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Formulation and Evaluation of Nanogel Containing Quercetin for Treatment of Alopecia

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ABSTRACT

The study aimed to develop and characterize Quercetin-loaded nanogel formulations for enhanced drug delivery and therapeutic efficacy. Initially, nine formulation batches viz., F1-F9 of Quercetin nanoparticles were prepared and evaluated for key parameters including particle size & zeta potential, entrapment efficiency, surface morphology, in-vitro drug release and stability. FT-IR spectra confirmed the chemical integrity of Quercetin. Zeta potential values ranged from -5.45 mV to -17.65 mV, indicating good colloidal stability. Scanning Electron Microscopy (SEM) revealed spherical nanoparticles with a rough surface. In-vitro drug release studies demonstrated a release profile ranging from 74.12% to 95.32% over 12 hours, with the optimized batch (Fopt) achieving the highest release of 95.32%. Prepared nanoparticles were further converted into gel formulation. The pH, viscosity, and spreadability of the nanogel were within acceptable ranges, ensuring suitability for topical application. Stability studies confirmed the optimized formulation's physical integrity and sustained drug release under accelerated storage conditions. These findings suggest that Quercetin-loaded nanogels can provide a promising platform for efficient drug delivery with enhanced stability for treatment of Alopecia.

Keywords: *Quercetin, nanogel, Zeta Potential, SEM, pH, viscosity and spreadability, Alopecia.*

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1. Introduction

Alopecia is a medical condition characterized by hair loss from the scalp or other body parts. It can occur due to various factors, including genetics, autoimmune disorders, hormonal changes, stress, or medical treatments. The most common types are androgenetic alopecia (pattern baldness) and alopecia areata (autoimmune hair loss). While it is not life-threatening, alopecia can significantly impact an individual's self-esteem and emotional well-being. Treatment options range from topical medications and oral drugs to advanced therapies like nanotechnology-based delivery systems and nano remedies.[1,2]

Quercetin, a natural flavonoid with antioxidant and anti-inflammatory properties, has shown promising results in managing alopecia. It helps protect hair follicles from oxidative stress and inflammation, which are key factors contributing to hair loss. By inhibiting pro-inflammatory cytokines and promoting the growth of dermal papilla cells, Quercetin supports hair regrowth and scalp health, making it a potential therapeutic agent in alopecia treatment.[3,4]

The stratum corneum is the primary barrier to drug delivery via the skin, with hair follicles, sebaceous glands, and sweat glands offering promising alternative pathways. Nanoparticles enhance drug penetration into hair follicles, leveraging their architecture and the rich capillary network for prolonged drug release. Particles sized between 300-600 nm are particularly effective, enabling extended depot periods of up to 10 days compared to non-particulate drugs. This targeted delivery increases drug bioavailability while requiring smaller doses, making it ideal for conditions like alopecia. While nanoparticle formulations of synthetic drugs show advantages, their side effects highlight the potential of nanoparticles as safer, effective alternatives. Further research on nanoparticle treatments for alopecia is needed.[5,6] Thus, our study is focused on the utilization of Quercetin, as a potential candidate for treating Alopecia.

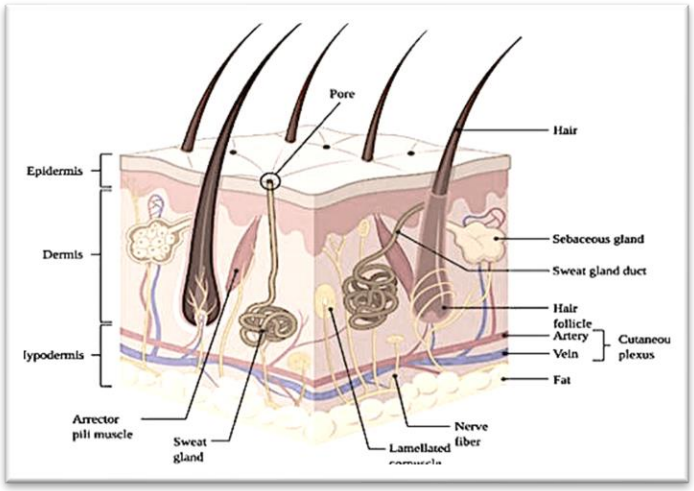


Figure 1: Drug distribution through the hair follicle

2. Material and Methods

2.1 Material

Quercetin, Chitosan, Sodium alginate, Calcium chloride, Acetic acid, Sodium hydroxide, Triethanolamine, Carbopol 940, Propylene glycol, Methyl paraben and propyl paraben were used for preparation of nanoparticles and nanogel and all ingredients used were procured from various reputed companies.

