin	75mg	75mg	75mg	75mg	75mg	75mg	75mg	75mg
Bess wax	3 gm	5 gm	5 gm	3 gm	5 gm	5 gm	3 gm	3 gm
Ceostearyl Alcohol	1.5 gm	1.5 gm	2 gm	1.5 gm	2 gm	1.5 gm	2 gm	2 gm
Cetyl Alcohol	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm
Liquid Paraffin	1.5 gm	1.5 gm	1.5 gm	2 gm	2 gm	2 gm	2 gm	1.5 gm

Factor A: Conc. of Bess wax (X1)
Factor B: Conc. of Ceostearyl Alcohol (X2)
Factor C: Conc. of Liquid Paraffin (X3)
In total, 8 experiments were conducted

Physiochemical Characterization of the Formulation

Physical examination

A visual examination was conducted on the prepared ointment formulations to assess their color, uniformity, and texture consistency.

Determination of Ph

The pH of the prepared ointment was determined using a pH meter, which had been previously calibrated with a pH 7 buffer solution before use. About 0.5g of the ointment was

weighed and dissolved in 50.0 ml of distilled water and set aside for 2 h and then the pH was measured by dipping electrode tip into the ointment. This measurement was conducted in triplicate, and the mean value was calculated.
The pH meter was calibrated using standard buffer solution.

Spreadability test

The spreadability of the ointment was evaluated by placing a measured sample between two glass slides and applying a 500 g weight for about five minutes. Once no further spreading occurred, the initial and final diameters of the spread circle were measured in centimetres to assess spreadability. The spreadability was determined using the formula provided below:

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

Where;
S = Spreadability
M = Weight tide to the upper slide
L = Length of glass slide
T = Time taken to separate the slides

Determination of Viscosity

The viscosity of the prepared ointment formulations was assessed using a Brookfield viscometer DV-III ULTRA (Brookfield Engineering Laboratories, USA) equipped with spindle no. 64. Viscosity measurements were conducted in centipoises (cps) at a speed of 10 rpm for 1 minute, with the temperature maintained at 25ºC. A 20-gram sample was used for each measurement[11,12].

***In-vitro* Drug release study**

The release rate of Curcumin Ointment encapsulated in capsule shells was examined in vitro using a USP class I dissolving apparatus (TDL-08L Electrolab, India). Each jar contained 900 milliliters of dissolving medium (pH 7.4), consisting of methanolic phosphate buffer, and one capsule was added to each jar. The mixture was continuously stirred at 50 revolutions per minute for eight hours at a temperature of 37.0±0.5 °C. At various intervals, a 5-milliliter sample was withdrawn as an aliquot, and an equal volume was replenished with fresh methanolic phosphate buffer. Spectrophotometric analysis of the emitted Curcumin was conducted by measuring absorbance at 425 nm, the λmax. Release data were then analyzed using various release kinetic models, including zero-order, first-order, and Higuchi, to determine the mechanism of drug release from the capsules[13].

Antifungal activity against Aspergillus species

The Potato Dextrose agar plate method is a common technique used to determine the zone of inhibition of antifungal ointments against fungal growth. The zone of inhibition test is a method to assess the effectiveness of antifungal ointments. It involves preparing Potato Dextrose Agar (PDA) plates, spreading the ointment evenly, labeling each plate, incubating at a temperature conducive to fungal growth, and measuring the zone of inhibition. The effectiveness of the ointment is evaluated by comparing it with known standards or previously tested agents. Replicating the experiment ensures reliability. A sample of ointment (0.013 g) equivalent to 1o mcg) was aseptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. In the control set, no extract was used. After the solidification of the media, one inoculum disc of the test fungus was aseptically inoculated upside down at the center of the petriplate and incubated at 25 $\pm 2^0$C
The average diameter of the fungal colonies were measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated as follows;

$$\%\ \boldsymbol{Growth\ Inhibition} = \frac{\boldsymbol{gc - gt}}{\boldsymbol{gc}} \times \mathbf{100}$$

Where;

gc : Growth of colony in control set after incubation period subtracting the diameter of inoculum disc.

gt : Growth of colony in treatment set after incubation period subtracting the diameter of inoculum disc.

