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RESEARCH ARTICLE

Design and Optimization of Quercetin-Loaded Polymeric Film Using Box–Behnken Design with HPLC and Antimicrobial Evaluation

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Abstract: The present study aimed to design and optimize Quercetin-loaded polymeric films using the Box–Behnken Design (BBD) for enhanced topical delivery and antimicrobial efficacy. Chitosan and polyvinyl alcohol (PVA) were employed as film-forming polymers, while glycerol served as a plasticizer. A three-factor, three-level BBD was used to investigate the effect of polymer ratio, glycerol concentration, and Quercetin loading on tensile strength, folding endurance, and in vitro drug release. The optimized formulation exhibited excellent mechanical strength (12.35 ± 0.18 N/mm²), high folding endurance (158 ± 2.3), and sustained drug release (77.02 ± 0.92% at 8 h) with an overall desirability of 0.923. HPLC analysis confirmed the presence of Quercetin at a retention time of 5.02 min, corresponding closely with the standard (4.52 min), indicating stability and purity of the encapsulated drug. The antimicrobial study against Staphylococcus aureus demonstrated a concentration-dependent increase in the zone of inhibition, with maximum activity at 200 µg/mL comparable to ciprofloxacin. These findings confirm the successful formulation of a biocompatible, mechanically stable, and antimicrobial Quercetin-loaded polymeric film, highlighting its potential for localized therapeutic applications in skin infections and wound management.

Keywords: Quercetin, Box–Behnken Design, Polymeric Film, HPLC, Chitosan, Antimicrobial Activity.

INTRODUCTION

Quercetin is a naturally occurring flavonoid widely distributed in fruits, vegetables, and medicinal plants, possessing diverse pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [1]. Despite its broad therapeutic potential, the clinical application of Quercetin remains limited due to its poor aqueous solubility, chemical instability, and low bioavailability [2]. These physicochemical constraints restrict its absorption and efficacy, particularly in topical and transdermal drug delivery systems, where consistent release and stability are crucial [3]. Polymeric films have gained significant attention in recent years as versatile carriers for the controlled and localized delivery of bioactive compounds [4]. Such films provide uniform drug distribution, flexibility, and intimate contact with the skin or mucosal surface, thereby improving therapeutic outcomes [5]. Among various polymers, chitosan a natural polysaccharide derived from chitin offers excellent film-forming ability, biocompatibility, and intrinsic antimicrobial activity [6]. However, chitosan films alone often exhibit brittleness and limited mechanical strength. To overcome these drawbacks, synthetic polymers such as polyvinyl alcohol (PVA) are frequently blended with chitosan to produce films with enhanced flexibility, mechanical integrity, and sustained release characteristics [7]. The Chitosan–PVA combination thus serves as a promising polymeric matrix for topical formulations of poorly soluble drugs like Quercetin. Formulation optimization plays a pivotal role in achieving desired mechanical and release properties in polymeric films [8]. Traditional trial-and-error approaches are often time-consuming and inefficient, leading to increased material consumption and unpredictable results. In contrast, statistical design of experiments (DoE) techniques, such as the Box–Behnken Design (BBD), enable systematic evaluation of multiple formulation variables and their interactions with minimal experimental runs [9]. This approach ensures the development of an optimized formulation with desirable characteristics while maintaining experimental efficiency [10-13]. In the present study, Quercetin-loaded polymeric films were designed and optimized using the Box–Behnken Design by varying three independent factors polymer ratio (Chitosan:PVA), glycerol concentration, and Quercetin loading [14-15]. The films were evaluated for tensile strength, folding endurance, and in vitro drug release to determine the optimal formulation using a desirability-based approach. Furthermore, high-performance liquid chromatography (HPLC) fingerprinting was employed to confirm the identity and purity of Quercetin within the optimized film. The antimicrobial potential of the developed films was assessed against selected microbial strains to explore their therapeutic applicability in topical infections.