2.2 Method

2.2.1 Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis (Spectrum RX, Perkin Elmer, USA) was performed to identify potential chemical interactions between drug and selected excipients in the nanoparticles. Formulation blend & pure drug samples were analyzed using KBr pellets, scanned in the 4000–400 cm−1 frequency range.

2.2.2 Preparation of Quercetin nanoparticles

Nanoparticles were prepared using the ionic gelation method. A chitosan solution (500 mg in 1% acetic acid) was prepared. Separately, sodium alginate and calcium chloride solutions were optimized and diluted. A calcium alginate pre-gel was formed by adding calcium chloride solution

to sodium alginate under stirring. Quercetin (100 mg) was incorporated into the chitosan solution, which was then added dropwise to the calcium alginate pre-gel under continuous stirring. The pH of the mixture was adjusted to 5.3 to facilitate nanoparticle formation. For comparison, blank nanoparticles were prepared following the same procedure but without adding Quercetin.

2.2.2.1 Optimization of nanoparticles: Design Expert Software, version 13 (Stat-Ease, Inc. Minneapolis, MN, USA) was used to generate polynomial equations with added interaction terms to correlate selected responses with selected variables. Particle size and entrapment efficiency were selected as response variables for systematic optimization. An optimum solution was also provided by the software using overlay plots. To find out the optimum concentration of sodium alginate and calcium chloride, central composite design face-centered was used.[6,7] The effect of two factors like the amount of sodium alginate and calcium chloride was investigated on two response variables *viz.* particle size (R1) and entrapment efficiency (R2). Factor levels were coded suitably. Table 1 represented the coded and actual values of independent variables.

Table 1: Coded and Actual values of selected independent variables

Coded Values	**Actual Values**	
	Sodium alginate (mg)	**Calcium chloride (mg)**
-1	0.5	0.1
0	1.0	0.3
+1	1.5	0.5

Table 2: Formulation chart of Nanoparticles of Quercetin as per Central composite design

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Quercetin (mg)	100	100	100	100	100	100	100	100	100

Chitosan (mg)	500	500	500	500	500	500	500	500	500
Sodium alginate (mg)	100	200	300	100	200	300	100	200	300
Calcium Chloride (mg)	100	100	100	125	125	125	150	150	150
Acetic acid 1% (ml)	100	100	100	100	100	100	100	100	100
Double distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

2.2.3 Evaluation of Nanoparticles

2.2.3.1 Particle Size and Zeta Potential: Nanoparticles were diluted in distilled water and analyzed using a zeta sizer operated in automatic mode at 25°C and a scattering angle of 90°.[8] Zeta potential was measured to assess long-term colloidal stability, with values greater than +25 mV or less than -25 mV indicating high stability.[9,10] Measurements were conducted in triplicate.

2.2.3.2 Entrapment Efficiency: Nanoparticles were separated by ultracentrifugation at 10,000 rpm and 40°C for 30 minutes, and free Quercetin was quantified by U.V spectrophotometer at 206 nm. Entrapment efficiency was calculated using the formula:

$$\textbf{Entrapment Efficiency} = \frac{T - F}{T} \times 100$$

Where T is the total drug amount and F is the free drug in the supernatant.

2.2.3.3 Scanning Electron microscopy (SEM):

A scanning electron microscopy was used to analyze the shape and surface morphology of the prepared nanoparticles.