Results and Discussion

Melting point:

The melting point of Curcumin was determined using the capillary method. The melting point was found to be in range between 181°C and 183°C, which is identical to the reported melting point.

Table3: *Melting Point of Drug by Capillary Method*

Sample	Melting point (Test)	Melting point (Reference)
Curcumin	182ºC±2ºC	179-182ºC

Solubility

The Saturated solubility of Curcumin was determined in distilled water and found to be 0.163µg/mL. According to the BCS classification system the solubility of drug in water is below the significant solubility value and hence the drug is said to be poorly water soluble. Curcumin is a BCS class 2 drug. Solubility of the Curcumin is pH dependent and solubility increases with increased pH. The aqueous solubility profile of Curcumin exhibits low solubility across pH range of 1 to 6.5.

UV Spectroscopy:

Calibration curve for the curcumin was plotted using uvabsorbances at different concentrations of standard curcumin solutions. Absorbances were recorded at wavelength of maximum absorbance that is 425 nm. Absorbances of different standard solutions of curcumin in recorded in ethanol are summarized in following table.

Table4: *UV absorbances of curcumin standard solutions*

Sr.	Concentration (mcg/mL)	Absorbance
1.	0	0
2.	2	0.07±0.0002
3.	4	0.187±0.0004
4.	6	0.312±0.0002
5.	8	0.47±0.002
6.	10	0.63±0.0003

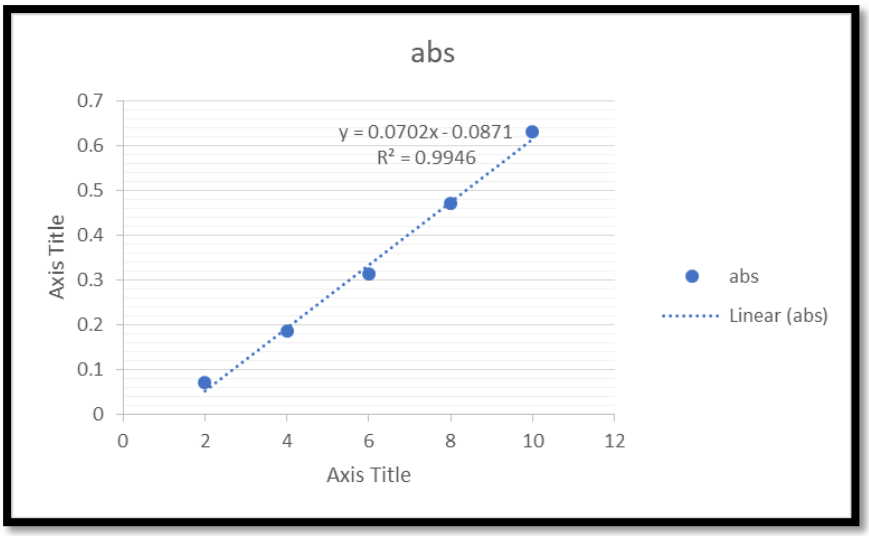

Figure1. Calibration Curve of Curcumin standard solution

FT IR Spectrum

FTIR of pure curcumin recorded in the range of 4000 to 400 cm^{-1}. Key bands observed in IR spectrum of curcumin are summarised in following table

Table5: *FTIR spectrum of curcumin*

Sr.	**Functional group**	**IR ranges**
1	-O-H stretching	3512 cm^{-1}
2	-C-H stretching aromatic	3014 cm^{-1}
3	-C-H stretching aliphatic	2872, 2958 cm^{-1}
4	C=O stretching	1631cm^{-1}
5	C=C stretching vibration	1597cm^{-1}
6	C-O stretching vibration	1315cm^{-1}

