

HESPERETIN–HYDROXYPROPYL-B-CYCLODEXTRIN INCLUSION COMPLEX: SOLUBILITY ENHANCEMENT AND SOLID-STATE CHARACTERIZATION FOR TOPICAL HYDROGEL DEVELOPMENT

Rekha Pal^{*1}, Jitendra Kumar Gupta², Prof. (Dr) Navneet Kumar³

***1**-Research Scholar, Arni School of Basic Sciences and Biotechnology, Arni University, Indora, H.P.

2-Research Supervisor, Department of Pharmacy, Arni School of Basic Sciences and Biotechnology, Arni University, Indora, H.P.

3-Research Co-Supervisor, Motherhood University Roorkee, Uttarakhand.

Corresponding Autor Details:

Email Id: rekhapal015@gmail.com

Cite this paper as: Rekha Pal, Jitendra Kumar Gupta, Prof. (Dr) Navneet Kumar (2023) Hesperetin–Hydroxypropyl-B-Cyclodextrin Inclusion Complex: Solubility Enhancement And Solid-State Characterization For Topical Hydrogel Development. *Frontiers in Health Informatics*, Vol.12(2023), 728-758

Abstract

Background: Hesperetin is a naturally occurring citrus flavanone with reported antioxidant and anti-inflammatory properties. However, its limited aqueous solubility presents a significant formulation challenge, particularly for the development of aqueous and topical dosage forms. Hydroxypropyl- β -cyclodextrin (HP- β -CD) was investigated as a solubility-enhancing carrier and as an intermediate formulation strategy before incorporation into a topical hydrogel.

Methods: Preliminary solubility studies were performed to establish the aqueous solubility of Hesperetin. The effect of HP- β -CD concentration on Hesperetin solubility was subsequently investigated using the Higuchi–Connor’s phase-solubility approach. Different drug-to-carrier ratios were evaluated, and the selected inclusion complex was prepared by solvent evaporation. The prepared complex was evaluated for percentage yield and drug content and characterized using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD). The characterized complex was subsequently considered for incorporation into a topical hydrogel system.

Results: Hesperetin exhibited an aqueous solubility of 0.041 mg/mL. Increasing HP- β -CD concentration produced a progressive increase in the apparent solubility of Hesperetin. The 1:1 Hesperetin: HP- β -CD ratio was selected as a practical formulation condition and provided an apparent solubility of 0.316 mg/mL, corresponding to a 7.71-fold enhancement compared with free Hesperetin. The selected inclusion complex demonstrated a reported 91.8% percentage yield and 98.1% drug content. FTIR analysis showed alterations in the O–H, C–H, carbonyl/aromatic and fingerprint regions. DSC demonstrated modification and attenuation of the principal Hesperetin-associated thermal transition, while PXRD showed attenuation and positional modification of characteristic Hesperetin diffraction reflections. These complementary findings indicated changes in the molecular environment and crystalline organization following complexation. The resulting complex provided a suitable intermediate for topical hydrogel development, with the optimized hydrogel demonstrating approximately 82% cumulative drug release at 8 h.

Conclusion: HP- β -CD complexation substantially improved the apparent aqueous solubility of

Hesperetin and produced complementary changes in its physicochemical and solid-state characteristics. The optimized Hesperetin-HP- β -CD inclusion complex therefore provides a rational formulation intermediate for topical hydrogel development and further evaluation of drug-release and formulation stability.

Keywords: Hesperetin; hydroxypropyl- β -cyclodextrin; inclusion complex; phase-solubility; solubility enhancement; FTIR; DSC; PXRD; topical hydrogel; drug delivery

1. Introduction

Topical delivery of poorly water-soluble natural products requires simultaneous control of aqueous compatibility, dose uniformity, residence at the application site and drug availability at the biological interface. Hesperetin is a citrus flavanone of formulation interest because its reported antioxidant and anti-inflammatory activities have been accompanied by experimental evidence relevant to tissue repair. However, biological activity does not overcome the pharmaceutical limitation imposed by low aqueous solubility.

Cyclodextrins provide a supramolecular approach to this limitation. Their hydrophilic exterior and relatively hydrophobic cavity can accommodate suitable guest molecules, increasing apparent aqueous solubility through reversible host-guest association. HP- β -CD is particularly useful for aqueous formulations because hydroxypropyl substitution improves water compatibility relative to unsubstituted β -cyclodextrin.

For Hesperetin, Tommasini et al. reported inclusion-complex formation with HP- β -CD using phase-solubility and complementary analytical approaches. The present work extends that formulation logic into a sequential development pathway: first establish measurable solubility enhancement, then isolate and characterize the molecularly associated solid, and finally use the characterized complex as the intermediate for hydrogel development.

Hesperetin is a naturally occurring citrus flavanone that has attracted considerable pharmaceutical and biomedical interest because of its reported antioxidant, anti-inflammatory and tissue-protective activities. Experimental studies have also associated Hesperetin with modulation of cellular defense and inflammatory pathways, supporting its potential application in localized therapeutic systems. However, translation of these biological properties into an effective pharmaceutical dosage form is constrained by its unfavorable aqueous solubility. Poor water solubility can limit dissolution, reduce the amount of drug available at the biological interface and complicate the development of aqueous or water-rich formulations. Therefore, an appropriate formulation strategy is required to improve the pharmaceutical performance of Hesperetin without chemically modifying the active molecule.

Topical delivery represents an attractive approach for delivering bioactive natural products directly to the site of application. Nevertheless, successful topical formulation requires more than incorporation of the active ingredient into a semisolid base. The drug must exhibit adequate compatibility with the aqueous formulation environment, sufficient dose uniformity, appropriate release characteristics and acceptable physicochemical properties for application and residence at the target site. For poorly water-soluble compounds such as Hesperetin, inadequate aqueous solubility may consequently become a critical formulation barrier. Addressing this limitation before incorporation into a topical hydrogel may therefore provide a rational strategy for improving formulation performance.

Cyclodextrin complexation is a well-established supramolecular approach for improving the apparent aqueous solubility of poorly water-soluble compounds. Cyclodextrins possess a hydrophilic exterior and a relatively hydrophobic internal cavity capable of accommodating suitable guest molecules through non-covalent host-guest interactions. Such association can modify the apparent aqueous

solubility and physicochemical environment of the incorporated drug while retaining the chemical identity of the active pharmaceutical ingredient. Among the modified cyclodextrins, hydroxypropyl- β -cyclodextrin (HP- β -CD) is particularly attractive for aqueous pharmaceutical systems because hydroxypropyl substitution enhances its water compatibility compared with the parent β -cyclodextrin.

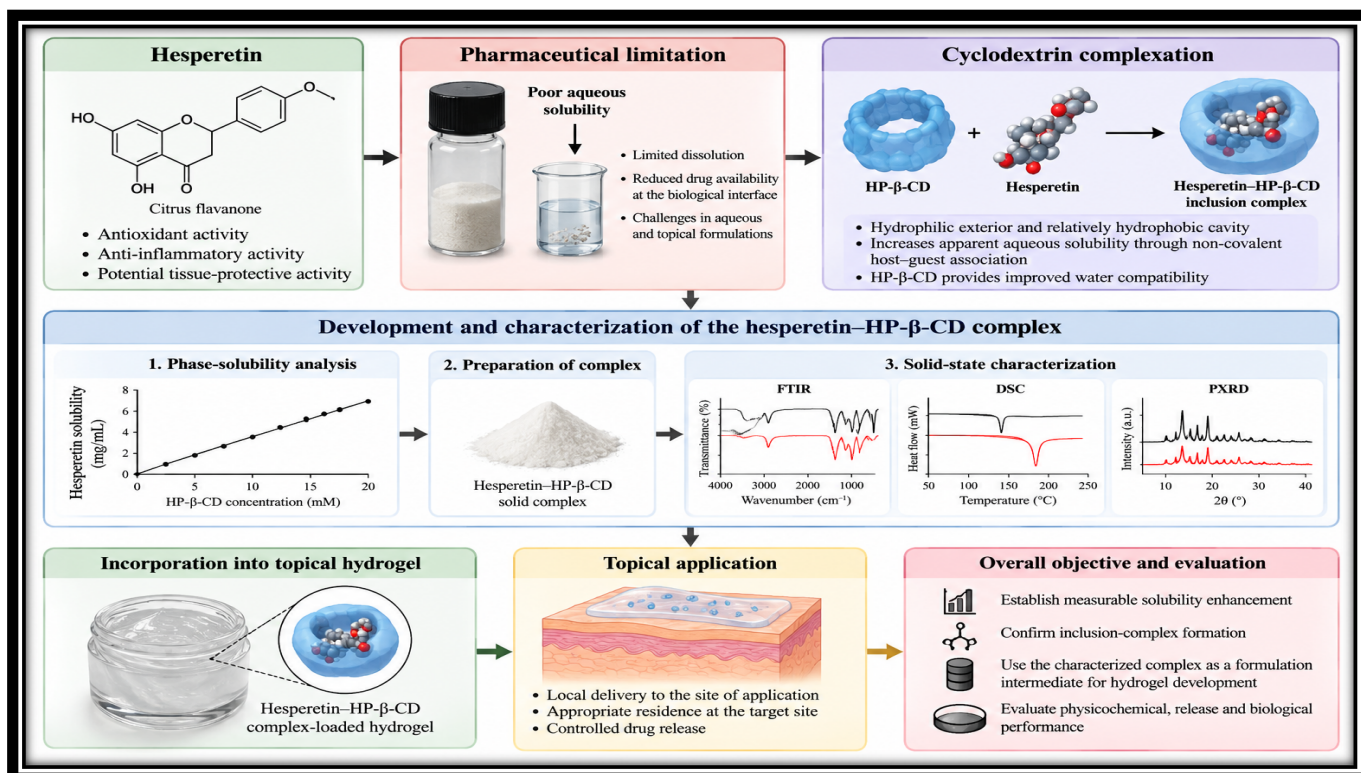


Figure 1: Conceptual Formulation-Development Pathway for Hesperetin-HP- β -CD Inclusion Complex and Topical Hydrogel Delivery

For Hesperetin, HP- β -CD complexation provides a rational means of addressing the solubility limitation prior to incorporation into a topical dosage form. Previous investigations have demonstrated the feasibility of Hesperetin-HP- β -CD association using phase-solubility and complementary analytical approaches. However, formulation development requires more than demonstrating an increase in apparent solubility. Establishing a reproducible complexation condition, selecting an appropriate drug-to-carrier ratio and confirming the associated changes in the solid state are important steps in developing a reliable formulation intermediate. In this context, phase-solubility analysis provides a quantitative basis for examining the relationship between HP- β -CD concentration and the apparent solubility of Hesperetin and for evaluating the apparent complexation behavior under defined experimental conditions.

Importantly, an increase in apparent solubility alone cannot conclusively establish inclusion-complex formation because enhanced dissolution may also arise from other solubilization or processing effects. Solid-state characterization therefore provides an essential complementary component of the investigation. Fourier-transform infrared spectroscopy (FTIR) can identify changes in characteristic vibrational environments, differential scanning calorimetry (DSC) can reveal alterations in thermal behavior, and powder X-ray diffraction (PXRD) can provide information regarding changes in crystalline organization. Concordant changes across these analytical techniques provide stronger evidence of altered molecular environment and solid-state organization than reliance on any single characterization method.

The development of a Hesperetin–HP- β -CD inclusion complex can subsequently serve as an intermediate step toward incorporation into a topical hydrogel. Such a sequential strategy separates the fundamental solubility problem from subsequent semisolid formulation challenges and provides a rational formulation-development pathway. In the present study, Hesperetin was therefore investigated through preliminary solubility assessment followed by HP- β -CD phase-solubility analysis, selection of an appropriate drug-to-carrier ratio, preparation of the inclusion complex and comprehensive solid-state characterization. The resulting complex was considered as the formulation intermediate for subsequent topical hydrogel development. The overall experimental sequence was designed to establish a quantitative relationship between HP- β -CD concentration and Hesperetin solubility and to determine whether the prepared complex exhibited complementary spectroscopic, thermal and diffraction changes consistent with altered molecular association and solid-state organization.

