

Development and Optimization of A Hesperetin–Hp- B -Cyclodextrin Hydrogel for Topical Delivery: Formulation Optimization, Release Kinetics and Stability Evaluation

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ABSTRACT

Background: Hesperetin is a poorly water-soluble flavanone with limited pharmaceutical availability, which may restrict its effective incorporation into topical dosage forms. Cyclodextrin complexation followed by incorporation into a polymeric hydrogel may provide a sequential approach to improve its formulation performance and regulate drug release. The present study aimed to develop and optimize a Hesperetin–hydroxypropyl- β -cyclodextrin (HP- β -CD) inclusion-complex-loaded Carbopol 934P hydrogel using a systematic design of experiments approach.

Methods: A three-factor, three-level Box–Behnken design was employed to investigate the effects of Carbopol 934P concentration (0.5–1.0%), Hesperetin–HP- β -CD inclusion-complex concentration (2.0–4.0% w/w), and propylene glycol concentration (5–15%) on pH, viscosity, spreadability, drug content, and cumulative drug release at 8 h. Seventeen experimental formulations were prepared and evaluated. Quadratic response-surface models were fitted, followed by multi-response desirability-based optimization. The optimized formulation was independently validated and further evaluated for physicochemical characteristics, swelling behavior, comparative in-vitro drug release, release kinetics, antioxidant activity, and accelerated stability.

Results: The experimental formulations showed pH values of 5.53–5.76, viscosity of 10,785–23,876 cP, spreadability of 6.51–8.06 g·cm/s, drug content of 95.74–99.07%, and 8-h drug release of 76.19–93.76%. Formulation F9 was selected as the optimized formulation, containing 0.75% Carbopol 934P, 4.0% w/w Hesperetin–HP- β -CD inclusion complex, and 15% propylene glycol, with a composite desirability of 0.942. The predicted responses were pH 5.64, viscosity 17,086 cP, spreadability 7.42 g·cm/s, drug content 97.92%, and 8-h release of 88.32%. Independent validation yielded pH 5.65 ± 0.01 , viscosity $17,016.7 \pm 116.8$ cP, spreadability 7.41 ± 0.04 g·cm/s, drug content $97.68 \pm 0.16\%$, and 8-h drug release of $88.32 \pm 0.23\%$, with prediction errors below 0.5%. The optimized hydrogel exhibited progressive swelling and drug release. In the extended-release dataset, drug release reached 80.4% at 8 h and 91.2% at 12 h. The Higuchi model showed a high correlation ($R^2 = 0.9969$), while the Korsmeyer–Peppas model provided a complementary empirical fit ($R^2 = 0.9998$; $n = 0.8702$). DPPH radical-scavenging activity increased with increasing concentration. Accelerated stability evaluation at 40 ± 2 °C/ $75 \pm 5\%$ RH demonstrated only minor changes in pH, drug content, and drug release over three months.

Conclusion: The findings demonstrate the feasibility of a sequential Hesperetin–HP- β -CD inclusion-complex-to-hydrogel strategy for developing a topical formulation with improved pharmaceutical availability and regulated drug release. The optimized Carbopol 934P hydrogel exhibited reproducible formulation characteristics, progressive drug release, concentration-dependent antioxidant activity, and short-term accelerated stability. Further studies involving longer-term stability, skin deposition, cytocompatibility, and appropriate biological evaluation are warranted.

Keywords: Hesperetin; HP- β -CD; Carbopol 934P; topical hydrogel; Box–Behnken design; formulation optimization; drug release; Higuchi model; Korsmeyer–Peppas model; stability.

INTRODUCTION

Topical hydrogels are attractive dosage forms for local delivery because their hydrated polymeric networks can accommodate active pharmaceutical ingredients while providing a soft and spreadable vehicle. For wound-oriented delivery, the formulation must simultaneously provide adequate hydration, application-site residence, acceptable handling and reproducible drug availability. These attributes are interdependent: increasing polymer concentration can strengthen the network and increase viscosity, but excessive viscosity can impair spreading and modify drug diffusion.

The Hesperetin-HP- β -CD inclusion complex provides a molecular approach to the aqueous-solubility limitation of Hesperetin, but complexation alone does not determine topical performance. After incorporation into a polymeric matrix, the formulation environment controls water uptake, drug mobility, diffusion path length and the rate at which the active becomes available.

Hydrogel development is therefore a multi-response formulation problem. A formulation may exhibit high drug content but inadequate spreadability, or excellent spreadability but insufficient viscosity. Similarly, a rapidly releasing formulation may not meet the intended controlled-delivery objective. Design of experiments permits simultaneous assessment of several formulation variables and responses and provides a rational basis for selecting a balanced composition.

The present work focuses on the formulation-development stage following Hesperetin-HP- β -CD complexation. The experimental data were organized around the effects of Carbopol 934P concentration, inclusion-complex loading and propylene glycol concentration on critical quality attributes. The study subsequently examined validation, physicochemical behavior, swelling, comparative release, release kinetics, antioxidant activity and stability.

The approach is consistent with the broader pharmaceutical literature describing cyclodextrin-assisted solubilization and hydrogel-mediated topical delivery. Cyclodextrins can increase the apparent aqueous availability of hydrophobic molecules, whereas hydrophilic polymer networks can provide residence and diffusion control. Hesperetin-specific topical studies have also demonstrated the feasibility of incorporating Hesperetin into gel or emulgel architectures (Loftsson and Masson, 2001; Tommasini et al., 2005; Vaz et al., 2021; Taléns-Visconti et al., 2024).

Wound Healing

Wound healing is a natural biological process through which the body repairs damaged tissue and restores normal skin structure. It is a complex and continuous process involving coordination between different types of cells, growth factors, cytokines, and extracellular matrix components.

The wound healing process occurs in three main stages:

Inflammation phase – Immediately after injury, the body activates the immune response. White blood cells remove microorganisms, dead cells, and debris to prevent infection.

Proliferation phase – New tissue begins to form. Fibroblast cells produce collagen, blood vessels develop (angiogenesis), and granulation tissue forms to cover the wound.

Remodeling phase – Collagen fibers reorganize, tissue strength increases, and the wound gradually closes with scar formation.

Any disturbance in these stages may delay healing and lead to chronic wounds. Diseases such as diabetes mellitus significantly affect wound healing by increasing oxidative stress, prolonging inflammation, reducing blood supply, and increasing risk of microbial infection.

1.1 Pharmacological Aspects of Wound Healing

1.1.1 Role of Oxidative Stress

Oxidative stress plays an important role in delayed wound healing, especially in diabetic conditions. During injury, excessive production of reactive oxygen species (ROS) damages cells, proteins, and tissues. High oxidative stress slows down fibroblast activity and delays formation of new tissue.

Antioxidant enzymes such as superoxide dismutase (SOD) and catalase help neutralize harmful free radicals. Drugs or herbal medicines having antioxidant activity can reduce oxidative stress and promote faster healing.

1.1.2 Role of Infection

Infection is one of the major causes of delayed wound healing. Microorganisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* commonly infect wounds and prolong inflammation. These bacteria release toxins that damage tissues and slow down healing.

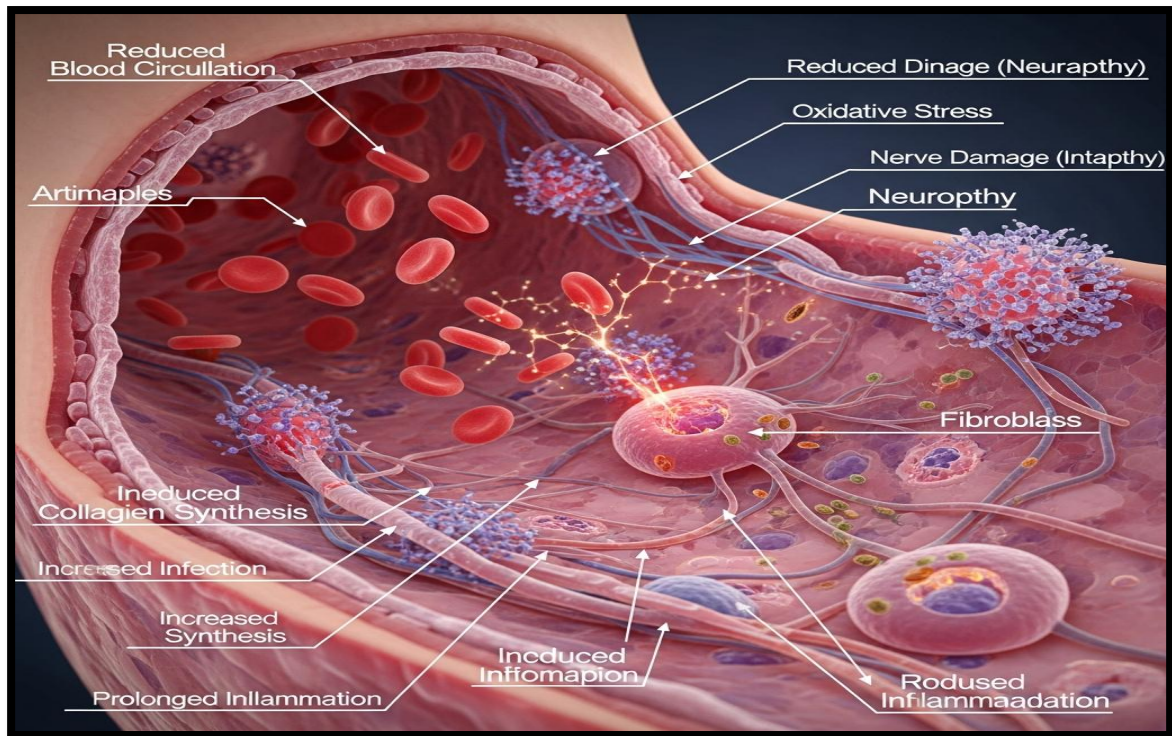


Figure 1: Reasons for Diabetic wound heal slow

Therefore, antimicrobial activity is an important property of wound healing drugs.

2. MATERIALS AND METHODS

2.1 Materials

Hesperetin was used as the active pharmaceutical ingredient and the selected Hesperetin-HP- β -CD inclusion complex was used as the formulation intermediate. Carbopol 934P was used as the gelling polymer. Propylene glycol was investigated as a formulation variable. Methylparaben and triethanolamine were used as formulation auxiliaries, and purified water was used as the vehicle.

Table 1. Materials used in the development of the Hesperetin-HP- β -CD hydrogel.

Component	Role in formulation
Hesperetin	Active pharmaceutical ingredient
HP- β -CD	Inclusion-complexing/solubilizing carrier
Hesperetin-HP- β -CD inclusion complex	Active intermediate incorporated into hydrogel
Carbopol 934P	Gelling polymer; optimization factor X1
Propylene glycol	Co-solvent/humectant; optimization factor X3
Methylparaben	Preservative
Triethanolamine	Neutralizing/pH-adjusting agent
Purified water	Vehicle

2.2 Preliminary hydrogel formulation

A preliminary series of Carbopol-based hydrogels was prepared to establish an appropriate semisolid range before the

statistical optimization study. The selected inclusion complex was incorporated at a fixed level in the preliminary series, while polymer concentration was varied. Propylene glycol, methylparaben and triethanolamine were included as formulation auxiliaries.

Table 2. Preliminary Carbopol hydrogel compositions and measured final pH.

