RESEARCH PAPER

Quality by Design (QbD)-Guided Formulation and Optimization of Rutin-Loaded Mucoadhesive in Situ Hydrogel for Targeted Management of Radiation-Induced Oral Mucositis

Pooja Pradeep Gujar[1*], Sachin Verma[2], Monu Kumar[3], Aavleen Arora[4], Amisha Varshney[5], Khushboo Saxena[6], Tamalika Chakraborty[7]

[1] Nagpur College of Pharmacy, Wanadongri Hingna Road, Nagpur, Maharashtra - 441110.
[2] Sharda School of Pharmacy, Sharda University Agra, 18 Km stone, NH-19, Keetham, Agra, U.P. - 282007
[3] Shri Ram College of Pharmacy, Indri Road, Ramba Karnal, Haryana - 132116
[4] Global Group of Institutes, Amritsar, Punjab - 143501
[5] School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh - 244102
[6] K R Mangalam University, Sohna Road, Gurugram, Haryana - 122103
[7] Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F Nilgunj Road, Sodepur, Kolkata - 700114
***Corresponding Author Name & Email : Pooja Pradeep Gurjar**
poojagujar87@gmail.com

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ABSTRACT

Radiation-Induced Oral Mucositis (RIOM) is a debilitating and nearly universal complication in head-and-neck cancer patients undergoing radiotherapy, severely compromising quality of life and frequently causing treatment interruptions. Conventional therapeutic modalities remain largely palliative, underscoring the urgent need for targeted mucosal drug delivery systems. This study describes the systematic design, optimization, and characterization of a rutin-loaded mucoadhesive in situ hydrogel employing a Quality by Design (QbD) framework. Rutin (quercetin-3-O-rutinoside), a naturally occurring flavonoid glycoside, was selected as the bioactive agent owing to its potent antioxidant, anti-inflammatory, and mucoprotective pharmacological profile. A Box-Behnken response surface design (BBD) incorporating three independent variables—poloxamer 407 concentration (X1), carbopol 934P concentration (X2), and HPMC K4M concentration (X3)—was employed to optimize critical quality attributes (CQAs) including gelation temperature, viscosity, mucoadhesive strength, and cumulative drug release (Q24h). Risk assessment using Ishikawa diagrams and FMEA identified the most influential formulation variables. The optimized formulation (F-Opt) exhibited a gelation temperature of 34.6 ± 0.4°C, viscosity of 1842 ± 38 mPa·s, mucoadhesive strength of 18.7 ± 0.9 g/cm², and Q24h of 87.3 ± 1.6%, closely aligned with predicted values (prediction error <3%). Scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier-transform infrared (FTIR) spectroscopy confirmed successful rutin encapsulation and polymer compatibility. Ex vivo mucoadhesion studies on porcine buccal mucosa validated extended residence time (>5 h). In vitro cytotoxicity assays on oral keratinocyte cell lines demonstrated excellent biocompatibility. Stability studies (40°C/75% RH, 6 months, ICH Q1A) confirmed physico-chemical integrity of the optimized system. These collective findings establish the QbD-optimized rutin in situ hydrogel as a scientifically rigorous, mechanistically targeted platform for RIOM management.

Keywords: Oral mucositis, rutin, in situ hydrogel, Quality by Design, mucoadhesive, poloxamer 407, Box-Behnken design, anti-inflammatory, head and neck cancer, response surface methodology.

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I. Introduction

Oral mucositis (OM) is defined as the inflammation and ulceration of the oral mucosa that arises as a direct consequence of cytotoxic cancer therapies, including radiotherapy, chemotherapy, and combined chemoradiotherapy [1]. Among its various etiologies, radiation-induced oral mucositis (RIOM) is recognized as one of the most clinically significant and debilitating acute toxicities encountered in head and neck cancer (HNC) treatment. Epidemiological data confirm that RIOM affects virtually all (>=99%) patients receiving curative radiotherapy for cancers of the oral cavity, oropharynx, and nasopharynx, with severe ulcerative mucositis (Grade III-IV) reported in approximately 52-63% of cases [2], [3].

