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DESIGN AND CHARACTERIZATION OF BIOADHESIVE ACYCLOVIR HYDROGEL INCORPORATING HONEY, CARBOPOL, AND CHITOSAN FOR PROLONGED SKIN DELIVERY

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Acyclovir, Hydrogel, Topical Delivery,Box–Behnken Design,Drug Release,Viscosity,Spreadability, Stability Study,Controlled Release,Antiviral Formulation

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Abstract

The present study aimed to formulate and evaluate acyclovir-loaded hydrogels for effective topical delivery using a Box–Behnken Design (BBD). Seventeen formulations (F1–F17) were developed and optimized based on drug content and spreadability. Among these, formulations F2, F9, and F16 were selected for further evaluation based on their favorable physicochemical properties. The pH, viscosity, extrudability, and in-vitro drug release of the optimized formulations were assessed to determine their suitability for topical use. The in-vitro drug release profile revealed sustained drug release over 12 to 24 hours, with F16 showing the highest release rate (98.45%), while F9 exhibited the most controlled release pattern. Kinetic analysis confirmed that the release followed first-order and Korsmeyer–Peppas models, indicating a non-Fickian diffusion mechanism. Stability studies over 90 days showed minimal changes in drug content and viscosity, confirming the stability of the optimized formulations under different storage conditions. These findings suggest that acyclovir hydrogels, particularly F9, offer a promising and stable system for sustained topical antiviral therapy.

INTRODUCTION

Topical drug delivery systems have gained substantial interest due to their ability to deliver drugs directly to the affected site, minimizing systemic side effects and improving patient compliance. Among these systems, hydrogels are considered promising carriers owing to their high water content, biocompatibility, and ease of application on the skin [1]. Hydrogels can efficiently encapsulate both hydrophilic and hydrophobic drugs and provide controlled or sustained drug release, making them suitable for the treatment of chronic skin conditions and viral infections [2].

Acyclovir, a synthetic purine nucleoside analogue, is widely used as an antiviral agent for the treatment of herpes simplex virus

(HSV) infections and varicella-zoster virus (VZV) infections [3]. Despite its efficacy, acyclovir suffers from poor oral bioavailability (15–30%) due to limited solubility and extensive first-pass metabolism [4]. Topical formulations can overcome these limitations; however, conventional creams and ointments often exhibit poor skin retention and rapid drug clearance from the site of action [5]. Therefore, developing a sustained-release topical delivery system such as a hydrogel becomes crucial for enhancing the therapeutic efficacy of acyclovir.

Natural polymers such as chitosan and honey are being increasingly integrated into pharmaceutical hydrogels due to their bioadhesive, wound-healing, and antimicrobial properties [6,7]. Chitosan, derived from chitin, offers excellent film-forming and controlled release capabilities, while honey acts not only as a humectant but also enhances healing due to its antimicrobial and antioxidant activity [8]. Combining synthetic polymers like Carbopol with natural bioactives enables the formulation of hybrid hydrogels with improved structural integrity and therapeutic performance.

In this study, an attempt was made to formulate and evaluate a hydrogel containing acyclovir using Carbopol 934P, chitosan, and honey, aiming to achieve sustained drug release, improved patient compliance, and enhanced skin penetration. The formulation was optimized using statistical design, and the prepared hydrogels were evaluated for physicochemical properties, drug release behavior, and stability.

Field of the Invention

The present invention relates to the field of pharmaceutical formulation and drug delivery systems. More particularly, it pertains to the development of a stable hydrogel formulation containing Acyclovir for topical application, using natural honey, Carbopol, and chitosan as excipients to enhance skin permeability, improve antiviral activity, and provide sustained drug release.

Background of the Invention

Acyclovir is a well-known antiviral agent used in the treatment of herpes simplex and varicella-zoster infections. However, its therapeutic efficacy is limited by poor skin permeability and low bioavailability when applied topically. Conventional ointments and creams often fail to maintain adequate drug concentration at the infection site. There is a need for a novel hydrogel-based delivery system that enhances drug permeation through the skin, ensures sustained release, and improves patient compliance.