2.2.3.4 *In-vitro* release study: Franz diffusion cell was used for *in-vitro* release study of nanoparticles. The study was performed to investigate the amount of drug release from the nanoparticles. A porous membrane of molecular weight cut off 50,000 Da (Sigma Aldrich, Japan) was used. The membrane was mounted between the donor and receiver compartments of the

instrument.[11] Donor compartment was filled with nanogel, receiver was filled with 10 ml of phosphate buffer (pH 7.4), stirred and maintained at 37^0C ± 0.5^0C using a circulator water bath. Top was covered by paraffin paper. At predetermined intervals (60, 120, 180, 240, 300, 360, 420, 480, 600 and 720 minutes), 1 ml of the sample was removed from receiver compartment and was replaced by fresh buffer solution. The collected samples were analyzed for Quercetin content using UV visible spectrophotometer at 26 6 nm. The amount of drug permeated through the porous membrane was calculated to determine the average percent release.[12]

2.2.4 Preparation of nanogel

Nanogel was prepared by dispersing 1 g of Carbopol 940 in 50 mL of distilled water and allowing it to swell for 30 minutes. The mixture was then stirred at 1200 rpm for another 30 minutes to ensure homogeneity. Separately, 5 mL of propylene glycol was mixed with weighed amounts of propyl and methyl parabens to form a preservative solution. Lyophilized nanoparticles and the preservative solution were subsequently added to the Carbopol dispersion under constant stirring. The volume of the mixture was adjusted to 100 mL using distilled water, and triethanolamine was added dropwise to adjust the pH to 6.8 and achieve the desired gel consistency.[13]

2.2.4.1 Composition of Quercetin nanogel

The prepared nanoparticles were added to Carbopol 940 gelling base to prepare the nanogel. Table 3 represented the composition of Quercetin nanogel.

Table 3: Composition of Quercetin Nanogel

Ingredients	**Quantity**
Quercetin Nanoparticles (mg)	equivalent to 100 mg Quercetin
Carbopol 940	1 g
Propylene glycol	5 ml

Methyl Paraben	50 mg
Propyl Paraben	50 mg
Triethanolamine	q.s
Distilled water	q.s upto 100 ml

2.2.5 Evaluation of Quercetin Nanogel: Different parameters like pH, color, appearance, homogeneity, viscosity, spreadability, *in vitro* release and stability studies were performed to evaluate the nanogel.[14]

2.2.5.1 Physical appearance: Physical appearance and homogeneity of the prepared gels were evaluated by visual perception.

2.2.5.2 Measurement of pH: The pH of nanogel formulations was measured by using digital pH meter. Electrode of digital pH meter was dipped into gel and readings were taken. The measurement of pH of each formulation was done in triplicate and average values were calculated.[15]

2.2.5.3 Viscosity study: The measurement of nanogel viscosity was performed with a Brookfield Viscometer using spindle no. 6 and shear rate was increased from 0.5 to 100 ml. Viscosity was performed in triplicate.

2.2.5.4 Spreadability: Two sets of standard-sized glass slides were taken. One of the slides was covered with the nanogel mixture. The gel was sandwiched between the two slides by the other slide, which was placed on top of it. A certain amount of gel was placed on the upper slides, and the gel was squeezed uniformly between the two slides to form a thin layer. The spreadability improves as the time it takes to separate two slides' decreases. The experiment was done in triplicate and the mean time was taken for calculation.[16] Spreadability was calculated by using the following formula:

S= m × l/t

where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec.[17]

2.2.5.5 *In-vitro* release study: The produced nanogel was tested for *in-vitro* diffusion in a Franz diffusion cell setup. In a Franz diffusion cell, 1g of gel was evenly placed across a cellophane membrane that had been soaked in phosphate buffer pH 7.4 for 8 hours and was sandwiched between the donor and receptor compartments. As the receptor compartment, 30 mL of phosphate buffer was employed. The temperature shall be kept at 37±0.5°C. The whole system was mounted on a magnetic stirrer, and the solution in the receptor compartment was constantly stirred at 50 rpm with a magnetic bead. At hourly intervals, a 1 mL sample was taken and replaced with 1 mL of the fresh buffer. The drug concentration in the receptor fluid was measured U.V visible spectrophotometrically at 266 nm against a blank. The percentage drug release was calculated using regression equation.[18]