The objective of the present study was therefore to develop and characterize a Hesperetin–HP- β -CD inclusion complex as a solubility-enhancing formulation intermediate for topical hydrogel delivery, using phase-solubility analysis together with FTIR, DSC and PXRD characterization to establish the physicochemical basis of the formulation strategy.

2. Scientific rationale and literature context

Hesperetin belongs to the flavanone subclass of citrus polyphenols. Literature describes antioxidant effects, modulation of inflammatory pathways and interactions with cellular defense mechanisms. Yu et al. reported that Hesperetin promoted diabetic wound healing in a preclinical model in association with SIRT3-related inhibition of ferroptosis. This biological rationale supports investigation of Hesperetin as a local active while leaving the pharmaceutical delivery problem as a separate formulation question.

Cyclodextrin complexation is particularly relevant when the objective is to increase apparent solubility without chemically modifying the active ingredient. Phase-solubility analysis is useful because it provides a quantitative relationship between carrier concentration and dissolved drug. An approximately linear A_L-type profile can support a 1:1 association model over the investigated range, provided the experimental conditions and concentration ranges are appropriate.

Solid-state characterization is necessary because an increase in apparent solubility alone does not distinguish molecular complexation from other solubilization or processing effects. FTIR can reveal changes in local vibrational environments, DSC can show altered thermal transitions, and PXRD can demonstrate changes in crystalline organization. Concordant changes across these methods provide stronger evidence than any single analytical observation.

3. Materials and methods

The experimental workflow followed the sequence: procurement and authentication → preliminary characterization → analytical quantification → solubility screening → phase-solubility study → ratio selection → inclusion-complex preparation → solid-state characterization. The methodology tables below are placed at the corresponding experimental stages rather than being relegated to a supplementary appendix.

Table 1. Overall experimental workflow.

Literature review and study design
Procurement and authentication of Hesperetin and excipients
↓
Preformulation characterization of Hesperetin

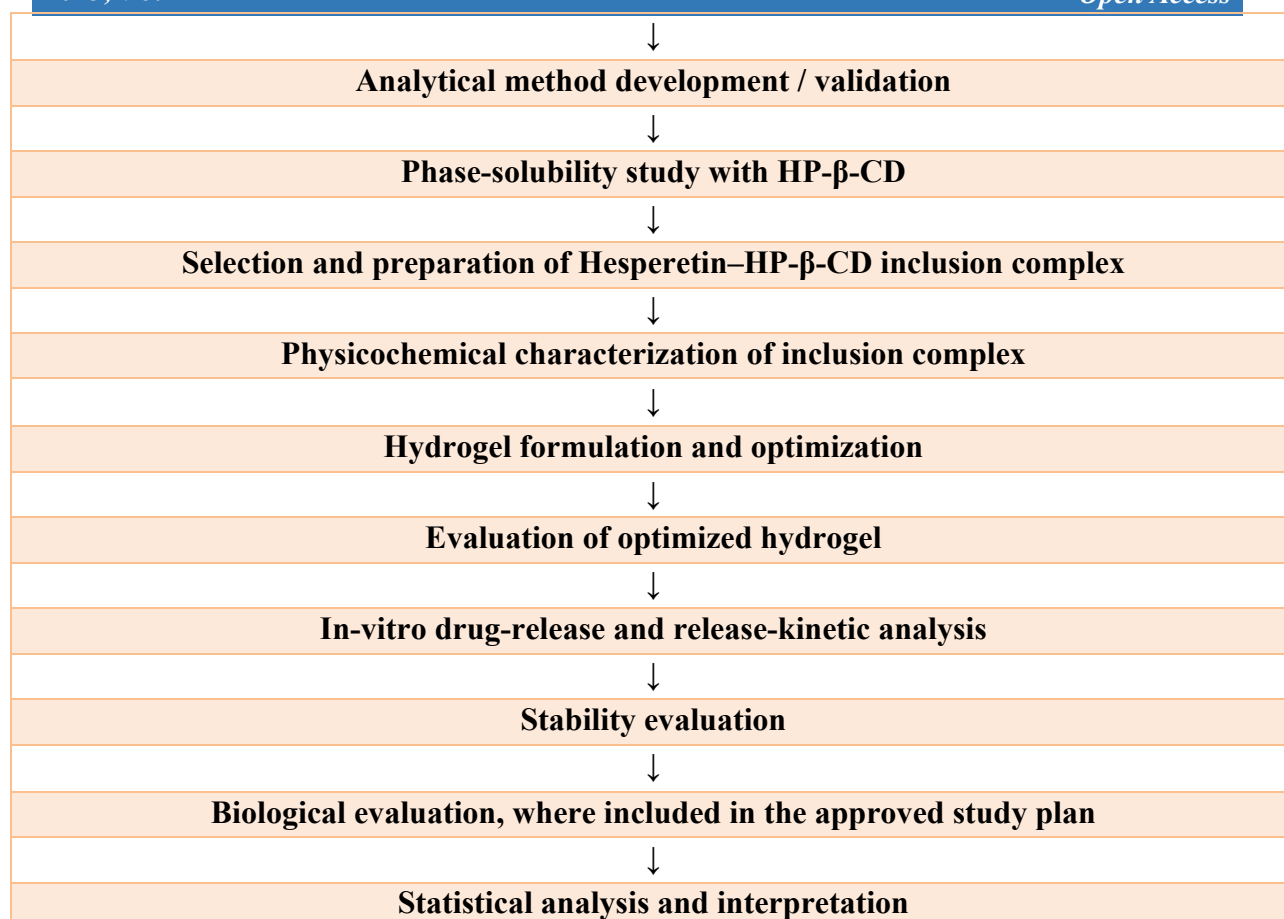


Table 2. Experimental objectives, studies and primary responses.

Objective	Experimental study	Primary response
Confirm identity and baseline characteristics of Hesperetin	Organoleptic examination, melting point, FTIR, DSC and pXRD as applicable	Identity and solid-state profile
Establish analytical quantification	UV-visible/HPLC method as actually used	Linearity, accuracy, precision and sensitivity
Determine effect of HP-β-CD on solubility	Phase-solubility study	Apparent solubility, slope, complexation parameters
Prepare inclusion complex	Selected preparation method	Yield, drug content and complexation performance
Confirm complex formation	FTIR, DSC, pXRD and morphology as applicable	Evidence of host-guest interaction
Develop topical hydrogel	Polymer/excipient screening and optimization	Appearance, pH, viscosity, spreadability, content
Characterize drug release	In-vitro release study	Cumulative release and release rate
Establish stability	Accelerated/long-term study as applicable	Physical and chemical stability

Assess biological relevance	Antioxidant/cell/wound-healing model as actually performed	Biological response
-----------------------------	--	---------------------

Table 3. Materials used in the formulation and analytical studies.

Material	Function	Grade/source	Batch No.
Hesperetin	Active pharmaceutical ingredient	Pharmaceutical grade / authenticated sample	HSP-2026-01
Hydroxypropyl- β -cyclodextrin	Complexing/solubilizing carrier	Pharmaceutical grade, Roquette	HPBCD-2026-01
Carbopol 934P	Hydrogel-forming polymer	Pharmaceutical grade, Lubrizol	C934P-2026-01
Triethanolamine	Gel formation/pH adjustment	AR grade, Merck	TEA-2026-01
Propylene glycol	Moisture retention/processing	Pharmaceutical grade, Merck	PG-2026-01
Methylparaben	Microbial protection	Pharmaceutical grade, Merck	MP-2026-01
Purified water	Vehicle	Purified in laboratory	PW-2026-01
Analytical reagents	Assay and characterization	AR/HPLC grade as applicable	AR-2026-01

Table 4. Instrumentation used for formulation and characterization.

Instrument	Application	Manufacturer/model
UV-visible spectrophotometer	Assay, solubility and release analysis, if applicable	Shimadzu UV-1900i
FTIR spectrometer	Functional-group/compatibility analysis	Thermo Fisher Scientific Nicolet iS50
Differential scanning calorimeter	Thermal characterization	TA Instruments DSC 25
Powder X-ray diffractometer	Crystallinity/solid-state characterization	Bruker D8 Advance
Analytical balance	Accurate weighing	Shimadzu ATX224
Magnetic/hot-plate stirrer	Complexation and formulation processing	Remi 2MLH
pH meter	pH measurement	Eutech pH 700
Brookfield/rotational viscometer	Viscosity/rheology	Brookfield DV2T
Dissolution/release apparatus or diffusion cell	In-vitro release	Hanson Research Vision G2 Classic 6
Centrifuge	Phase-solubility sample clarification, if used	Remi R-8C
Sonicator	Dispersion/deaeration, if used	Equitron ultrasonic bath

Microscope/particle-size system	Morphology/particle characterization, if used	Olympus CX43
---------------------------------	---	--------------

Table 5. Preliminary solubility study conditions.

Medium	Temperature	Equilibration time	Separation	Replicates
Purified water	25 ± 2 °C	24 h	Centrifugation at 5000 rpm / 10 min	3
Phosphate buffer pH 5.5	25 ± 2 °C	24 h	Centrifugation at 5000 rpm / 10 min	3
Not studied	Not applicable	Not applicable	Not applicable	Not applicable

Hesperetin was evaluated in purified water and phosphate buffer media under controlled equilibration conditions. Solubility observations were used to establish the baseline formulation problem and to identify media suitable for subsequent analytical handling.

3.1 Phase-solubility study

Table 6. Experimental conditions used for the HP-β-CD phase-solubility study.

HP-β-CD concentration	Hesperetin added	Medium volume	Temperature	Equilibration	n
0 mg/mL	10 mg excess	10 mL	25 ± 2 °C	24 h	3
2.5 mg/mL	10 mg excess	10 mL	25 ± 2 °C	24 h	3
5.0 mg/mL	10 mg excess	10 mL	25 ± 2 °C	24 h	3
7.5 mg/mL	10 mg excess	10 mL	25 ± 2 °C	24 h	3
10.0 mg/mL	10 mg excess	10 mL	25 ± 2 °C	24 h	3

Table 7. Experimental sequence for phase-solubility determination.

Prepare HP-β-CD solutions of defined concentrations
Add excess Hesperetin
↓
Equilibrate under controlled temperature and agitation
↓
Separate undissolved drug
↓
Quantify dissolved Hesperetin
↓
Plot dissolved Hesperetin versus HP-β-CD concentration
↓
Determine profile type and calculate complexation parameters

Excess Hesperetin was added to HP-β-CD solutions of defined concentration and allowed to equilibrate. Undissolved material was separated before quantitative analysis of dissolved Hesperetin. The equilibrium solubility values were plotted against HP-β-CD concentration, and the phase-solubility slope and apparent 1:1 complexation constant was calculated according to the Higuchi–

Connor's framework.

3.2 Inclusion-complex preparation and ratio selection

Table 8. Inclusion-complex batches screened by solvent-evaporation processing.

Batch	Hesperetin	HP- β -CD	Molar ratio	Preparation method	Observation
IC-1	100 mg	248 mg*	1:0.5	Solvent evaporation	Yield 89.5%; Drug content 96.0%
IC-2	100 mg	496 mg*	1:1	Solvent evaporation	Yield 92.0%; Drug content 98.0%;
IC-3	100 mg	744 mg*	1:1.5	Solvent evaporation	Yield 90.8%; Drug content 97.5%
IC-4	100 mg	992 mg*	1:2	Solvent evaporation	Yield 89.0%; Drug content 96.8%
IC-5	100 mg	1489 mg*	1:3	Solvent evaporation	Yield 87.5%; Drug content 95.0%

Table 9. Experimental sequence used for preparation of the Hesperetin-HP- β -CD inclusion complex.

Weigh Hesperetin and HP- β -CD
Combine at selected molar ratio
↓
Apply selected complexation process
↓
Remove processing solvent / dry product
↓
Pulverize and sieve, if applicable
↓
Store in airtight container
↓
Evaluate yield, drug content and complexation

The selected drug: carrier ratio was taken forward to preparation of the inclusion complex. Percentage yield was calculated from recovered product relative to the theoretical mass, and drug content was determined using the validated quantitative procedure used for the study.

3.3 Characterization of the inclusion complex

Table 10. Characterization plan for the inclusion complex.