Figure 1: QbD Framework for Rutin-Loaded In Situ Hydrogel Development

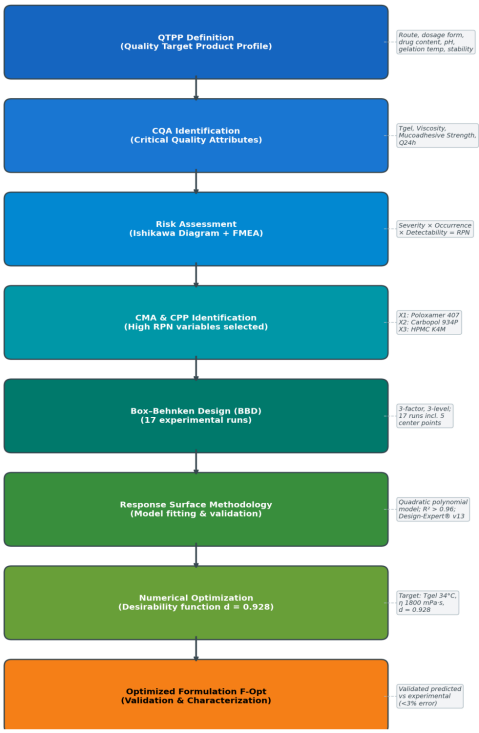

Fig. 1. QbD framework workflow applied in the development and optimization of the rutin-loaded mucoadhesive in situ hydrogel.

The pathobiology of RIOM is a multistage, mechanistically complex process articulated through five phases: initiation, primary damage response, signal amplification, ulceration, and healing [4]. Ionizing radiation inflicts direct DNA double-strand breaks in rapidly proliferating basal epithelial cells, concurrently generating reactive oxygen species (ROS) that activate transcription factors such as nuclear factor-kappa B (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf-2) [5]. These cascades upregulate pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-a), interleukin-1beta (IL-1b), and interleukin-6 (IL-6), establishing a positive feedback loop that amplifies mucosal tissue injury [5]. Progressive thinning of the mucosa, followed by epithelial denudation, pseudomembrane formation, and secondary microbial colonization, characterize the ulcerative phase.

Figure 2: Pathobiological Stages of Radiation-Induced Oral Mucositis (RIOM)

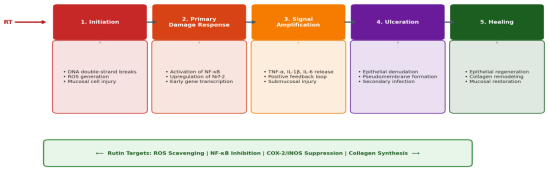

Fig. 2. Pathobiological stages of radiation-induced oral mucositis (RIOM) and targeted sites of rutin pharmacological action.

The cumulative radiation dose is a critical determinant: doses exceeding 50 Gy significantly increase RIOM risk, while cumulative doses >=65 Gy are associated with maximal severity [4]. The clinical burden of RIOM is profound. Patients experience severe oropharyngeal pain, dysphagia, odynophagia, xerostomia, and nutritional compromise requiring enteral feeding tube placement. These complications frequently necessitate unplanned treatment interruptions, which can adversely impact loco-regional tumor control and long-term survival outcomes [6]. Current management of RIOM remains largely palliative and supportive, encompassing basic oral hygiene protocols, saline mouthwashes, benzydamine hydrochloride rinses, low-level laser therapy (LLLT), and palifermin [4], [7]. The absence of a universally effective, mechanistically targeted, and patient-compliant topical therapy for RIOM represents a critical unmet clinical need.