2.2.5.6 Stability study: Nanogel formulations were filled in scintillation glass vials and incubated as per ICH guidelines for accelerated stability study *viz.* $40^0C \pm 2^0C/75\% \pm 5\%$ RH for three months and evaluated for physical appearance, homogeneity, pH, viscosity and spreadability.[19, 20]

3. Results and discussion

3.1 FT-IR

The drug-excipient compatibility was accessed by FTIR analysis. From the results it was found that the selected drug was compatible with the excipients as the prominent peaks of drug were seen in the graph obtained with the excipients. Thus, the selected drug and excipients can be further used together to formulate the nanogel. The FTIR spectra of the Quercetin (pure drug) and formulation blend was obtained and shown in figure 2A & 2B.

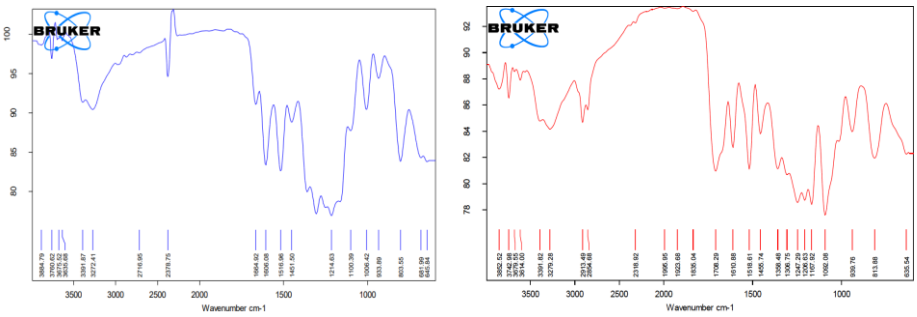


Figure 2: FT-IR spectra of Quercetin (pure drug) (2A) and FT-IR spectra of Formulation blend (2B)

3.2 Particle size and Zeta potential

Particle size of formulated nanoparticles was ranged between 159.83-415.85nm. Zeta potential of drug loaded nanoparticles was determined and found to be in the range of -5.45 to -17.65mV. Results were shown in table 3.

Table 3: Particle size and Zeta potential of Quercetin nanoparticles (Formulation batch F1-F9)

Formulation Batch	Particle size (nm)	Zeta Potential (mV)
F1	415.85	-12.67
F2	312.44	-13.89
F3	198.17	-11.65
F4	399.11	-10.98
F5	278.95	-10.75
F6	174.78	-15.87
F7	358.35	-14.34
F8	224.62	-5.45

F9	159.83	-17.65

3.3 Entrapment efficiency (%EE)

The percentage entrapment efficiency of prepared nanoparticles was determined and represented in table 4. The % EE of prepared nanoparticles ranged between 56.71%-88.47%.

Table 4: Entrapment efficiency of

Formulation Batch	Entrapment efficiency (%)
F1	56.71
F2	71.88
F3	82.29
F4	61.28
F5	74.83
F6	85.55
F7	67.56
F8	79.37
F9	88.47

Quercetin Nanoparticles

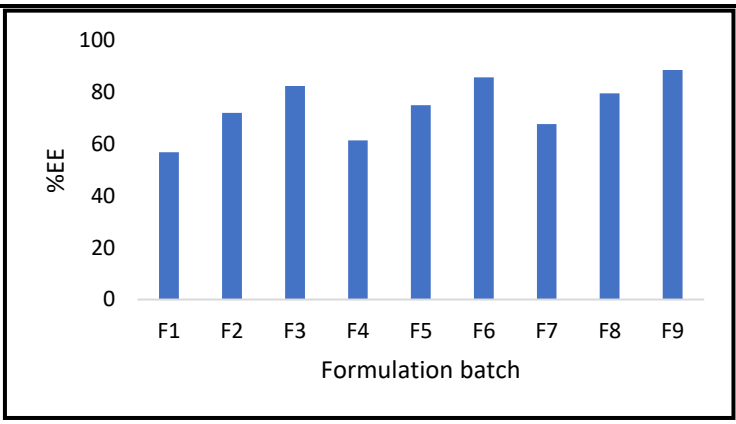


Figure 3: Entrapment efficiency of Quercetin nanoparticles Formulations F1-F9

3.4 Optimization study:

In the present work, two formulation parameters i.e., Factor A (Sodium alginate) and Factor B (Calcium chloride) were optimized to formulate nanoparticles with desired particle size and entrapment efficiency. Table 5 represented the Responses 1 (Particle size) and Response 2 (Entrapment efficiency).