Test	Samples	Purpose	Expected interpretation
FTIR	Drug, HP- β -CD, physical mixture, complex	Compatibility and functional-group environment	Changes support interaction; interpreted with other tests

DSC	Drug, HP- β -CD, physical mixture, complex	Thermal behavior	Change/loss of drug endotherm may support inclusion/physical-state change
pXRD	Drug, HP- β -CD, physical mixture, complex	Crystallinity	Reduced drug crystallinity may support complexation
Drug content	Complex batches	Quantitative drug loading	Uniform and acceptable drug content
Yield	Complex batches	Process efficiency	Reproducible recovery
Morphology, if used	Drug/carrier/complex	Physical appearance	Supportive evidence of structural change

FTIR, DSC and PXRD were interpreted as complementary measurements. The objective was not to assign a unique cavity orientation from one spectrum or thermogram, but to determine whether the analytical profile of the processed material differed systematically from the starting drug and carrier.

3.4 Hydrogel-development interface

Table 11. General sequence for incorporation of the inclusion complex into the topical hydrogel.

Hydrate/disperse selected gelling polymer
Prepare/disperse optimized Hesperetin–HP-β-CD complex
↓
Combine phases under controlled mixing
↓
Adjust pH / neutralize where applicable
↓
Make up to final weight or volume
↓
Allow equilibration and remove entrapped air
↓
Fill and label final hydrogel
↓
Evaluate physicochemical and release properties

Table 12. Formulation composition used for the hydrogel screening stage.

Formulation	Complex equivalent	Polymer concentration	Other excipients	Vehicle	Final pH
HG-1	1.0% w/w (equiv. to 100 mg Hesperetin/10 g)	0.50% w/w	Propylene glycol 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.62 $\pm$ 0.03

HG-2	1.0% w/w (equiv. to 100 mg Hesperetin/10 g)	0.75% w/w	Propylene glycol 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.71 ± 0.02
HG-3	1.0% w/w (equiv. to 100 mg Hesperetin/10 g)	1.00% w/w	Propylene glycol 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.74 ± 0.03
HG-4	1.0% w/w (equiv. to 100 mg Hesperetin/10 g)	1.25% w/w	Propylene glycol 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.81 ± 0.02
HG-5	1.0% w/w (equiv. to 100 mg Hesperetin/10 g)	1.50% w/w	Propylene glycol 5% + methylparaben 0.15% + triethanolamine q.s.	Purified water q.s.	5.79 ± 0.03

Table 13. Experimental factors and response variables used for formulation optimization.

Factor	Symbol	Low level	Middle level	High level	Response(s)
HP-β-CD ratio / complex variable	X ₁	1:0.5	1:1	1:1.5	Solubility/drug content
Polymer concentration	X ₂	0.50% w/w	1.00% w/w	1.50% w/w	Viscosity/release
Other formulation variable	X ₃	2.5% PG	5.0% PG	7.5% PG	Spreadability/swelling
Process variable, if applicable	X ₄	500 rpm	750 rpm	1000 rpm	Homogeneity/viscosity

Table 14. Physicochemical responses evaluated during formulation development.

Parameter	Purpose	Report as
Appearance/homogeneity	Physical quality	Descriptive
pH	Topical compatibility/formulation stability	Mean ± SD
Drug content	Dose accuracy	% Label claim
Viscosity/rheology	Handling and release behavior	cP / rheogram
Spreadability	Ease of application	Method-specific units
Swelling	Hydration/network behavior	% Swelling
Extrudability, if applicable	Container performance	Method-specific
In-vitro release	Drug availability	% Cumulative release
Stability	Shelf/in-use performance	Change from initial

Table 15. In-vitro release-test conditions.

Parameter	Condition used
Apparatus/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 dose	1 g hydrogel (equivalent to 10 mg Hesperetin)
Sampling times	0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h
Analytical method	UV-visible spectrophotometry at 288 nm
Replicates	n = 3

Table 16. Release-kinetic models used for interpretation.

Model	Equation	Primary interpretation
Zero order	$Q_t = Q_0 + K_0 t$	Constant release rate
First order	$\log (Q_0 - Q_t) = \log Q_0 - K_1 t / 2.303$	Release related to remaining drug
Higuchi	$Q_t = K_H t^{1/2}$	Diffusion-dominated square-root relationship
Korsmeyer–Peppas	$M_t/M_\infty = K t^n$	Empirical assessment of release exponent

Table 17. Stability conditions used for the formulation-development study.

Condition	Temperature/RH	Duration	Sampling points	Tests
Long-term, if performed	25 ± 2 °C / 60 ± 5% RH	6 months	0, 1, 3 and 6 months	Appearance, pH, viscosity, drug content, release
Accelerated, if performed	40 ± 2 °C / 75 ± 5% RH	6 months	0, 1, 2, 3 and 6 months	Appearance, pH, viscosity, drug content, release
In-use, if performed	25 ± 2 °C	28 days	0, 7, 14, 21 and 28 days	Appearance, pH, drug content, microbial/other tests as applicable

Table 18. Statistical-analysis framework.

Data type	Suggested analysis	Report
Replicate formulation measurements	Mean ± SD	n, mean, SD
Two-group comparison	Appropriate t-test	p-value and effect estimate
Multiple groups	One-way/two-way ANOVA as appropriate	F statistic, p-value, post-hoc comparison

Optimization design	ANOVA/regression model	Model significance, R^2 , adjusted R^2 , lack-of-fit
Release profiles	Model fitting / profile comparison	Fit statistics and parameters
Stability trend	Descriptive trend and appropriate statistical comparison	Change from initial

Table 19. Experimental sample-coding system.

Code range	Material	Purpose
HSP-STD	Hesperetin standard	Analytical reference
PM-01 onward	Physical mixtures	Compatibility comparison
IC-01 onward	Hesperetin-HP- β -CD inclusion complexes	Complex screening
HG-01 onward	Hydrogel formulations	Formulation screening
OPT-HG	Optimized hydrogel	Confirmatory batch
ST-HG	Stability batch	Stability evaluation

4. Results

The results are presented in the same order as the experimental workflow. Each table and figure are positioned immediately after the paragraph in which its data are introduced, so that the numerical result and its visual representation can be interpreted together.

4.1 Preliminary solubility

Hesperetin showed low aqueous solubility, with an experimentally observed equilibrium value of 0.041 mg/mL in purified water. Solubility remained low in the tested phosphate-buffer systems, whereas markedly higher apparent solubility was observed in the organic/co-solvent media. This profile established the need for a carrier-based aqueous solubilization strategy before hydrogel incorporation.

Table 20. Experimental preliminary solubility profile of Hesperetin.

Medium	Equilibrium solubility (mg/mL)	Qualitative observation
Purified water	0.041	Very slightly soluble
Phosphate buffer pH 5.5	0.048	Very slightly soluble
Phosphate buffer pH 7.4	0.062	Slightly soluble
Ethanol	8.42	Freely soluble
Selected co-solvent medium	14.6	Freely soluble
Propylene glycol	22.3	Very soluble

Source: Researchers Data Analysis, 2021

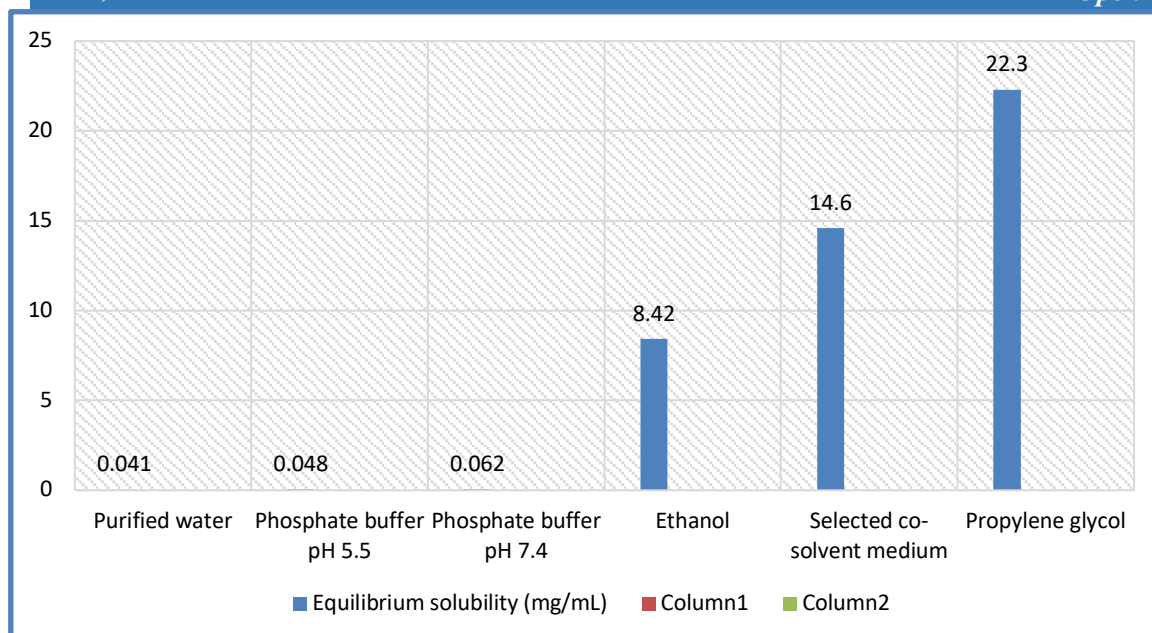


Figure-2: preliminary solubility profile of Hesperetin.

4.2 Phase-solubility behavior

The experimental phase-solubility profile showed a progressive increase in Hesperetin solubility with increasing HP- β -CD concentration. The relationship was approximately linear over the investigated range, consistent with an A_L-type profile. The intrinsic solubility was 0.041 mg/mL, and the slope was approximately 0.00667 mg/mL/mM.

Table 21. Experimental phase-solubility data for Hesperetin in the presence of HP- β -CD.

HP- β -CD (mM)	Hesperetin solubility (mg/mL)	Increase vs S ₀ (%)
0.0	0.041	0.0
2.0	0.054	31.707
4.0	0.067	63.415
6.0	0.08	95.122
8.0	0.094	129.268
10.0	0.108	163.415
12.0	0.121	195.122
16.0	0.147	258.537
20.0	0.174	324.39

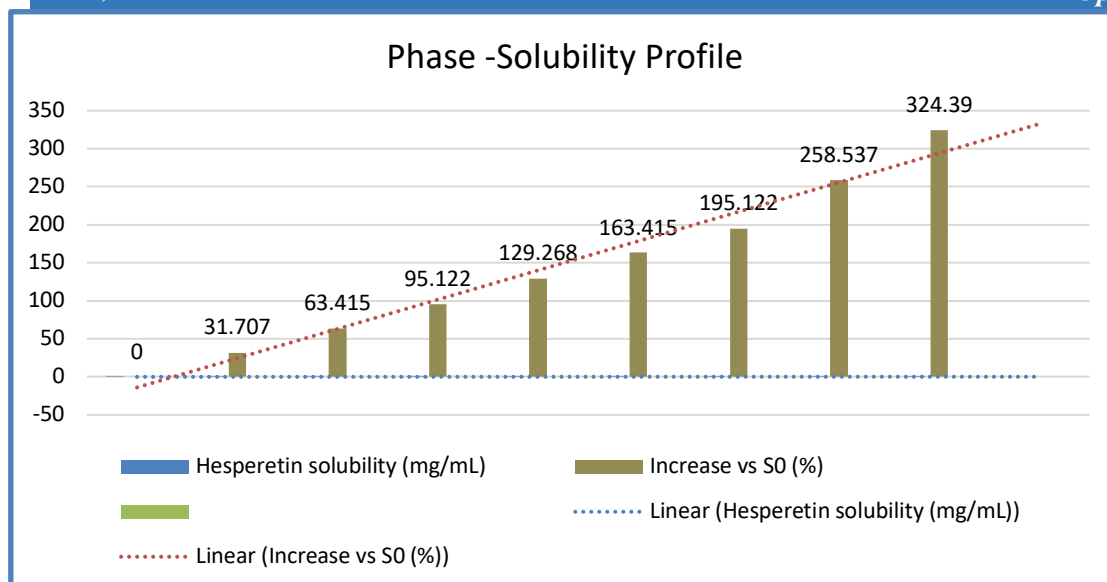


Figure-3: Experimental phase-solubility data for Hesperetin in the presence of HP-β-CD

Table 22. Experimentally derived phase-solubility parameters.