Natural polymers such as honey and chitosan, in combination with Carbopol, offer an excellent biocompatible and bioadhesive matrix for hydrogel formulation. Honey provides natural humectant, antioxidant, and antimicrobial properties, while chitosan improves mucoadhesion and permeability, thereby synergistically enhancing the therapeutic potential of Acyclovir.

Material and Methods

Material

The following chemicals and reagents were utilized in the formulation and evaluation of the acyclovir-loaded hydrogel. Acyclovir was procured from Bioplus Life Sciences Pvt. Ltd., Bangalore. Di-potassium hydrogen orthophosphate, sodium chloride, Carbopol 934P, and propylene glycol were sourced from S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform were obtained from Qualigens Fine Chemicals, Mumbai. Honey, used as a natural therapeutic agent, was supplied by Vindhya Herbal, Bhopal. Chitosan, a natural polymer used for gel formation, was purchased from Himedia Pvt. Ltd., Thane, Maharashtra.

Methods

Optimization of Acyclovir Loaded Hydrogel

The Box–Behnken Design, using Design Expert® software (version 11.0.4.0, Minneapolis, MN, USA), assessed the relationships between independent variables, i.e., Chitosan (A), Carbopol (B), and Honey (C), at three levels (−1, 0, +1), and dependent responses, i.e., Drug Content (R_1), Spreadability (R_2). Quadratic models were found to be the best fit for both the independent and dependent responses. According to Box–Behnken Design, the compositions of various trial runs of Chitosan, Carbopol, and Honey are tabulated 4.3. These findings confirm this study's independent variables. The quadratic model describes the link between independent factors and dependent responses.

Formulation of Acyclovir-Honey Chitosan Hydrogel

Acyclovir-honey chitosan hydrogels were prepared using the cold mechanical method. The formulation process began with the preparation of a hydrogel base [9]. Appropriate amounts of Carbopol 934 and chitosan (ranging from 50 mg to 150 mg for chitosan and 250 mg to 500 mg for Carbopol, depending on the formulation) were dispersed in 20 ml of purified water. The dispersion was stirred continuously using a magnetic stirrer for one hour to allow complete hydration and dispersion of the polymers. To adjust the pH

of the hydrogel and facilitate gel formation, triethanolamine (TEA, 0.5 mg) was added dropwise under continuous stirring. A preservative, methyl paraben (20 mg), was then incorporated to prevent microbial growth. The mixture was left undisturbed for 24 hours at room temperature to allow for complete swelling and equilibration of the polymers.

After the swelling period, the active drug acyclovir and varying concentrations of honey (ranging from 10 mg to 30 mg) were added to the gel base. The mixture was stirred thoroughly to ensure uniform dispersion of the drug and honey throughout the hydrogel matrix. The final volume of each formulation was brought up to 100 g with purified water to standardize the batch weight.

The prepared hydrogels were transferred into wide-mouthed glass containers with screw-cap lids and stored in a refrigerator. This step ensured the completion of hydrogel formation and maintained the physical stability of the formulation during storage.

Formulation design using box-behnken design

A total of 17 hydrogel formulations were developed as per the Box-Behnken Design (BBD) to study the influence of three independent variables chitosan, Carbopol 934, and honey concentrations on the formulation characteristics. All formulations included constant amounts of triethanolamine (0.5 mg), methyl paraben (20 mg), and purified water (up to 100 ml or g), with only the polymer and honey concentrations varied.

The formulation codes (F1 to F17) represented different combinations of chitosan (50 mg, 100 mg, and 150 mg), Carbopol 934 (250 mg, 375 mg, and 500 mg), and honey (10 mg, 20 mg, and 30 mg) [10]. These combinations were arranged in randomized runs to eliminate bias and ensure robust statistical analysis. For example, F1 contained 100 mg chitosan, 375 mg Carbopol, and 20 mg honey, whereas F5 contained 50 mg chitosan, 375 mg Carbopol, and 30 mg honey. Each formulation was prepared following the same procedure described above, and the prepared batches were analyzed further for their physicochemical and biological properties.