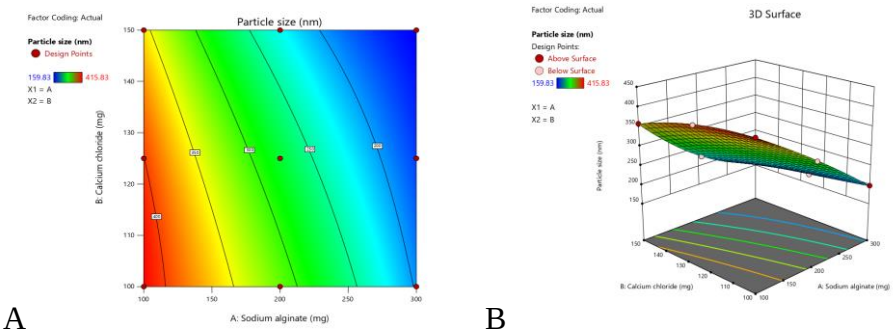
Table 5: Independent and Dependent factors of Quercetin nanoparticles Formulation batches (F1-F9)

Formulation batch	**Factor A: Sodium alginate**	**Factor B: Calcium chloride**	**Response 1: Particle size (nm)**	**Response 2: Entrapment efficiency (%)**
F1	100	100	415.83	56.71
F2	200	100	312.44	71.88
F3	300	100	198.17	82.29
F4	100	125	399.11	61.28
F5	200	125	278.95	74.83
F6	300	125	174.78	85.55
F7	100	150	358.35	67.56
F8	200	150	224.62	79.37
F9	300	150	159.83	88.47

The selected independent factors, concentration of Sodium alginate and Calcium chloride were undergone ANOVA analysis to check out the effect of selected factors individually and collectively on dependent factors i.e., Particle size (R1) and Entrapment efficiency (R2). The ANOVA analysis of factor 1 (Particle size) was depicted in Table 6 & Figure 4.

Table 6: ANOVA for Reduced Cubic model for Response 1: Particle size

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	75084.96	7	10726.42	567.73	0.0323	significant
A-Sodium alginate	25161.97	1	25161.97	1331.78	0.0174	
B-Calcium chloride	3856.18	1	3856.18	204.10	0.0445	
AB	91.58	1	91.58	4.85	0.2714	
A^2	304.63	1	304.63	16.12	0.1554	
B^2	73.77	1	73.77	3.90	0.2983	
A^2B	530.94	1	530.94	28.10	0.1187	
AB^2	87.91	1	87.91	4.65	0.2764	
Residual	18.89	1	18.89			
Cor Total	75103.85	8				



A B

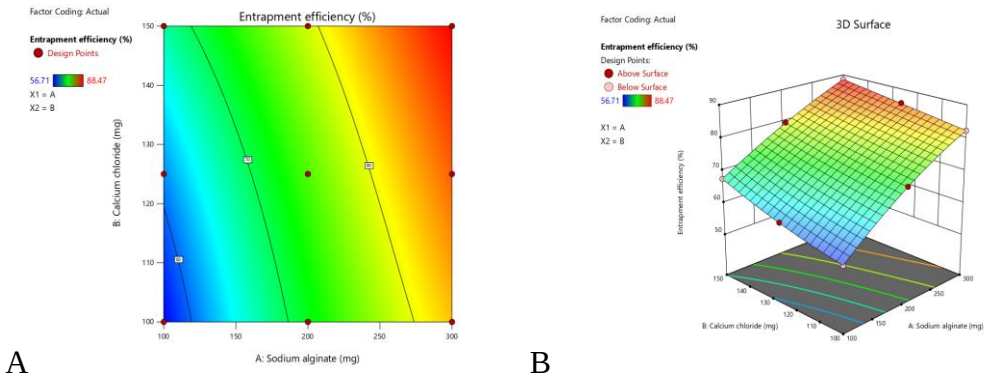
Figure 4: Graph showing the effect of Sodium alginate and calcium chloride on Response 1 (Particle size); (A) Contour Graph and (B) 3D graph

Similarly, the ANOVA analysis of factor 2 (Entrapment efficiency) was depicted in Table 7 & Figure 5.