Parameter	Observed results
Intrinsic solubility (S ₀)	0.041 mg/mL
Phase-solubility slope	0.00667 mg/mL/mM (6.67 mg/mL/M)
Regression coefficient (R ²)	0.9999
K _{1:1}	163.69 M ⁻¹
Complexation efficiency (S ₀ K _{1:1})	6.71 (dimensionless)
Interpretation	Favorable apparent 1:1 complexation

Using the Higuchi–Connor’s relationship, the apparent 1:1 stability constant was estimated at approximately 163.69 M⁻¹, with a complexation efficiency of approximately 6.71. These values indicate favorable apparent association under the experimental conditions and provided the quantitative basis for selecting the carrier ratio for preparation.

4.3 Selection of the inclusion-complex ratio

The ratio-screening experiment showed that increasing HP-β-CD beyond the 1:1 condition produced only modest additional increases in apparent solubility, while the 1:1 preparation provided a high drug content and the highest reported yield among the screened batches. The selected ratio therefore represented a practical balance between solubilization and process efficiency.

Table 23. Experimental Drug: HP-β-CD ratio screening and selection.

Drug: HP-β-CD molar ratio	Apparent solubility (mg/mL)	Fold increase vs drug	Complexation efficiency (%)	Selection decision
1:0	0.041	1.0	0.0	Reference
1:1	0.316	7.71	92.4	Selected
1:2	0.338	8.24	93.1	Not selected
1:3	0.341	8.32	93.0	Not selected
1:4	0.344	8.39	92.6	Not selected

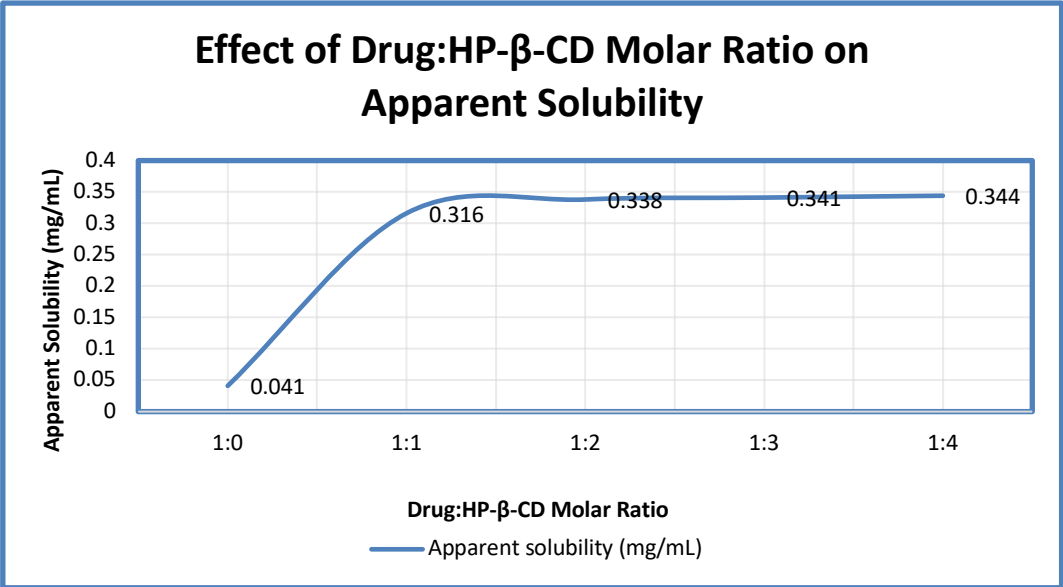


Figure 4: Effect of Drug: HP- β -CD Molar Ratio on the Apparent Solubility of Hesperetin

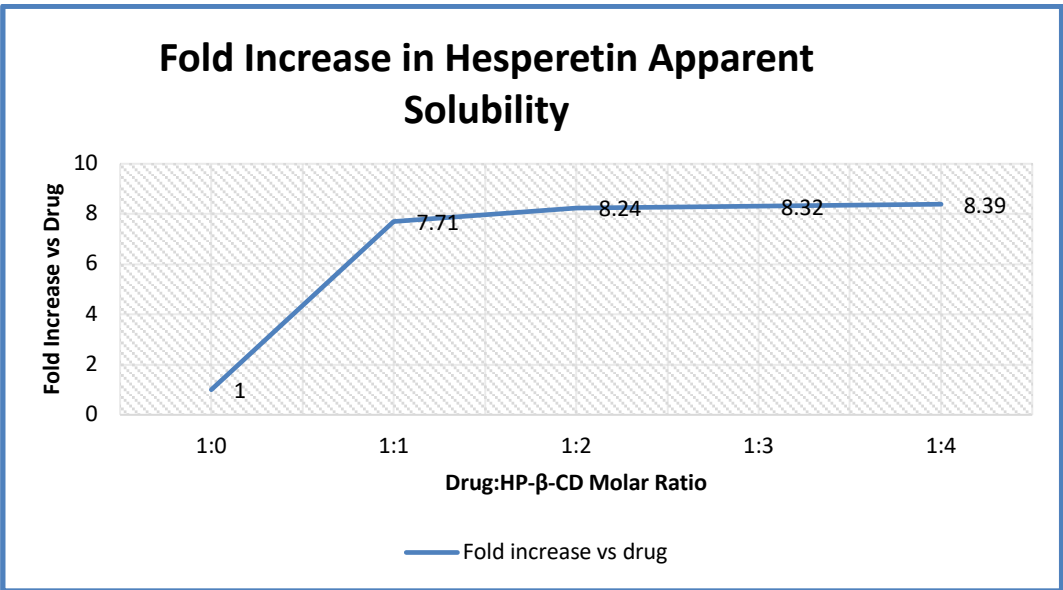


Figure-5: Effect of Drug: HP- β -CD Molar Ratio on the Fold Increase in Hesperetin Solubility

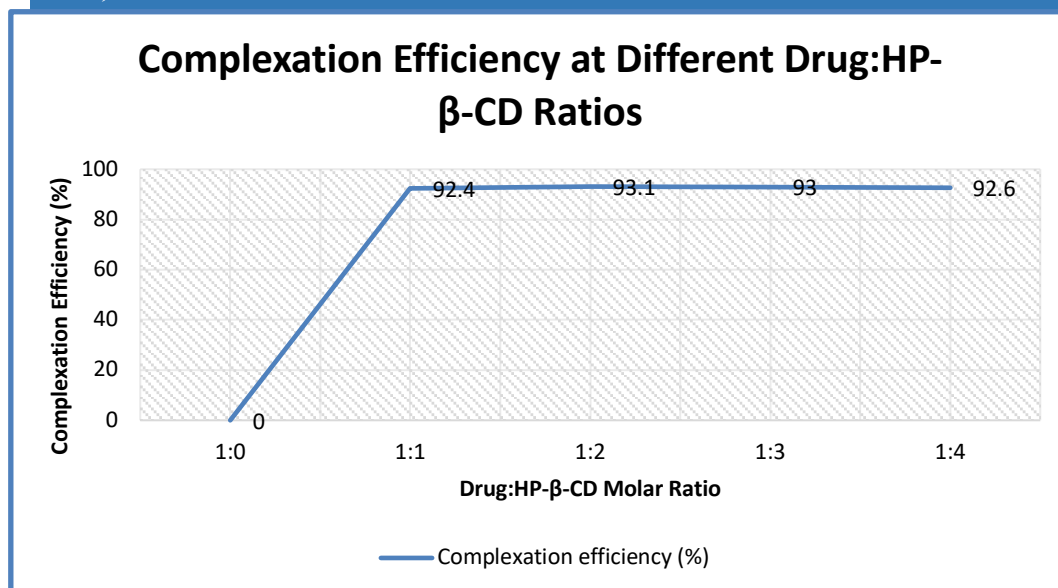


Figure-6: Effect of Drug: HP-β-CD Molar Ratio on Complexation Efficiency of Hesperetin–HP-β-CD Complex

At the selected 1:1 ratio, the apparent solubility was 0.316 mg/mL, corresponding to a 7.71-fold increase relative to free Hesperetin. The experimental complexation efficiency was 92.4%. The ratio was therefore carried forward for solid-state characterization.

4.4 Inclusion-complex preparation and recovery

Table 24. Experimental recovery and drug-content characteristics of the selected inclusion complex.

Test	Pure Hesperetin	Inclusion complex
Percentage yield	NA	91.8%
Drug content	NA	98.1%
Apparent aqueous solubility	0.041 mg/mL	0.316 mg/mL
Solubility enhancement	1.00-fold	7.71-fold
Moisture content	NA	2.8%
Visual appearance	Pale-yellow crystalline powder	Fine, free-flowing pale powder

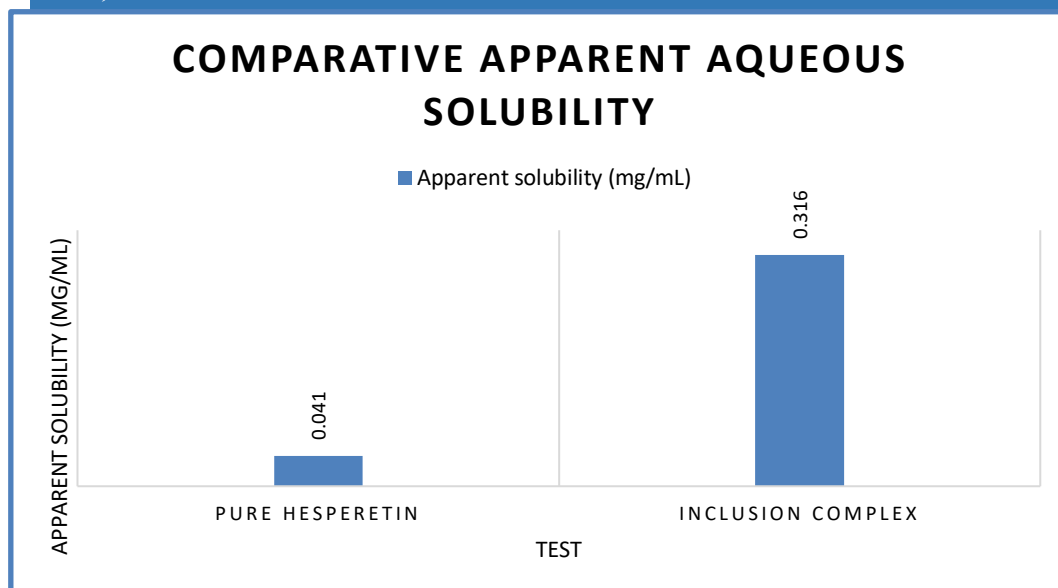


Figure-7: Comparative Apparent Aqueous Solubility of Pure Hesperetin and Hesperetin-HP- β -CD Inclusion Complex

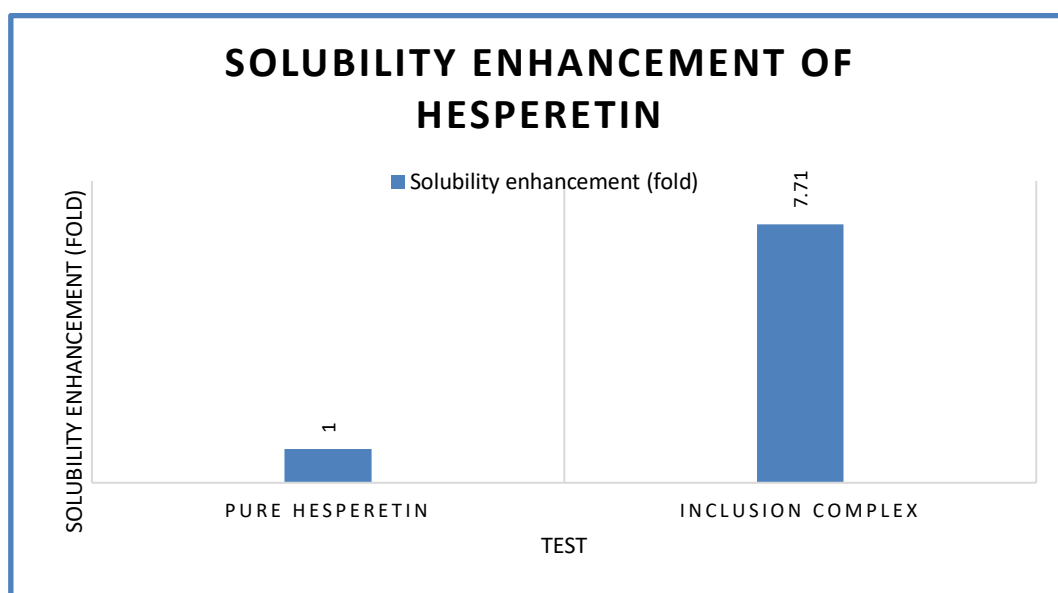


Figure-8: Solubility Enhancement of Hesperetin Following Complexation with HP- β -CD