This systematic design approach allowed for the identification of optimal concentrations of polymers and honey required to produce a hydrogel with desired drug release characteristics, consistency, and therapeutic efficacy.

Table 1: Design matrix in Box-Behnken design for hydrogel preparation

F. Code	Std	Run	Factor 1: Chitosan (mg)	Factor 2: Carbopol 934(mg)	Factor 3: Honey (mg)
F1	5	1	-1	0	-1
F2	7	2	-1	0	1
F3	4	3	1	1	0
F4	16	4	0	0	0
F5	14	5	0	0	0
F6	2	6	1	-1	0
F7	13	7	0	0	0
F8	1	8	-1	-1	0
F9	15	9	0	0	0
F10	12	10	0	1	1
F11	3	11	-1	1	0
F12	6	12	1	0	-1
F13	10	13	0	1	-1
F14	8	14	1	0	1
F15	9	15	0	-1	-1
F16	11	16	0	-1	1
F17	17	17	0	0	0

Formulation runs and responses of various compositions as per BBD

Table 2: Formulation runs and responses of various compositions as per BBD

F. Code	Std	Run	Factor 1: Chitosan (mg)	Factor 2: Carbopol 934 (mg)	Factor 3: Honey (mg)	TEA (mg)	Methyl Paraben (mg)	Water (ml)
F1	15	1	100	375	20	0.5	20	100
F2	3	2	50	500	20	0.5	20	100

F3	4	3	150	500	20	0.5	20	100
F4	17	4	100	375	20	0.5	20	100
F5	7	5	50	375	30	0.5	20	100
F6	14	6	100	375	20	0.5	20	100
F7	1	7	50	250	20	0.5	20	100
F8	2	8	150	250	20	0.5	20	100
F9	12	9	100	500	30	0.5	20	100
F10	11	10	100	250	30	0.5	20	100
F11	6	11	150	375	10	0.5	20	100
F12	5	12	50	375	10	0.5	20	100
F13	13	13	100	375	20	0.5	20	100
F14	9	14	100	250	10	0.5	20	100
F15	8	15	150	375	30	0.5	20	100
F16	10	16	100	500	10	0.5	20	100
F17	16	17	100	375	20	0.5	20	100

Final equation in terms of coded factors

Drug Content = +93.49-0.0800 A+1.72 B-0.2837 C-1.91 AB-0.3000 AC+0.2975 BC+0.8102 A^2+1.44 B^2+1.05 C^2

Final equation in terms of actual factors

Drug Content = +98.63500+0.059880 Chitosan-0.029782 Carbopol-0.476725 Honey-0.000305 Chitosan * Carbopol-0.000600 Chitosan * Honey+0.000238 Carbopol * Honey+0.000324 $Chitosan^2$+0.000092 $Carbopol^2$+0.010478 $Honey^2$.

Selection of factors and responses

Factors								
Factor	**Name**	**Unit**	**Minimum**	**Maximum**				
A	Chitosan	mg	50.00	150.00				
B	Carbopol 934	mg	250.00	500.00				
C	Honey	mg	10.00	30.00				
Responses								
Response	**Name**	**Unit**	**Observ**	**Minimu**	**Maximu**	**Mea**	**SD**	**Model**

			ations	m	m	n		
R1	Drug Content	%	17	92.56	98.85	95.05	1.94	Quadratic
R2	Spreadability	g.cm/sec	17	5.22	8.02	6.83	0.8495	Quadratic

Build Information			
File Version	11.0.4.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	22.00		

Final equation in terms of coded factors

Spreadability = +7.34-0.3063 A-0.5438 B+0.2500 C+0.4225 AB+1.04 AC -0.1750 BC-0.0808 A^2 -0.5658 B^2-0.4533C^2