Table 7: ANOVA for Reduced Cubic model for Response 2: Entrapment efficiency

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	947.23	7	135.32	1486.98	0.0200	significant
A-Sodium alginate	294.52	1	294.52	3236.35	0.0112	
B-Calcium chloride	28.05	1	28.05	308.23	0.0362	
AB	5.45	1	5.45	59.91	0.0818	
A^2	5.89	1	5.89	64.77	0.0787	
B^2	0.4868	1	0.4868	5.35	0.2598	
A^2B	0.3502	1	0.3502	3.85	0.3001	
AB^2	0.3502	1	0.3502	3.85	0.3001	

Residual	0.0910	1	0.0910			
Cor Total	947.33	8				



A B

Figure 5: Graph showing the effect of Sodium alginate and calcium chloride on Response 2 (Entrapment efficiency); (A) Contour Graph and (B) 3D graph

P-values was less than 0.0500, which indicated model terms were significant. In this case A, B both were significant model terms. From the results obtained from optimization study, it was observed that factors A & B i.e., Sodium alginate and Calcium chloride showed negative effect on Particle size individually. If independent factors increased, the response particle size was decreased (Figure 4). The response was positive when observed combinely. The polynomial equation for Response 1 (Particle size) was presented in Eq. 1.

R1= 276.05–112.17*A– 43.91*B + 4.79*AB + 12.34*A2- 6.07*B2+19.96*A2B +8.12*AB2 Eq. 1

However, the independent factors showed positive effect on Entrapment efficiency. If the concentration of Sodium alginate and Calcium chloride were increased, the entrapment efficiency also increased (Figure 5). The polynomial equation for Response 2 (Entrapment efficiency) was depicted in Eq. 2.

R2= 75.03 +12.13*A+3.75*B –1.17*AB –1.72*A2+0.4933*B2+0.5125*A2B –0.5125*AB2 Eq. 2

After conducting ANOVA study on prepared batches, an optimized formula was obtained which was presented in Figure 6 and used for further study.

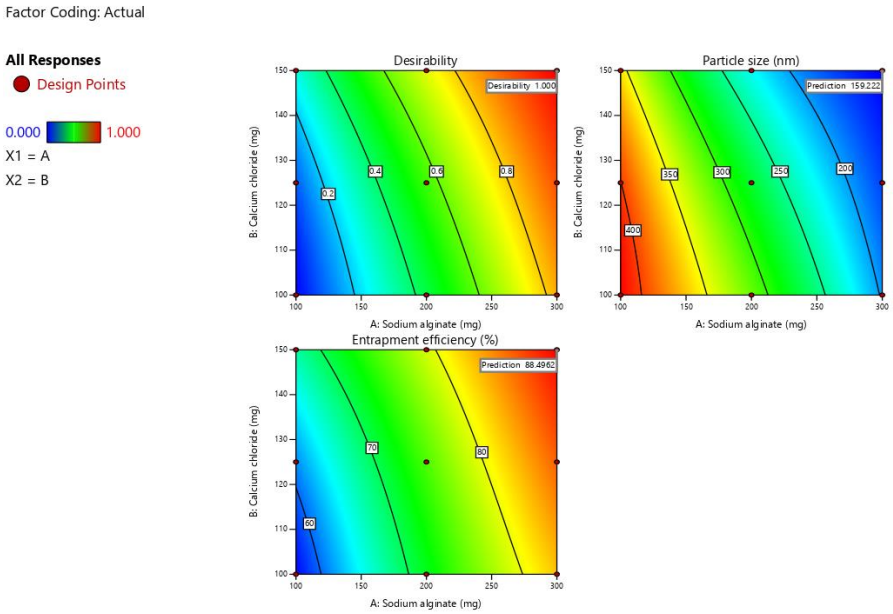


Figure 6: Predicted solution obtained from Optimization study

3.5 Scanning Electron Microscope (SEM)

The surface morphology study of prepared formulation batches of Quercetin nanoparticles showed that the nanoparticles were spherical with a rough surface.