MATERIALS AND METHODS

Materials

Quercetin (≥98% purity) was purchased from Sigma-Aldrich, Chitosan (medium molecular weight, 85% deacetylated) and polyvinyl alcohol (PVA, Mw 89,000–98,000) were procured from HiMedia Laboratories (Mumbai, India). Glycerol (analytical grade) was obtained from Merck (Germany). Methanol (HPLC grade), acetonitrile, and orthophosphoric acid were supplied by Fisher Scientific (India). Dialysis membranes (MWCO 12–14 kDa), phosphate-buffered saline (PBS), and nutrient agar were obtained from SRL Chemicals (Mumbai, India). All chemicals were of analytical or HPLC grade and used without further purification.

Preparation of Quercetin-Loaded Polymeric Film

Quercetin-loaded chitosan–PVA films were prepared by the solvent casting method. Chitosan was dissolved in 1% (v/v) acetic acid under magnetic stirring until a clear solution was obtained. PVA was dissolved separately in distilled water at 80 °C until complete solubilization. Both polymeric solutions were blended in specified ratios (1:1, 2:1, and 3:1) according to the experimental design [16]. Glycerol was incorporated as a plasticizer at varying concentrations (0.5–1.5% w/w), followed by addition of Quercetin at predetermined levels (10–15 mg/mL). The mixture was stirred for 30 minutes to achieve uniform dispersion and degassed under vacuum to remove air bubbles.The prepared solution was poured into glass Petri dishes and dried at 45 °C for 24 hours. Dried films were peeled carefully, cut into 2 cm circular discs, and stored in a desiccator for further analysis [17].

Experimental Design (Box–Behnken Design)

A three-factor, three-level Box–Behnken Design (BBD) was applied using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, USA) to optimize the formulation variables [18]. The independent factors were polymer ratio (A: Chitosan:PVA) Coded Factor 1-1:1, 2- 2:1 and 3-3:1, glycerol concentration (B, % w/w), and Quercetin concentration (C, mg/mL). The dependent responses were tensile strength (R_1), folding endurance (R_2), and percentage drug release (R_3). A total of 15 experimental runs were generated, as shown in Table 1.

Table 1. Formulation batches for Quercetin-loaded polymeric films based on Box–Behnken Design

Run	Polymer Ratio (Chitosan:PVA)	Glycerol Conc. (% w/w)	Quercetin Conc. (mg/mL)
1	2	0.5	10
2	3	0.5	12.5
3	2	1.5	15
4	2	1	12.5
5	3	1	15
6	2	1	12.5
7	2	1	12.5
8	1	1.5	12.5
9	3	1.5	12.5
10	1	0.5	12.5
11	1	1	10
12	2	1.5	10
13	3	1	10
14	1	1	15
15	2	0.5	15

Evaluation of Polymeric Films

Thickness and Weight Uniformity

Film thickness was measured using a digital micrometer at five different points, and mean ± SD was calculated. For weight uniformity, individual discs (2 cm diameter) were weighed using an analytical balance [19].

Folding Endurance

Films were repeatedly folded at the same point until breaking, and the number of folds required to break was recorded as folding endurance [20].

In Vitro Drug Release

The release of Quercetin from the films was evaluated using a Franz diffusion cell with a dialysis membrane. The receptor compartment contained PBS (pH 7.4) maintained at 37 ± 0.5 °C and stirred at 100 rpm. Samples were withdrawn at predetermined intervals up to 8 hours and analyzed spectrophotometrically at 370 nm [21].

Optimization and Validation of Model

Experimental data were fitted to a quadratic polynomial model using Design-Expert® software. The significance of the model and individual terms was assessed through Analysis of Variance (ANOVA). 3D surface response plots were generated to visualize the effects of variables on responses. Optimized formulation was selected based on the highest desirability value (0.924), targeting maximum tensile strength, optimum folding endurance, and sustained drug release [22].

HPLC Analysis

The quantitative estimation of Quercetin in the standard solution and the optimized polymeric film formulation was performed using a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method. The analysis was carried out on a C18 column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of methanol and water (70:30 v/v), adjusted to pH 3.5 with orthophosphoric acid. The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 µL. The detection was carried out at λmax 370 nm using a UV detector, and the total run time was set to 10 minutes.Standard Quercetin solution was prepared by dissolving accurately weighed Quercetin in methanol to obtain a concentration of 10 µg/mL. The extract of the optimized polymeric film was prepared by dissolving a film equivalent to 10 mg of drug in 10 mL of methanol, followed by sonication for 10 minutes and filtration through a 0.45 µm membrane filter before injection. All analyses were performed at ambient temperature, and each sample was injected in triplicate to ensure reproducibility [17].