Final equation in terms of actual factors

Spreadability = +7.50500-0.066615 Chitosan+0.018846 Carbopol+0.050800 Honey+0.000068 Chitosan * Carbopol+0.002080 Chitosan * Honey-0.000140 Carbopol * Honey-0.000032 $Chitosan^2$ -0.000036 $Carbopol^2$ -0.004533 $Honey^2$

Characterization of Hydrogel

pH measurements

The pH of the selected optimized formulations was measured using a digital pH meter. Prior to each measurement, the pH meter was calibrated using standard buffer solutions with pH values of 4.0, 7.0, and 9.0 to ensure accuracy [11]. Following calibration, the electrode was carefully immersed into the formulation until it was fully submerged. The pH value of the formulation was then recorded directly from the digital display of the instrument.

Measurement of viscosity

The viscosity of the formulated topical hydrogels was evaluated using a Brookfield viscometer, which is a standard instrument widely used for rheological characterization of semi-solid formulations. For the measurement, spindle number 63 was employed, which is suitable for moderately viscous materials like hydrogels [12]. The

instrument was operated at a constant speed of 10 revolutions per minute (rpm), ensuring uniform shear conditions across all samples. Prior to analysis, the hydrogel samples were allowed to equilibrate at room temperature to avoid temperature-induced variations in viscosity. Approximately 30–50 grams of each formulation were transferred into the sample container of the viscometer, ensuring that the spindle was adequately immersed in the gel without touching the bottom or sides of the container. Each sample was subjected to rotational shear at the specified speed, and the resistance offered by the hydrogel to the spindle's movement was recorded as viscosity in centipoise (Cps). This parameter is significant for evaluating the spreadability, stability, and ease of application of the gel on the skin. The results of the viscosity measurements for all selected formulations are summarized in Table.

Drug content

Accurately weighed amount of gel formulation equivalent to 100 mg of topical Hydrogel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using calibration curve method [13]. Drug content of topical Hydrogel based gel is shown in table.

Extrudability study

Extrudability of the formulated gels was assessed to evaluate their ease of application from collapsible tubes, which is an important parameter influencing patient compliance and product usability [14]. The test was conducted by filling the gel into a standard collapsible aluminum tube, which was then sealed properly to prevent leakage. To determine extrudability, a specific weight was gradually applied to the sealed tube, and the amount of gel extruded from the nozzle was measured. The force required to extrude the gel and the quantity of gel expelled under a fixed weight were noted. A higher quantity of gel extruded under a constant load indicates better extrudability, reflecting the gel's suitability for effortless dispensing and application.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response [15]. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 5 g of

gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadibility show lesser time to spread.

$$Spreadibil\,ity\,(g.cm/\sec) = \frac{Weight\ tide\,to\,Upper\ Slide \times Lenth\ moved\,on\,the\,glass\ slide}{Time\ taken\,to\,slide}$$

***In-vitro* diffusion study**

The in-vitro diffusion study was conducted using a Franz Diffusion Cell to evaluate the drug release characteristics of the formulated hydrogel [16-17]. An egg membrane was used as a semi-permeable barrier to simulate biological conditions.

The Franz diffusion apparatus consisted of a receptor compartment with an approximate volume of 60 mL and a diffusion surface area of 3.14 cm^2.

To initiate the study, the egg membrane was carefully positioned between the donor and receptor compartments. A pre-weighed patch of the hydrogel formulation, with an area of 2 cm^2, was placed on the membrane in the donor compartment, ensuring that the drug-loaded side was in direct contact with the membrane. The receptor compartment was filled with phosphate buffer (pH 5.5), chosen to mimic physiological conditions relevant for topical application. The entire setup was maintained at a constant temperature of 32 ± 0.5°C by circulating water through the jacketed receptor compartment, using a thermostatically controlled hot plate. Continuous stirring of the receptor medium was achieved using a Teflon-coated magnetic bead to ensure uniform drug distribution throughout the solution.

At predetermined time intervals, aliquots were withdrawn from the receptor compartment for analysis. Each withdrawn sample was immediately replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. The collected samples were analyzed using a UV-visible spectrophotometer at a wavelength of 243 nm to determine the concentration of drug diffused across the membrane. This procedure provided valuable insights into the drug release profile and permeation characteristics of the hydrogel formulation.