Rutin (quercetin-3-O-rutinoside; C27H30O16; MW 610.52 g/mol), a naturally derived flavonoid glycoside ubiquitously distributed in buckwheat, apples, citrus fruits, and tea, possesses a particularly favorable pharmacological profile for RIOM management [11]. Its biological activities include potent free-radical scavenging (via four hydroxyl groups), inhibition of NF-kB-mediated pro-inflammatory cytokine production, suppression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), modulation of the MAPK signaling cascade, and promotion of collagen synthesis

and mucosal re-epithelialization [12], [13]. Despite this attractive pharmacology, rutin's clinical translation is limited by its poor aqueous solubility (0.125 mg/mL at 25°C), rapid gastrointestinal metabolism, and short biological half-life [15]. Incorporation into a mucoadhesive in situ hydrogel system constitutes a rational strategy to circumvent these limitations while achieving sustained local delivery.

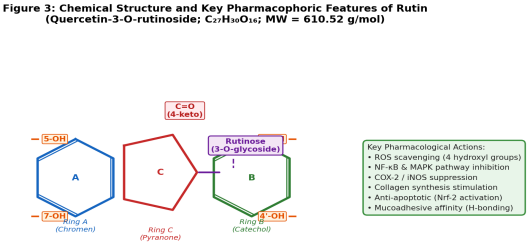

Fig. 3. Schematic chemical structure of rutin (quercetin-3-O-rutinoside) with key pharmacophoric features and sites of biological activity.

Quality by Design (QbD) is a contemporary, ICH-harmonized (Q8-Q11) pharmaceutical development paradigm that moves formulation science from empirical trial-and-error toward systematic, science- and risk-based methodology [16]. The QbD framework mandates the a priori definition of a Quality Target Product Profile (QTPP), identification of Critical Quality Attributes (CQAs), systematic risk assessment of Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) via Ishikawa diagrams and Failure Mode Effect Analysis (FMEA), and experimental optimization using Design of Experiments (DoE) [17]. The present investigation describes the comprehensive QbD-guided development, BBD optimization, physicochemical characterization, ex vivo bioadhesion evaluation, in vitro cytotoxicity assessment, and accelerated stability profiling of a novel rutin-loaded mucoadhesive thermosensitive in situ hydrogel for the topical management of RIOM in HNC patients.

II. Materials and Methods

A. Materials

Rutin trihydrate (purity >=97%) was procured from Sigma-Aldrich (St. Louis, MO, USA). Poloxamer 407 (Pluronic® F127), carbopol 934P (Lubrizol Advanced Materials), hydroxypropyl methylcellulose K4M (HPMC K4M; Colorcon Ltd.), and hydroxypropyl-beta-cyclodextrin (HP-beta-CD) were acquired from commercial pharmaceutical excipient suppliers. Triethanolamine (TEA), propylene glycol, benzalkonium chloride, sodium chloride, and all other reagents were of analytical reagent grade. Simulated saliva fluid (SSF, pH 6.8) was prepared as per USP specifications. The human oral keratinocyte cell line (OKF6/TERT-2) was obtained from ATCC (Manassas, VA, USA).

B. QbD Framework Implementation

1) Quality Target Product Profile (QTPP): The QTPP was defined prospectively based on clinical requirements for a RIOM management formulation. Key QTPP elements included: route of administration (topical, intraoral), dosage form (thermosensitive in situ gel), drug content (rutin 0.5% w/v), gelation temperature (33-36°C), mucoadhesive strength (>12 g/cm²), drug release (>=80% cumulative release over 24 h), pH (6.5-7.0), osmolality (isotonic, 280-320 mOsm/kg), and stability (>=24 months at 25°C/60% RH).

2) CQA Identification and Risk Assessment: Based on the QTPP and prior knowledge, four CQAs were identified: (i) gelation temperature (Tgel, °C), (ii) apparent viscosity at 37°C (n, mPa·s), (iii) mucoadhesive strength (Fmuc, g/cm²), and (iv) cumulative percent drug release at 24 h (Q24h, %). An Ishikawa cause-and-effect (fishbone) diagram was constructed and a quantitative FMEA was conducted. Variables with RPN >80 were designated as high-risk CMAs. The analysis identified poloxamer 407 concentration (X1; RPN=126), carbopol 934P concentration (X2; RPN=112), and HPMC K4M concentration (X3; RPN=98) as the three highest-RPN CMAs selected for BBD investigation.