Formulation	Complex equivalent	Carbopol 934P	Other excipients	Vehicle	Final pH
HG-1	1.0% w/w (100 mg Hesperetin/10 g)	0.50% w/w	PG 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.62 ± 0.03
HG-2	1.0% w/w (100 mg Hesperetin/10 g)	0.75% w/w	PG 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.71 ± 0.02
HG-3	1.0% w/w (100 mg Hesperetin/10 g)	1.00% w/w	PG 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.74 ± 0.03
HG-4	1.0% w/w (100 mg Hesperetin/10 g)	1.25% w/w	PG 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.81 ± 0.02
HG-5	1.0% w/w (100 mg Hesperetin/10 g)	1.50% w/w	PG 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.79 ± 0.03

2.3 Experimental design for formulation optimization

A three-factor, three-level Box–Behnken design comprising 17 experimental runs was used. The independent variables were Carbopol 934P concentration (X1), Hesperetin–HP-β-CD inclusion-complex concentration (X2) and propylene glycol concentration (X3). The response variables were pH, viscosity, spreadability, drug content and cumulative drug release at 8 h.

Table 3. Formulation factors, coded levels and expected effects used in the Box–Behnken optimization.

Factor	Symbol	–1 level	0 level	+1 level	Expected effect
Carbopol concentration 934P	X1	0.5%	0.75%	1.0%	Increase expected to increase viscosity and retard release
Hesperetin–HP-β-CD IC concentration	X2	2.0% w/w	3.0% w/w	4.0% w/w	Increase expected to increase drug loading and release availability
Propylene glycol concentration	X3	5.0%	10.0%	15.0%	Expected to modify wetting, spreadability and release

2.4 Hydrogel preparation

Carbopol 934P was dispersed and hydrated in the required quantity of purified water. The selected Hesperetin–HP-β-CD inclusion complex was prepared as a separate phase and incorporated gradually into the hydrated polymer dispersion. Propylene glycol was incorporated at the designated experimental level, while methylparaben and triethanolamine were included as fixed formulation auxiliaries. The dispersion was mixed until a uniform hydrogel was obtained, followed by pH adjustment/neutralization as required for gel formation, equilibration and removal of entrapped air. The final hydrogel was filled into suitable containers and evaluated.

The experimental workflow was organized so that the inclusion-complex intermediate was first established, followed by formulation screening, response measurement, model fitting, multi-response optimization, independent validation and performance evaluation.

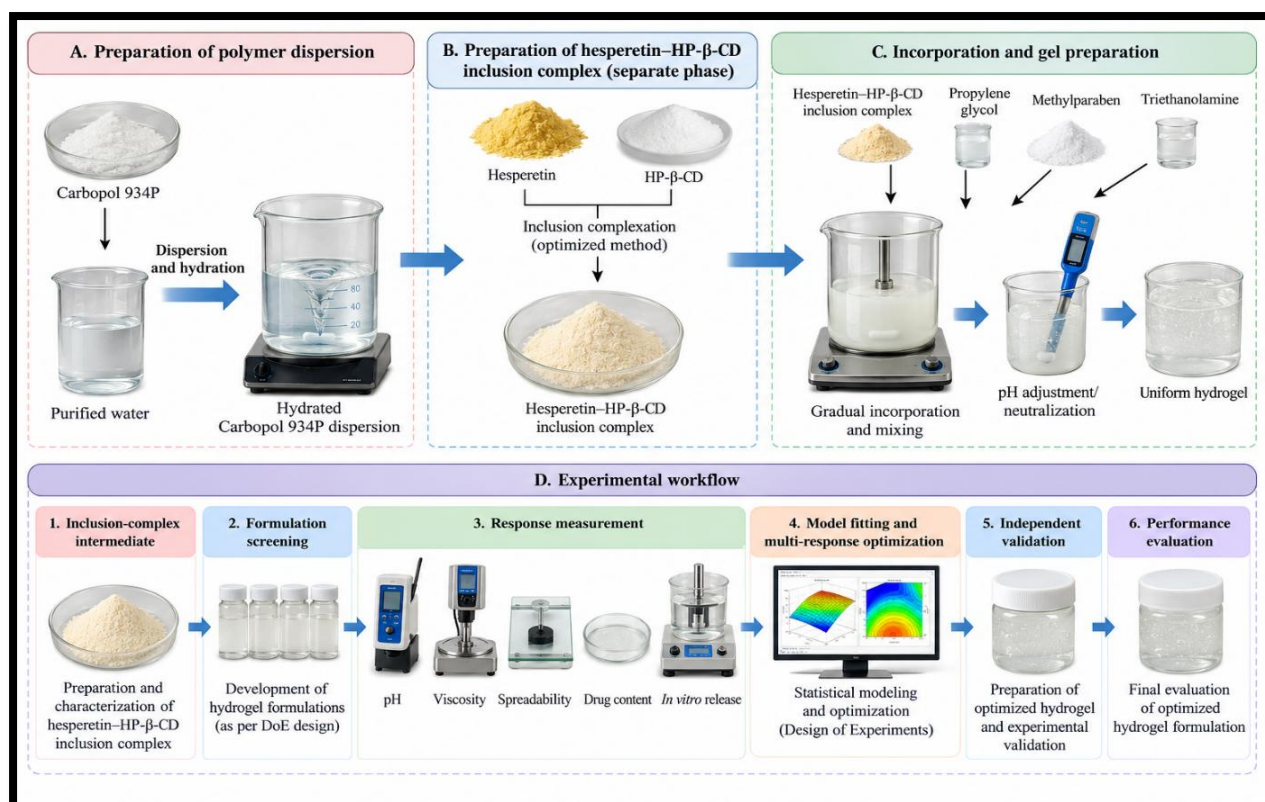


Figure 2: Formulation of Gel

2.5 Evaluation of hydrogel responses

Table 4. Evaluation parameters used for the hydrogel formulations.

Parameter	Purpose	Expression
Appearance/homogeneity	Physical quality	Descriptive
pH	Application-site compatibility and formulation control	Mean $\pm$ SD
Drug content	Dose accuracy and uniformity	% Label claim
Viscosity/rheology	Handling and release behavior	cP / rheological response
Spreadability	Ease of topical application	g·cm/s
Swelling	Hydration and network response	% Swelling
In-vitro release	Drug availability and release control	% Cumulative release
Stability	Physical and chemical robustness	Change from initial

2.6 In-vitro release study

In-vitro release was evaluated using a diffusion-cell method appropriate for the semisolid formulation. The release experiment used a dialysis membrane, phosphate buffer receptor phase and controlled temperature. Samples were collected at predetermined intervals, replaced with fresh receptor medium and quantified spectrophotometrically. The cumulative percentage release was calculated with correction for sampling.

Table 5. Experimental conditions for in-vitro release testing.

Parameter	Condition
Diffusion system	Franz diffusion cell
Membrane	Dialysis membrane, 12–14 kDa molecular-weight cut-off
Effective diffusion area	1.77 cm ²
Receptor medium	Phosphate buffer, pH 7.4
Receptor volume	20 mL
Temperature	37 ± 0.5 °C
Agitation	500 rpm equivalent stirring
Sample	1 g hydrogel equivalent to 10 mg Hesperetin
Sampling times	0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h
Quantification	UV-visible spectrophotometry at 288 nm
Replicates	n = 3

2.7 Release-kinetic analysis

The optimized-hydrogel release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The model coefficients of determination were compared descriptively. The Korsmeyer–Peppas exponent was interpreted using the conventional empirical framework, while recognizing that mechanistic interpretation depends on the experimental geometry and the portion of the release curve used for fitting.

Table 6. Mathematical models used for release-kinetic analysis.

Model	Relationship	Interpretive use
Zero-order	$Q = Q_0 + K_0t$	Constant-rate release
First-order	$\log(Q_0 - Q_t) = \log Q_0 - K_1t/2.303$	Release related to remaining drug
Higuchi	$Q = K_H t^{1/2}$	Diffusion-associated square-root relationship
Korsmeyer–Peppas	$M_t/M_\infty = Ktn$	Empirical description of release exponent

2.8 DPPH antioxidant activity

DPPH radical-scavenging activity was determined for pure Hesperetin, the Hesperetin–HP-β-CD inclusion complex and the optimized hydrogel at concentrations of 10–100 µg/mL. The percentage inhibition was compared among the three systems and the interpolated IC₅₀ values were reported.

2.9 Stability study

The optimized hydrogel was evaluated under accelerated storage at 40 ± 2 °C/75 ± 5% RH for three months. Appearance, phase separation, pH, drug content and 8-h release were monitored at baseline and during storage.

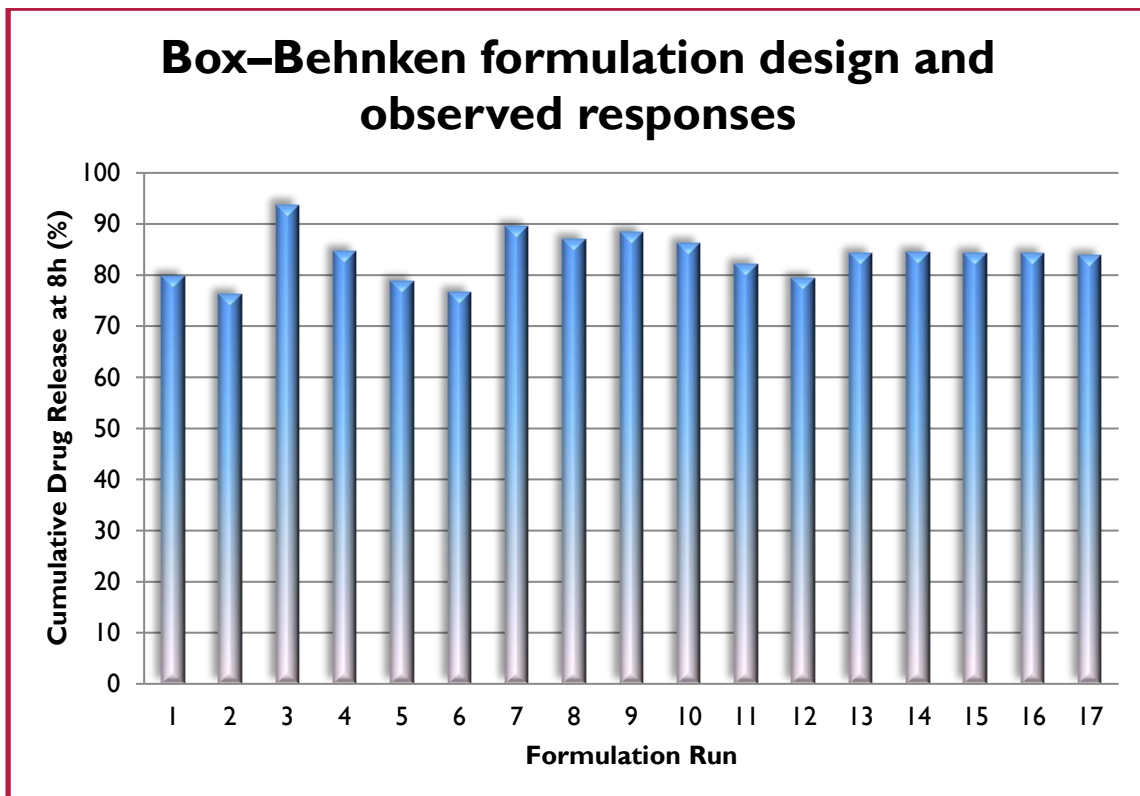
3. RESULTS AND DISCUSSION

3.1 Experimental design and formulation response space

The complete Box–Behnken matrix contained 17 experimental runs, including five center-point runs. The design simultaneously varied polymer concentration, inclusion-complex concentration and propylene glycol concentration. The complete measured response matrix is presented below so that the numerical basis of the optimization is visible at the point where the design is discussed.