Antimicrobial Activity

The antimicrobial activity of the optimized Quercetin-loaded polymeric film (F_6 batch) was determined using the agar well diffusion method against Staphylococcus aureus. Nutrient agar medium was sterilized and poured into Petri plates, followed by inoculation with a bacterial suspension equivalent to 0.5 McFarland standard (≈1 × 10^8 CFU/mL). After solidification, five wells of uniform diameter were aseptically bored into each plate.Cavity 1 was filled with ciprofloxacin (10 µg/mL) as the positive control, while Cavities 2–5 received the film extract at concentrations of 50, 100, 150, and 200 µg/mL, respectively. The plates were incubated at 37 °C for 24 hours, and the zone of inhibition (ZOI) around each well was measured in millimeters.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical evaluation was carried out using Design-Expert® software (v13). Significance was considered at $p< 0.05$.

RESULTS AND DISCUSSION

Experimental Design and Model Fitting

The Box–Behnken design was employed to investigate the influence of three independent variables polymer ratio (A; Chitosan:PVA), glycerol concentration (B), and quercetin concentration (C) on three critical quality attributes: tensile strength (Response 1), number of folds (Response 2), and % drug release after 8 h (Response 3). The observed responses for all 15 runs are summarized in Table 2, and ANOVA results for each response are presented in Table 3. The high F-values and very low p-values (< 0.05) confirmed the statistical significance of the models, indicating strong correlation between experimental and predicted responses. The non-significant lack-of-fit values (> 0.05) suggest that all models adequately fit the data, demonstrating good predictability of the design.

Table 2. Experimental design matrix with measured responses

Run	Tensile Strength (N/mm²)	Number of Folds	% Drug Release (8 h)
1	10.5	130	82.65
2	12.6	125	79.50
3	9.8	170	77.12
4	10.2	150	80.36
5	12.5	145	76.50
6	9.8	148	79.24
7	10.3	151	78.50
8	7.2	165	83.67
9	12.2	160	78.09
10	7.5	110	81.50
11	7.8	140	84.18
12	10.6	168	85.27
13	12.4	148	80.50
14	6.9	135	78.17
15	9.5	128	76.33

Table 3. ANOVA summary of model significance

Response	Model Type	F-value	p-value	Significant Factors	Lack of Fit
Tensile Strength	Linear	225.94	3.67×10^{-10}	A, C	Not significant
Number of Folds	Quadratic	68.48	1.06×10^{-4}	A, B, AB, A^2	Not significant
% Drug Release (8 h)	Linear	25.11	3.20×10^{-5}	A, C	Not significant

Effect of Formulation Variables

Tensile Strength

The tensile strength was primarily influenced by the polymer ratio (A) and quercetin concentration (C). As shown in Figure 1, increasing the Chitosan proportion enhanced the tensile strength due to stronger intermolecular interactions and hydrogen bonding, which imparted rigidity to the film matrix. In contrast, higher quercetin concentration slightly reduced tensile strength beyond an optimum level, possibly due to drug–polymer interference reducing crosslink density. The model predicted tensile strength in the range of 6.9–12.6 N/mm².