Stability Study

The optimized formulation of acyclovir hydrogel was subjected to accelerated stability studies under two storage conditions: refrigeration at 4 ± 0.5°C and ambient room temperature at 28 ± 0.5°C. The formulations were stored in screw-capped, amber-colored glass bottles, and evaluated over a period of 0, 15, 30, 60, and 90 days. At each time interval,

samples were analyzed for changes in drug content and viscosity. Drug content was determined spectrophotometrically to indirectly assess the amount of acyclovir retained in the hydrogel, indicating the stability and entrapment efficiency over time. Additionally, the viscosity of the formulations was measured using a Brookfield viscometer to observe any rheological changes due to storage temperature and duration. These evaluations helped assess the effect of storage conditions on the physical and chemical stability of the hydrogel formulation.

Results and Discussion

The prepared acyclovir-loaded hydrogels were evaluated for various physicochemical and performance parameters to assess their suitability for topical application. Seventeen formulations (F1–F17) were developed based on a Box–Behnken design, and their drug content and spreadability were initially assessed. As per the results (Table 3), the drug content ranged from 92.56% to 98.85%. Among these, formulation F2 showed the highest drug content (98.85%), which indicates effective entrapment and uniform drug dispersion in the hydrogel matrix. The spreadability values varied between 5.22 and 8.02 g·cm/sec, with formulation F7 exhibiting the highest spreadability. An ideal topical formulation should have sufficient spreadability to ensure ease of application, and F2, F9, and F16 demonstrated balanced properties in terms of both drug content and spreadability, making them suitable candidates for further evaluation.

To validate the design model, a comparison of experimental and predicted values was performed (Table 4). The results confirmed that the actual and predicted values were in close agreement, with minimal percentage error. This validated the reliability and predictability of the response surface methodology used for formulation optimization.

Further characterization of the selected formulations F2, F9, and F16 revealed pH values within the skin-friendly range of 6.76 to 7.12 (Table 5), ensuring that the hydrogels would not cause skin irritation. Viscosity values indicated that F9 had the highest viscosity (6698 cps), contributing to greater gel strength and sustained release, while F16 exhibited a relatively lower viscosity (5369 cps), promoting better spreadability. Extrudability tests showed that F2 had the best performance (210 g), suggesting ease of dispensing from containers without excessive force.

The in-vitro drug release profiles (Table 6)

showed that all selected formulations achieved more than 98% drug release over 24 hours. F16 demonstrated the fastest release (98.45% in 12 hours), likely due to its lower viscosity, which facilitates faster diffusion of the drug. In contrast, F2 and F9 provided a more prolonged release, indicating their potential use in controlled delivery systems.

Kinetic modeling of the drug release data (Table 7) revealed that the first-order release model best fit the release profile of F9 (R^2 = 0.9716), implying that drug release is concentration-dependent. Furthermore, the Korsmeyer-Peppas model also showed a good fit (R^2 = 0.966), suggesting an anomalous (non-Fickian) transport mechanism, which involves both drug diffusion and polymer erosion.

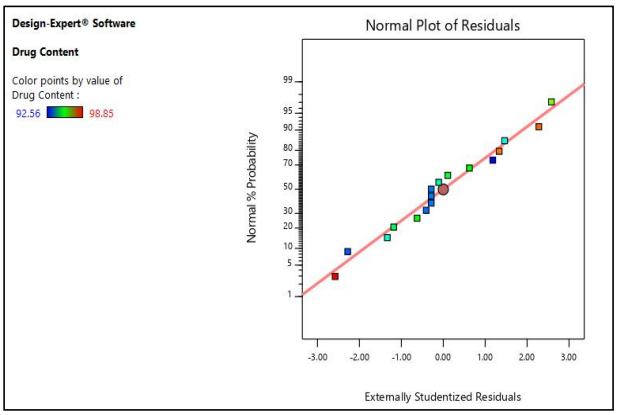
Stability studies (Tables 8 and 9) were conducted on formulation F9 to assess changes in drug content and viscosity under storage conditions (4°C and 28°C) over 90 days. Results showed minimal degradation, with drug content remaining above 95% in both conditions, affirming the chemical stability of the hydrogel. A slight increase in viscosity was noted at room temperature, possibly due to minor water loss or further cross-linking in the polymeric network, yet the changes were within acceptable limits.