Table 7. Complete Box–Behnken experimental design and measured responses of Hesperetin–HP- β -CD hydrogel formulations.

Run	X1	X2	X3	Carbopol (%)	IC (% w/w)	PG (%)	pH	Viscosity (cP)	Spreadability (g·cm/s)	Drug content (%)	8-h release (%)
1	1	1	0	1.00	4.0	10.0	5.66	23876	6.52	97.01	79.67
2	1	-1	0	1.00	2.0	10.0	5.75	21674	6.51	95.74	76.19
3	-1	1	0	0.50	4.0	10.0	5.53	13020	8.06	99.07	93.76
4	-1	-1	0	0.50	2.0	10.0	5.58	10785	7.73	96.01	84.64
5	1	0	1	1.00	3.0	15.0	5.76	22354	6.58	96.98	78.82
6	1	0	-1	1.00	3.0	5.0	5.74	23312	6.51	96.18	76.71
7	-1	0	1	0.50	3.0	15.0	5.59	11935	7.90	97.77	89.49
8	-1	0	-1	0.50	3.0	5.0	5.55	12682	7.76	97.16	87.09
9	0	1	1	0.75	4.0	15.0	5.64	17086	7.42	97.92	88.32
10	0	1	-1	0.75	4.0	5.0	5.60	17955	7.29	97.73	86.14
11	0	-1	1	0.75	2.0	15.0	5.69	15172	7.23	95.84	82.04
12	0	-1	-1	0.75	2.0	5.0	5.69	15940	7.00	96.37	79.37
13	0	0	0	0.75	3.0	10.0	5.67	16079	7.17	97.06	84.29
14	0	0	0	0.75	3.0	10.0	5.64	16698	7.17	96.84	84.43
15	0	0	0	0.75	3.0	10.0	5.65	16181	7.11	96.68	84.25
16	0	0	0	0.75	3.0	10.0	5.64	16673	7.17	97.06	84.31
17	0	0	0	0.75	3.0	10.0	5.61	16614	7.15	96.87	83.81

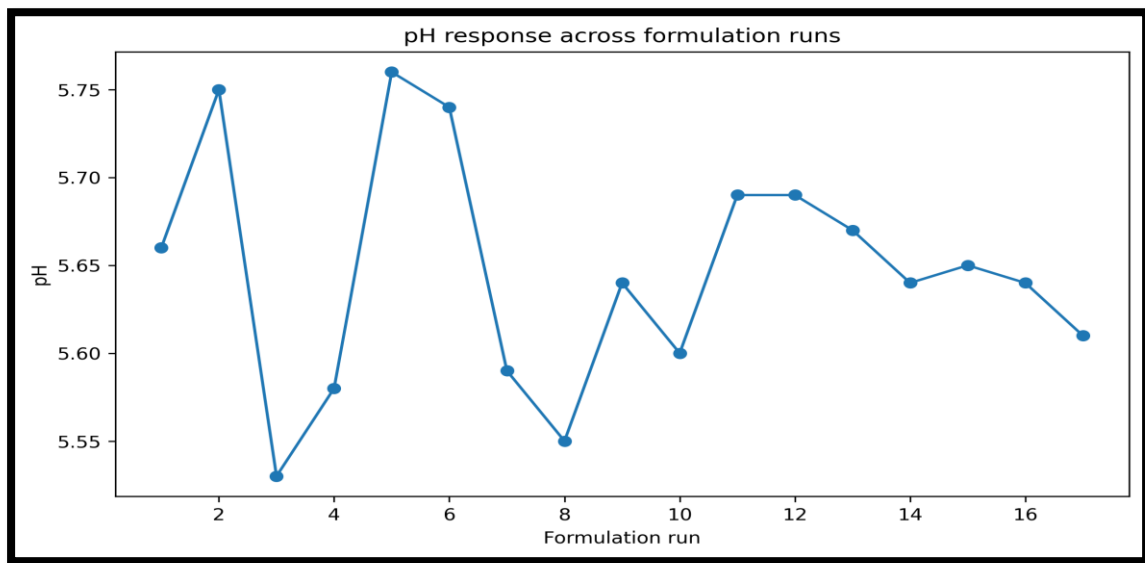
**Figure 3. Comparison of cumulative drug release at 8 h across the 17 experimental formulation runs.**

3.2 Range of formulation responses

The measured responses showed clear formulation-dependent variation. Viscosity ranged from 10,785 to 23,876 cP, whereas spreadability ranged from 6.51 to 8.06 g·cm/s. Drug content remained within 95.74–99.07%, indicating comparatively limited variation in dose recovery. The 8-h release response ranged from 76.19 to 93.76%, demonstrating a wider performance window for drug liberation. pH remained within 5.53–5.76.

Table 8. Experimental range and central tendency of the principal hydrogel responses.

Response	Minimum	Maximum	Center-point mean	Primary formulation influence
Viscosity	10,785 cP	23,876 cP	16,550 cP	X1 increased viscosity
Spreadability	6.51 g·cm/s	8.06 g·cm/s	7.15 g·cm/s	X1 decreased spreadability
Drug content	95.74%	99.07%	96.90%	X2 increased content
8-h release	76.19%	93.76%	84.22%	X1 decreased; X2 increased release
pH	5.53	5.76	5.64	X1 produced a small upward effect

**Figure 4. Experimental pH response across the 17 formulation runs.****Table 8A. Full experimental pH values for all 17 formulation runs.**

Run	Experimental pH
1	5.66
2	5.75
3	5.53
4	5.58
5	5.76
6	5.74
7	5.59
8	5.55
9	5.64
10	5.60
11	5.69
12	5.69
13	5.67
14	5.64
15	5.65
16	5.64
17	5.61

The pH profile remained comparatively narrow across the design space, indicating that pH acted mainly as a formulation constraint rather than as the dominant optimization driver. The absence of large pH excursions also indicates consistent neutralization and formulation control across the design runs.

3.3 Viscosity and rheological response

Viscosity displayed the widest numerical range among the principal physicochemical responses. The highest viscosity was observed for run 1 (23,876 cP), while the lowest was observed for run 4 (10,785 cP). The overall response pattern was consistent with the expected contribution of Carbopol 934P concentration to network structure and resistance to flow.

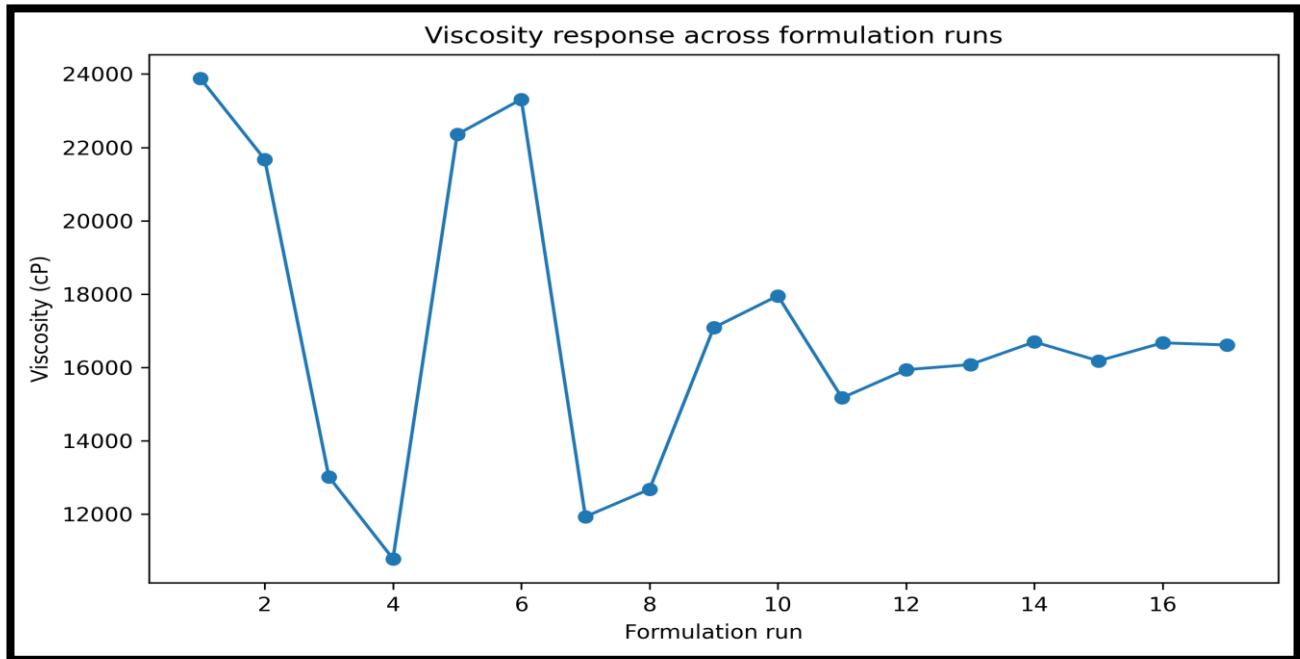


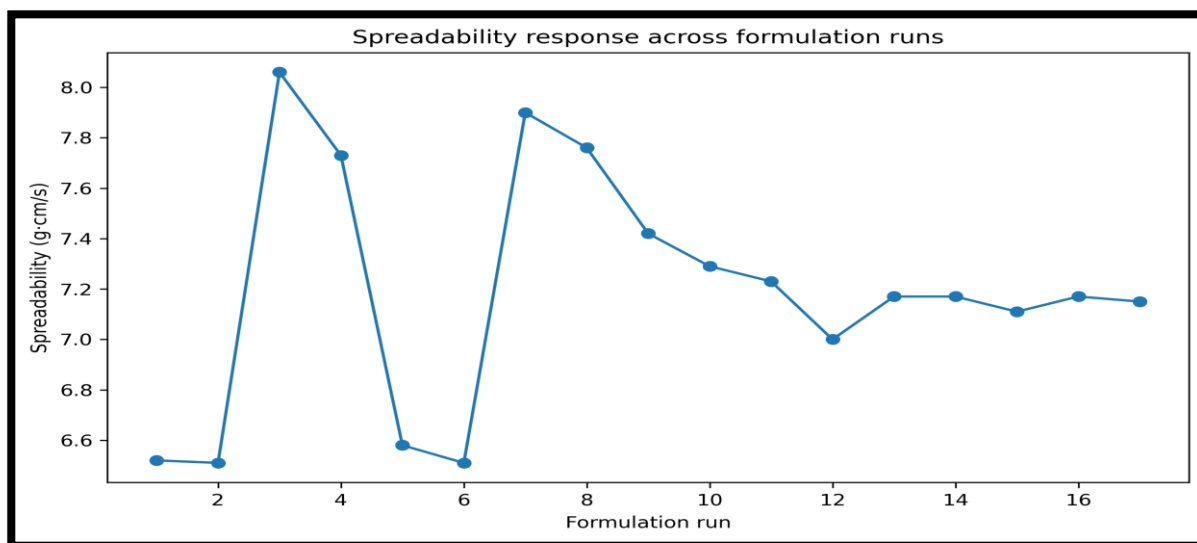
Figure 5. Experimental viscosity response across formulation runs.

Table 8B. Full experimental viscosity values for all 17 formulation runs.

