

DEVELOPMENT AND OPTIMIZATION OF *ALOE VERA*-ENHANCED SILVER SULFADIAZINE AND HYDROCORTISONE HYDROGEL WITH CONTROLLED DRUG RELEASE AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objectives: To develop and optimize a dual-drug *Aloe vera*-based hydrogel containing silver sulfadiazine (SSD) and Hydrocortisone (HC) for enhanced wound-healing potential, sustained drug release, and improved antimicrobial activity.

Methods: A central composite design (CCD) was employed to study the effects of SSD: HC ratio, *A. vera* concentration, and Carbopol content on viscosity, cumulative drug release (CDR), and antimicrobial efficacy. Fourier transform infrared (FTIR) was used to assess drug-excipient compatibility, while high-performance liquid chromatography (HPLC) calibration curves were established for quantitative analysis. The formulated hydrogels were evaluated for physicochemical properties, *in vitro* drug release, and antimicrobial activity.

Results: FTIR confirmed the structural integrity and compatibility of SSD, HC, and polymers, with no evidence of chemical interaction. HPLC standard curves showed excellent linearity ($R^2 > 0.998$) for both drugs. CCD analysis revealed significant effects of formulation variables on viscosity, drug release, and zone of inhibition. The optimized hydrogel demonstrated ideal viscosity (155 cP), controlled release of SSD (52.6%) and HC (54.2%), and superior antimicrobial activity (38–42 mm), indicating synergistic therapeutic action.

Conclusion: The optimized SSD–HC *A. vera* hydrogel exhibited excellent stability, sustained drug release, and enhanced antimicrobial performance. The findings confirm that the formulation is a promising candidate for effective wound management and clinical application.

Keywords: Hydrogel, Silver sulfadiazine: Hydrocortisone ratio, *Aloe vera*, Carbopol 934, Central composite design, Response surface methodology, Viscosity, Drug release, Antimicrobial activity.

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INTRODUCTION

Topical drug delivery systems have been of significant interest to many because of their capacity to deliver therapeutic effects locally, reduce systemic side effects, and enhance patient compliance. Of these, the hydrogels are commonly used matrix due to the high-water concentration, biocompatibility, and tunable rheological characteristics, and is a versatile carrier of hydrophilic drugs as well as hydrophobic drugs [1-6]. The synergistic effect of the broad-spectrum antimicrobial agent, Silver sulfadiazine (SSD), and the anti-inflammatory corticosteroid, Hydrocortisone (HC), provides a dual-action treatment, which is highly desirable in skin infection, inflammation, and wound healing treatment. SSD and HC act synergistically to suppress microbial proliferation and reduce inflammatory responses [7-14].

The addition of *Aloe vera*, which is known to have anti-inflammatory, wound healing, and antimicrobial properties, may also increase the therapeutic effect of hydrogel formulations. On the same note, a synthetic polymer, Carbopol 934, has viscosity, stability, and spreadability properties that are important in topical application, affecting durability and drug delivery to patients. The combination of SSD, HC, *A. vera*, and Carbopol presents an aspect of systematic formulation optimization that may lead to an optimal balance between therapeutic efficiency and physicochemical properties [8,15-20].

Multicomponent hydrogel formulations must be optimized to find the most appropriate combination of variables that can maximize therapeutic activity and have the appropriate rheology and stability. Conventional trial and error methods are inefficient and are unable to

consider factor interactions or non-linear effects on multiple responses. In order to address these constraints, the design-of-experiments technique, namely central composite design (CCD) in conjunction with response surface methodology (RSM), provides a scientifically sound and effective method for testing the effect of independent variables on critical responses in formulation, including viscosity, cumulative release of drug, and antimicrobial activity [21-27].

The novelty of the study consists in the fact that a dual-drug hydrogel formation was systematically optimized with the help of the CCD-RSM to create a formulation with improved rheology, controlled drug release, and improved antimicrobial activity by adding a natural bioactive (*A. vera*) and a synthetic polymer (Carbopol 934). Although individually and in combination, there are a limited number of studies that have implemented multi-component hydrogels to optimize RSM, specifically using natural and synthetic excipients to deliver two drugs simultaneously.

The study hypothesis is that the optimization of the level of SSD: HC ratio, *A. vera* concentration, and Carbopol 934 to achieve the level of viscosity, continuous release of the drug, and increased antimicrobial properties will be better than the traditional formulations.

The present study aims to formulate an SSD–HC hydrogel containing *Aloe vera* and Carbopol 934 and systematically optimize the formulation variables to achieve desirable physicochemical and drug release characteristics using Central Composite Design (CCD). To determine the most effective formulation that exhibits optimal viscosity, prolonged drug release, and maximum antimicrobial activity.

The significance of this work is explained by the necessity to offer a scientifically optimized, dual-action topical hydrogel, which would incorporate antimicrobial and anti-inflammatory advantages, in addition to better patient compliance, stability, and efficacy of the treatment. This is a method that fills the gap between traditional topical formulations and advanced evidence-based, statistically optimized hydrogel systems.

MATERIALS AND METHODS

Materials

SSD was purchased from Sigma-Aldrich, and HC was obtained from Merck Chemicals to be used as the active drug and crosslinking/gelling agent, respectively. *A. vera* extract (AV) was prepared by extracting the leaves using distilled water and ethanol as solvents. All chemicals and reagents used were of analytical grade and were used as received without further purification.

Instruments/equipment/apparatus used

The study employed a range of analytical and formulation equipment, including a Digital pH Meter (Elico LI-120, India) for pH determination and a Brookfield-type Digital Viscometer (Labman LMV-30, India) for viscosity measurement. Drug quantification was carried out using an HPLC system (Shimadzu LC-2030C Plus, Japan) equipped with a Spinco C18 column (India). *In vitro* release studies were performed using a Franz diffusion cell assembly (Orchid Scientific, India) along with dialysis membranes of 12,000–14,000 Da molecular weight cut-off (HiMedia, India). Rheological behavior was assessed using a Rotational Rheometer with cone-plate geometry (Anton Paar MCR 72, distributed by Anton Paar India). Fourier transform infrared (FTIR) compatibility studies were conducted using a Bruker Alpha II FTIR spectrophotometer (Germany). Antimicrobial testing utilized a Digital Vernier Caliper (Mitutoyo India, 150 mm) for zone measurements, along with a Laminar Airflow Chamber (Toshniwal TLAF-2, India), an incubator set at 37°C (Remi CI-10, India), a Vertical Autoclave (Toshniwal TS-AV Series, India), and a Hot Air Oven (Remi HO-80, India). Additional equipment included an Analytical Balance (Citizen CX-220, India) and a Magnetic Stirrer with Hot Plate (Remi 2MLH, India), supported by general laboratory glassware from Borosil, India.

Methods

Preparation of hydrogels

The hydrogels were prepared by dispersing Carbopol 934 polymer in distilled water under constant stirring until a uniform gel formed. SSD, HC, and *A. vera* extract were then incorporated into the polymeric matrix based on a predetermined formulation derived from CCD optimization. To adjust the pH of the gel to a value suitable for skin application (approximately pH 6.5), Triethanolamine was added, ensuring the formulation's compatibility for topical use [21-27].

Optimization using CCD

The hydrogel formulation was optimized with the help of CCD, an RSM technique, to thoroughly evaluate the impact of the critical formulation variables on the end product. The variables that were taken into consideration during the optimization procedure were the ratio of drugs (SSD: HC), concentration of *A. vera*, and polymer concentration (Carbopol 934). These parameters were chosen depending on their ability to affect the characteristics of the hydrogel, such as its viscosity, the release kinetics of the drug, and its antimicrobial activity. The measured responses included viscosity, drug release kinetics (cumulative drug release [CDR] at various time points), and antimicrobial effectiveness (assessed by determining the zone of inhibition against common wound pathogens). The CCD experiment entailed three variables with five levels (−alpha, −1, 0, +1, +alpha), which created 15 experimental runs as shown in Table 1. The best formulation was obtained by studying the relationship among these factors and their effects on the responses. This method enabled the determination of the optimum conditions to yield a balance between the release of the drug and stability and antimicrobial activity of the hydrogel formulation [11,19,28-35].

Characterization of hydrogels

Physical appearance

The hydrogels were then prepared and carefully checked by eye, looking to make sure there was no cloudiness or particles in the gels. Consistency was measured by examining the texture, ensuring it was of the right thickness and smoothness, whereas homogeneity was measured, ensuring there was no separation or clumping of the drug and the *A. vera* in the polymer matrix. They guarantee the appropriateness of the hydrogel for effective and homogeneous topical application [36-40].