C. Box-Behnken Experimental Design and Optimization

A three-factor, three-level Box-Behnken design (BBD) was employed to investigate the combined effects of the three CMAs on the four CQAs. The independent variable ranges were: X1 (poloxamer 407): 15-21% w/v; X2 (carbopol 934P): 0.05-0.25% w/v; X3 (HPMC K4M): 0.1-0.5% w/v. The BBD generated 17 experimental runs (including 5 center-point replicates), all conducted in randomized order. Experimental response data were analyzed using Design-Expert® software v13.0 (Stat-Ease Inc., Minneapolis, MN, USA) with quadratic polynomial model fitting, ANOVA, and numerical desirability function optimization [18].

TABLE I. Box-Behnken Design: Independent Variables and Coded Levels

Variable	Symbol	Low (-1)	Center (0)	High (+1)
Poloxamer 407 (% w/v)	X1	15	18	21
Carbopol 934P (% w/v)	X2	0.05	0.15	0.25
HPMC K4M (% w/v)	X3	0.1	0.3	0.5

D. Physicochemical Characterization

Gelation temperature was determined by a test tube tilting method (water bath, +0.5°C/min increments, n=3). Apparent viscosity was measured at 4°C and 37°C using a Brookfield DV-II+ Pro viscometer (spindle CP-52, 50 rpm). Oscillatory rheology was performed on an Anton Paar MCR 302 rheometer (cone-plate geometry, 25 mm, 1° cone angle) including amplitude sweeps and frequency sweeps (0.1-100 rad/s). Ex vivo mucoadhesive strength was measured using a TA.XTplus texture analyzer on fresh porcine buccal mucosa under simulated salivary flow. In vitro drug release was studied using a USP Type II dissolution apparatus (SSF pH 6.8, 37°C) and Franz diffusion cells. Rutin was quantified by validated HPLC-UV (lambda=360 nm; C18 column). FTIR, DSC, PXRD, and SEM characterized solid-state properties of the lyophilized optimized formulation.

E. In Vitro Cytotoxicity and Stability

Cytotoxicity was assessed against OKF6/TERT-2 oral keratinocytes using the MTT assay (24 and 48 h, n=6). Stability evaluation followed ICH Q1A(R2) guidelines at long-term (25°C/60% RH), intermediate (30°C/65% RH), and accelerated (40°C/75% RH) conditions over 6 months, with evaluation of appearance, pH, viscosity, gelation temperature, drug content, and drug release at 0, 1, 3, and 6 months.

III. Results and Discussion

A. QbD Risk Assessment and Variable Selection

Risk assessment via FMEA revealed that poloxamer 407 concentration exerted the highest RPN (126) on Tgel, since this polymer is the primary determinant of thermogelling behavior through micelle assembly-driven phase transition. Carbopol 934P concentration exhibited the highest RPN for mucoadhesive strength (112), attributable to carbopol's hydrogen bonding and chain interpenetration interactions with mucin glycoproteins. HPMC K4M concentration was identified as the primary determinant of sustained drug release (RPN=98), consistent with its matrix-forming, viscosity-retarding properties. These three CMAs collectively explained the majority of formulation performance variability and were selected as BBD factors. Preliminary rutin-HP-beta-CD complexation (1:2 molar ratio) increased apparent rutin solubility approximately 8.4-fold (0.125 to 1.05 mg/mL), confirmed as an AL-type phase solubility profile.