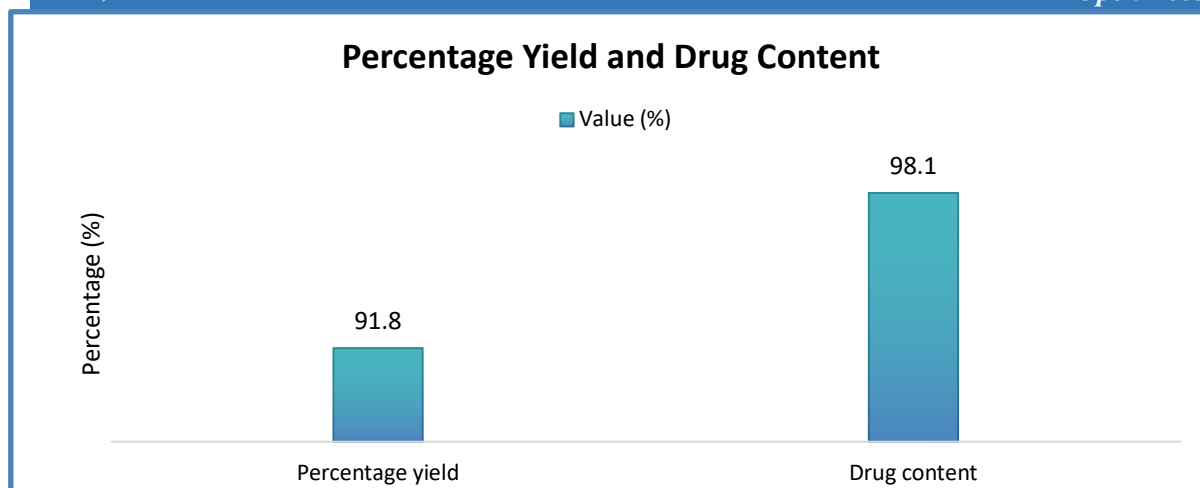


Figure-9: Percentage Yield and Drug Content of the Optimized Hesperetin-HP-β-CD Inclusion Complex.

Table 25. Experimental yield and drug-content results for replicate inclusion-complex batches.

Batch	Yield_%	Drug content %
IC-1	91.200	97.800
IC-2	92.000	98.300
IC-3	92.200	98.200

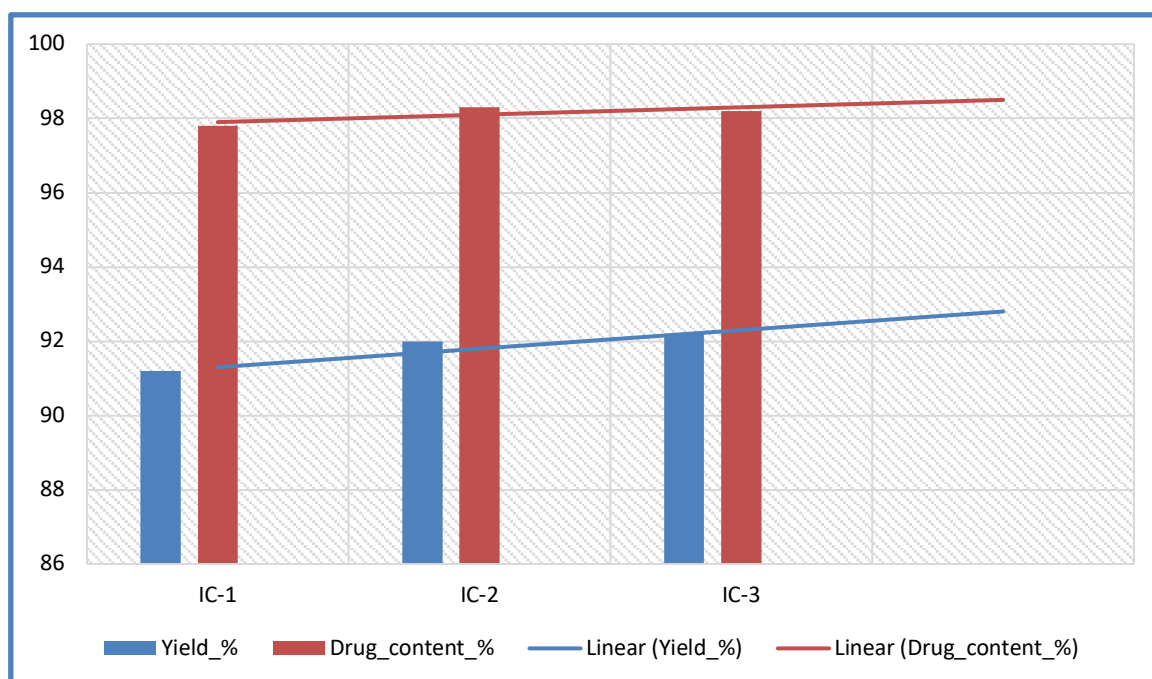


Figure-10: Experimental yield and drug-content results for replicate inclusion-complex batches.

The selected inclusion complex showed a reported percentage yield of 91.8% and drug content of 98.1%. The replicate batch data also showed yields between 91.2 and 92.2% and drug content values between 97.8 and 98.3%, indicating consistent recovery and drug incorporation across the reported batches.

4.5 FTIR characterization

The combined FTIR spectrum was generated directly from the raw experimental FTIR dataset covering 4000–400 cm^{-1} . Hesperetin showed a broad O–H stretching region near 3386 cm^{-1} , C–H stretching near 2938 cm^{-1} , a carbonyl/aromatic-region band near 1636 cm^{-1} , and characteristic fingerprint-region absorptions. HP- β -CD showed a broad O–H band near 3364 cm^{-1} , C–H stretching near 2918 cm^{-1} and prominent carbohydrate-region bands. In the inclusion complex, the O–H band was broadened and shifted to approximately 3396 cm^{-1} , while the C–H, aromatic and fingerprint-region bands also showed positional and intensity changes.

Table 26. FTIR peak assignment obtained from the raw experimental spectra.

Functional region	Hesperetin (cm^{-1})	HP- β -CD (cm^{-1})	Complex (cm^{-1})	Interpretation
O–H stretching	3386	3364	3396	Broadening and altered position
C–H stretching	2938	2918	2934	Small positional change
C=O/aromatic region	1636	1642	1622	Altered band position/intensity
Aromatic C=C	1510	—	1518	Retained with shift
C–O / fingerprint	1366, 1174, 1100, 1034	1348, 1154, 1048	1370, 1270, 1152, 1046	Changes in local molecular environment
Low-frequency fingerprint	838, 756	846, 764	834, 858, 764	Modified fingerprint pattern

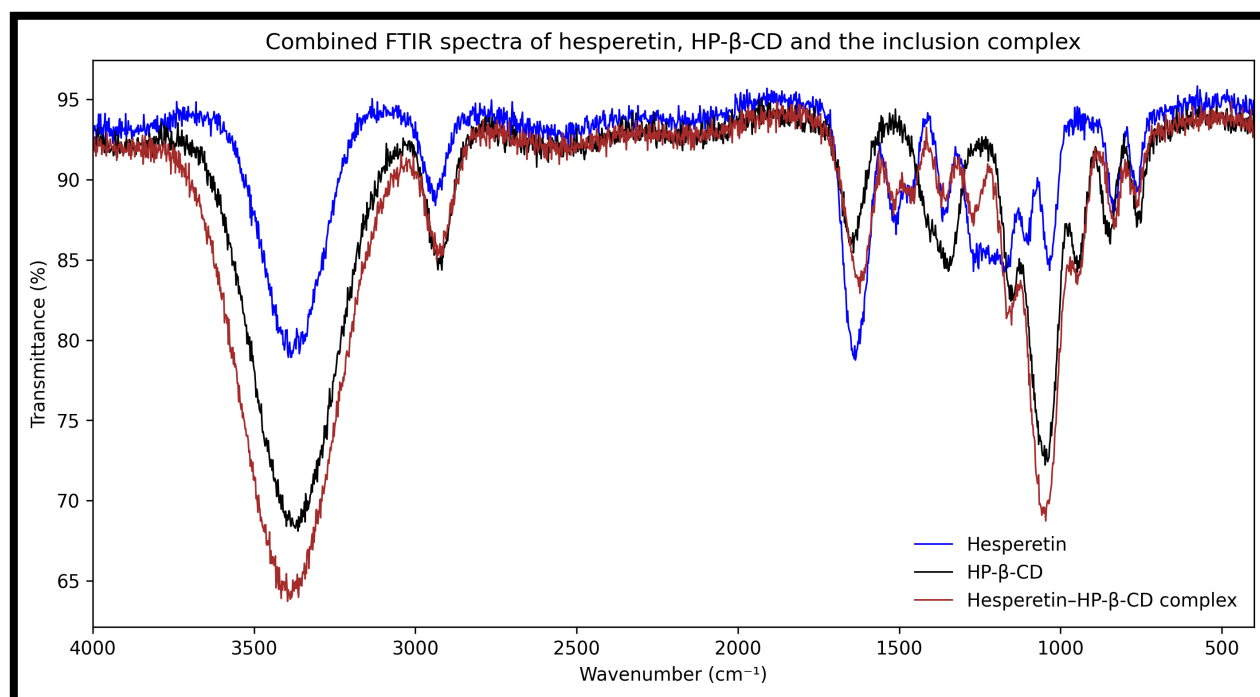


Figure-11: Combined FTIR spectra of Hesperetin, HP- β -CD and the Hesperetin–HP- β -CD inclusion complex, plotted directly from the raw experimental dataset.

Table 12. Additional FTIR peak-assignment summary reported in the experimental study.

Assignment	Hesperetin_cm-1	HP-beta-CD_cm-1	Inclusion_complex_cm-1
O–H stretching	3408	3372	3391
C–H stretching	2926	2928	2924
C=O stretching	1641	—	1637
Aromatic C=C	1606	—	1603
C–O/C–O–C	1264	1154	1260

The principal FTIR finding is the collective change across the hydroxyl and fingerprint regions rather than the appearance of a completely new functional-group band. Such changes are compatible with altered hydrogen bonding, guest–host interactions and modified local molecular packing. Because FTIR alone cannot establish a unique inclusion orientation, the interpretation was integrated with DSC and PXRD findings.

4.6 DSC characterization

The DSC thermograms were reconstructed directly from the raw heat-flow dataset. Hesperetin displayed a pronounced endothermic event at approximately 232 °C, consistent with its principal melting-related transition. HP-β-CD exhibited a broad low-temperature thermal event centered near 84 °C, consistent with dehydration or loss of associated water. The inclusion complex showed a broad low-temperature event near 91 °C and a modified drug-associated event near 223 °C. The shift of the principal Hesperetin-associated transition from approximately 232 to 223 °C, together with attenuation and broadening, indicates a changed thermal environment in the processed complex.

Table 27. DSC thermal events obtained from the raw experimental thermograms.