3.6 *In-vitro* drug release

The dissolution profile of all the batches of Quercetin nanoparticles was obtained in phosphate buffer pH 7.4. The *in-vitro* dissolution testing was performed for 12 hr. (720 minutes). The *in-vitro* drug release from Quercetin nanoparticles ranged in between 74.12% to 92.35%. The maximum *in-vitro* drug release was found to be 92.35 % from F9 at the end of 720 minutes as shown in figure 7.

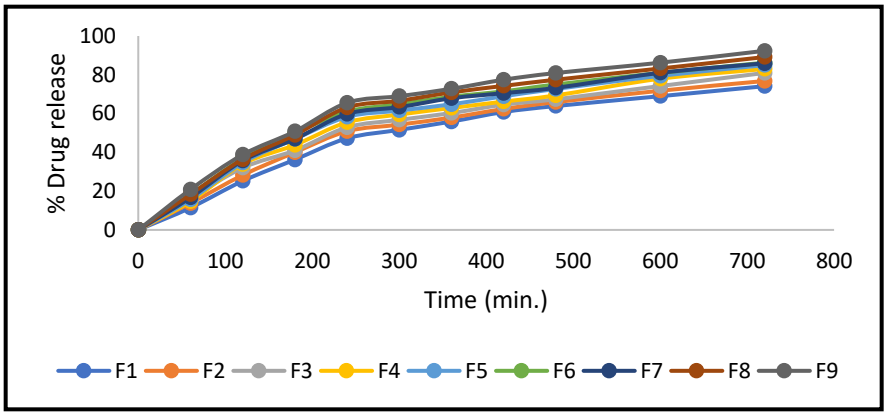
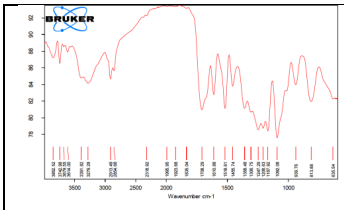
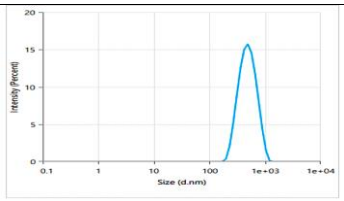
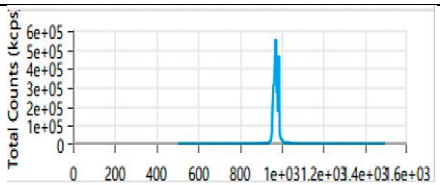
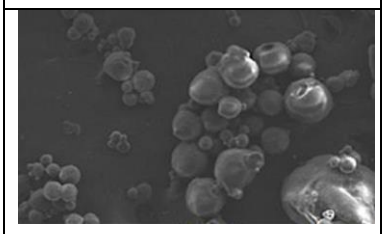
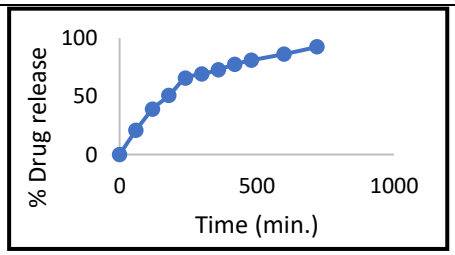


Figure 7: *In-vitro* drug release profile of Quercetin nanoparticles (Formulation batches F1-F9)

Based on the result obtained from optimization and *in-vitro* evaluation study, it was observed that the batch F9 showed better results when compared with other batches. The F9 batch comprised of similar concentration of Sodium alginate and Calcium chloride as suggested by optimization study, thus it was considered as an optimized batch and further used to prepare nanogel. Figure 8 is showing the evaluation parameters of optimized batch (F9).