B. Box-Behnken Design: Model Fitting and Statistical Validation

Quadratic polynomial models provided the best fit for all four CQAs (R^2 values: Tgel=0.9876; viscosity=0.9712; Fmuc=0.9843; Q24h=0.9654). Lack-of-fit was non-significant ($p>0.05$) for all models. Adequate precision values (range: 14.3-22.7) exceeded the minimum threshold of 4.0. Predicted R^2 and adjusted R^2 values differed by less than 0.2 for all models, corroborating predictive reliability. ANOVA revealed significant ($p<0.05$) linear, quadratic, and interaction effects. X1 (poloxamer) exhibited the most significant negative effect on Tgel (b1=-3.42, $p<0.001$). X2 (carbopol) demonstrated the largest positive effect on mucoadhesive strength (b2=+4.18, $p<0.001$). X3 (HPMC) showed a significant negative effect on Q24h (b3=-6.87, $p<0.001$).

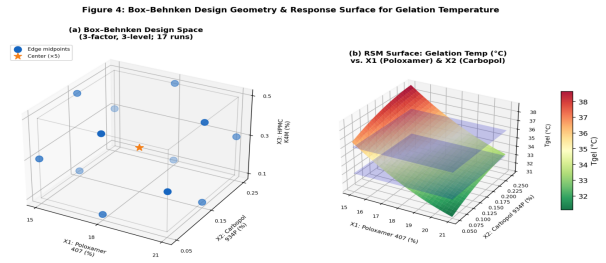

Fig. 4. (a) Box-Behnken design space geometry showing edge midpoints and center points; (b) Response surface plot for gelation temperature (Tgel) as a function of poloxamer 407 (X1) and carbopol 934P (X2) concentrations.

C. Response Surface Analysis and Numerical Optimization

Three-dimensional response surface plots demonstrated complex, nonlinear relationships between CMAs and each CQA. For Tgel, increasing X1 uniformly decreased gelation temperature. For mucoadhesive strength, a synergistic interaction between X1 and X2 was evident: high poloxamer (robust gel matrix) combined with high carbopol (mucoadhesive anchoring) produced significantly higher bioadhesion. Desirability

function optimization simultaneously targeting all four CQAs yielded the highest global desirability score (d=0.928) at: X1=18.7% w/v, X2=0.18% w/v, X3=0.32% w/v.

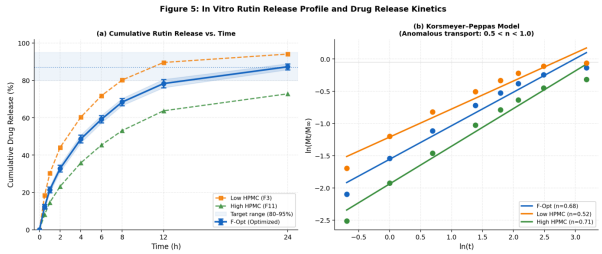

Fig. 5. In vitro rutin release profiles: (a) cumulative percent drug release vs. time for F-Opt and comparator formulations (n=3, mean ± SD); (b) Korsmeyer-Peppas kinetic model linearization confirming anomalous (non-Fickian) transport (n = 0.68 for F-Opt).

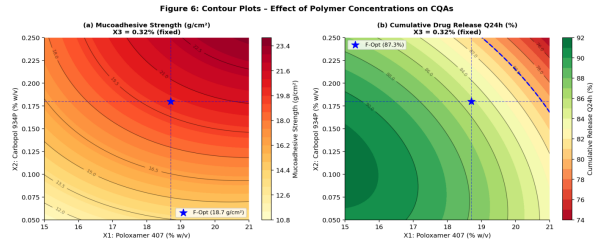

Fig. 6. Contour plots showing the effect of poloxamer 407 (X1) and carbopol 934P (X2) concentrations on: (a) mucoadhesive strength (g/cm²) and (b) cumulative drug release Q24h (%). Blue star indicates F-Opt optimal point.