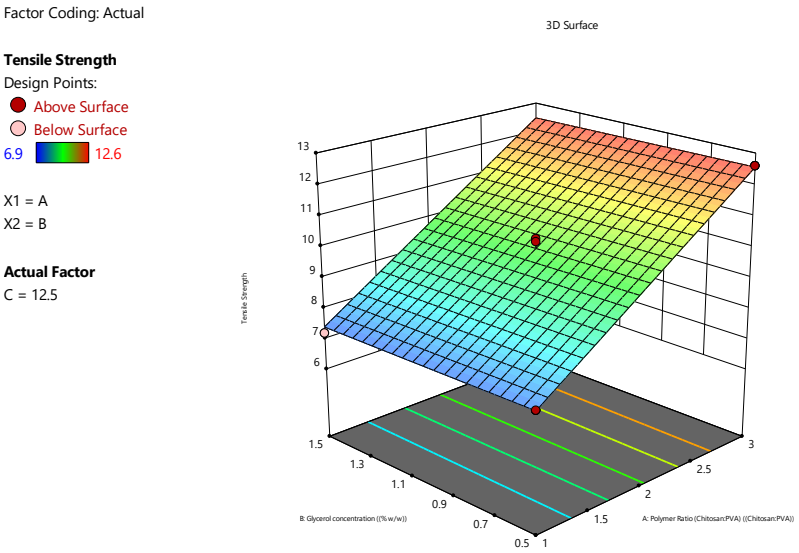

Figure 1. Response surface plot showing the effect of polymer ratio and quercetin concentration on tensile strength.

Folding Endurance

Folding endurance was used as a measure of flexibility. The quadratic model (Figure 2) revealed that glycerol concentration (B) and its interaction with the polymer ratio (AB) were the most significant factors. Increasing the plasticizer content enhanced polymer chain mobility, resulting in improved flexibility and a higher number of folds (up to 170). However, excessive plasticizer beyond the optimal concentration led to film softening and reduced mechanical integrity.

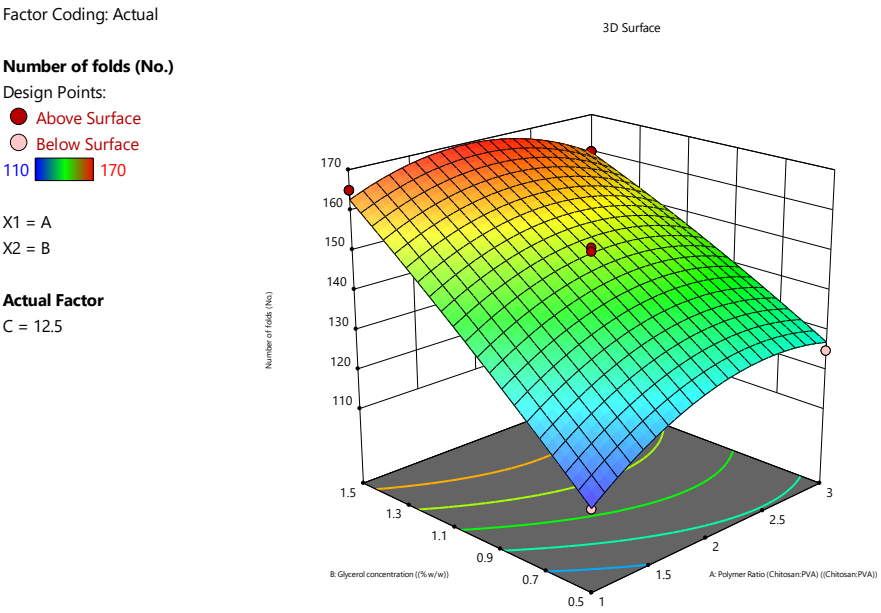

Figure 2. Response surface plot showing the combined effect of polymer ratio and glycerol concentration on folding endurance.

% Drug Release

As depicted in Figure 3, % drug release after 8 h was significantly affected by the polymer ratio (A) and quercetin concentration (C). Formulations with lower Chitosan content and higher drug loading exhibited a faster release rate due to decreased matrix density and enhanced diffusion. The release values ranged between 76.33 % and 85.27 %, indicating controlled and sustained release characteristics.