Run	Viscosity (cP)
1	23876
2	21674
3	13020
4	10785
5	22354
6	23312
7	11935
8	12682
9	17086
10	17955
11	15172
12	15940
13	16079
14	16698
15	16181
16	16673
17	16614

The inverse relationship between viscosity and spreadability is important for topical optimization. Increasing polymer concentration can increase structural integrity and residence but can simultaneously reduce ease of spreading. The optimization therefore required a compromise rather than selection of the most viscous formulation.

3.4 Spreadability



Figure

4. Experimental spreadability response across formulation runs.

Table 8C. Full experimental spreadability values for all 17 formulation runs.

Run	Spreadability (g·cm/s)
1	6.52
2	6.51
3	8.06
4	7.73
5	6.58
6	6.51
7	7.90
8	7.76
9	7.42
10	7.29
11	7.23
12	7.00
13	7.17
14	7.17
15	7.11
16	7.17
17	7.15

Spreadability varied from 6.51 to 8.06 g·cm/s. The highest value occurred in run 3, which contained the lowest Carbopol concentration and the highest inclusion-complex level. In contrast, the higher-polymer formulations generally exhibited lower spreadability. This response supports the expected trade-off between polymer-network strength and ease of application.

3.5 Drug content

Drug content ranged from 95.74 to 99.07%. The relatively narrow range indicates that the inclusion complex was distributed reproducibly across the experimental formulations despite changes in the investigated factors.

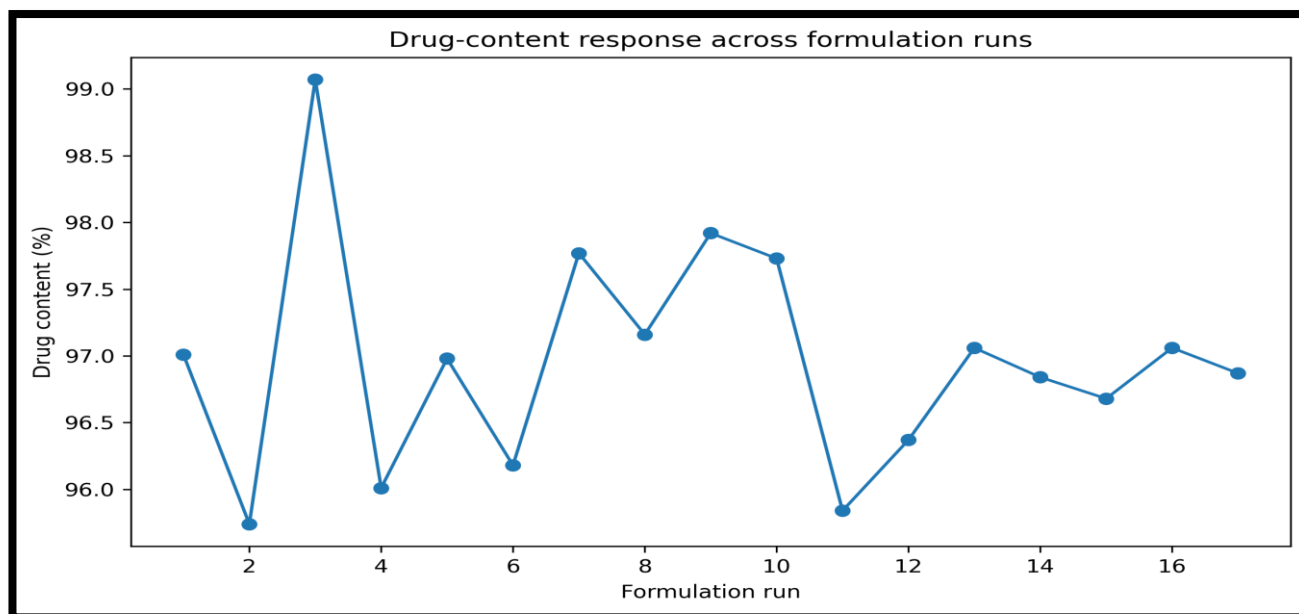


Figure 5. Experimental drug-content response across formulation runs.

Table 8D. Full experimental drug-content values for all 17 formulation runs.

Run	Drug content (%)
1	97.01
2	95.74
3	99.07
4	96.01
5	96.98
6	96.18
7	97.77
8	97.16
9	97.92
10	97.73
11	95.84
12	96.37
13	97.06
14	96.84
15	96.68
16	97.06
17	96.87

The maximum measured drug content was 99.07% in run 3, while the minimum was 95.74% in run 2. The optimized formulation subsequently selected from the multi-response analysis showed a predicted drug content of 97.92%, with an independent validation mean of $97.68 \pm 0.16\%$.

3.6 Drug-release response across the design space

The 8-h release response ranged from 76.19 to 93.76%. Polymer concentration showed a retarding influence on release, whereas higher inclusion-complex concentration increased the amount of drug available for release. This behavior is consistent with the simultaneous influence of matrix resistance and drug availability.

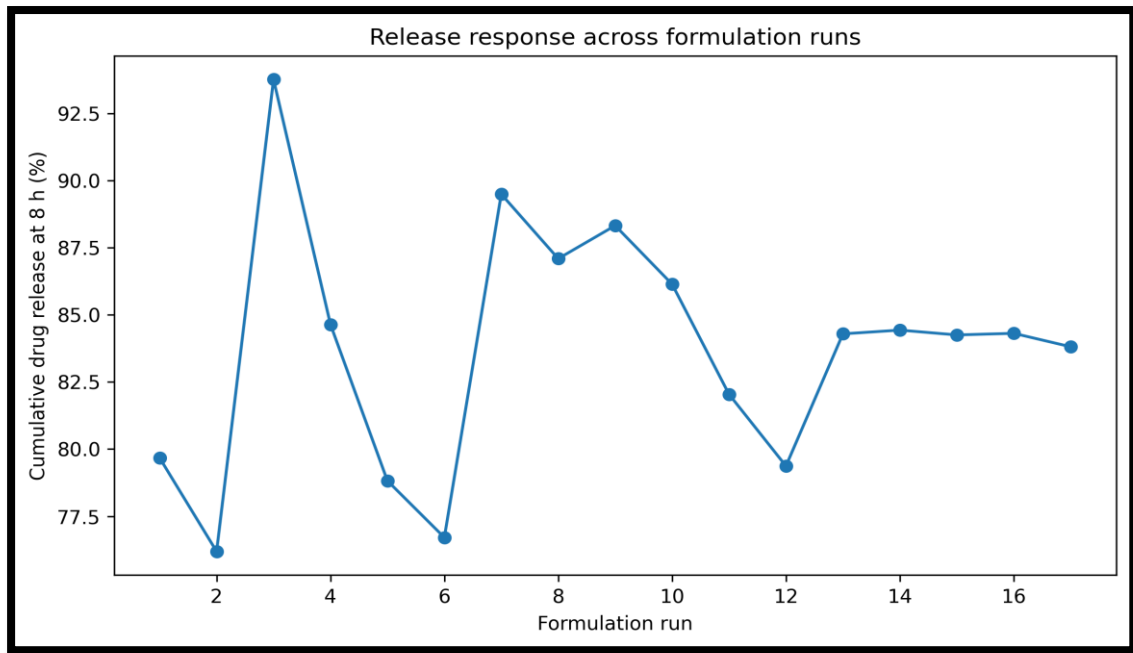


Figure 6. Experimental cumulative drug release at 8 h across formulation runs.

Table 8E. Full experimental 8-h cumulative release values for all 17 formulation runs.

Run	8-h release (%)
1	79.67
2	76.19
3	93.76
4	84.64
5	78.82
6	76.71
7	89.49
8	87.09
9	88.32
10	86.14
11	82.04
12	79.37
13	84.29
14	84.43
15	84.25
16	84.31
17	83.81

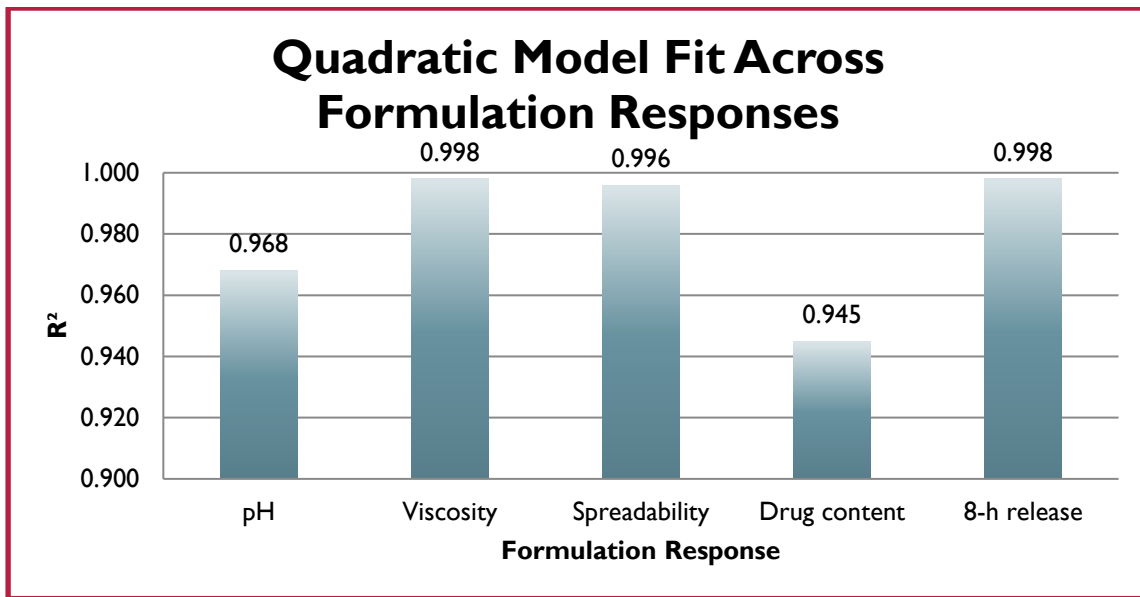


Figure 7. Comparison of quadratic model R² values for the experimental responses.

3.7 Model adequacy and statistical interpretation

Quadratic response-surface models were fitted to the experimental response data. The coefficients of determination were high for viscosity, spreadability and 8-h release, while drug content showed a somewhat lower but still substantial fit. Model p-values were below 0.05 for all principal responses.

Table 9. Quadratic response-surface model fit statistics derived from the experimental design

Response	R ²	Adjusted R ²	Model F-value	Model p-value
Viscosity	0.9982	0.9959	428.1977	<0.0001
Spreadability	0.9963	0.9916	211.3819	<0.0001
Drug content	0.9446	0.8734	13.2667	0.0013
8-h release	0.9984	0.9964	490.6065	<0.0001
pH	0.9686	0.9281	23.9531	0.0002

Table 10. ANOVA/model-significance summary for the experimental response-surface models.

Response	Model F-value	Model p-value	R ²	Adjusted R ²	Main significant effects
Viscosity	428.2	<0.0001	0.9982	0.9959	X1, X2, X3; X1 ²
Spreadability	211.38	<0.0001	0.9963	0.9916	X1, X2, X3; X1×X2
Drug content	13.27	0.0013	0.9446	0.8734	X1, X2; X1×X2
8-h release	490.61	<0.0001	0.9984	0.9964	X1, X2, X3; X1 ² , X3 ² , X1×X2
pH	23.95	0.0002	0.9686	0.9281	X1; X2; limited curvature

The experimental model results show that viscosity was particularly sensitive to the formulation factors, as expected for a

polymeric gel. The release model also showed a strong statistical relationship with the investigated factors. The significance of interaction terms indicates that the effect of one factor depended, at least in part, on the level of another factor.