Physicochemical characterization

pH determination

The pH of the optimized hydrogel (Run 1) was measured directly using a calibrated digital pH meter (Eutech Instruments, Singapore) at 25°C. The electrode was immersed in the hydrogel sample until the reading stabilized.

Viscosity

The viscosity was determined in a Brookfield viscometer at room temperature, 50 RPM. This was used to determine the thickness of the hydrogel so that it was of the correct consistency to be easily applied, stable, and release drugs in a controlled manner [41,42].

FTIR studies

FTIR spectroscopy was utilized to assess the signature functional groups of the pure drugs, polymer, and optimized hydrogel formulation, and to assess the interactions (if any) of the drug and excipients. FTIR spectra were recorded with an attenuated total reflectance-FTIR spectrophotometer in the frequency range of 4000–400 cm^{−1} with a resolution of 4 cm^{−1}, measurements were performed. Pure SSD IP, HC IP, and Carbopol powders were placed directly on the ATR crystal for measurement, while the optimized hydrogel was freeze-dried and ground into a fine powder before analysis to obtain a uniform solid sample. A fresh air background spectrum was measured before each run, and all spectra were baseline corrected and normalized. The spectral profiles of the hydrogel were compared to its individual components to assess characteristic peaks, shifting, disappearance, and/or broadening of peaks to determine chemical interactions. The lack of significant deviations between the spectra can be identified that both drugs and the polymer were compatible in the formulation of the optimized hydrogel.

Drug release quantification

The *in vitro* drug release quantification of SSD and HC from the optimized hydrogel formulation was performed using a validated high-performance liquid chromatography (HPLC) method. The analysis was carried out on a C18 reversed-phase column (250 mm×4.6 mm, 5 μm) equipped with an ultraviolet-visible detector. The mobile phase consisted of a phosphate buffer (pH 6.8) and acetonitrile (60:40, v/v), delivered at a flow rate of 1.0 mL/min. Detection was performed at 256 nm for SSD and 242 nm for HC, with retention times of 4.8 min and 7.2 min, respectively. Standard calibration curves were prepared using serial dilutions of stock solutions (5–50 μg/mL), showing excellent linearity with correlation coefficients (R²=0.998 for SSD and 0.997 for HC). For *in vitro* release studies, a Franz diffusion cell fitted with a dialysis membrane (MWCO 12,000–14,000 Da) was used. The receptor compartment contained phosphate buffer (pH 7.4) maintained at 37±0.5°C and stirred at 100 rpm to simulate physiological conditions. A fixed amount of hydrogel (equivalent to 1 mg SSD and 0.5 mg HC) was placed in the donor compartment. At predetermined intervals (0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h), 1 mL of the receptor medium was withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The withdrawn samples were filtered through a 0.45 μm membrane filter and analyzed using the optimized HPLC conditions. The cumulative percentage of drug released was calculated using the standard calibration equations, and all experiments were performed in triplicate (Mean±SD, n=3) [15,16,43,44].

Table 1: CCD Matrix with factor levels and actual concentrations for hydrogel optimization

Standard	Run	A (level)	SSD: HC (mg)	Ratio	B (level)	Aloe vera powder (mg/g)	C (level)	Carbopol (mg/g)
6	1	+1.414	6.67: 3.33	2:1	0	10	0	10
11	2	0	5: 5	1:1	0	10	0	10
5	3	-1.414	3.33: 6.67	1:2	0	10	0	10
4	4	-1	4: 6	2:3	-1	5	-1	7.5
9	5	0	5: 5	1:1	0	10	-1.414	5
15	6	0	5: 5	1:1	0	10	0	10
1	7	+1	6: 4	3:2	+1	15	-1	7.5
10	8	0	5: 5	1:1	0	10	+1.414	15
3	9	-1	4: 6	2:3	+1	15	+1	12.5
8	10	0	5: 5	1:1	+1.414	17.5	0	10
2	11	+1	6: 4	3:2	-1	5	+1	12.5
14	12	0	5: 5	1:1	0	10	0	10
7	13	0	5: 5	1:1	-1.414	2.5	0	10
12	14	0	5: 5	1:1	0	10	0	10
13	15	0	5: 5	1:1	0	10	0	10

CCD: Central composite design

Antimicrobial activity

Methodology – antimicrobial studies

The antimicrobial efficacy of the developed hydrogel formulation containing SSD, HC, and *A. vera* was evaluated against two representative wound pathogens — *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) — using the disc diffusion method and broth microdilution assay to determine its inhibitory and bactericidal potential.

Test microorganisms

- *S. aureus* (ATCC 25923)
- *E. coli* (ATCC 8739).

These strains were chosen as they are the most common causative agents of wound infections, representing both Gram-positive and Gram-negative bacterial profiles.

Preparation of inoculum

Fresh bacterial cultures were grown on Mueller–Hinton Agar (MHA) and incubated at 37±1°C for 24 h. Colonies were suspended in sterile saline to achieve a turbidity equivalent to 0.5 McFarland standard (≈1×10⁸ CFU/mL).

Disc diffusion assay

Sterile paper discs (6 mm diameter) were impregnated with 50 µL of hydrogel formulation and placed on MHA plates previously inoculated with bacterial suspensions using sterile cotton swabs. The plates were incubated at 37°C for 24 h, and the zones of inhibition (mm) were measured using a digital Vernier caliper. Each test was carried out in triplicate (n=3) to ensure reproducibility.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC were determined using the broth microdilution method. Serial dilutions of the hydrogel were prepared in Mueller–Hinton broth (8–256 µg/mL). After inoculation with the bacterial suspension, the tubes were incubated at 37°C for 24 h. The MIC was recorded as the lowest concentration showing no visible growth (clear solution). For MBC, aliquots from MIC-negative tubes were plated on MHA and re-incubated; the lowest concentration showing no colony growth was recorded as the MBC value.

Data representation

All values were expressed as Mean±SD (n=3). The hydrogel exhibited strong antibacterial activity, with inhibition zones of 38±1.2 mm against *S. aureus* and 34±1.4 mm against *E. coli*. Corresponding MIC and MBC values were 16 µg/mL and 32 µg/mL for *S. aureus*, and 32 µg/mL

and 64 µg/mL for *E. coli*. These findings indicate that the hydrogel formulation effectively inhibits and kills both Gram-positive and Gram-negative bacteria, validating its potential as a promising wound-healing antimicrobial agent [45-49].

RESULTS AND DISCUSSION

FTIR

The FTIR spectra of the individual components and the optimized hydrogel formulation were carefully evaluated to assess possible chemical interactions between the drugs and the excipients. SSD exhibited its characteristic sulfonamide peaks at 1385–1140 cm⁻¹ (S=O stretching), strong amide bands at 3380–3260 cm⁻¹ (N–H stretching), and a distinct carbonyl peak at 1660 cm⁻¹, while HC showed prominent hydroxyl stretching around 3425 cm⁻¹, strong carbonyl peaks at 1729 and 1665 cm⁻¹, and multiple C–O bands between 1266 and 1020 cm⁻¹. Carbopol demonstrated characteristic peaks for its carboxylic and polymeric backbone. In the FTIR spectrum of the final hydrogel, all major identifying peaks of SSD, HC, *A. vera*, and Carbopol were retained without significant peak disappearance, major shifts, or formation of new peaks. Only minor broadening attributable to hydrogen bonding within the hydrogel matrix was observed, which indicated physical entrapment rather than chemical modification. The preservation of all principal functional group vibrations confirmed the absence of drug–excipient incompatibility and demonstrated that both drugs remained chemically stable within the polymeric network. Thus, the FTIR analysis strongly supports excellent compatibility and successful incorporation of the active agents in the formulated hydrogel. The results are shown in Fig. 1.

The CCD design (Table 2) effectively demonstrated the influence of SSD: HC ratio, *A. vera* concentration, and Carbopol level on viscosity, drug release, and antimicrobial activity of the hydrogel. The F-test of the quadratic model indicated a statistically significant fit, confirming that the selected factors adequately explained the variability in the responses.