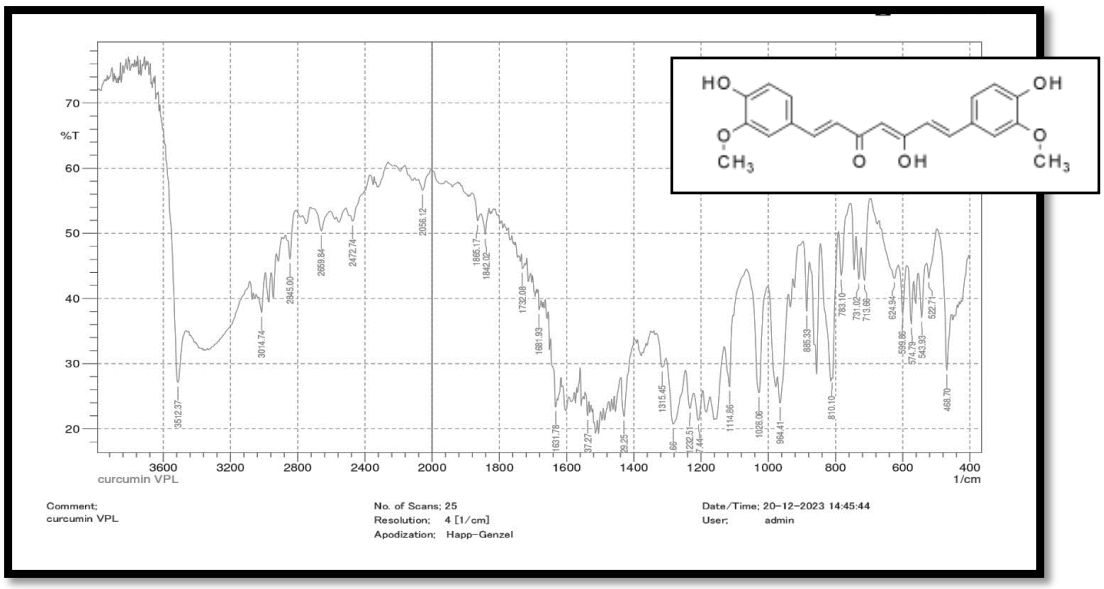

Figure2. FT IR spectrum of Curcumin

It is evident from the infra red spectrum of curcumin that band appearing at 3512 cm^{-1} is may be due to -O-H group (alcoholic -OH), whereas aromatic C=C stretching vibrations at 1631 cm-1 and a high intensity band at 1537 cm-1 are ascribed to mixed vibrations, which also include stretching carbonyl bond vibrations ν (C=O). Additionally, a notable strong band at 1315 cm^{-1} is ascribed to the ν (C-O) phenolic band's bending vibration.

Formulation of Curcumin Ointment:

The fusion method is a traditional method for creating ointments, which involves weighing out the necessary ingredients, melting the base over a heat source, incorporating the Curcumin (API), and adding any additional excipients. Curcumin ointment was prepared by the following formula

Preparation of Curcumin Ointment by Fusion method-

Table6: Ingredients of ointment

Sr No.	Ingredients	Quantity
1	Curcumin	75mg
2	Bess Wax	5gm
3	Cetostearyl Alcohol	1.5gm
4	Cetyl Alcohol	1.5gm
5	Liquid Paraffin	2gm
	Total	10gm

2^3 Factorial Design-

Table7: *Possible Combinations*

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Curcumin	75mg	75mg	75mg	75mg	75mg	75mg	75mg	75mg
Bess wax	3 gm	5 gm	5 gm	3 gm	5 gm	5 gm	3 gm	3 gm
Ceostearyl Alcohol	1.5 gm	1.5 gm	2 gm	1.5 gm	2 gm	1.5 gm	2 gm	2 gm
Cetyl Alcohol	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm	1.5 gm
Liquid Paraffin	1.5 gm	1.5 gm	1.5 gm	2 gm	2 gm	2 gm	2 gm	1.5 gm

Factorial design is an experimental design technique by which the factor involved and their relative importance can be assessed. A 2^3 full factorial design containing 3 factors evaluated at two levels and the experimental trials were performed at all possible combinations.
The two independent formulation variables evaluated included:

Factor A: Conc. of Bess wax (X1)
Factor B: Conc. of Ceostearyl Alcohol (X2)
Factor C: Conc. of Liquid Paraffin (X3)
In total, 8 experiments were conducted.