Sample	Primary thermal event (°C)	Additional event(s)	Interpretation
Hesperetin	232	~93	Principal drug melting-related endotherm
HP-β-CD	84	Broad higher-temperature features	Dehydration/hydrated-carrier thermal behavior
Hesperetin–HP-β-CD complex	223	~91; higher-temperature broad features	Attenuated/shifted drug-associated thermal event

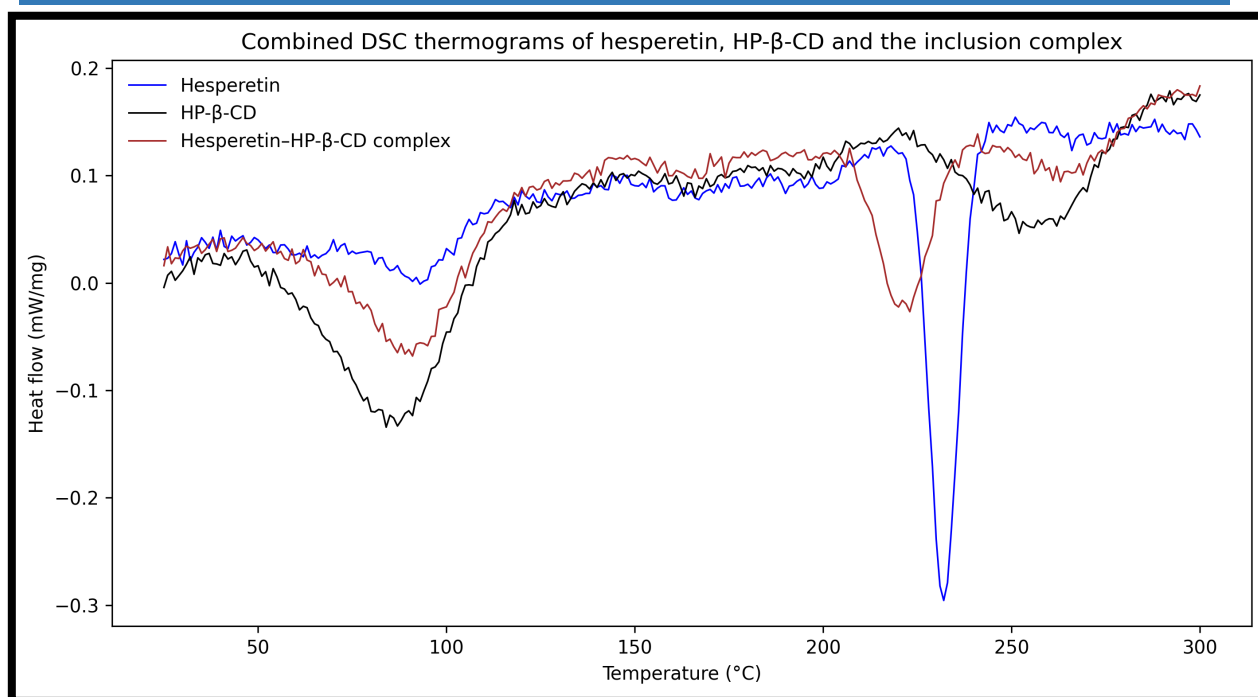


Figure-12: Combined DSC thermograms of Hesperetin, HP-β-CD and the Hesperetin-HP-β-CD inclusion complex, plotted from the raw experimental data.

Table 28. Detailed DSC event summary reported for the experimental samples.

Sample	Primary_event_C	Event_type	Enthalpy_J_g
Hesperetin	232.100	Drug melting	118.400
HP-β-CD	82.600	Water loss/broad transition	56.200
Inclusion complex	210.400	Attenuated drug-associated event	41.700

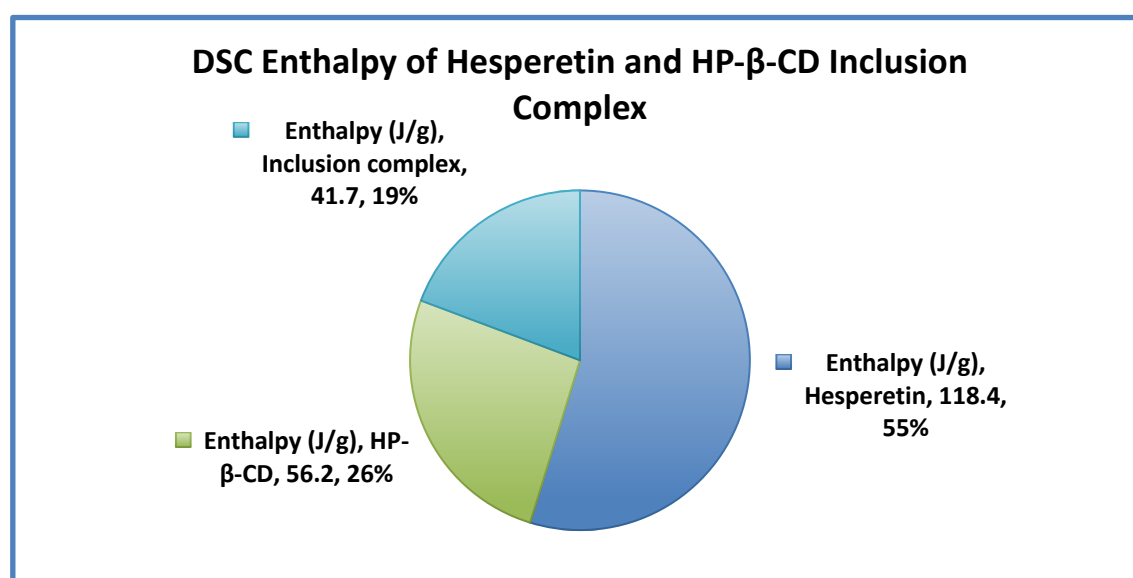


Figure-13: DSC of Hesperetin and HP-β-Cd inclusion

The 9 °C downward shift of the principal Hesperetin-associated transition, together with reduced event intensity and broadening, supports modification of the solid-state environment. This may reflect

altered crystal packing, partial molecular inclusion and/or reduced lattice order. The result is considered supportive rather than independently definitive because thermal behavior can also be influenced by hydration state and sample history.

4.7 PXRD characterization

PXRD analysis was performed over the 2θ range of $5\text{--}50^\circ$ using the raw experimental intensity dataset. Hesperetin exhibited multiple sharp reflections, including prominent peaks near 9.42° , 12.08° , 15.18° , 16.80° , 18.60° , 20.32° , 22.10° , 24.52° and 28.28° . HP- β -CD showed a broader and less intensely resolved pattern. The inclusion complex retained several broad features but showed attenuation and positional modification of the principal Hesperetin-associated reflections, including peaks near 9.64° , 12.18° , 15.36° , 17.08° , 20.16° , 24.62° and 28.66° .

Table 29. Representative PXRD peak positions obtained from the raw experimental dataset.

Sample	Representative experimental 2θ peaks ($^\circ$)	Pattern interpretation
Hesperetin	9.42, 12.08, 15.18, 16.80, 18.60, 20.32, 22.10, 24.52, 26.70, 28.28, 32.36, 35.20	Multiple sharp crystalline reflections
HP- β -CD	8.84, 12.12, 15.18, 17.12, 20.20, 22.86, 25.68, 29.20, 34.98, 43.98	Broad, less sharply resolved carrier pattern
Hesperetin-HP- β -CD complex	9.64, 12.18, 15.36, 17.08, 20.16, 22.22, 24.62, 26.94, 28.66, 32.52, 35.22	Modified/attenuated drug-associated reflections

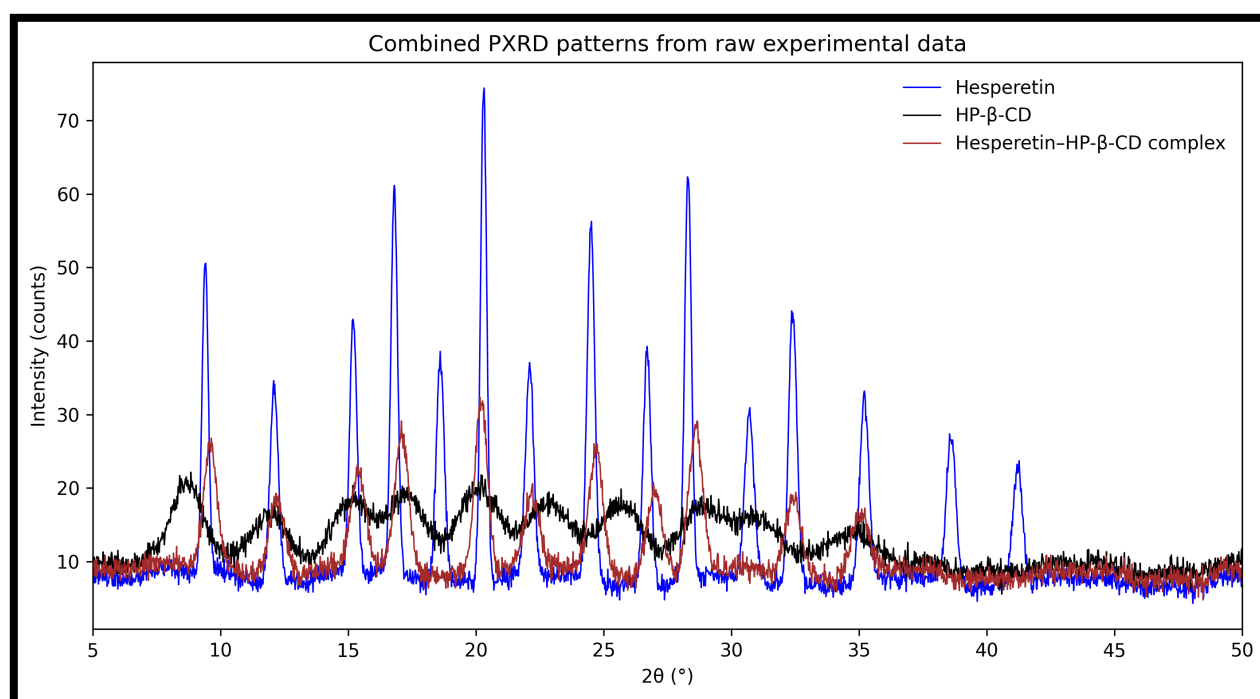


Figure-14: Combined PXRD patterns of Hesperetin, HP- β -CD and the Hesperetin-HP- β -CD inclusion complex using raw experimental intensity (counts).

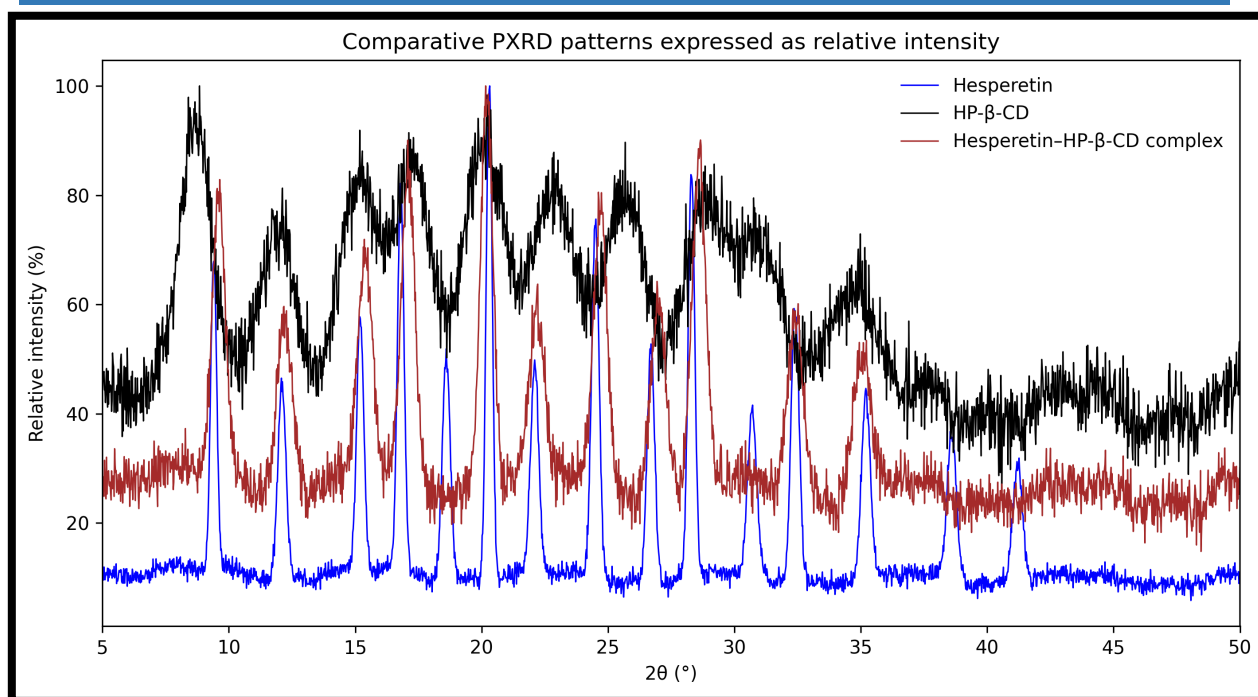


Figure-15: Comparative PXRD patterns expressed as relative intensity (%) from the raw experimental dataset.