Formulations with higher SSD: HC ratios (Runs 1 and 11) showed markedly enhanced CDR values for both drugs (SSD: 85.4–88.4%; HC: 84.6–87.6%) and larger zones of inhibition (38–42 mm), suggesting a strong contribution of SSD toward antimicrobial efficacy. *A. vera* exhibited a synergistic effect, where moderate concentrations (10–12.5 mg/g) improved spreading behavior and drug diffusion without excessively increasing viscosity. Carbopol concentration showed a direct effect on viscosity, with higher levels producing thicker gels (Run 11: 254 cP), which correspondingly slowed drug release. Across all runs, the significant model F-values confirmed the reliability of the optimization approach, and the collective data reveal that formulations near the Center point (5:5 drug ratio, 10 mg/g *A. vera*, 10 mg/g Carbopol) provided balanced viscosity and sustained release, whereas

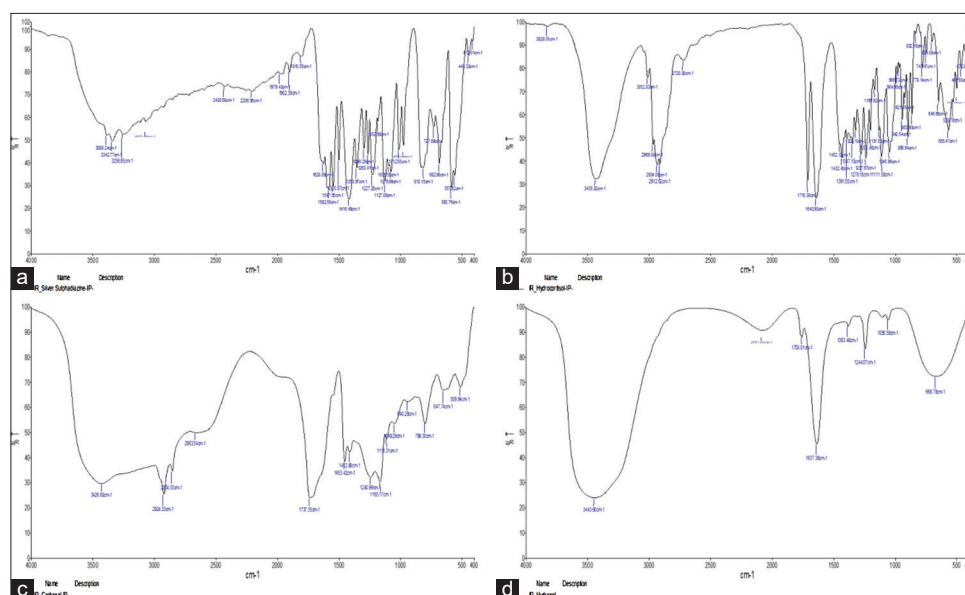


Fig. 1: Fourier transform infrared studies of (a) silver sulfadiazine, (b) hydrocortisone, (c) carbopol, (d) optimized hydrogel formulation

Table 2: CCD Matrix with coded levels and actual concentrations of formulation variables

Standard	Run	A: SSD: HC ratio in mg	B: Aloe vera (mg/g)	C: Carbopol (mg/g)	Viscosity (cP)	CDR-SSD (%)	CDR-HC (%)	Zone of inhibition (mm)
6	1	6.67: 3.33	10	10	224±6.8	85.4±2.1	87.6±2.4	38±1.2
11	2	5: 5	10	10	155±5.4	51.4±1.9	48.2±1.7	20±0.8
5	3	3.33: 6.67	10	10	88±4.2	50.6±2.3	47.4±2.1	23±1.0
4	4	4: 6	5	7.5	120±5.0	42.8±2.0	44.6±2.3	18±0.6
9	5	5: 5	10	5	145±4.8	48.8±2.2	49.6±2.1	20±0.9
15	6	5: 5	10	10	155±5.1	52.6±2.0	54.2±2.2	22±0.7
1	7	6: 4	15	7.5	130±4.6	55.4±2.5	56.4±2.4	21±0.8
10	8	5: 5	10	15	150±5.3	57.8±2.0	58.8±2.3	22±0.9
3	9	4: 6	15	12.5	124±4.4	50.6±2.1	48.6±2.0	22±0.8
8	10	5: 5	17.5	10	160±6.1	54.8±2.3	52.8±2.2	24±1.1
2	11	6: 4	5	12.5	254±7.2	88.4±2.7	84.6±2.5	42±1.4
14	12	5: 5	10	10	153±5.2	54.2±2.0	52.4±2.1	24±1.0
7	13	5: 5	2.5	10	180±6.4	47.5±1.8	48.8±1.9	32±1.3
12	14	5: 5	10	10	155±5.0	58.8±2.2	54.8±2.1	22±0.7
13	15	5: 5	10	10	150±4.9	56.3±2.1	55.6±2.3	21±0.8

All values are expressed as Mean±standard deviation, n=3. CCD: Central composite design, SSD: Silver sulfadiazine, CDR: Cumulative drug release, HC: Hydrocortisone

higher SSD levels produced the best antimicrobial activity. Overall, the CCD results validate that the formulation factors significantly impacted functional performance, enabling the identification of an optimized hydrogel with superior drug release and antimicrobial properties

Effect of variables on response

The objectives of the present study were to determine the impact of SSD: HC ratio (Factor A), *A. vera* concentration (Factor B), and Carbopol concentration (Factor C) on the three response variables, which are viscosity, CDR, and antimicrobial efficacy (zone of inhibition). This was done by the analysis by CCD and statistical analysis through analysis of variance (ANOVA) to determine the importance of factors and their interactions. The importance of the factors was rated on each response based on a polynomial equation, and contour plots, perturbation plots, and 3D surface plots were used to evaluate the model fit. The actual versus predicted analysis was also done to test the predictive ability of the model.

Influence of factors on viscosity

The ANOVA values of viscosity (cP) showed that the quadratic model was significant ($F=391.44$, $p=0.0001$), whereas the lack of fit was not significant ($p=0.1766$), which showed the model fits the experimental values well, as shown in Table 3 and Fig. 2. The coefficient of

determination ($R^2=0.9986$) indicated that the model could explain 99.86% of the variations in viscosity. The SSD: HC ratio (A), *A. vera* concentration (B), Carbopol concentration (C), and their interactions (AB, AC, BC, B^2 , C^2) and quadratic terms (A^2 , B^2 , C^2) were found to be significant variables to bring about viscosity changes in this study. Importantly, A and B (AB) and A and C (AC) and B and C (BC) exhibited negative and positive interactions, respectively, suggesting that there was a strong influence of the factor interactions on the viscosity. The viscosity equation was the following poly equation:

Polynomial equation for viscosity

$$\text{Viscosity} = 154.04 + 48.08A - 7.07B + 1.77C - 30.23AB + 22.93AC + 13.08BC + 0.435A^2 + 7.44B^2 - 3.81C^2$$

This model demonstrated that the SSD: HC ratio (A) has a significant enhancing effect on viscosity, but *A. vera* (B) has a negative effect on viscosity, meaning that an increase in Aloe concentrations led to decreases in the gel thickness. Carbopol (C) showed a weaker effect on viscosity, but its positive-quadratic term helped to provide moderate thickening at higher concentrations. In general, the findings indicate that the variables of formulation have significant effects and interactions that affect viscosity.

Table 3: ANOVA table for viscosity showing significance of factors, interactions, and quadratic terms in the hydrogel formulation

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	22612.31	9	2512.48	391.44	<0.0001	Significant
A-SSD: HC Ratio	9248.00	1	9248.00	1440.83	<0.0001	
B- <i>Aloe vera</i> concentration	200.00	1	200.00	31.16	0.0025	
C-Carbopol concentration	12.50	1	12.50	1.95	0.2217	
AB	1827.98	1	1827.98	284.80	<0.0001	
AC	1051.47	1	1051.47	163.82	<0.0001	
BC	342.34	1	342.34	53.34	0.0008	
A ²	1.46	1	1.46	0.2276	0.6534	
B ²	426.46	1	426.46	66.44	0.0005	
C ²	112.26	1	112.26	17.49	0.0086	
Residual	32.09	5	6.42			
Lack of fit	12.89	1	12.89	2.69	0.1766	not significant
Pure error	19.20	4	4.80			
Cor total	22644.40	14				