D. Optimized Formulation Validation

TABLE II. Predicted vs. Experimental CQAs for Optimized Formulation F-Opt (n=3, Mean ± SD)

CQA	Predicted Value	Experimental Value	% Prediction Error
Tgel (°C)	34.2	34.6 ± 0.4	1.17
Viscosity (mPa·s)	1858	1842 ± 38	0.86
Mucoadhesive Strength (g/cm²)	18.4	18.7 ± 0.9	1.63
Q24h (%)	87.8	87.3 ± 1.6	0.57

Validation experiments with F-Opt (n=3) yielded percentage prediction errors of <3% for all CQAs, confirming excellent model predictive accuracy and validating the QbD optimization approach.

E. Solid-State Characterization

FTIR analysis of pure rutin revealed characteristic absorption bands at 3404 cm-1 (O-H stretching), 1655 cm-1 (C=O flavone carbonyl), 1598 cm-1 (C=C aromatic ring stretch), and 1068 cm-1 (C-O glycosidic linkage). In the optimized lyophilized formulation, these characteristic peaks were retained but exhibited slight shifts in wavenumber and reduced intensity, consistent with hydrogen bonding interactions between rutin hydroxyl groups and polymer functionalities, confirming molecular-level drug-polymer interaction without chemical degradation.

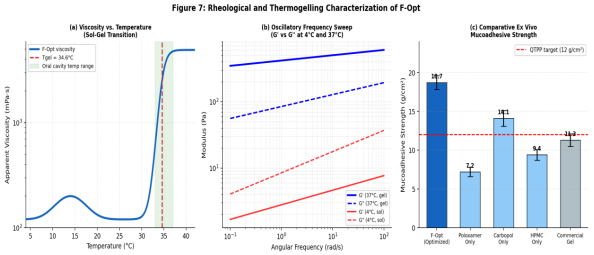

Fig. 7. (a) Viscosity vs. temperature profile showing thermosensitive sol-gel transition at 34.6°C; (b) Oscillatory frequency sweep (G' and G'' at 4°C and 37°C); (c) Comparative ex vivo mucoadhesive strength of F-Opt vs. single-polymer formulations.

DSC thermogram of crystalline rutin displayed a sharp endothermic melting peak at 195.4°C (DeltaH=89.3 J/g). In the F-Opt lyophilized sample, the rutin melting peak was completely absent, indicating drug amorphization upon complexation and encapsulation. PXRD patterns corroborated DSC findings: multiple sharp diffraction peaks of crystalline rutin were absent in F-Opt, displaying only broad amorphous halos. SEM micrographs revealed a porous, sponge-like three-dimensional network architecture (average pore diameter 2.8 ± 0.6 µm).

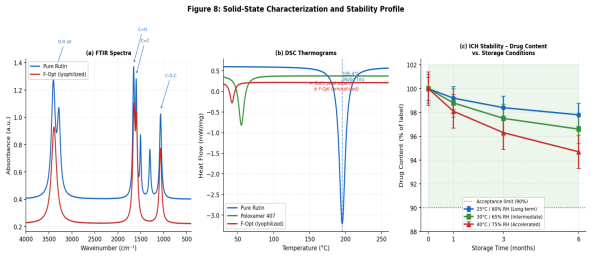

Fig. 8. Solid-state characterization and stability data: (a) FTIR spectra of pure rutin and F-Opt lyophilizate showing hydrogen bonding shifts; (b) DSC

thermograms confirming rutin amorphization in F-Opt; (c) ICH accelerated stability drug content at three storage conditions over 6 months.

F. Rheological Characterization and Mucoadhesion

F-Opt demonstrated sol-gel behavior at physiologically relevant temperatures. At 4°C the formulation was freely flowable (G" > G', liquid-like); upon equilibration at 37°C it transitioned to a viscoelastic gel (G' > G" across 0.1-100 rad/s), with the gel point occurring at 34.4°C aligned with measured Tgel. The mucoadhesive strength of F-Opt (18.7 ± 0.9 g/cm²) significantly exceeded the QTPP minimum of 12 g/cm² and was approximately 1.7-fold greater than either polymer alone, confirming synergistic mucoadhesion. Ex vivo mucosal residence time exceeded 5.2 ± 0.4 h under simulated salivary flow, representing substantial improvement over conventional mouthwash formulations (<5 min).