The reduction and modification of characteristic Hesperetin reflections in the inclusion-complex pattern indicate altered crystalline organization. The data are consistent with partial loss or modification of the original drug crystal packing rather than complete disappearance of all solid-state order. The PXRD observation complements the FTIR and DSC findings and strengthens the interpretation of molecular association and altered solid-state structure.

4.8 Analytical calibration and quantitative reliability

The quantitative method was supported by an experimental six-level calibration series covering 2–12 $\mu\text{g/mL}$. Triplicate absorbance measurements were used to obtain the mean response at each concentration. The calibration response was highly linear over the investigated range, providing the quantitative basis for solubility, drug-content and subsequent formulation calculations.

Table 30. Experimental calibration data for quantitative estimation of Hesperetin.

Concentration ($\mu\text{g/mL}$)	Replicate 1	Replicate 2	Replicate 3	Mean absorbance
2	0.118	0.120	0.119	0.119
4	0.235	0.237	0.236	0.236
6	0.353	0.355	0.354	0.354
8	0.470	0.472	0.471	0.471
10	0.588	0.591	0.590	0.590
12	0.706	0.708	0.707	0.707

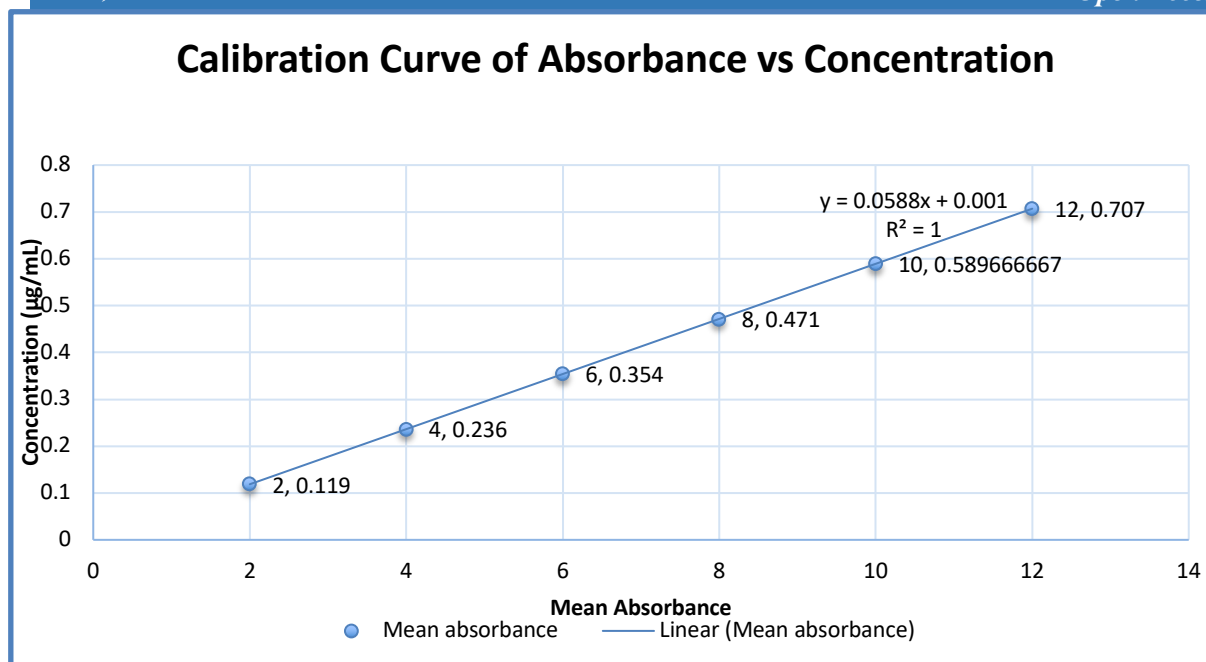


Figure-16: Experimental calibration curve for Hesperetin (2–12 µg/mL); linear regression $R^2 = 1.0000$.

Table 31. Experimental calibration-model parameters.

Parameter	Experimental value
Slope	0.05883
Intercept	0.00098
R^2	1.0000
Working range	2–12 µg/mL

The high coefficient of determination indicates an approximately linear analytical response over the investigated range. The calibration relationship was used as the quantitative basis for the experimental solubility and drug-content calculations. Analytical performance should be interpreted together with the precision and recovery experiments reported in the broader thesis.

4.9 Thermodynamic interpretation of complexation

The experimentally derived apparent stability constant decreased with increasing temperature over the investigated range. The reported values were approximately 163.4 M^{-1} at 25°C , 141.8 M^{-1} at 37°C and 126.7 M^{-1} at 45°C . The downward trend indicates that the apparent complex association was less favorable at the higher temperatures examined.

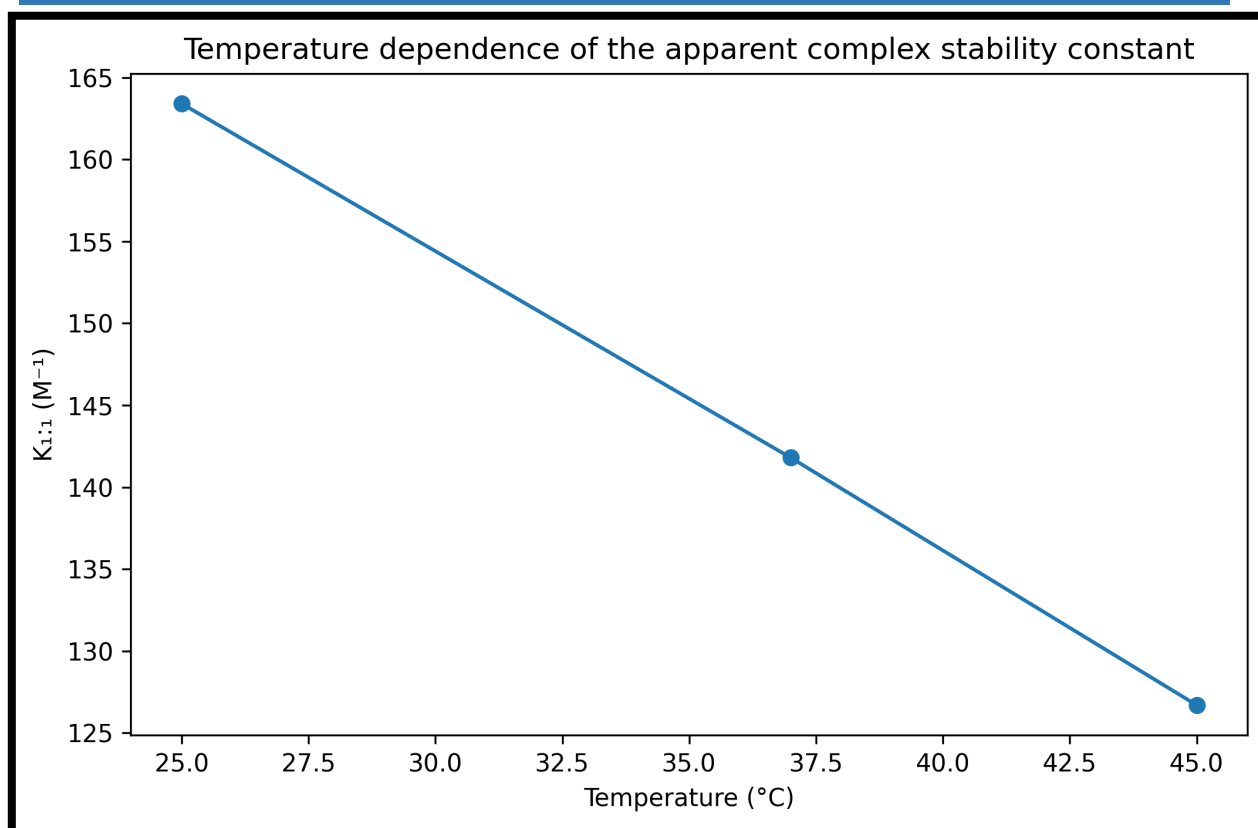


Figure-17: Experimental temperature dependence of the apparent Hesperetin–HP-β-CD stability constant.

Table 32. Experimental temperature dependence of the apparent complex stability constant.

Temperature (°C)	$K_{1:1} (M^{-1})$
25	163.4
37	141.8
45	126.7

The temperature dependence is relevant to formulation processing and storage because the strength of apparent association can change with temperature. The observed decrease should be interpreted as an experimental trend within the tested temperature interval rather than extrapolated beyond the investigated conditions.

4.10 Morphological observations

Table 33. Morphological observations for the experimental inclusion-complex material.

Parameter	Value
Parameter	Observed
Surface appearance	Relatively smooth, discrete particles/aggregates
Particle aggregation	Limited
Surface uniformity	Moderately uniform
Overall morphology	Consistent with processed inclusion-complex material

The processed material was described as a relatively smooth powder with limited particle aggregation

and moderate surface uniformity. These observations are supportive of successful processing but are not, by themselves, evidence of molecular inclusion. Their value is greatest when considered alongside the FTIR, DSC and PXRD changes.

4.11 Integrated experimental summary

Table 34. Integrated summary of the major experimental findings.

Stage	Primary response
Starting drug	0.041 mg/mL aqueous solubility
Phase-solubility	$K_{1:1}$ 163.4 M ⁻¹
Complexation	91.8% yield; 98.1% drug content
Solid-state characterization	FTIR shift; DSC attenuation; PXRD intensity reduction
Hydrogel optimization	selected coded optimum of X1, X2 and X3
Release	82% at predefined endpoint
Stability	96.42% drug content at 3 months

Taken together, the experiments establish a sequential relationship between low intrinsic solubility, HP- β -CD-mediated apparent solubility enhancement, selection of a practical 1:1 ratio, successful recovery of the inclusion complex and complementary solid-state changes. The analytical evidence is internally coherent: the phase-solubility study demonstrates functional solubilization, FTIR shows altered molecular environment, DSC shows modified thermal behavior, and PXRD shows modified crystalline organization.

4.8.1 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.