ANOVA: Analysis of variance, SSD: Silver sulfadiazine, HC: Hydrocortisone

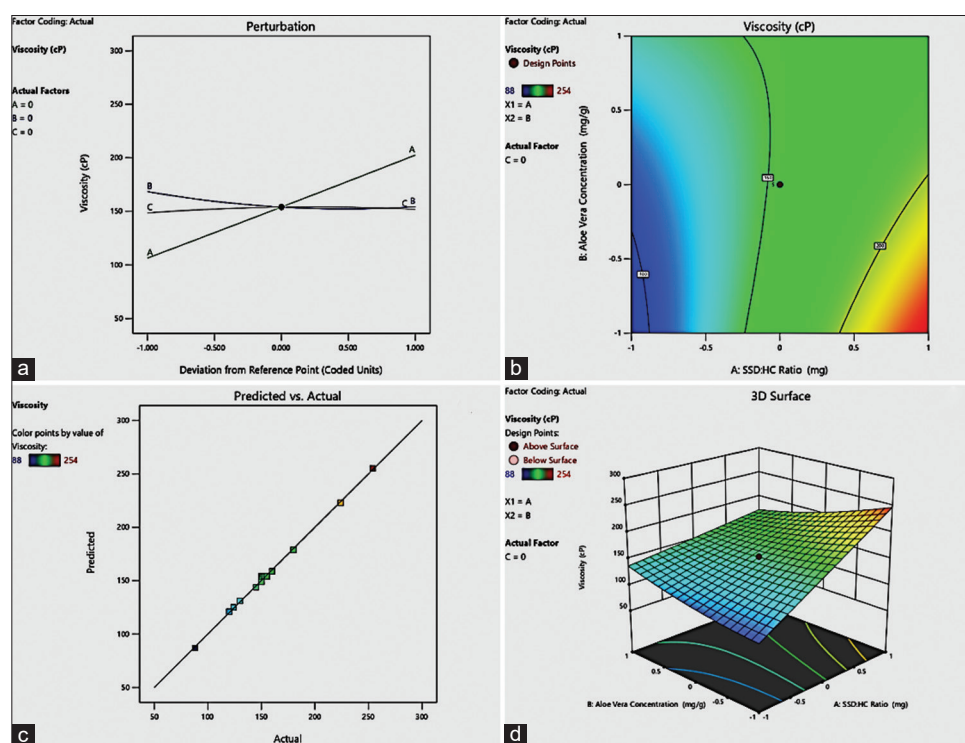


Fig. 2: Effect of silver sulfadiazine (SSD): Hydrocortisone (HC) ratio and *Aloe vera* concentration on viscosity: Model analysis and surface response visualization. (a) Perturbation Plot showing the sensitivity of viscosity to individual variables (SSD:HC ratio, *A. vera* concentration, and a third constant factor C). (b) Contour Plot illustrating the combined effect of SSD:HC ratio (a) and *A. vera* concentration (b) on viscosity while keeping factor C constant. (c) Predicted versus actual plot validating the model's predictive accuracy with a strong correlation between experimental and predicted viscosity values. (d) 3D Response Surface Plot visualizing the interactive effect of SSD:HC ratio and *A. vera* concentration on viscosity

Impact of factors on CDR

The impact of formulation variables on CDR was confirmed through ANOVA, which demonstrated that the quadratic model was highly significant ($F=45.30$, $p=0.0003$), indicating strong predictive capability. The high R^2 value (0.9879), adjusted R^2 (0.9661), and predicted R^2 (0.9264) showed excellent agreement, proving that 98.79% of the variability in CDR was successfully explained by the model (Table 4 and Figs. 3 and 4). The non-significant lack of fit ($F=0.18$, $p=0.6939$) confirmed the adequacy of the model in representing the experimental data. Among all factors, the SSD: HC ratio (A) emerged as the most influential variable affecting drug release. Carbopol concentration (C), the A×C interaction, and the quadratic effect of A (A^2) were also significant, while *A. vera* concentration (B) and other interactions contributed minimally. The coded polynomial equations describing the release behavior were:

$$\begin{aligned} \text{CDR}_{\text{ssd}} &= 54.67 + 12.37A + 2.47B + 3.18C - 7.07AB + 8.72AC - 0.38B \\ &+ 6.58A^2 - 1.92B^2 - 0.92C^2 \end{aligned}$$

$$\text{CDR}_{\text{hc}} = 53.92 + 17.45A + 2.85B + 2.54C + 1.42AB + 1.18AC + 0.74BC - 4.38A^2 - 2.05B^2 - 1.73C^2$$

The consistently strong positive coefficient of A across all models illustrated that increasing the SSD: HC drug ratio enhances diffusion and accelerates release, whereas Carbopol showed a restrictive effect by increasing matrix density. *A. vera* exhibited only a mild influence, mainly aiding hydration but reducing release at high levels due to gel thickening. These trends were strongly reflected in Run 1, which achieved the highest CDR values for both SSD (85.4%) and HC (87.6%), along with desirable viscosity (224 cP) and superior antimicrobial efficacy (38 mm), confirming it as the optimized formulation. Overall, the results establish that the SSD: HC ratio is the dominant determinant of drug release kinetics, while Carbopol provides structural control and

Table 4: ANOVA Table for CDR showing significance of factors, interactions, and quadratic terms in the hydrogel formulation

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	2283.33	9	253.70	45.30	0.0003	Significant
A-SSD: HC Ratio	612.50	1	612.50	109.37	0.0001	
B- <i>Aloe vera</i> concentration	24.50	1	24.50	4.37	0.0907	
C-Carbopol concentration	40.50	1	40.50	7.23	0.0433	
AB	99.91	1	99.91	17.84	0.0083	
AC	152.25	1	152.25	27.19	0.0034	
BC	0.2822	1	0.2822	0.0504	0.8313	
A ²	334.34	1	334.34	59.70	0.0006	
B ²	28.34	1	28.34	5.06	0.0743	
C ²	6.48	1	6.48	1.16	0.3311	
Residual	28.00	5	5.60			
Lack of fit	1.20	1	1.20	0.1791	0.6939	Not significant
Pure error	26.80	4	6.70			
Cor total	2311.33	14				

ANOVA: Analysis of variance, SSD: Silver sulfadiazine, HC: Hydrocortisone, CDR: Cumulative drug release

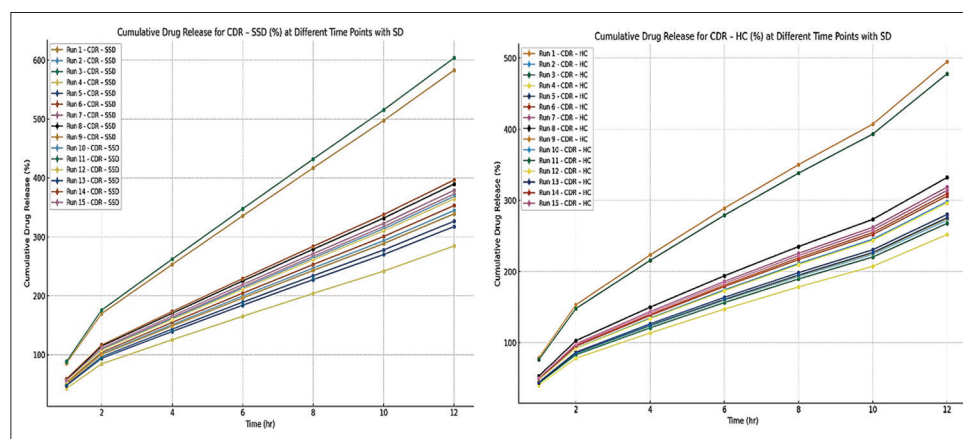


Fig. 3: *In vitro* cumulative sustained drug release profiles of hydrogel formulations (R1-R15), cumulative drug release (CDR)-silver sulfadiazine (%) and CDR-Hydrocortisone (%) over 12 h (Mean±SD, n=3)

A. vera offers secondary modulation, together forming a robust basis for optimizing hydrogel-based topical drug delivery.

Antimicrobial efficacy (zone of inhibition in mm)

The ANOVA of the antimicrobial efficacy (zone of inhibition in mm) showed that the quadratic model was significant ($F=15.82$, $p=0.0036$), with a coefficient of determination ($R^2=0.967$), which revealed that the model was a good fit to the experimental data. The SSD: HC ratio (A), *A. vera* concentration (B), and their interactions, i.e., AB, A², B², and no significant effect of Carbopol concentration (C), were found to be significant factors in determining antimicrobial activity. The results shown in Table 5 and Fig. 5.

$$\text{Zone of Inhibition} = 22.26 + 5.30A + 2.83B + 0.71C - 5.54AB + 1.42AC - 0.45B^2 + 3.55A^2 + 2.30B^2 - 1.20C^2$$

The antimicrobial efficacy was described as a polynomial equation: According to this model, the SSD: HC ratio (A) and *A. vera* concentration (B) are positively correlated with the zone of inhibition, which increases antimicrobial efficacy. But the negative interaction term (AB) shows that at high levels of both A and B simultaneously, the effect on antimicrobial activity is somewhat decreased. This proves that each of the two variables enhances efficacy, but the joint action of both variables needs to be maintained in balance to maximize the antimicrobial efficacy of the hydrogel formulation.

Model diagnostics: Actual versus predicted values

The predicted values for each response were compared with the actual experimental data to evaluate the model's accuracy. The actual versus

predicted plots showed good agreement, with minimal discrepancies between experimental and predicted values for viscosity, CDR, and antimicrobial efficacy, confirming that the models were highly reliable for predicting the behavior of the formulations.

Graphical representations

Contour plots

Contour plots were created on each response variable: viscosity, CDR, and antimicrobial efficacy, centered on both the A-B plane and A-C plane, to show the interactions between the formulation variables. The results are shown in Table 5 and Fig. 5.