Experimental domain-

Table8: Experimental domain

Model	**Actual Values**		**Coded Values**	
Factor	Low level	High level	Low level	High level
A	3	5	-	+
B	1.5	2	**-**	**+**
C	1.5	2	**-**	**+**

Response Surface Graph:
Interpretation:
Response 1 %CDR
ANOVA for selected factorial model

Table9:*Analysis of variance table [Partial sum of squares - Type III]*

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob> F**	
Model	541.37	1	541.37	10.74	0.0169	significant
BC	541.37	1	541.37	10.74	0.0169	
Residual	302.34	6	50.39			
Cor Total	843.71	7				

Factor coding is **coded**.
Sum of squares is **Type III - Partial**
The **Model F-value** of 7.87 implies the model is significant.
There is only a 3.74% 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, 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.

Table10: Fit Statistics

Std. Dev.	7.1	R^2	0.6417
Mean	81.85	Adjusted R^2	0.5819
C.V.%	8.67	Predicted R^2	0.3629
		Adeq Precision	46354

The **Predicted R^2** of 0.3629 is not as close to the **Adjusted R^2** of 0.5819 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 4.635 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

$$\%CDR = +81.85-8.23BC$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

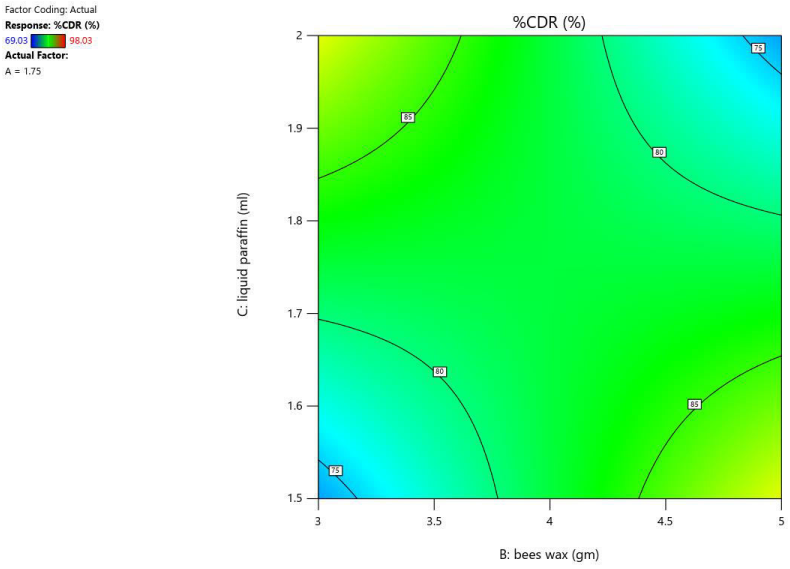

Figure3. Contour plot showing the influence of Crosspovidone: Liquid Paraffin and Bess wax on %CDR