Table 3: Results of drug content and spreadability

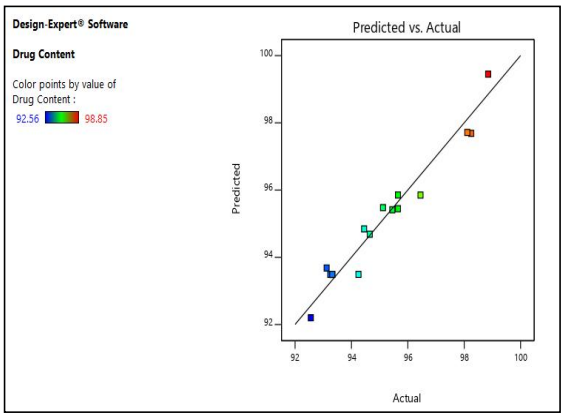
F. Code	Response 1: Drug Content (%)	Response 2: Spreadability (g.cm/sec)
F1	93.25	7.85
F2	98.85	6.05
F3	95.12	6.22
F4	93.32	7.25
F5	95.65	6.32
F6	94.25	7.15
F7	92.56	8.02
F8	96.45	6.5
F9	98.11	5.85
F10	93.12	7.25
F11	95.65	5.22
F12	95.45	7.85

F13	93.32	7.32
F14	94.45	6.45
F15	94.65	7.85
F16	98.25	5.75
F17	93.32	7.15

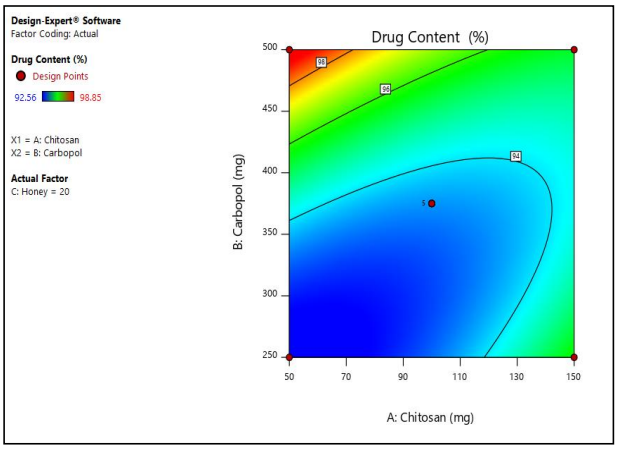
Different graphs for drug content obtained by DOE