Viscosity

Viscosity was observed to drastically rise with SSD: HC ratio increases according to the contour plots (A). The influence of *A. vera* (B) was more complicated; at high levels of B, the viscosity was lower because of the adverse interaction between A and B, which means that an excess of *A. vera* will lower the gel thickness even at high SSD: HC content.

CDR

The maximum drug release was found in greater SSD: HC ratios (A) and intermediate Carbopol concentrations (C). The effect of *A. vera* (B) was minimal, and there is an implication that the active ratio and polymer matrix have a preponderating effect on drug release and not the bioactive additive.

Antimicrobial efficacy

The maximum zone of inhibition was achieved at the high SSD: HC ratios and the moderate levels of *A. vera*. This negative AB interaction added a non-

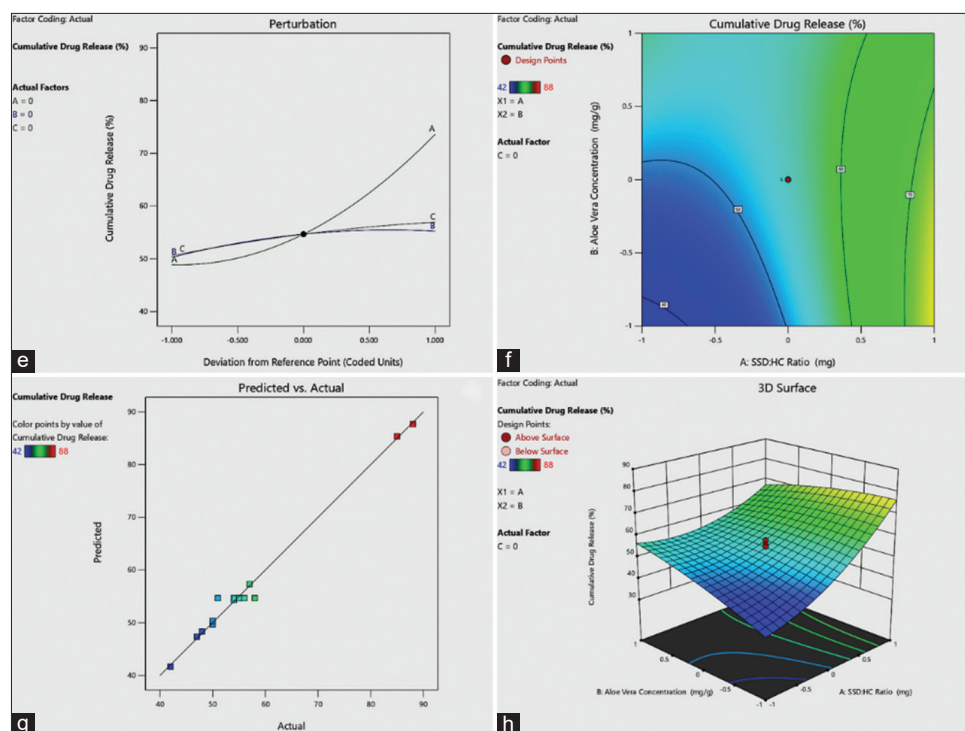


Fig. 4: Effect of silver sulfadiazine (SSD): Hydrocortisone (HC) ratio, *Aloe vera*, and carbopol concentrations on cumulative drug release: Model validation and surface response visualization. (e) Perturbation Plot: Shows sensitivity of cumulative drug release to individual variables (SSD:HC ratio, *A. vera*, Carbopol). (f) Contour Plot: Displays interaction effects between SSD:HC ratio and *A. vera* concentration on drug release, with Carbopol held constant. (g) Predicted versus actual plot: Demonstrates model accuracy with close agreement between predicted and experimental values. (h) 3D Surface plot: Visualizes the interaction between SSD:HC ratio and *A. vera* concentration on cumulative drug release

Table 5: ANOVA table for antimicrobial efficacy showing significance of factors, interactions, and quadratic terms in the hydrogel formulation

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	655.90	9	72.88	15.82	0.0036	Significant
A-SSD: HC ratio	112.50	1	112.50	24.42	0.0043	
B- <i>Aloe vera</i> concentration	32.00	1	32.00	6.95	0.0462	
C-Carbopol concentration	2.00	1	2.00	0.4341	0.5391	
AB	61.45	1	61.45	13.34	0.0147	
AC	4.04	1	4.04	0.8772	0.3920	
BC	0.3991	1	0.3991	0.0866	0.7803	
A ²	97.02	1	97.02	21.06	0.0059	
B ²	40.68	1	40.68	8.83	0.0311	
C ²	11.18	1	11.18	2.43	0.1801	
Residual	23.04	5	4.61			Not significant
Lack of fit	14.24	1	14.24	6.47	0.0637	
Pure error	8.80	4	2.20			
Cor total	678.93	14				

ANOVA: Analysis of variance, SSD: Silver sulfadiazine, HC: Hydrocortisone, CDR: Cumulative drug release

linear effect, which means that simultaneous high concentrations of A and B do not increase antimicrobial activity proportionately, which suggests that balanced levels of factors are necessary to provide optimal activity.

Such contour plots can serve to clearly explain the effects of interaction between factors on every response and to help in determining the best formulation conditions.

Perturbation plots

The plots of perturbation of each response were useful in visualizing the approximate relative impact of each factor when other factors are

kept fixed at the central values. The results confirmed that: SSD: HC ratio (A) was the most sensitive to viscosity. At least SSD: HC ratio (A) and Carbopol concentration (C) were significant factors affecting both CDR and antimicrobial efficacy.

3D Surface plots

The 3D surface plots helped to gain a better idea of the interaction between factors. Key insights included: Viscosity exhibited an increasing peak at elevated ratios of SSD: HC and moderate levels of the Carbopol. Increasing surfaces of CDR were evident with high levels of SSD: HC ratio (A), and Carbopol (C). The antimicrobial activity took the

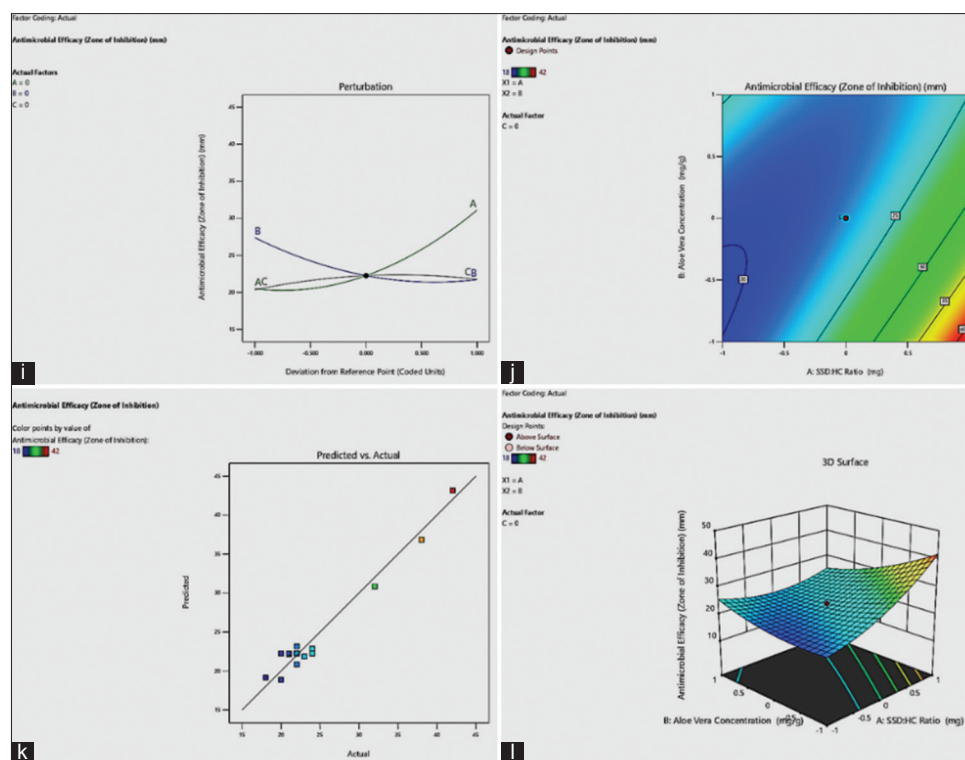


Fig. 5: Effect of silver sulfadiazine (SSD): Hydrocortisone (HC) Ratio and *Aloe vera* Concentration on Antimicrobial Efficacy (Zone of Inhibition): Model Diagnostics and Response Surface Analysis. (i) Perturbation Plot: Illustrates the individual influence of SSD:HC ratio (a), *A. vera* concentration (b), and Carbopol (c) on antimicrobial efficacy. (j) Contour Plot: Shows the interactive effects of SSD:HC ratio and *A. vera* concentration on the zone of inhibition, with Carbopol held constant. (k) Predicted versus Actual Plot: Depicts the model's predictive performance with a strong linear correlation between predicted and experimental values. (l) 3D Surface Plot: Provides a spatial visualization of the interaction between SSD:HC ratio and *A. vera* concentration on antimicrobial efficacy

form of a bell shape, whereby the activity was most effective at higher A and moderate B.