 FTIR spectra of optimized formulation	 Particle size: 159.83 nm	 Zeta Potential: -17.65 mV
	Entrapment efficiency: 88.47%	 % Drug release

Surface morphology: Spherical shaped nanoparticles		

Figure 8: Evaluation parameters of Optimized batch (F9)

3.7 Preparation and Evaluation of Quercetin Nanogel

The optimized batch F9 was transformed into nanogel utilizing Carbopol 940 as gelling agent. The prepared nanogel was evaluated for various evaluation parameters. Different parameters like pH, color, appearance, homogeneity, viscosity, spreadability, *in-vitro* release, stability studies were studied for nanogel.

3.7.1 Physical appearance: The Quercetin nanogel was tested for clarity and homogeneity. It was found that the prepared gel was clear, transparent and homogeneous.

3.7.2 Measurement of pH: The Quercetin nanogel formulation was checked for pH. The pH of Quercetin nanogel was found to be in the range of 6.8, similar to skin pH.

3.7.3 Viscosity study: The measurement of nanogel viscosity was performed with a Brookfield Viscometer using spindle no. 6 and was found to be 258cP.

3.7.4 Spreadability: Spreadability of nano gel was found to be 17.1±0.12 g.cm/sec. which showed good spreading ability.

3.8 Stability Study of Quercetin nanogel

The Quercetin nanogel was kept on accelerated conditions to check the stability of formulated gel. The physical appearance and *in-vitro* drug release studies were observed during stability testing. The results of appearance, and *in-vitro* drug release from nanogel on 0 day, after 30 days, 60 days and 90 days of storage were shown in table 8 and figure 9.

Table 8: Effect of stability conditions on different parameters of optimized formulation (F9)

	Parameters	**Stability study**

S. No		Day 0	Day 30	Day 60	Day 90
1	Appearance	Clear and Transparent	Clear and Transparent	Clear and Transparent	Clear and Transparent
2	*In-vitro* Drug release at 12 hr	92.35%	92.18%	91.85%	91.28%

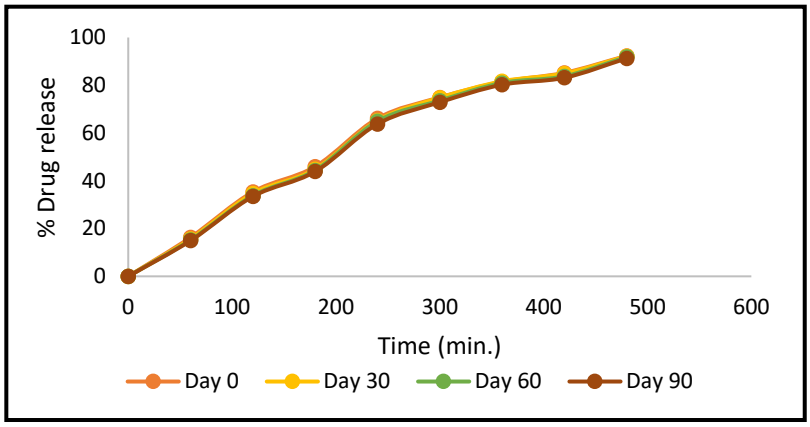


Figure 9: Effect of stability conditions on drug release from Quercetin nanogel

4. Conclusion

The developed Quercetin-loaded nanoparticles demonstrated excellent physicochemical properties, including optimal zeta potential, uniform spherical morphology, and sustained drug release. The optimized batch (F9) showed the highest drug release of 92.35% within 12 hr. The Prepared Quercetin nanogel showed favorable pH, viscosity, and spreadability characteristics. Stability studies under accelerated conditions confirmed the formulation's robustness, maintaining physical appearance and significant drug release profiles over three months. These results highlighted the potential of Quercetin nanogel as an effective and stable drug delivery system, paving the way for further clinical evaluations and applications in targeted therapy.

5. Conflict of interest

The authors have no conflict of interest.

6. Acknowledgement

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