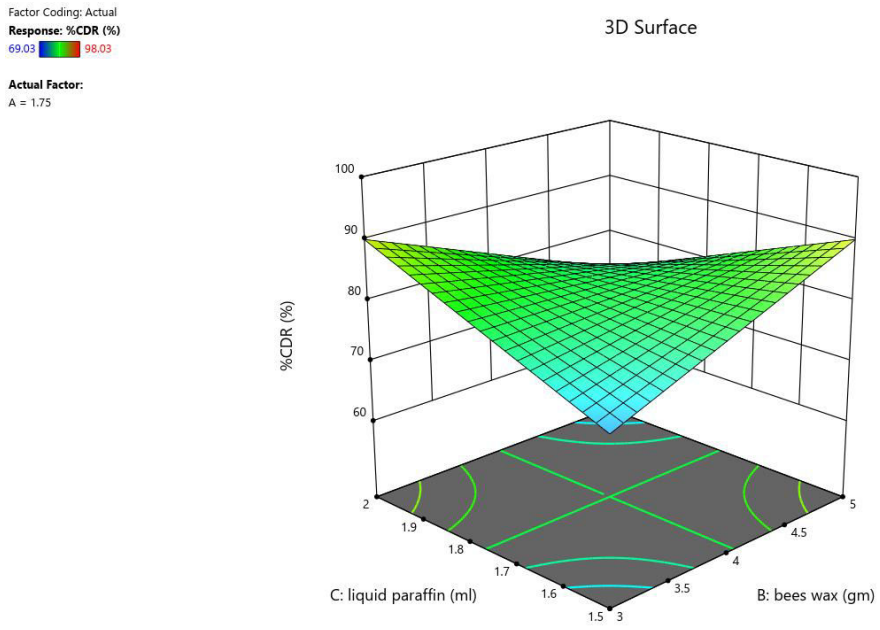

Figure9. Response surface plot showing the influence of retention period of Ointment

Table11: *Analysis of variance table [Partial sum of squares - Type III]*

Source	**Sum of Squares**	**Df**	**Mean Square**	**F- value**	**P-value**	
Model	9.123E+07	3	3.041E+07	7.87	0.0374	**Significant**
A-cetostearyl alcohol	3.653E+07	1	3.653E+07	9.45	0.0372	
C- liquid paraffin	1.867E+07	1	1.867E+07	4.83	0.0929	
AB	3.603E+07	1	3.603+07	9.32	0.0379	
Residual	1.546E+07	4	3.866E+06			
Cor Total	1.067E+08	7				

Factor coding is **coded**.Sum of squares is **Type III - Partial**
The **Model F-value** of 7.87 implies the model is significant. There is only a 3.74% 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, 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.

Table12: Fit Statistics

Std. Dev.	1966.24	**R^2**	0.8551
Mean	35764.25	**Adjusted R^2**	0.7464
C.V. %	5.50	**Predicted R^2**	0.4202
		Adeq Precision	8.3242

The **Predicted R^2** of 0.4202 is not as close to the **Adjusted R^2** of 0.7464 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or

a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.324 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

Viscosity = +35764.25+2136.75A+1527.75-2122.25AB

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

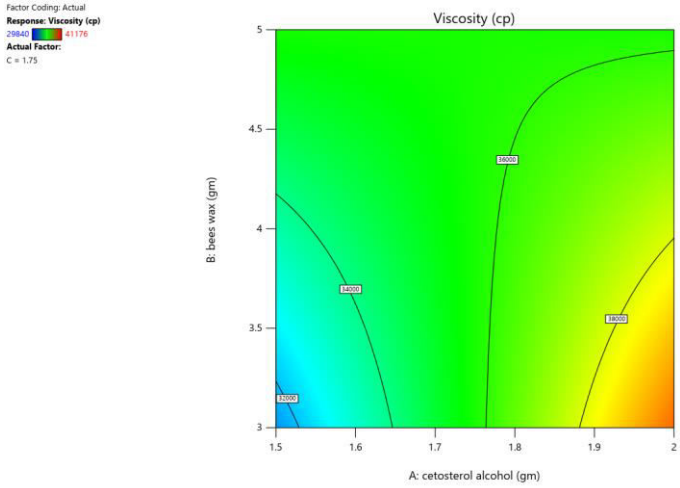

Figure4. Contour plot showing the influence of Viscosity

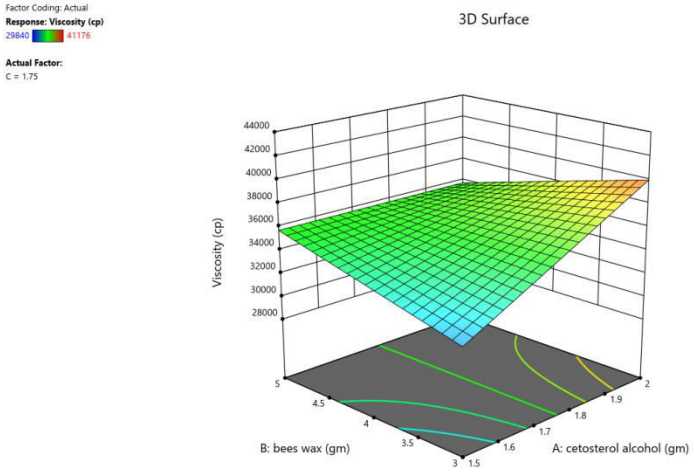

Figure5. Response surface plot showing the Viscosity

Evaluation ointment

Physical Examination:

The Prepared ointment formulations were inspected visually for their colour, homogeneity, and consistency.