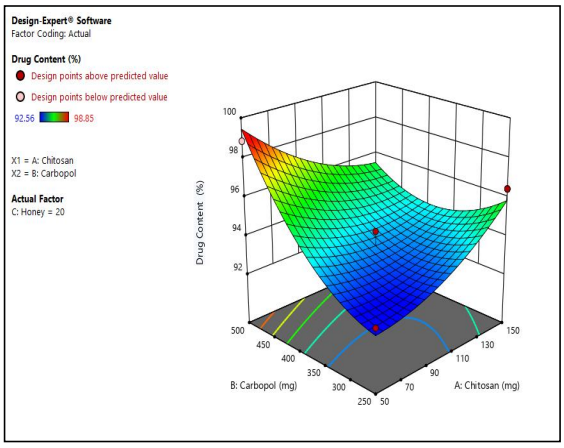
Normal Plots of Residuals



Predicted vs Actual

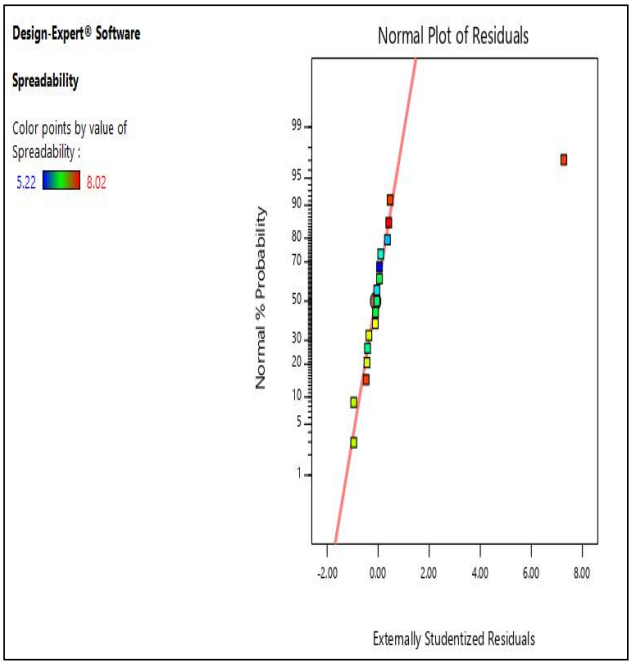


Contour plots (Chitosan vs Carbopol)

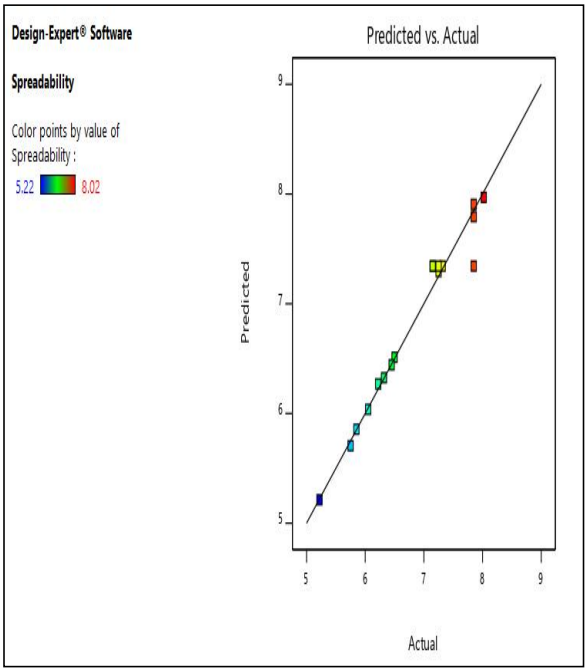


3D surface plots (Carbopol vs Chitosan)

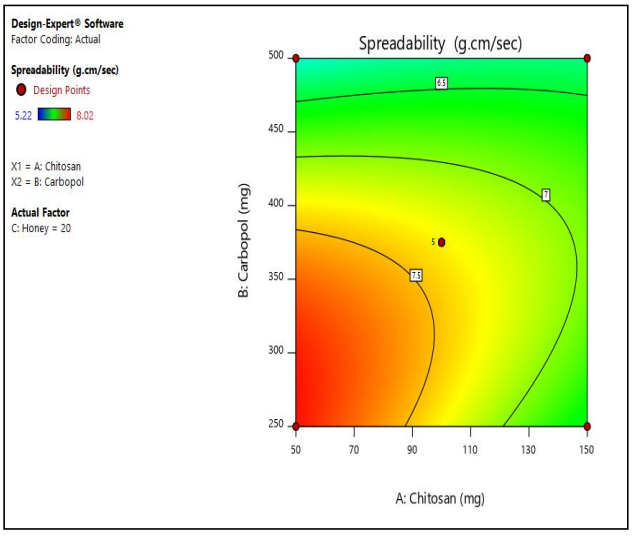
Figure 1: Different graphs for drug content obtained by DOE