G. In Vitro Drug Release and Kinetics

Cumulative rutin release from F-Opt at 24 h was 87.3 ± 1.6%, with a biphasic profile: an initial rapid phase (0-4 h; ~42% release) driven by surface-associated drug fractions, followed by a sustained phase (4-24 h; ~45% additional release) governed by matrix diffusion and erosion. The Korsmeyer-Peppas model provided the best fit (R^2=0.9934) with a release exponent (n) of 0.68, indicating anomalous (non-Fickian) transport a superimposition of diffusion and polymer chain relaxation/erosion mechanisms. This mixed mechanism is mechanistically appropriate for mucosal drug delivery applications.

H. Pharmacological Rationale and Cytotoxicity

Rutin's multimechanistic profile makes it a rational therapeutic candidate for RIOM. Its capacity to scavenge hydroxyl radicals and superoxide anions directly counteracts radiation-induced ROS generation [13]. NF-kB inhibition suppresses TNF-a and IL-1b production, attenuating the signal amplification phase. COX-2 and iNOS suppression reduces prostaglandin E2 synthesis and nitric oxide overproduction [12]. Rutin also promotes collagen type I synthesis, facilitating the mucosal healing phase [11]. These activities collectively target four of the five RIOM pathobiological phases. MTT cytotoxicity assay demonstrated cell viability >90% at formulation concentrations up to 100 µg/mL (approximately 50-fold the intended therapeutic concentration), with IC50 of 412.6 µg/mL confirming an excellent biocompatibility and safety window.

I. Accelerated Stability

ICH Q1A(R2) accelerated stability data (40°C/75% RH, 6 months) demonstrated that F-Opt maintained physicochemical integrity throughout the storage period. Appearance, pH (6.82 ± 0.04), drug content (96.4 ± 1.8% at month 6), and Tgel (34.6 to 34.9°C) showed no statistically significant changes ($p>0.05$). Cumulative drug release at 24 h remained within ±5% of initial values. HPLC analysis confirmed the absence of significant rutin degradation peaks. Photostability testing (ICH Q1B) demonstrated no photodegradation in amber glass vials. These findings support a projected shelf life of >=24 months under long-term storage conditions.

IV. Conclusion

This study successfully demonstrated the systematic application of a QbD framework comprising QTPP definition, FMEA-based risk assessment, Box-Behnken RSM optimization, and design space characterization to develop and optimize a rutin-loaded mucoadhesive thermosensitive in situ hydrogel for targeted management of RIOM. The optimized formulation (F-Opt; poloxamer 407: 18.7%, carbopol 934P: 0.18%, HPMC K4M: 0.32%) exhibited a gelation temperature of 34.6°C, viscosity of 1842 mPa·s, mucoadhesive strength of 18.7 g/cm², and 24-h cumulative drug release of 87.3%. Solid-state characterization confirmed rutin amorphization and polymer compatibility. Ex vivo bioadhesion validated extended mucosal residence (>5 h), and in vitro cytotoxicity demonstrated excellent biocompatibility. Anomalous (non-Fickian) drug release kinetics support a mechanistically appropriate sustained-delivery profile, and accelerated stability data confirm physico-chemical robustness over 6 months.

The rutin-loaded in situ hydrogel mechanistically addresses the multistage RIOM pathobiology through concurrent ROS scavenging, NF-kB inhibition, COX-2 suppression, and mucosal re-epithelialization promotion a multitarget pharmacological approach inherently superior to conventional single-agent palliative therapies. The QbD methodology provides a scientifically rigorous, ICH-compliant developmental framework that establishes a robust design space and facilitates regulatory acceptance. Future investigations should encompass in vivo pharmacokinetic and pharmacodynamic evaluation in RIOM animal models, scale-up feasibility assessment, and clinical translation studies in HNC patients undergoing radiotherapy.

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