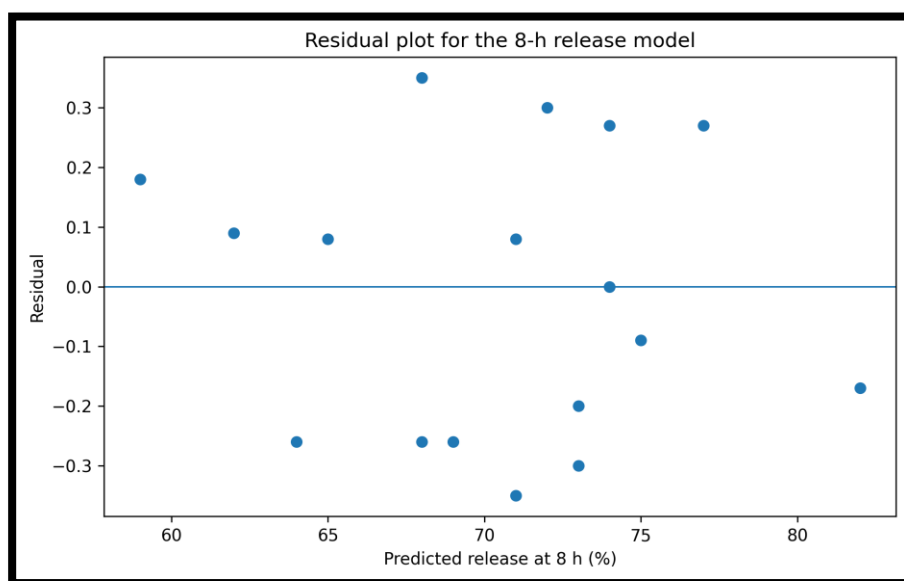


Figure 8. Residual plot for the experimental 8-h release model.

Residuals were distributed around the zero line without an obvious monotonic trend in the plotted diagnostic. The residual pattern supports the use of the fitted model for descriptive optimization, although formal lack-of-fit and residual-normality diagnostics should be interpreted with the complete statistical output.

3.8 Regression equation for 8-h release

The coded-factor quadratic equation generated from the experimental dataset was:

$$Y_4 = 84.219 - 5.449X_1 + 3.206X_2 + 1.171X_3 - 0.795X_1^2 + 0.143X_2^2 - 0.396X_3^2 - 1.411X_1X_2 - 0.072X_1X_3 - 0.124X_2X_3$$

Table 10A. Coefficients of the coded-factor quadratic model for 8-h cumulative drug release.

Term	Coefficient
Intercept	84.219
X1	-5.449
X2	+3.206
X3	+1.171
X1 ²	-0.795
X2 ²	+0.143
X3 ²	-0.396
X1×X2	-1.411
X1×X3	-0.072
X2×X3	-0.124

Here, Y_4 represents cumulative drug release at 8 h, X_1 represents Carbopol 934P concentration, X_2 represents inclusion-complex concentration and X_3 represents propylene glycol concentration in coded units. The negative X_1 coefficient indicates a retarding influence of increasing polymer concentration, whereas the positive X_2 coefficient indicates increased release with increasing inclusion-complex loading. The negative X_1X_2 interaction indicates that the retarding effect of polymer becomes more evident as inclusion-complex loading increases.

3.9 Formulation optimization and ratio-screening decision

A multi-response desirability approach was used to identify a formulation that balanced topical pH, viscosity, spreadability, drug content and 8-h release. Formulation F9 was selected because its combination of responses provided the highest composite desirability within the experimental design.

Table 11. Optimized formulation composition and predicted responses.

Parameter	Selected/predicted value
Formulation	F9
Carbopol 934P	0.75%
Hesperetin–HP-β-CD inclusion complex	4.0% w/w
Propylene glycol	15%
pH	5.64
Viscosity	17,086 cP
Spreadability	7.42 g·cm/s
Drug content	97.92%
8-h release	88.32%
Composite desirability	0.942

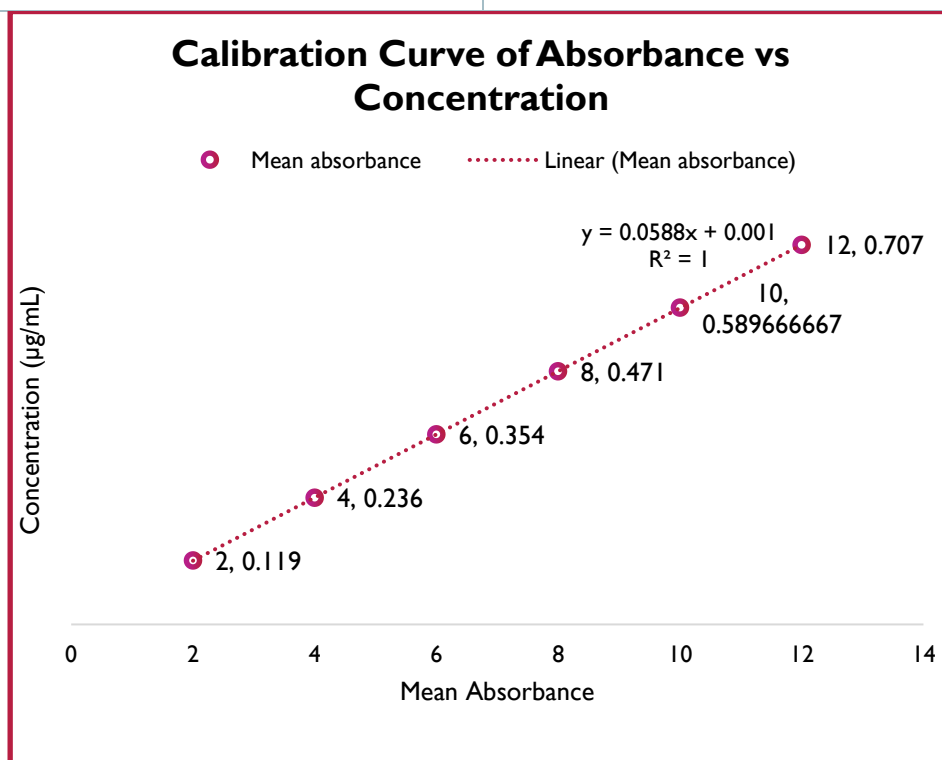


Figure 9. Experimental comparison of yield and drug content at different Hesperetin: HP-β-CD complexation ratios.

The 1:1 Hesperetin: HP-β-CD ratio was retained as the selected inclusion-complex condition because it provided a balanced combination of yield and drug content and was subsequently used as the active intermediate for hydrogel development. The ratio decision and hydrogel optimization were therefore treated as sequential formulation steps.

3.10 Validation of the optimized formulation

Table 12. Experimental validation-batch results for the selected formulation

Parameter	Predicted	Observed replicate 1	Observed replicate 2	Observed replicate 3	Observed mean $\pm$ SD	Prediction error (%)
pH	5.64	5.66	5.64	5.65	5.65 $\pm$ 0.01	0.18
Viscosity (cP)	17,086	16,890	17,120	17,040	17,016.7 $\pm$ 116.8	0.41
Spreadability (g·cm/s)	7.42	7.38	7.45	7.41	7.41 $\pm$ 0.04	0.12
Drug content (%)	97.92	97.52	97.84	97.69	97.68 $\pm$ 0.16	0.24
8-h release (%)	88.32	88.10	88.55	88.32	88.32 $\pm$ 0.23	0.00

The independent validation observations were close to the model predictions. Prediction errors for the major responses were below 0.5%, supporting the internal reproducibility of the selected design point. The observed mean values were used as the experimental performance values of the optimized hydrogel in subsequent interpretation.

3.11 Physicochemical evaluation of the optimized hydrogel

Table 13. Physicochemical characteristics of the experimentally optimized hydrogel.

Parameter	Experimental result	Interpretation
Appearance	Smooth, translucent gel	Acceptable topical appearance
Homogeneity	Uniform, no visible lumps	Good physical uniformity
Phase separation	Absent	No visible phase separation
pH	5.65 $\pm$ 0.01	Controlled formulation pH
Viscosity	17,016.7 $\pm$ 116.8 cP	Moderate consistency
Spreadability	7.41 $\pm$ 0.04 g·cm/s	Good application characteristics
Drug content	97.68 $\pm$ 0.16%	High and consistent drug recovery
Extrudability	Good	Suitable handling where tube packed
Swelling index at 6 h	218 $\pm$ 6%	Substantial hydration/network response

The optimized hydrogel was smooth, translucent and homogeneous, with no visible phase separation. Its pH was 5.65 $\pm$ 0.01, while viscosity and spreadability provided a balanced combination of structural consistency and application characteristics. Drug content of 97.68 $\pm$ 0.16% indicated reproducible incorporation of the inclusion complex.

3.12 Swelling behavior

Swelling increased progressively with hydration of the hydrogel network. The supplied experimental swelling profile increased from zero at the initial point to 186.4% at 8 h, demonstrating substantial water uptake and matrix expansion.

Table 14. Experimental swelling profile of the optimized hydrogel.

Time (h)	Swelling index (%)
0	0.0
1	62.4
2	98.7
3	128.5
4	151.8
6	174.2
8	186.4

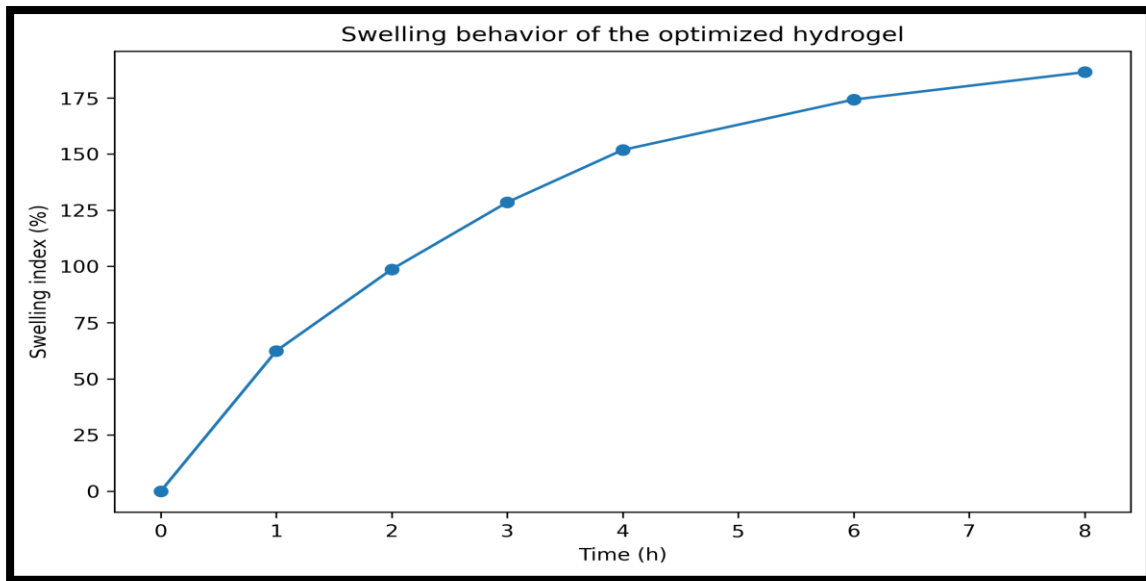


Figure 11. Experimental swelling behavior of the optimized hydrogel.