Measurement of viscosity:
The viscosity of the prepared ointment formulations was determined using Brook Field viscometer DV-III ULTRA (Brookfield Engineering laboratories, USA) using spindle no. 64. The viscosity was measured in centipoises (cps) at 10 rpm for 1 minute and temperature 25ºC using 20 gram sample.

Spread ability:
The ointment's spreadability was assessed by sandwiching a weighed sample between two glass slides and covering them with a 500 g weight for approximately five minutes. There was to be no more spreading after that. The initial and final spread circle diameters were measured in centimeters and used as benchmarks for spreadability[15].

Table13: *Results of pH, Viscosity, Spread ability*

Formulation	pH	Viscosity	Spread ability
F1	7.2±0.2	29840±7.1	36.31±1.3
F2	6.36±0.3	32646±6.4	28.49±0.6
F3	6.8±0.9	33284±7.8	39.54±1.02
F4	7.09±0.1	34028±6.7	18.7±1.07
F5	6.53±0.5	37416±1.3	29.64±0.74
F6	7.12±0.4	37996±6.3	22.15±1.22
F7	6.8±0.9	39728±7.1	32.87±1.8
F8	6.22±0.18	41176±7.4	42.38±0.75

***In-vitro* Drug release study:**
Curcumin Ointment filled in a capsule shell was studied for release rate in vitro using a USP class I dissolving equipment (TDL-08L Electrolab, India). A spectrophotometric analysis of the Curcumin emitted was conducted by measuring the absorbance at 425 nm. By plugging the release data into various release kinetic models, including zero-order, first-order, and Higuchi, the mechanism of drug release from the capsules was ascertained.

Table14. *In vitro* drug release study of optimized batch of Curcumin Ointment

Time (hr.)	Abs	Conc.	Conc. 25ml	Cf.	µ/cm²	mg/mL	Dose % in mL	%CDR
0.25	0.06	2.095442	52.38604	52.74217	47.94743	0.047947427	0.5	9.589485
0.5	0.072	2.266382	56.65954	109.4017	99.4561	0.099456104	0.5	19.89122
1	0.084	2.437322	60.93305	170.3348	154.8498	0.154849784	0.5	30.96996
1.5	0.097	2.622507	65.56268	235.8974	214.4522	0.214452219	0.5	42.89044
2	0.112	2.836182	70.90456	306.802	278.9109	0.278910908	0.5	55.78218
4	0.122	2.978632	74.46581	381.2678	346.6071	0.346607101	0.5	69.32142
6	0.135	3.163818	79.09544	460.3633	418.512	0.418512048	0.5	83.70241
8	0.144	3.292023	82.30057	542.6638	493.3307	0.493330748	0.5	98.66615

Antifungal activity
The Potato Dextrose agar plate method is a common technique used to determine the zone of inhibition of antifungal ointments against fungal growth. It is evident from the antifungal activity data that,curcumin exhibited higher inhibition of growth[16].

Table15: Antifungal activity of formulated ointment

Sr.	Sample	Zone of inhibition (mm) Against Fusariumsolani
01	Curcumin Ointment	18
02	Nystatin	19
03	DMSO solvent	02

Conclusion:

The study demonstrated that the developed formulation (F8) was found the best formulation based on optimization of drug content uniformity and exhibited potential antifungal potential against the survivability of the Fusariumsolani. strains. F_8 formulation batch showed acceptable results. The in-vitro drug release was studied with USP class 1 dissolution apparatus in pH 7.4 buffer solution. Results showed that formulations F_8 (98.6%) at 8 hr.

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