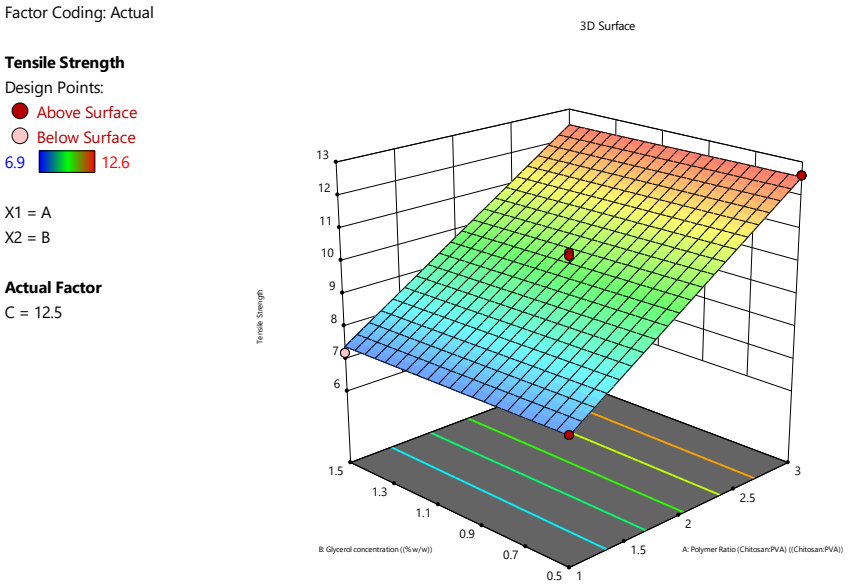

Figure 3. Response surface plot illustrating the effect of polymer ratio and quercetin concentration on % Drug Release.

Optimization and Validation of the Formulation

Numerical optimization was conducted using the **Design Expert® 13 software**, targeting **maximum tensile strength, maximum folding endurance**, and **controlled % drug release (around 80%)**. The software-generated desirability function provided the best combination of formulation variables that simultaneously satisfied all response criteria.The high desirability value (>0.90) confirmed that the model effectively balanced mechanical strength and sustained drug release behaviour. Thus, this formulation was selected for experimental validation.

Preparation and Experimental Validation of Optimized Batch

To validate the model predictions, an **extra batch** of the optimized quercetin-loaded polymeric film ($\mathbf{F_{opt}}$) was prepared using the same process parameters described earlier (Section 2.5). The composition was adjusted according to the optimized levels: **Chitosan:PVA = 3:1.5, glycerol = 1.5%**, and **quercetin = 14.69 mg/mL**.The experimentally observed results for this validation batch were compared with the predicted values, as shown in **Table 4**.The experimental results were found to be in close agreement with the predicted values, exhibiting less than **2% deviation** for all responses. This confirmed the **predictive capability and reliability** of the Box–Behnken design model in optimizing the formulation parameters.

Table 4. Comparison between predicted and experimental values of optimized batch

Response	Predicted	Desirability	Experimental (Mean ± SD, n = 3)	% Error
Tensile Strength (N/mm²)	12.202	**0.923**	12.35 ± 0.18	1.21 %
Number of Folds	160.70		158 ± 2.3	1.68 %
% Drug Release (8 h)	76.33		77.02 ± 0.92	0.90 %

HPLC Analysis

The HPLC chromatogram of the standard Quercetin solution showed a sharp and symmetrical peak at a retention time of 4.52 min, confirming its purity and stability. The chromatogram of the optimized polymeric film extract exhibited a corresponding peak at 5.02 min, as shown in Figure 4 and 5, indicating the presence of Quercetin in the formulation. The slight shift in retention time may be attributed to the influence of polymeric excipients or minor variations in the mobile phase composition. No additional or interfering peaks were observed in the chromatogram, confirming the absence of

degradation products or impurities. These findings demonstrate that Quercetin remained chemically stable and compatible within the developed film matrix.