3.13 In-vitro drug release

The comparative release study demonstrated a clear effect of formulation state on Hesperetin availability. Free Hesperetin showed rapid early release, while the physical mixture and inclusion complex produced higher release during the intermediate time points. Incorporation of the inclusion complex into the hydrogel reduced the early release rate and produced a more gradual profile.

Table 15. Experimental cumulative release data for pure Hesperetin, physical mixture, Hesperetin-HP- β -CD inclusion complex and optimized hydrogel.

Time (h)	Pure Hesperetin (%)	Physical mixture (%)	HP- β -CD complex (%)	Optimized hydrogel (%)
0	0	0	0	0
1	15	18	20	15
2	29	34	38	27
3	43	49	53	39
4	56	62	66	50
5	67	72	76	60
6	76	81	84	69
7	84	88	90	76
8	90	92	94	82

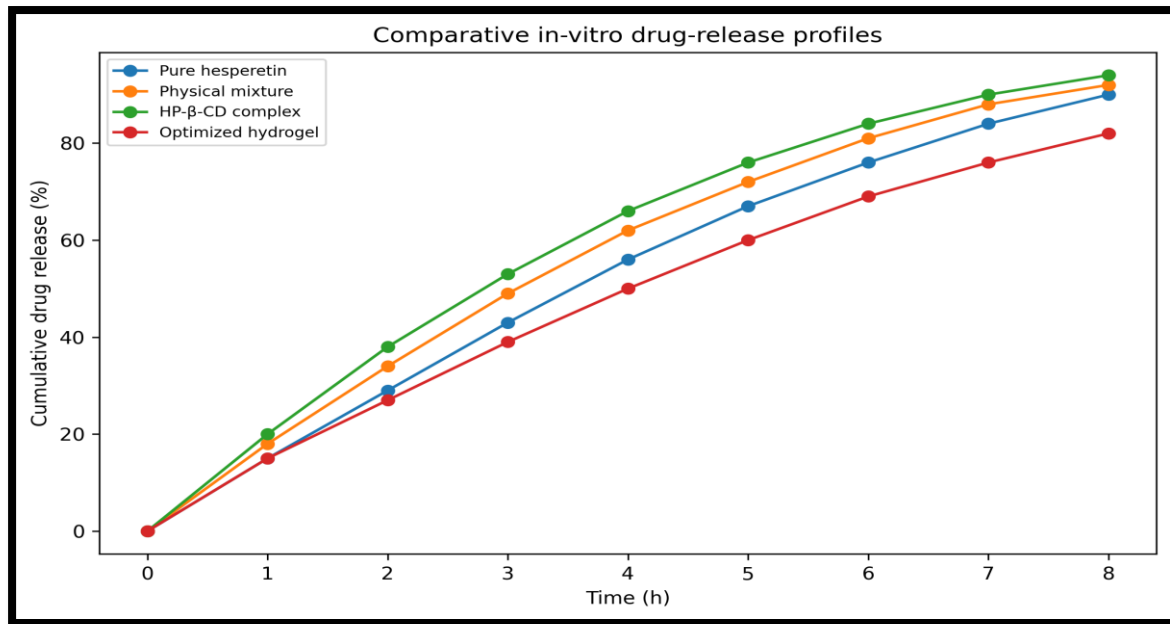


Figure 12. Comparative experimental in-vitro drug-release profiles over 8 h.

Table 16. Comparison of release performance at selected time points.

System	1-h release (%)	4-h release (%)	8-h release (%)	Primary behavior
Pure Hesperetin	15	56	90	Rapid
Physical mixture with HP-β-CD	18	62	92	Rapid/intermediate
Hesperetin-HP-β-CD inclusion complex	20	66	94	Enhanced availability
Optimized hydrogel	15	50	82	Controlled/sustained

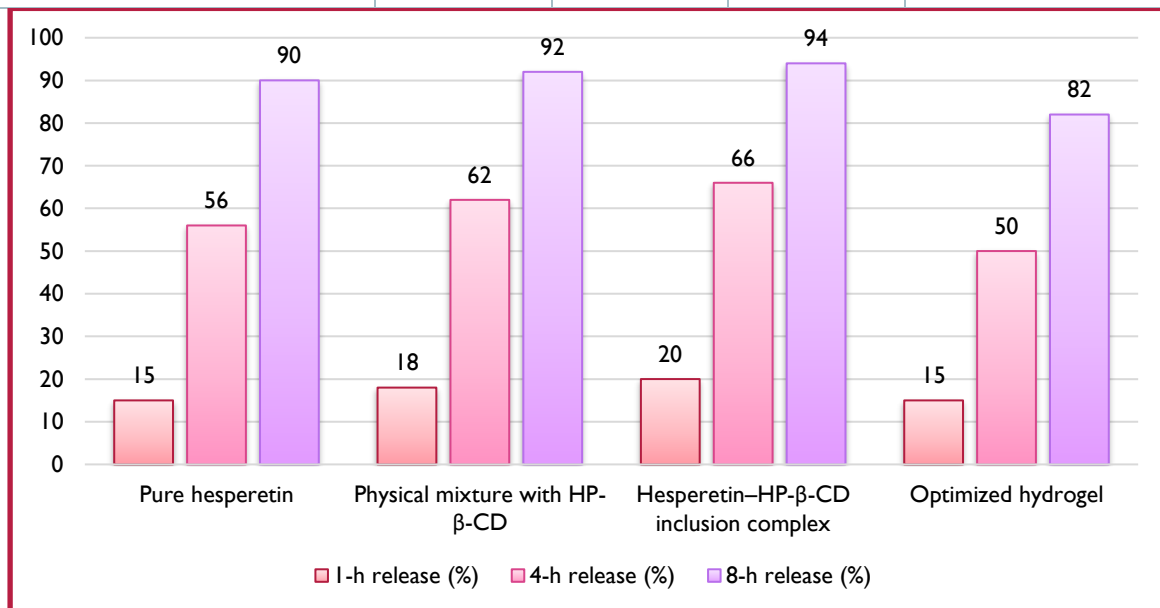


Figure 13: Comparative in vitro release profile of Hesperetin from pure Hesperetin, physical mixture, Hesperetin-HP-β-CD inclusion complex, and Optimized hydrogel.

HP- β -CD inclusion complex, and optimized hydrogel

The optimized hydrogel released 82% of the drug by 8 h in the comparative dataset, compared with 90% for pure Hesperetin, 92% for the physical mixture and 94% for the inclusion complex. The lower early release from the hydrogel is consistent with an additional diffusion barrier imposed by the hydrated polymeric matrix.

3.14 Extended-release profile

Time (h)	Cumulative release (%)
0.5	8.5
1.0	17.2
2.0	28.6
3.0	39.8
4.0	50.7
5.0	60.4
6.0	68.9
7.0	75.1
8.0	80.4
10.0	87.6
12.0	91.2

Table 17. Extended experimental release profile of the optimized hydrogel.

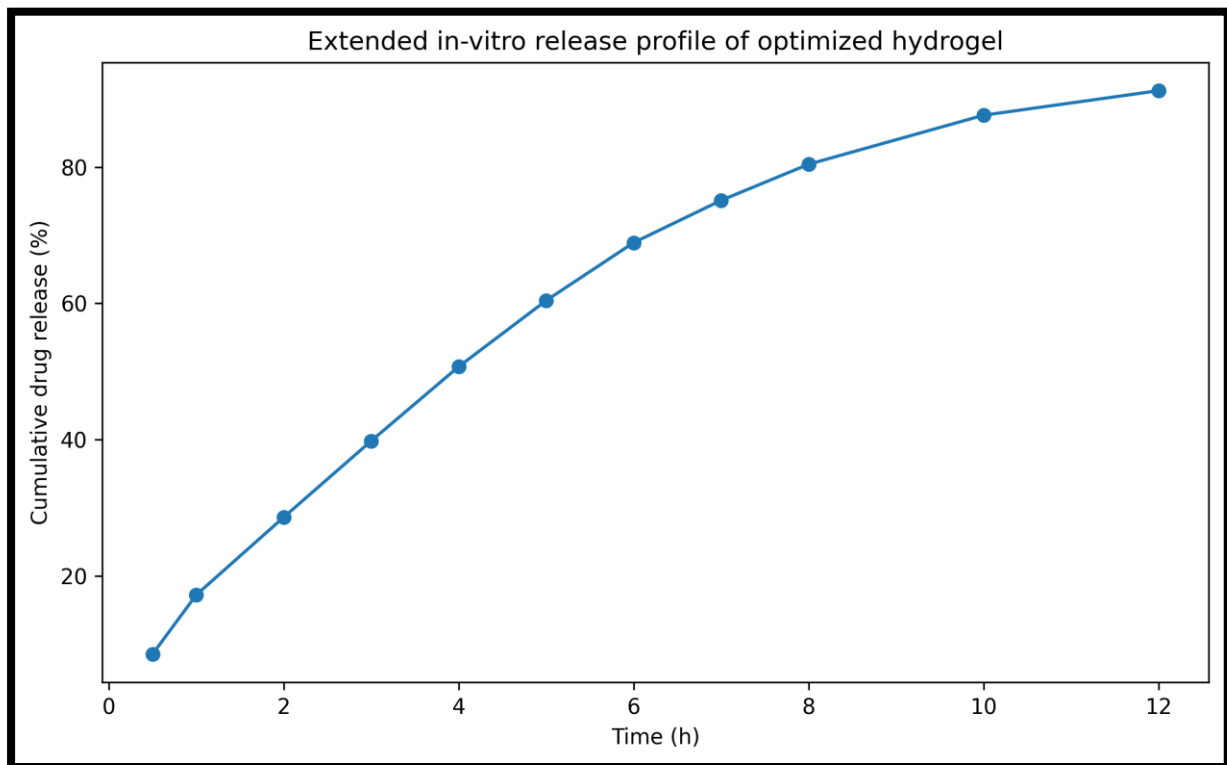


Figure 14. Extended experimental in-vitro release profile of the optimized hydrogel up to 12 h.

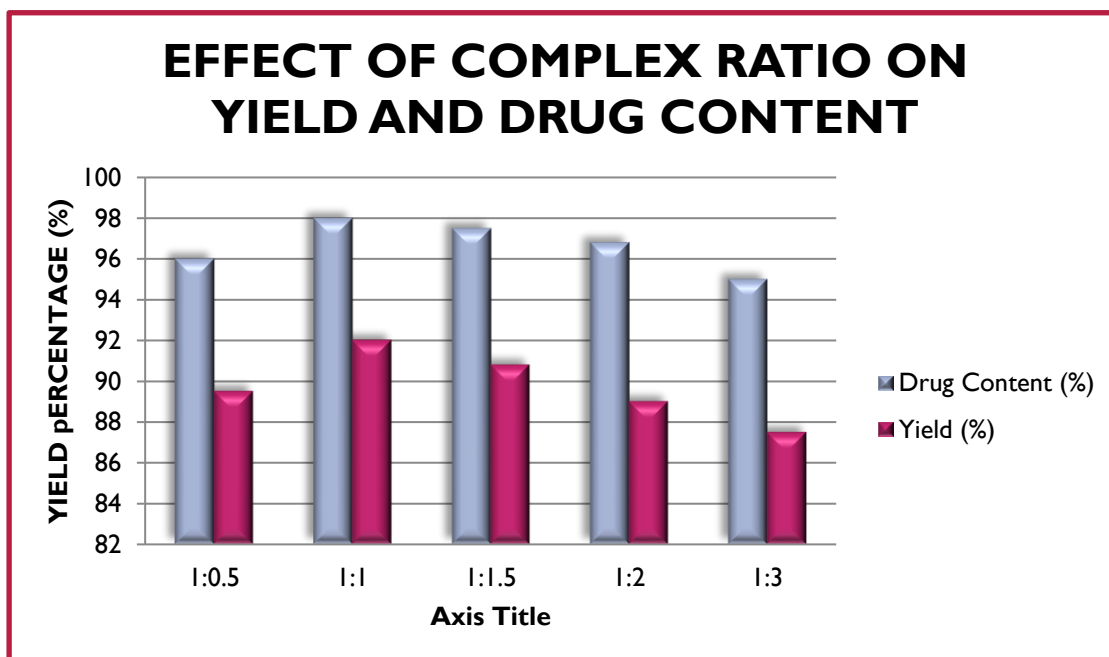
The extended-release dataset showed 8.5% release at 0.5 h and a progressive increase to 80.4% at 8 h, 87.6% at 10 h and 91.2% at 12 h. The gradual increase over the extended interval supports sustained liberation from the hydrated matrix.