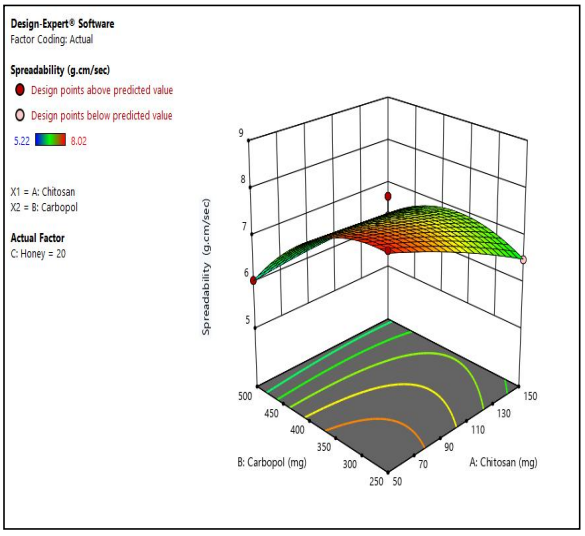
Normal Plots of Residuals



Predicted vs Actual



Contour plots (Chitosan vs Carbopol)



3D surface plots (Carbopol vs Chitosan)

Table 4: Experimental data with predicted response

Run Order	Formulation Code	Std Order	Parameters	Actual Value	Predicted Value
2	F2	7	Drug Content (%)	98.85	99.45
			Spreadability (g.cm/sec)	6.05	6.04

9	F9	15	Drug Content (%)	98.11	97.72
			Spreadability (g.cm/sec)	5.85	5.86
16	F16	11	Drug Content (%)	98.25	97.69
			Spreadability (g.cm/sec)	5.75	5.71

Table 5: Results of pH hydrogel formulations

S. No.	Code	pH	Viscosity (cps)	Extrudability (g)
1	F2	6.76±0.1	5584±12	210±12
2	F9	6.85±0.1	6698±18	196±10
3	F16	7.12±0.2	5369±14	184±11

*Average of 03 readings

Table 6: *In-vitro* drug release data for formulation F2, F9 and F16

Time (h)	Cumulative* % Drug Release		
	F2	F9	F16
0.5	14.56±0.15	12.25±0.45	21.25±0.65
1	22.36±0.32	20.32±0.36	36.65±0.47
2	36.85±0.96	32.25±0.74	45.63±0.32
3	49.98±0.74	45.65±0.65	59.98±0.74
4	55.65±0.65	53.12±0.85	68.85±0.69
6	69.98±0.82	56.65±0.74	73.32±0.85
8	73.32±0.96	68.89±0.63	86.65±0.74
10	92.25±0.74	73.32±0.75	96.65±0.32
12	96.65±0.36	91.12±0.69	98.12±0.84
24	98.12±0.92	98.45±0.74	98.45±0.77

*Average of 03 readings

Table 7: Regression analysis data of Hydrogel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
F9	0.7954	0.9716	0.9415	0.966

*Average of 03 readings

Table 8: Effect of storage temperature on the % drug content of Acyclovir Hydrogel Formulation F9

Time (Days)	Drug Content (%)	
	4.0±0.5°C	28±0.5°C
0	98.11±0.85	98.11±0.76
15	97.98±0.36	97.45±0.84
30	97.45±0.48	96.65±0.36
60	97.12±0.65	96.12±0.47
90	97.05±0.76	95.45±0.75

*Average of 03 readings

Table 9: Effect of storage temperature on the Viscosity of Acyclovir Hydrogel Formulation F9

Time (Days)	Viscosity (Cps)	
	4.0 ± 1°C	28 ± 1°C
0	6698±18	6698±18
15	6750±25	6798±23
30	6785±20	6815±18
60	6799±32	6895±20
90	6815±30	6945±36

*Average of 03 readings

Conclusion

In conclusion, formulation F16 is most suitable for rapid drug delivery due to its high release rate and adequate mechanical properties. However, formulations F2 and F9 demonstrated better viscosity, sustained release, and long-term stability, making them excellent candidates for the controlled topical

delivery of acyclovir. These hydrogels offer promising potential for clinical application in the management of viral skin infections.

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