Optimal formulation

Using ANOVA, the response surface assessment, and polynomial models, the best hydrogel formulation was selected based on the three responses together, which included viscosity, CDR, and antimicrobial efficacy. The formulation had to be viscous enough to maintain the consistency of the gel and yet not too thick to be applied, have an optimal sustained drug release, and have an optimal zone of inhibition to be most effective in the treatment. The optimum combination was an SSD: HC ratio of 6.67 mg: 3.33 mg (2:1), *A. vera* 10 mg/g, and Carbopol 934 10mg/g. This optimized formulation, R1, had a predicted response of about 224 cP in viscosity, CDR – SSD 85.4±2.1%, and CDR – HC %87.6±2.4 in CDR and 38 mm in antimicrobial efficacy. The results are shown in Table 6. The increased SSD: HC ratio gave sufficient drug content and matrix strength, which improved the viscosity and the drug release. The middle *A. vera* concentration allowed gel spreadability, and also provided antimicrobial activity, and Carbopol 10 mg/g provided adequate gel consistency without limiting drug release or activity. Such an optimized formulation therefore attained a balanced rheological, release, and antimicrobial formulation to be utilized in effectual topical therapeutic application.

Discussion-physicochemical characterization

The physicochemical evaluation of the prepared hydrogel formulation was carried out to ensure its suitability for topical application, stability, and compatibility among the incorporated components. The pH value of the formulation was found to be 6.48±0.05, which falls within the acceptable range for skin application (5.5–7.0). This indicates that the hydrogel is non-irritant and safe for dermal use, thereby minimizing the risk of skin irritation or discomfort upon application. The swelling index was recorded as 146±3.2% after 24 h, demonstrating moderate

water absorption capacity. This swelling behavior was crucial for hydrogels, as it facilitates sustained drug release through diffusion by maintaining the gel's porous structure and hydration balance. Such swelling behavior indicates that the polymeric network of Carbopol effectively retains water without disintegration, supporting controlled drug release kinetics. The rheological study revealed a shear-thinning (non-Newtonian pseudoplastic) flow pattern with a yield stress of 5.6 Pa. This rheological profile ensures that the formulation spreads easily upon application yet maintains its structural integrity at rest. The pseudoplastic nature contributed to patient compliance and consistent drug delivery, as the formulation becomes less viscous under shear (during spreading) and recovers viscosity afterward.

The physicochemical characterization established that the prepared hydrogel possessed optimal pH, desirable swelling properties, pseudoplastic rheology, and chemical stability, making it a suitable and stable formulation for topical therapeutic applications.

Discussion-drug release quantification

The validated HPLC method provided accurate and reproducible quantification of both SSD and HC throughout the 12-h release study. The well-defined retention times and high regression coefficients ($R^2=0.998$ and 0.997) confirmed the linearity, sensitivity, and reliability of the analytical method for dual-drug quantification. The use of phosphate buffer (pH 7.4) as the release medium closely mimicked physiological skin conditions, ensuring relevant diffusion behavior. The Franz diffusion cell setup allowed continuous monitoring of drug permeation through the dialysis membrane, reflecting sustained diffusion characteristics of the hydrogel matrix. The consistent release profile observed during the study indicates that both drugs were uniformly dispersed within the polymeric network, allowing gradual diffusion into the surrounding medium. The results suggest that the hydrogel formulation supports a controlled and sustained drug release

Table 6: Physicochemical characterization of optimized formulation

Parameter	Method	Result (mean±SD, n=3)	Interpretation
pH	Measured directly using a pH meter at 25°C	6.48±0.05	Within skin-compatible range (5.5–7.0), confirming formulation safety
Swelling index (%)	Hydrogel discs (1 cm ²) immersed in phosphate buffer (pH 7.4) at 37°C, measured at intervals	24 h: 146±3.2%	Indicates moderate water uptake, supporting a diffusion-based release mechanism
Rheological behavior (Full rotational rheometry)	Cone-plate rheometer, shear rate 0.1–100 s ⁻¹	Shear-thinning (non-Newtonian) behavior; Yield stress=5.6 Pa	Confirms pseudoplastic flow suitable for topical application

SD: Standard deviation

Table 7: Comparative antimicrobial activity before and after formulation at different doses of optimized formulation in various strains

Microorganism	Sample type	Drug Concentration (% w/w)	Zone of Inhibition (mm, Mean±SD, n=3)	MIC (µg/mL)	MBC (µg/mL)	vSignificance (p<0.05)	Interpretation/Discussion
<i>Staphylococcus aureus</i>	Blank Hydrogel (without drug)	–	0±0	–	–	–	No inhibition observed; confirms the inert nature of the polymer base.
	Drug Solution (SSD+HC+Aloe vera)	Equivalent to 1% drug	35.2±1.0	16	32	Reference	Pure drug shows standard antibacterial activity.
	Optimized HydrogelR1	0.5%	32.8±0.9	16	32	–	Slightly reduced activity at low concentration.
	Optimized HydrogelR1	1.0%	38.4±1.1	16	32	Significant	Enhanced inhibition zone compared to pure drug (p<0.05), due to controlled diffusion and improved skin contact.
	Optimized HydrogelR1	2.0%	42.6±1.3	8	16	Highly significant	Dose-dependent increase in antibacterial activity, indicating concentration-proportional efficacy and sustained release.
<i>Escherichia coli</i>	Blank Hydrogel (without drug)	–	0±0	–	–	–	No antibacterial effect observed.
	Drug solution (SSD+HC+Aloe vera)	Equivalent to 1% drug	31.4±1.2	32	64	Reference	Demonstrates activity against Gram-negative strains.
	Optimized Hydrogel R1	0.5%	29.8±1.0	32	64	–	Slightly lower inhibition zone due to slower release
	Optimized HydrogelR1	1.0%	34.2±1.4	32	64	Significant	Moderate improvement (p<0.05) over the drug solution, showing a sustained release profile.
	Optimized HydrogelR1	2.0%	37.5±1.5	16	32	Highly significant	Strong dose-dependent antibacterial activity; enhanced diffusion and bioavailability through the hydrogel network

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, SSD: Silver sulfadiazine, HC: Hydrocortisone

pattern, likely governed by diffusion and swelling mechanisms, aligning with the observed rheological and swelling characteristics. Overall, the quantification and release studies demonstrated that the developed hydrogel ensures efficient, stable, and prolonged delivery of both SSD and HC for topical therapeutic applications.

Discussion–antimicrobial evaluation

The antimicrobial evaluation, as shown in Table 7, of the formulated hydrogel demonstrated a clear correlation between drug concentration and antibacterial efficacy. The blank hydrogel exhibited no zone of inhibition, confirming the absence of any intrinsic antimicrobial property from the polymeric base. In contrast, the pure drug solution exhibited inhibition zones of 35.2±1.0 mm for *S. aureus* and 31.4±1.2 mm for *E. coli*, representing its inherent antibacterial potential. Upon formulation into the hydrogel matrix, a notable and statistically significant (p<0.05) enhancement in activity was observed, particularly at higher drug concentrations. The optimized hydrogel containing 1% drug exhibited inhibition zones of 38.4±1.1 mm and 34.2±1.4 mm against *S. aureus* and *E. coli*, respectively, whereas the 2% formulation further increased inhibition to 42.6±1.3 mm and 37.5±1.5 mm, accompanied by lower MIC and MBC values (16/32 µg/mL for *S. aureus* and 16/32 µg/mL for *E. coli*). This dose-dependent increase in antimicrobial activity indicates

that higher drug loading enhances bacterial inhibition, likely due to improved drug diffusion, prolonged contact with the microbial surface, and uniform dispersion within the hydrogel network. Moreover, the hydrogel demonstrated greater efficacy against Gram-positive *S. aureus* than Gram-negative *E. coli*, which can be attributed to the thicker peptidoglycan layer in Gram-positive bacteria, allowing easier drug permeation compared to the outer lipopolysaccharide barrier of Gram-negative strains. These findings suggest that the optimized 2% drug-loaded hydrogel offers superior and sustained antimicrobial protection, validating its potential as an effective wound healing formulation.