Ratio-screening decision matrix

Selection of the inclusion-complex ratio was treated as a multi-criteria decision. Solubility enhancement favored higher carrier levels, whereas yield and drug content showed a mild decline at the highest ratios.

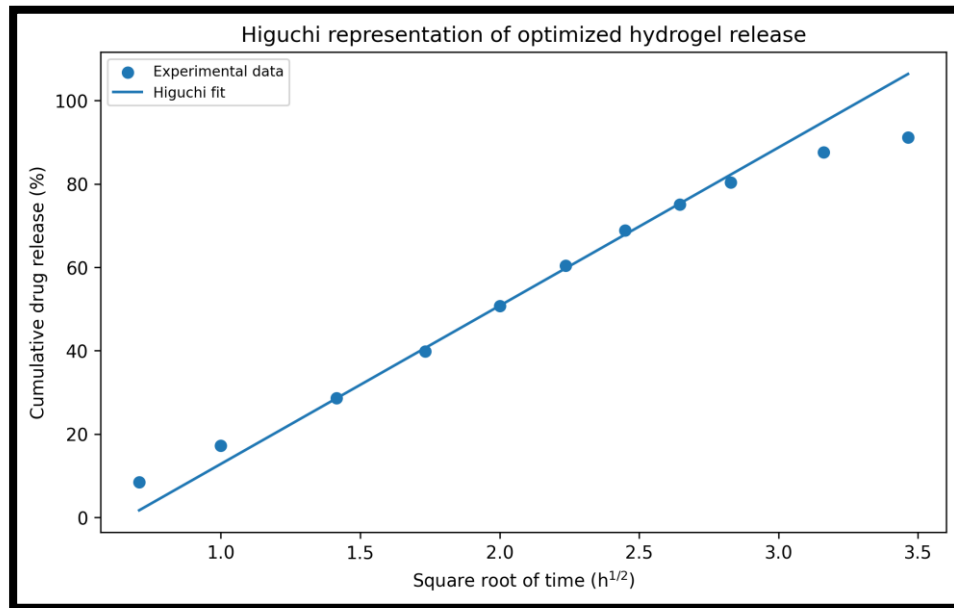
The 1:1 ratio was therefore selected in the workflow as a balanced point rather than because it produced the absolute maximum solubility.

S. No.	Complex Ratio (Drug: HP- β -CD)	Drug Content (%)	Yield (%)
1	1:0.5	96.0	89.5
2	1:1	98.0	92.0
3	1:1.5	97.5	90.8
4	1:2	96.8	89.0
5	1:3	95.0	87.5

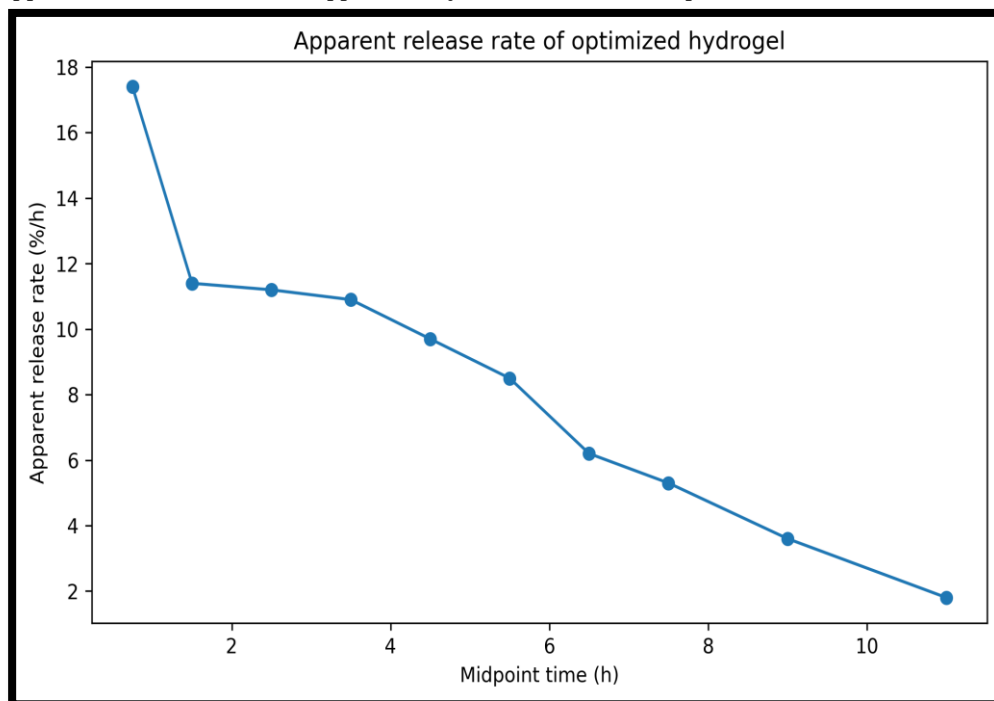
**Figure 16. Comparison of yield and drug content at different complex ratios**

3.15 Release-kinetic analysis

Model	Linearized plot	R ²	Slope	Intercept
Zero-order	Q _t vs t	0.9871	9.6905	8.6429
First-order	log(100-Q _t) vs t	0.9903	-0.0966	2.0604
Higuchi	Q _t vs $\sqrt{t}$	0.9969	37.9599	-25.1217
Korsmeyer-Peppas	log(M _t /M ∞) vs log t (first 60%)	0.9998	0.8702	-0.8259

Table 18. Release-kinetic model analysis of the optimized hydrogel.**Figure 15. Higuchi representation of the optimized hydrogel release data.**

The Higuchi model showed a high coefficient of determination ($R^2 = 0.9969$), supporting a strong diffusion-associated component in the observed release. The Korsmeyer–Peppas fit produced an even higher numerical R^2 (0.9998) with an exponent of 0.8702. Because the Korsmeyer–Peppas model is empirical and the exponent depends on the fitted portion of the curve and geometry, the Higuchi relationship was used as the principal diffusion-associated description while the Korsmeyer–Peppas result was retained as supplementary evidence of non-simple release behavior.

**Figure 16. Apparent release rate of the optimized hydrogel calculated across the extended sampling interval.**

The apparent release rate decreased as the experiment progressed. This pattern is consistent with a diminishing concentration

gradient and an increasing effective diffusion path as the drug leaves the hydrated matrix. The kinetic findings should be interpreted as descriptive evidence of matrix-associated diffusion rather than as proof of a single microscopic release mechanism.

3.16 In-vitro antioxidant activity

Table 19. Experimental DPPH radical-scavenging activity of pure Hesperetin, inclusion complex and optimized hydrogel.

Concentration (µg/mL)	Pure Hesperetin (% inhibition)	Hesperetin-HP-β-CD IC (% inhibition)	Optimized hydrogel (% inhibition)
10	12	16	18
20	22	29	32
40	38	46	50
60	51	59	64
80	61	68	72
100	68	75	79