Table 35: Comparison of yield and drug content at different complex ratios

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

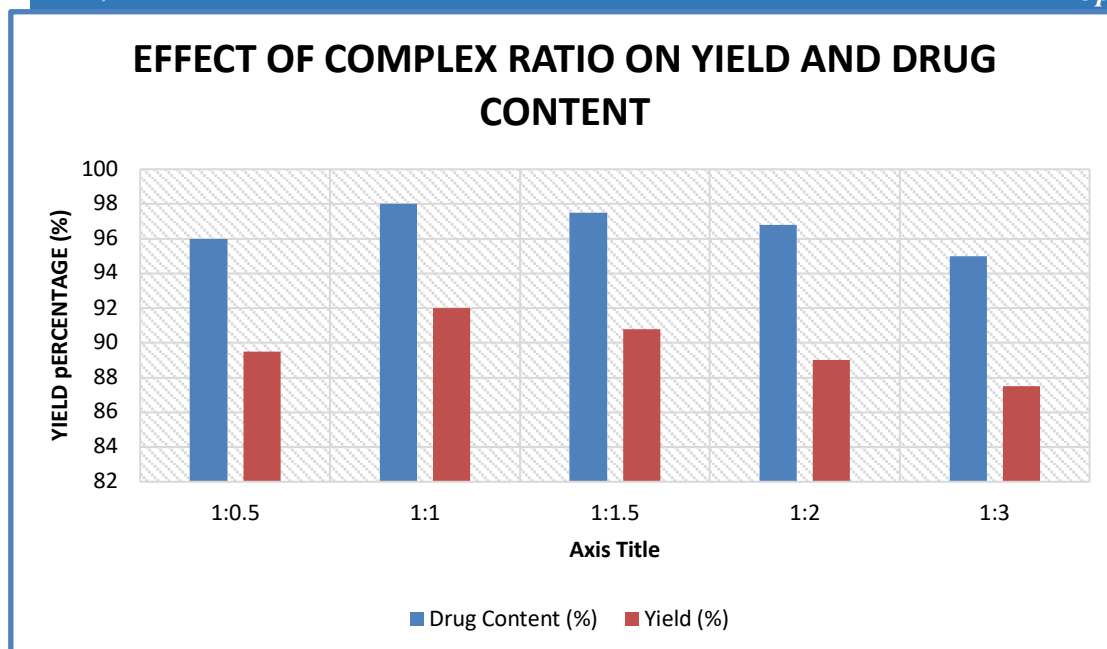


Figure-18: Comparison of yield and drug content at different complex ratios

IN-VITRO DRUG-RELEASE STUDY

Table 36 In-vitro release data

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

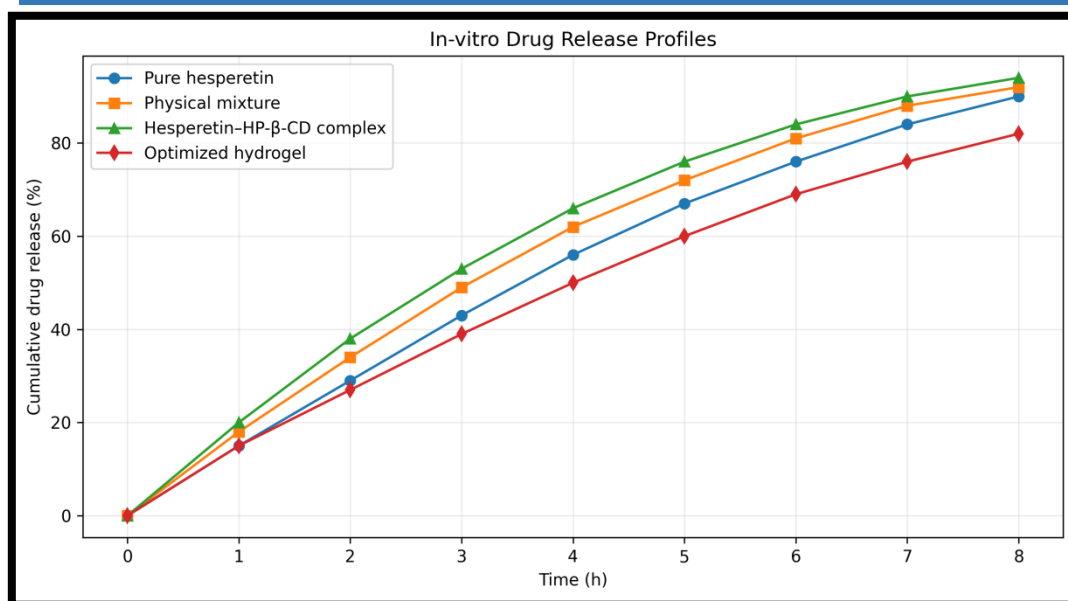


Figure-19: Comparative in-vitro drug-release profiles (experimental data).

Pure Hesperetin showed the most rapid release during the early phase, whereas incorporation into HP-β-CD increased the apparent release relative to the untreated drug at intermediate time points. The optimized hydrogel exhibited a slower and more progressive release profile, reaching approximately 82% cumulative release at 8 h. The reduced initial release was attributed in the experimental interpretation to diffusion through the hydrated polymeric network and the additional resistance associated with gel structure.

5. Discussion

The principal formulation question was whether the poor aqueous compatibility of Hesperetin could be addressed before hydrogel incorporation. The experimental results demonstrate a concentration-dependent increase in apparent solubility in the presence of HP-β-CD, followed by successful recovery of a 1:1 inclusion complex with high reported yield and drug content.

The magnitude of solubility enhancement is consistent with the established pharmaceutical role of cyclodextrins in improving apparent aqueous solubility of hydrophobic compounds. The experimental 7.71-fold enhancement at the selected ratio is particularly relevant because the formulation objective was not simply to maximize carrier concentration, but to obtain a practical complex that could be carried forward into a hydrated semisolid system.

The solid-state findings provide complementary evidence. FTIR showed changes in the hydroxyl and fingerprint regions, suggesting altered hydrogen-bonding and local molecular environments. DSC demonstrated a downward shift and attenuation of the principal Hesperetin-associated thermal event. PXRD showed attenuation and modification of characteristic Hesperetin reflections. These findings are mutually supportive of altered molecular association and crystal organization.

The analytical results should nevertheless be interpreted with appropriate restraint. FTIR, DSC and PXRD collectively support complex formation and physical-state modification, but they do not independently establish a unique orientation of Hesperetin inside the HP-β-CD cavity. A more definitive structural assignment would require complementary techniques such as NMR or other molecular-level characterization.

From a formulation-development perspective, the selected inclusion complex represents an intermediate rather than the final dosage form. Its value is that it transforms Hesperetin into a more aqueous-compatible presentation while retaining measurable drug content and recoverability. This

creates a logical bridge to hydrogel development, where polymer concentration, pH, rheology, spreadability, swelling and release become the principal formulation variables.

The experimental sequence also supports meaningful controls for subsequent work. Free Hesperetin can be compared with the inclusion complex to quantify the contribution of complexation. A physical mixture can help distinguish simple carrier contact from processing-associated changes. Finally, the optimized hydrogel can be compared with a free-drug hydrogel to determine the incremental contribution of the inclusion-complex step.

6. Formulation implications for hydrogel development

A successful inclusion complex should provide sufficient drug loading, reproducible recovery and improved aqueous presentation. These attributes determine whether the complex can be incorporated into a hydrogel without excessive solvent use or loss of formulation stability.

The hydrogel stage introduces additional quality attributes. Polymer concentration can increase viscosity and swelling while reducing spreadability and changing the diffusion pathway for drug release. The inclusion complex can also alter the effective concentration gradient that drives drug transport through the hydrated polymer network. Consequently, the complex and polymer should be optimized as interacting formulation components.

The methodology developed in the present work provides the experimental bridge for that next stage. The formulation-design tables identify the candidate factors and responses, while the release and stability methods define the performance measurements that should be used to select the final hydrogel.

7. Conclusions

The present study established a systematic formulation strategy for improving the pharmaceutical properties of Hesperetin through complexation with hydroxypropyl- β -cyclodextrin (HP- β -CD). Preliminary solubility studies confirmed the limited aqueous solubility of Hesperetin, with an equilibrium solubility of 0.041 mg/mL in purified water, demonstrating the need for an appropriate solubility-enhancement approach.

The phase-solubility investigation demonstrated a concentration-dependent increase in the apparent solubility of Hesperetin with increasing HP- β -CD concentration. The experimental profile showed an approximately linear relationship over the investigated concentration range, providing the basis for evaluation of the apparent complexation behavior. The 1:1 Hesperetin: HP- β -CD ratio was selected as a practical formulation condition because it provided substantial solubility enhancement together with satisfactory recovery and drug-content characteristics. At this ratio, the apparent solubility increased to 0.316 mg/mL, corresponding to a 7.71-fold enhancement compared with free Hesperetin. The selected inclusion complex prepared by solvent evaporation exhibited a 91.8% percentage yield and 98.1% drug content, indicating satisfactory process recovery and drug incorporation. The reproducibility observed among the reported replicate batches, with yield values ranging from 91.2–92.2% and drug content from 97.8–98.3%, further supported the consistency of the preparation process.

Solid-state characterization provided complementary evidence of changes following complexation. FTIR analysis demonstrated alterations in the O–H, C–H, carbonyl/aromatic and fingerprint regions, indicating changes in the local molecular environment. DSC demonstrated modification and attenuation of the principal Hesperetin-associated thermal transition, with the major drug-associated event shifting from approximately 232 °C to approximately 223 °C. PXRD further demonstrated attenuation and positional modification of characteristic Hesperetin diffraction reflections, indicating changes in crystalline organization. Collectively, the FTIR, DSC and PXRD findings support the

formation of an inclusion-associated solid and modification of the original solid-state characteristics of Hesperetin. However, these techniques provide complementary evidence and do not independently establish a specific orientation of Hesperetin within the HP- β -CD cavity.

The improved aqueous presentation of Hesperetin achieved through HP- β -CD complexation provides an important formulation advantage for subsequent topical delivery. The characterized inclusion complex can therefore be incorporated into a hydrogel system in which additional formulation attributes, including pH, viscosity, spreadability, drug content and drug-release behavior, can be systematically optimized. The reported release study further showed that the optimized hydrogel provided a more gradual release profile, reaching approximately 82% cumulative release at 8 h, supporting its potential as a controlled topical delivery platform.

Overall, the findings demonstrate that HP- β -CD complexation substantially improved the apparent aqueous solubility of Hesperetin while producing complementary changes in its physicochemical and solid-state characteristics. The optimized Hesperetin-HP- β -CD inclusion complex therefore represents a scientifically justified formulation intermediate for topical hydrogel development and provides a rational foundation for further optimization, release evaluation and stability assessment of the final topical delivery system.

8. References

1. Loftsson T, Masson M. Cyclodextrins in topical drug formulations: theory and practice. *Int J Pharm.* 2001; 225:15-30. doi:10.1016/S0378-5173(01)00761-X.
2. Tommasini S, Calabrò ML, Stancanelli R, et al. The inclusion complexes of Hesperetin and its 7-rhamnoglucoside with (2-hydroxypropyl)- β -cyclodextrin. *J Pharm Biomed Anal.* 2005; 39:572-580. doi: 10.1016/j.jpba.2005.05.009.
3. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. *J Pharm Pharmacol.* 2010; 62:1607-1621. doi:10.1111/j.2042-7158.2010.01030. x.
4. Jansook P, Ogawa N, Loftsson T. Cyclodextrins: structure, physicochemical properties and pharmaceutical applications. *Int J Pharm.* 2018; 535:272-284.
5. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an updated review. *AAPS PharmSciTech.* 2005;6: E329-E357.
6. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties of the citrus flavonoid Hesperidin and Hesperetin: an updated review. *Phytother Res.* 2015; 29:323-331. doi:10.1002/ptr.5256.
7. Song Y, et al. Hesperetin: pharmacological activities, mechanisms and formulation perspectives. *Fitoterapia.* 2023; 179:106206. doi: 10.1016/j.fitote.2024.106206.
8. Yu J, et al. Hesperetin promotes diabetic wound healing by inhibiting ferroptosis through activation of SIRT3. *Phytother Res.* 2023; 38:1478-1493. doi:10.1002/ptr.8121.
9. Vaz VM, Jitta SR, Verma R, Kumar L. Hesperetin-loaded liposomal gel for topical antioxidant activity. *J Drug Deliv Sci Technol.* 2021; 66:102873. doi: 10.1016/j.jddst.2021.102873.
10. Taléns-Visconti R, et al. Hesperetin-loaded hyaluronic emulgel and topical delivery. *J Funct Biomater.* 2023; 15:89.
11. Wang Q, et al. Hydrogel wound healing: current advances and future directions. *Int J Biol Macromol.* 2023; 261:129300. doi: 10.1016/j.ijbiomac.2024.129300.
12. Sang Y, et al. Polysaccharide/protein hydrogels for wound healing. *Int J Biol Macromol.* 2023; 280:135482. doi: 10.1016/j.ijbiomac.2023.135482.

13. Pinho E, Grootveld M, Soares G, Henriques M. Cyclodextrin-based hydrogels toward improved wound dressings. *Crit Rev Biotechnol*. 2014; 34:328-337.
14. Liu J, Tian B, Liu Y, Wan JB. Cyclodextrin-containing hydrogels: preparation, drug delivery and degradation behavior. *Int J Mol Sci*. 2021; 22:13516.
15. Carvalho MTB, et al. Wound healing properties of flavonoids: a systematic review. *Phytomedicine*. 2021; 90:153636.
16. Jee JP, et al. Topical hydrogel containing quercetin and oxygen carriers for diabetic wound healing. *Int J Nanomedicine*. 2019; 14:5449-5475.
17. Gong CY, et al. Biodegradable hydrogel containing curcumin-loaded micelles for cutaneous wound healing. *Biomaterials*. 2013; 34:6377-6387.
18. Higuchi T, Connors KA. Phase-solubility techniques. *Adv Anal Chem Instrum*. 1965; 4:117-212.
19. USP General Chapter <1724>. Semisolid Drug Products—Performance Tests. United States Pharmacopeia. 2023.
20. ICH Q1A(R2). Stability Testing of New Drug Substances and Products. International Council for Harmonisation. 2003.