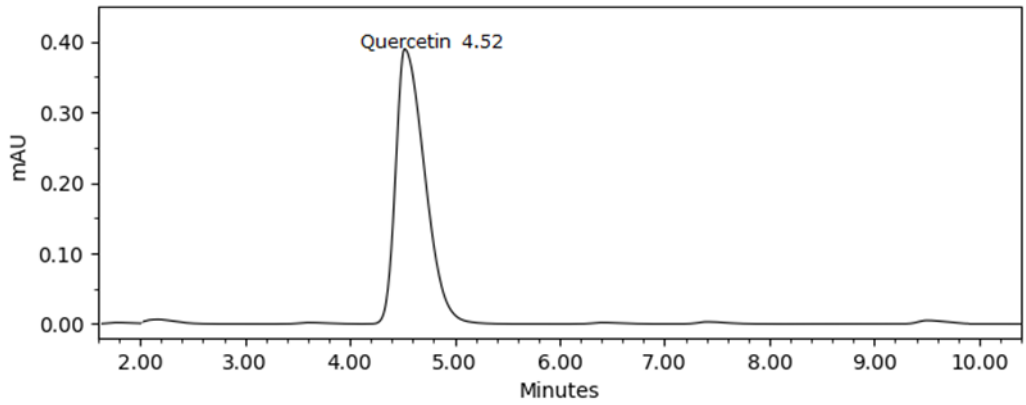

Figure 4.*HPLC chromatogram of standard Quercetin*

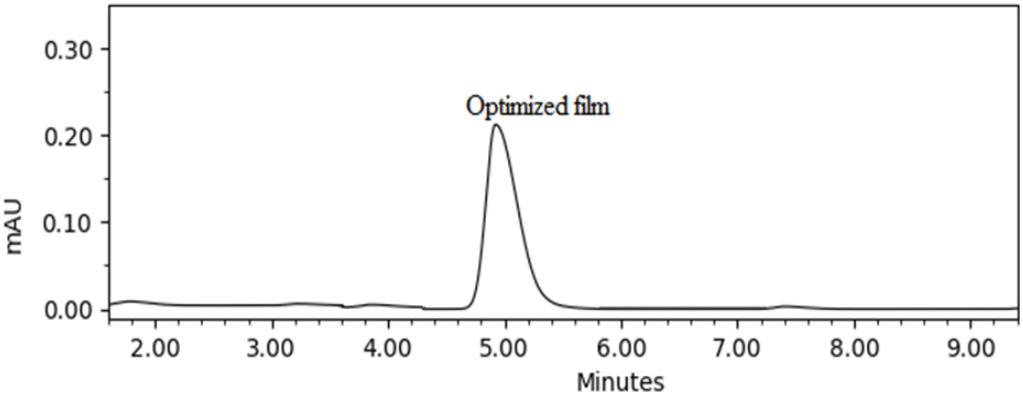

Figure 5.*HPLC chromatogram of optimized polymeric film extract*

Antimicrobial Activity

The antimicrobial activity of the optimized Quercetin-loaded polymeric film was evaluated against *Staphylococcus aureus* using the agar well diffusion method. A clear concentration-dependent increase in the zone of inhibition (ZOI) was observed, confirming the formulation's antibacterial potential. The 200 µg/mL sample exhibited the highest inhibitory effect, comparable to the standard ciprofloxacin (10 µg/mL). This enhanced activity may be attributed to the synergistic effect of Quercetin and the chitosan–PVA matrix, which promotes sustained drug diffusion and microbial membrane interaction (**Figure 6**).

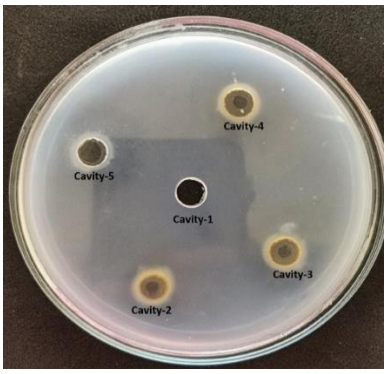

Figure 6.* Antimicrobial Activity of optimized*Quercetin-loaded film

CONCLUSION

The Quercetin-loaded chitosan–PVA polymeric film developed through Box–Behnken optimization demonstrated an optimal balance of mechanical strength, flexibility, and sustained drug release. The close agreement between experimental and predicted responses validated the robustness of the statistical design model. HPLC analysis confirmed the chemical integrity and uniform distribution of Quercetin within the

optimized matrix, while antimicrobial evaluation established significant, dose-dependent inhibition of *Staphylococcus aureus*. Overall, the study successfully established a reproducible and efficient design framework for developing polymeric film-based delivery systems of poorly soluble phytoconstituents like Quercetin, suggesting strong potential for further development in transdermal and wound-healing applications.

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Conflict of Interest
No conflict of interest.

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NIL

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