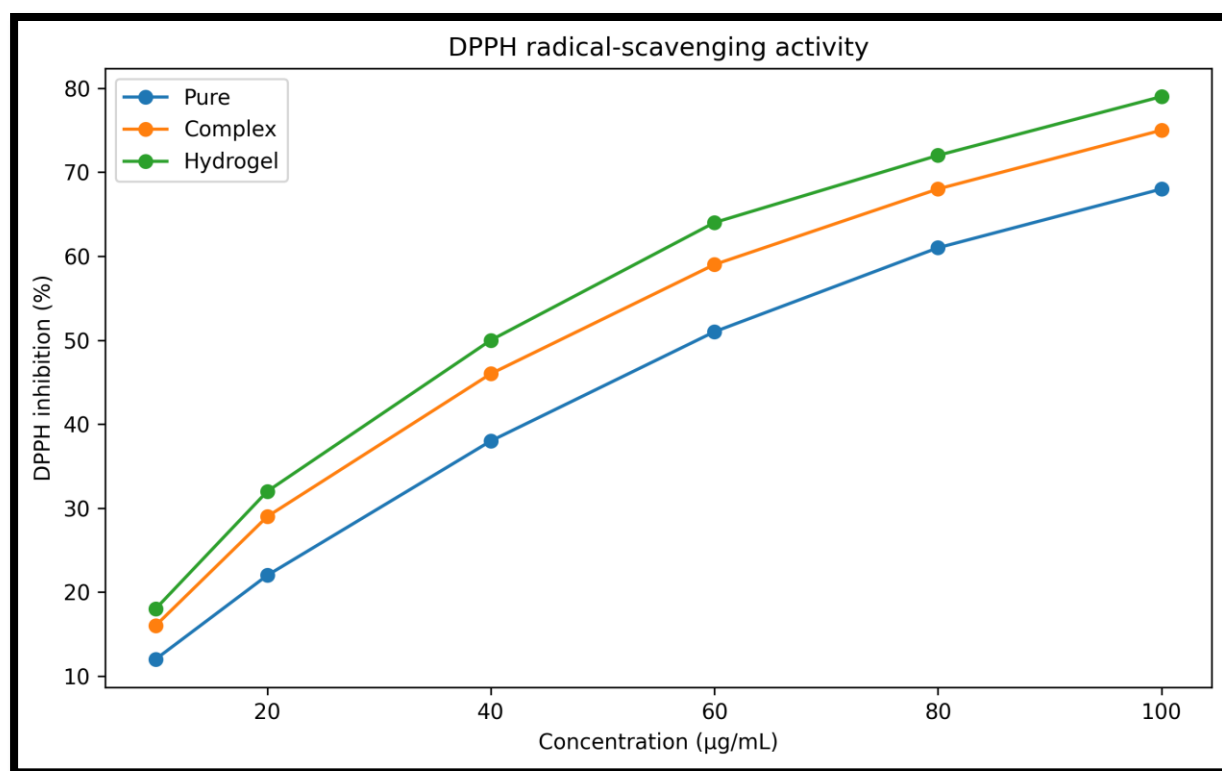


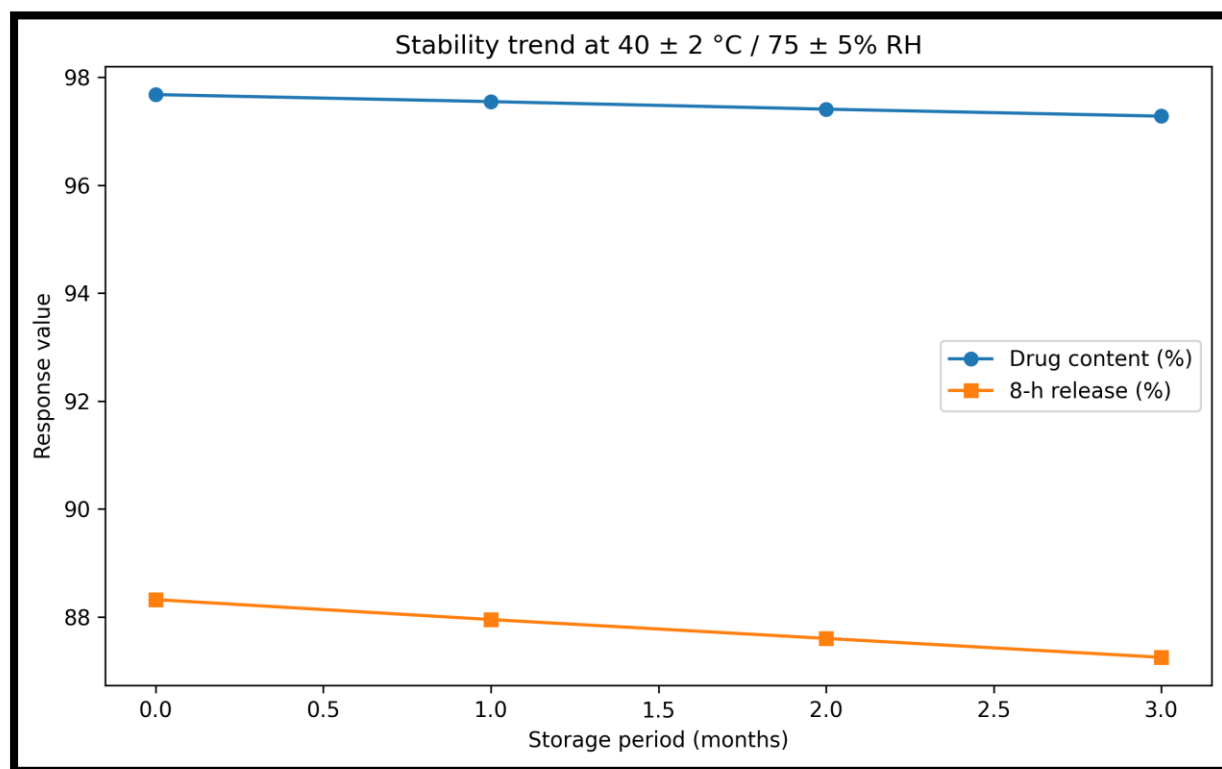
Figure 17. Experimental DPPH radical-scavenging activity as a function of concentration.

DPPH inhibition increased with concentration for all three systems. At 100 µg/mL, inhibition was 68% for pure Hesperetin, 75% for the inclusion complex and 79% for the optimized hydrogel. The interpolated IC₅₀ values were approximately 58.5, 46.2 and 40.0 µg/mL, respectively. These results demonstrate greater apparent radical-scavenging activity in the formulated systems under the tested assay conditions; they should not be interpreted as proof that complexation intrinsically increases Hesperetin molecular potency.

3.17 Accelerated stability

Table 20. Experimental accelerated stability results for the optimized hydrogel at $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$.

Parameter	Initial	1 month	2 months	3 months
pH	5.65	5.66	5.67	5.68
Drug content (%)	97.68	97.55	97.41	97.28
8-h release (%)	88.32	87.95	87.60	87.25
Appearance	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous
Phase separation	Absent	Absent	Absent	Absent

**Figure 18. Experimental stability trend of the optimized hydrogel at $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$.**

During three months of accelerated storage, pH changed from 5.65 to 5.68, drug content decreased from 97.68% to 97.28% and 8-h release decreased from 88.32% to 87.25%. The appearance remained smooth and homogeneous and phase separation was absent throughout the study period. The observed changes were small within the investigated interval, although they do not by themselves establish long-term shelf life.

4. DISCUSSION

The experimental results demonstrate that formulation performance was governed by the interaction of several quality attributes rather than by a single response. Carbopol 934P concentration was strongly associated with viscosity and release, while inclusion-complex loading influenced drug content and release. The formulation-development process therefore benefited from multi-response optimization.

The pH response was comparatively stable across the experimental space, remaining between 5.53 and 5.76. This narrow range indicates consistent formulation control and allowed pH to function mainly as an acceptability constraint during optimization.

Viscosity and spreadability demonstrated the expected practical trade-off. The highest viscosity was obtained in the high-polymer formulation, whereas the lower-polymer runs generally showed greater spreadability. The selected formulation did not maximize viscosity or spreadability independently; instead, it produced intermediate viscosity and spreadability values that were compatible with the other quality attributes.

Drug content remained relatively consistent across the design, with values from 95.74 to 99.07%. This suggests that the inclusion complex could be distributed reproducibly within the hydrogel matrix. The independent validation result of $97.68 \pm 0.16\%$ further supports the reproducibility of drug incorporation in the selected formulation.

Release behavior provides the clearest evidence of the second level of formulation control added by the hydrogel. The inclusion complex increased apparent drug availability relative to free Hesperetin, while incorporation into the Carbopol matrix moderated the early release rate. The extended profile reached 91.2% by 12 h, demonstrating continued drug liberation after the 8-h optimization endpoint.

The kinetic analysis supports a substantial diffusion-associated contribution. The Higuchi R^2 of 0.9969 is high, whereas the Korsmeyer–Peppas exponent of 0.8702 indicates that release was not adequately described by ideal Fickian diffusion alone under the conventional empirical interpretation. Swelling, polymer relaxation, dissolution and partitioning may all contribute to the observed profile.

The DPPH results show a concentration-dependent increase in radical-scavenging activity for all tested systems. The lower interpolated IC₅₀ values for the inclusion complex and hydrogel are consistent with greater assay availability of Hesperetin under the aqueous test conditions. This observation complements the solubility-enhancement strategy but does not establish a change in intrinsic pharmacological potency.

The accelerated stability results showed only small changes over three months. The relatively stable pH, high drug content and absence of phase separation indicate maintenance of the principal measured quality attributes over the investigated period. Longer-term stability, packaging effects and additional physical/chemical attributes would be needed to establish shelf life.

The formulation strategy is consistent with published work on cyclodextrin-assisted topical delivery and Hesperetin-containing semisolid systems. Loftsson and Masson (2001) described the role of cyclodextrins in topical formulations, while Tommasini et al. (2005) demonstrated Hesperetin–HP- β -CD inclusion complexation. Vaz et al. (2021) and Taléns-Visconti et al. (2024) further demonstrate that Hesperetin can be incorporated into topical gel/emulgel architectures. The present experimental sequence combines these concepts by using the inclusion complex as the active intermediate before hydrogel optimization.

The results should nevertheless be interpreted within the scope of the experiments performed. In-vitro release and antioxidant activity establish pharmaceutical performance under the tested conditions but do not by themselves establish wound closure, skin retention, systemic exposure or therapeutic efficacy. Those endpoints require appropriately designed ex-vivo, cell-based or in-vivo studies.

5. INTEGRATED FORMULATION-QUALITY-ATTRIBUTE RELATIONSHIP

Stage	Experimental finding	Role in formulation pathway
Starting Hesperetin	Poor aqueous solubility; 0.041 mg/mL	Defines solubility challenge
HP-β-CD complexation	Selected 1:1 complex; 7.71-fold apparent solubility enhancement	Improves molecular availability
Solid-state characterization	FTIR/DSC/PXRD changes supportive of complex formation	Confirms altered molecular/solid-state environment
Hydrogel optimization	F9 selected; composite desirability 0.942	Balances multiple critical responses
Optimized hydrogel	pH 5.65; viscosity 17,016.7 cP; spreadability 7.41 g·cm/s; drug content 97.68%	Provides practical topical dosage form
Drug release	82% at 8 h in comparative dataset; 91.2% at 12 h in extended dataset	Provides controlled/progressive availability
Stability	Drug content 97.28%; 8-h release 87.25% after 3 months	Demonstrates short-term accelerated robustness

Table 21. Integrated experimental formulation-quality-attribute map.

The overall formulation pathway is hierarchical. The inclusion complex addresses the molecular availability problem, whereas the hydrogel controls the dosage-form properties and release behavior. Optimization then balances these attributes, and validation demonstrates that the selected composition can be reproduced experimentally. Stability provides a further test of whether the optimized profile is retained under accelerated storage.

6. CONCLUSION

The present study successfully developed and optimized a Hesperetin–HP- β -CD inclusion-complex-loaded Carbopol 934P topical hydrogel using a systematic Box–Behnken experimental design. The formulation development followed a sequential approach in which the inclusion complex served as the intermediate for subsequent hydrogel development and optimization. The three formulation variables—Carbopol 934P concentration, Hesperetin–HP- β -CD inclusion-complex concentration, and propylene glycol concentration—produced measurable effects on the critical formulation responses.

Among the 17 experimental formulations, F9 was identified as the optimized formulation through multi-response desirability analysis. The optimized composition consisted of 0.75% Carbopol 934P, 4.0% w/w Hesperetin–HP- β -CD inclusion complex, and 15% propylene glycol, with a composite desirability of 0.942. The optimized formulation demonstrated a pH of 5.65 ± 0.01 , viscosity of $17,016.7 \pm 116.8$ cP, spreadability of 7.41 ± 0.04 g·cm/s, and drug content of $97.68 \pm 0.16\%$. The experimentally observed values were closely comparable with the predicted responses, with prediction errors below 0.5%, supporting the reproducibility of the selected formulation.

The optimized hydrogel exhibited progressive hydration and swelling, indicating development of a hydrated polymeric network capable of supporting drug release. The formulation also demonstrated progressive drug liberation during the extended-release experiment, reaching 80.4% at 8 h and 91.2% at 12 h. The release-kinetic analysis showed a strong relationship with the Higuchi model ($R^2 = 0.9969$), while the Korsmeyer–Peppas model ($R^2 = 0.9998$; $n = 0.8702$) provided complementary empirical information regarding the release behavior. The release profile therefore suggests a substantial diffusion-associated contribution together with possible effects of swelling, polymer relaxation, and drug partitioning within the hydrogel matrix.

The DPPH assay demonstrated a concentration-dependent increase in radical-scavenging activity for Hesperetin, the inclusion complex, and the optimized hydrogel. These findings support the antioxidant activity of the developed formulation under the tested in-vitro conditions. However, the antioxidant findings should be interpreted as assay-based pharmaceutical performance and not as direct evidence of enhanced therapeutic efficacy.

The accelerated stability study further demonstrated that the optimized hydrogel maintained its principal measured quality attributes during storage at 40 ± 2 °C/ $75 \pm 5\%$ RH for three months, with only minor changes in pH, drug content, and drug release. Drug content remained 97.28%, while the 8-h release remained 87.25% at the end of the three-month accelerated study. These findings indicate short-term robustness of the optimized formulation under the investigated accelerated conditions.

Overall, the experimental findings support the feasibility of combining HP- β -CD complexation with Carbopol-based hydrogel incorporation as a sequential formulation strategy for Hesperetin. The optimized hydrogel provided a balanced combination of formulation characteristics, drug content, swelling behavior, progressive drug release, antioxidant activity, and short-term accelerated stability. Nevertheless, the present findings are limited to the experimental conditions investigated. Longer-term stability studies, ex-vivo skin permeation/deposition studies, cytocompatibility assessment, and appropriate in-vivo studies are required to establish the translational potential of the developed topical formulation.

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