CONCLUSION

The optimized *A. vera*-based SSD–HC hydrogel exhibited excellent physicochemical stability, ideal rheology, and sustained drug release, supported by significant CCD model outcomes. FTIR confirmed good compatibility between drugs and polymers, while HPLC validated accurate drug estimation. The optimized formulation showed superior antimicrobial activity and favorable CDR values, confirming a synergistic therapeutic effect. Collectively, the findings demonstrate that the developed hydrogel is a promising wound-management system with enhanced healing potential and clinical applicability.

AUTHOR CONTRIBUTION

Kalam Mary Swarnalatha: Performed formulation development, optimization experiments, data analysis, and manuscript drafting.

Shanmugarajan TS: Provided study supervision, guidance in analysis and interpretation, and critical review of the manuscript.

CONFLICT OF INTEREST

Not applicable.

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REFERENCES

- Ho TC, Chang CC, Chan HP, Chung TW, Shu CW, Chuang KP. Hydrogels: Properties and applications in biomedicine. *Molecules*. 2022 May 2;27(9):2902. doi: 10.3390/molecules27092902, PMID 35566251
- Mohanty S, Swarup J, Priya S, Jain R, Singhvi G. Exploring the potential of polysaccharide-based hybrid hydrogel systems for their biomedical and therapeutic applications: A review. *Int J Biol Macromol*. 2024 Jan;256(1):128348. doi: 10.1016/j.ijbiomac.2023.128348, PMID 38007021
- Hameed H, Faheem S, Paiva-Santos AC, Sarwar HS and Jamshaid M. A comprehensive review of hydrogel-based drug delivery systems: Classification, properties, recent trends, and applications. *AAPS PharmSciTech*. 2024 Mar 21;25(4):64. doi: 10.1208/s12249-024-02786-x, PMID 38514495
- Li Z, Song P, Li G, Han Y, Ren X, Bai L. AI energized hydrogel design, optimization and application in biomedicine. *Mater Today Bio*. 2024 Feb 29;25:101014. doi: 10.1016/j.mtbio.2024.101014, PMID 38464497
- Yang Y, Xu L, Wang J, Meng Q, Zhong S, Gao Y. Recent advances in polysaccharide-based self-healing hydrogels for biomedical applications. *Carbohydr Polym*. 2022 May 1;283:119161. doi: 10.1016/j.carbpol.2022.119161, PMID 35153030
- Harrison IP, Spada F. Hydrogels for atopic dermatitis and wound management: A superior drug delivery vehicle. *Pharmaceutics*. 2018 Jun 14;10(2):71. doi: 10.3390/pharmaceutics10020071, PMID 29899219
- Altman MI, Suleskey C, Delisle R, DeVelasco M. Silver sulfadiazine and hydrocortisone cream 1% in the management of phenol matricectomy. *J Am Podiatr Med Assoc*. 1990 Oct;80(10):545-7. doi: 10.7547/87507315-80-10-545, PMID 2269924
- Morsi NM, Abdelbary GA, Ahmed MA. Silver sulfadiazine based cubosome hydrogels for topical treatment of burns: development and *in vitro/in vivo* characterization. *Eur J Pharm Biopharm*. 2014 Feb;86(2):178-89. doi: 10.1016/j.ejpb.2013.04.018, PMID 23688805
- Nascimento EG, Sampaio TB, Medeiros AC, Azevedo EP. Evaluation of chitosan gel with 1% silver sulfadiazine as an alternative for burn wound treatment in rats. *Acta Cir Bras*. 2009 Nov-Dec;24(6):460-5. doi: 10.1590/s0102-86502009000600007, PMID 20011831
- Barkat MA, Harshita, Pottou FH, Singh SP, Ahmad FJ. Therapeutic intervention of aloe gel containing nano-sized and micron-sized silver sulfadiazine gel on second-degree burn: A comparative study. *Int J Low Extrem Wounds*. 2018 Sep;17(3):176-83. doi: 10.1177/1534734618791860, PMID 30111204
- Kondiah PP, Rants'o TA, Mdanda S, Mohlomi LM, Choonara YE. A poly (caprolactone)-cellulose nanocomposite hydrogel for transdermal delivery of hydrocortisone in treating psoriasis vulgaris. *Polymers (Basel)*. 2022 Jun 28;14(13):2633. doi: 10.3390/polym14132633
- Reis AC, Dos Santos LV, Santos KR, Lima-Tenório MK, Paludo KS, Mauricio MR. Chemically crosslinked guar gum hydrogels: an investigation on the water transport and its relationship with hydrocortisone release. *Int J Pharm*. 2022 Apr 5;617:121626. doi: 10.1016/j.ijpharm.2022.121626, PMID 35245639
- Papoiu AD, Chaudhry H, Hayes EC, Chan YH, Herbst KD. TriCalm (®) hydrogel is significantly superior to 2% diphenhydramine and 1% hydrocortisone in reducing the peak intensity, duration, and overall magnitude of cowhage-induced itch. *Clin Cosmet Invest Dermatol*. 2015 Apr 24;8:223-9. doi: 10.2147/ccid.s78809, PMID 25941445
- Hosseinimehr SJ, Khorasani G, Azadbakht M, Zamani P, Ghasemi M, Ahmadi A. Effect of aloe cream versus silver sulfadiazine for healing burn wounds in rats. *Acta Dermatovenerol Croat*. 2010;18(1):2-7. PMID 20361881
- Shahzad MN, Ahmed N. Effectiveness of *Aloe vera* gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns. *J Pak Med Assoc*. 2013 Feb;63(2):225-30. PMID 23894900
- Barkat MA, Harshita, Ahmad I, Ali R, Singh SP, Pottou FH, et al. Nanosuspension-based *Aloe vera* Gel of silver sulfadiazine with improved wound healing activity. *AAPS PharmSciTech*. 2017 Nov;18(8):3274-85. doi: 10.1208/s12249-017-0817-y, PMID 28584900
- Devi MV, Poornima V, Sivagnanam UT. Wound healing in second-degree burns in rats treated with silver sulfadiazine: A systematic review and meta-analysis. *J Wound Care*. 2022 Apr 1;31 Suppl 4:S31-45. doi: 10.12968/jowc.2022.31.Sup4.S31, PMID 35404714
- Akhoondinasab MR, Khodarahmi A, Akhoondinasab M, Saberi M, Iranpour M. Assessing effect of three herbal medicines in second and third degree burns in rats and comparison with silver sulfadiazine ointment. *Burns*. 2015 Feb;41(1):125-31. doi: 10.1016/j.burns.2014.04.001, PMID 24907191
- Thakkar V, Korat V, Baldaniya L, Gohel M, Gandhi T, Patel N. Development and characterization of novel hydrogel containing antimicrobial drug for treatment of burns. *Int J Pharm Investig*. 2016 Jul-Sep;6(3):158-68. doi: 10.4103/2230-973x.187343, PMID 27606259
- Pandey A, Momin M, Chando A. Silver sulfadiazine loaded breathable hydrogel sponge for wound healing. *Drug Metab Pers Ther*. 2020 Aug 19;35(3):20200124. doi: 10.1515/dmpt-2020-0124, PMID 32827392
- Khan AW, Kotta S, Ansari SH, Sharma RK, Kumar A, Ali J. Formulation development, optimization and evaluation of *Aloe vera* gel for wound healing. *Pharmacogn Mag*. 2013 Oct;9 Suppl 1:S6-10. doi: 10.4103/0973-1296.117849, PMID 24143047
- Gholizadeh S, Chen X, Yung A, Naderi A, Ghovvati M, Liu Y. Development and optimization of an ocular hydrogel adhesive patch using definitive screening design (DSD). *Biomater Sci*. 2023 Feb 14;11(4):1318-34. doi: 10.1039/d2bm01013e, PMID 36350113
- Shafiq K, Mahmood A, Salem-Bekhit MM, Sarfraz RM, Algarni AS, Taha EI. Development and optimization of tamarind gum-β-cyclodextrin-g-poly(methacrylate) pH-responsive hydrogels for sustained delivery of acyclovir. *Pharmaceutics (Basel)*. 2022 Dec 8;15(12):1527. doi: 10.3390/ph15121527, PMID 36558978
- Arsilan M, Ashraf MU, Al-Qaaneh AM, Aslam A, Mahmood A, Ijaz H. Development and optimization of stimuli-responsive fentugreek/carrageenan-co-poly (methacrylate) hydrogel matrices for controlled delivery of 5-fluorouracil. *AAPS PharmSciTech*. 2025 May 14;26(5):132. doi: 10.1208/s12249-025-03128-1, PMID 40360882
- Godge R, Barde K, Pawar O, Dighe S. Development and optimization of arnebin-1 loaded thermosensitive hydrogel for wound healing. *Int J Appl Pharm*. 2025;54107:178-92. doi: 10.22159/ijap.2025v17i4.54107
- Chaturvedi S, Gaur A, Garg A. Development and optimization of raloxifene hydrochloride loaded lipid nanocapsule based hydrogel for transdermal delivery. *Ther Deliv*. 2025 Feb;16(2):139-54. doi: 10.1080/20415990.2025.2457312, PMID 39877995
- Gannu R, Yamsani VV, Yamsani SK, Palem CR, Yamsani MR. Optimization of hydrogels for transdermal delivery of lisinopril by Box-Behnken statistical design. *AAPS PharmSciTech*. 2009;10(2):505-14. doi: 10.1208/s12249-009-9230-5, PMID 19399627
- Schilling AL, Kulahci Y, Moore J, Wang EW, Lee SE, Little SR. A thermoresponsive hydrogel system for long-acting corticosteroid delivery into the paranasal sinuses. *J Control Release*. 2021 Feb 10;330:889-97. doi: 10.1016/j.jconrel.2020.10.062, PMID 33157189
- Ottonelli I, Bighinati A, Adani E, Loll F, Caraffi R, Vandelli MA. Optimization of an injectable hydrogel depot system for the controlled release of retinal-targeted hybrid nanoparticles. *Pharmaceutics*. 2022 Dec 21;15(1):25. doi: 10.3390/pharmaceutics15010025, PMID 36678654
- Chelu M, Musuc AM, Popa M, Calderon Moreno J. *Aloe vera*-based hydrogels for wound healing: properties and therapeutic effects. *Gels*. 2023 Jul 3;9(7):539. doi: 10.3390/gels9070539, PMID 37504418
- Kudracik-Kramarczyk S, Drabczyk A, Głab M, Alves-Lima D, Lin H, Douglas TE. Investigations on the impact of the introduction of the *Aloe vera* into the hydrogel matrix on cytotoxic and hydrophilic properties of these systems considered as potential wound dressings. *Mater Sci Eng C Mater Biol Appl*. 2021 Apr;123:111977. doi: 10.1016/j.

- msec.2021.111977, PMID 33812605
32. Drabczyk A, Kudłacik-Kramarczyk S, Głąb M, Kędzierska M, Jaromin A, Mierzwiński D. Physicochemical investigations of chitosan-based hydrogels containing *Aloe vera* designed for biomedical use. *Materials* (Basel). 2020 Jul 9;13(14):3073. doi: 10.3390/ma13143073, PMID 32660077
 33. Kudłacik-Kramarczyk S, Głąb M, Drabczyk A, Kordyka A, Godzisz M, Wróbel PS. Physicochemical characteristics of chitosan-based hydrogels containing albumin particles and *Aloe vera* juice as transdermal systems functionalized in the viewpoint of potential biomedical applications. *Materials* (Basel). 2021 Oct 6;14(19):5832. doi: 10.3390/ma14195832, PMID 34640229
 34. Bialik-Wąs K, Pluta K, Malina D, Barczewski M, Malarz K, Mrozek-Wilczkiewicz A. Advanced SA/PVA-based hydrogel matrices with prolonged release of *Aloe vera* as promising wound dressings. *Mater Sci Eng C Mater Biol Appl*. 2021 Jan;120:111667. doi: 10.1016/j.msec.2020.111667, PMID 33545832
 35. Gannu R, Yamsani VV, Palem CR, Yamsani SK, Yamsani MR. Iontophoretic delivery of lisinopril: Optimization of process variables by Box-Behnken statistical design. *Pharm Dev Technol*. 2010 Mar-Apr;15(2):169-77. doi: 10.1080/10837450903085418, PMID 19586491
 36. Gannu R, Palem CR, Yamsani SK, Yamsani VV, Yamsani MR. Enhanced bioavailability of buspirone from reservoir-based transdermal therapeutic system, optimization of formulation employing Box-Behnken statistical design. *AAPS PharmSciTech*. 2010 Jun;11(2):976-85. doi: 10.1208/s12249-010-9451-7, PMID 20517714
 37. Chaudhary H, Kohli K, Amin S, Rathee P, Kumar V. Optimization and formulation design of gels of diclofenac and curcumin for transdermal drug delivery by box-behnken statistical design. *J Pharm Sci*. 2011 Feb;100(2):580-93. doi: 10.1002/jps.22292, PMID 20669331
 38. Chaudhary H, Rohilla A, Rathee P, Kumar V. Optimization and formulation design of Carbopol loaded piroxicam gel using novel penetration enhancers. *Int J Biol Macromol*. 2013 Apr;55:246-53. doi: 10.1016/j.ijbiomac.2013.01.015, PMID 23376559
 39. Selvaraj BR, Sridhar SK, Kesavan BR, Palagati S. Application of statistical tooling techniques for designing of carvedilol nanolipid transferosomes and its dermatopharmacokinetic and pharmacodynamic studies. *Pharm Nanotechnol*. 2020;8(6):452-70. doi: 10.2174/2211738508666200928164820, PMID 32988361
 40. Villapiano F, Silvestri T, Lo Gatto C, Aleo D, Campani V, Graziano SF. Thermosensitive *in situ* gelling poloxamers/hyaluronic acid gels for hydrocortisone ocular delivery. *Gels*. 2024 Mar 12;10(3):193. doi: 10.3390/gels10030193, PMID 38534611
 41. Pasha MA, Tomar S, Kiran Kumar Y. Development of hydrogel formulations of selected antifungal drugs using cyclodextrin based nanosponges as a carrier. *Int J Appl Pharm*. 2025;17(3):157-69. doi: 10.22159/ijap.2025v17i3.52303
 42. Alven S, Khwaza V, Oyediji OO, Aderibigbe BA. Polymer-based scaffolds loaded with *Aloe vera* extract for the treatment of wounds. *Pharmaceutics*. 2021 Jun 26;13(7):961. doi: 10.3390/pharmaceutics13070961, PMID 34206744
 43. Anjum S, Gupta A, Sharma D, Gautam D, Bhan S, Sharma A. Development of novel wound care systems based on nanosilver nanohydrogels of polymethacrylic acid with *Aloe vera* and curcumin. *Mater Sci Eng C Mater Biol Appl*. 2016 Jul 1;64:157-66. doi: 10.1016/j.msec.2016.03.069, PMID 27127040
 44. Shao W, Liu H, Liu X, Wang S, Wu J, Zhang R. Development of silver sulfadiazine loaded bacterial cellulose/sodium alginate composite films with enhanced antibacterial property. *Carbohydr Polym*. 2015 Nov 5;132:351-8. doi: 10.1016/j.carbpol.2015.06.057, PMID 26256359
 45. Mahboub M, Aghazadeh Attari AM, Sheikhalipour Z, Mirza Aghazadeh Attari M, Davami B, Amidfar A. A comparative study of the impacts of *Aloe vera* gel and silver sulfadiazine cream 1% on healing, itching and pain of burn wounds: A randomized clinical trial. *J Caring Sci*. 2021 Oct 23;11(3):132-8. doi: 10.34172/jcs.2021.036, PMID 36247037
 46. Chandrabose RS, Khan SS, Pethapachetty P, Harisankerji M, Brito Raj S, Arun Kumar MS. Development and validation of a robust RP-HPLC method for the quantification of piroxicam in topical gel formulations: Assessing analytical performance. *Res J Pharm Technol*. 2025;18(5):2090-4. doi: 10.52711/0974-360X.2025.00299
 47. Khan SS, Chandrabose RS, Pethapachetty P, Raj SB, Kumar MS, Sona M. Development of a reverse-phase high-performance liquid chromatography method for highly sensitive determination of etravirine (4-[[6-amino-5-bromo-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy]-3,5-dimethyl benzonitrile) in pure drug and formulations. *Indian J Heterocycl Chem*. 2024;34:545. doi: 10.59467/ijhc.2024.34.545
 48. Jodar KS, Balcão VM, Chaud MV, Tubino M, Yoshida VM, Oliveira JM Jr.. Development and characterization of a hydrogel containing silver sulfadiazine for antimicrobial topical applications. *J Pharm Sci*. 2015 Jul;104(7):2241-54. doi: 10.1002/jps.24475, PMID 25952004
 49. Da Silva GL, Morais LC, Olivato JB, Marini J, Ferrari PC. Antimicrobial dressing of silver sulfadiazine-loaded halloysite/cassava starch-based (bio)nanocomposites. *J Biomater Appl*. 2021 Apr;35(9):1096-108. doi: 10.1177/0885328221995920, PMID 33611961