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Formulation, FTIR Characterization and Anti-microbial Evaluation of Quercetin and Hyaluronic Acid-Infused Mouth Dissolving Film for the Treatment of Oral Ulcer

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sulopenem and Probenecid in bulk and pharmaceutical dosage form. Chromatogram was run through Agilent 250 x 4.6 mm, 5µm. Mobile phase containing 0.01N Ammonium acetate: Acetonitrile taken in the ratio 65:35 % v/v was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 272.0 nm. Retention time of Sulopenem and Probenecid were found to be 2.165 min and 2.918 min. %RSD of standard of the Sulopenem and Probenecid were and found to be 0.5% and 0.6% respectively. %Assay was obtained as 99.70% and 99.63% for Sulopenem and Probenecid respectively. %Recovery was obtained as 99.66% and 99.65% for Sulopenem and Probenecid respectively. LOD, LOQ values obtained from regression equations of Sulopenem and Probenecid were 0.1, 0.31 and 0.07, 0.2 respectively. Regression equation of Probenecid is y = 5534.5x + 4985.3, y = 5484.4x + 282.8 of Sulopenem. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Probenecid, Sulopenem, RP-HPLC, Validation, Development

1. INTRODUCTION

Oral ulcers are painful lesions of the oral mucosa that commonly affect individuals of all age groups worldwide. These lesions, often referred to as aphthous ulcers or canker sores, are characterized by localized inflammation, discomfort, and difficulty in performing routine activities such as eating, speaking, and maintaining oral hygiene. The etiology of oral ulcers is multifactorial and includes immune dysregulation, microbial infections, nutritional deficiencies, stress, hormonal fluctuations, genetic predisposition, and local trauma. Despite being a common condition, recurrent oral ulcers continue to pose a significant clinical challenge due to their frequent recurrence and impact on patient well-being.

Figure.1 Mouth ulcer

Current treatment modalities primarily focus on alleviating symptoms through topical corticosteroids, analgesics, antiseptic mouthwashes, and systemic medications in severe cases. However, these conventional therapies often provide only temporary relief and are associated with limitations such as inadequate drug retention, poor patient compliance, frequent administration, and potential adverse effects. The dynamic environment of the oral cavity, including constant salivary flow and tongue movement, further reduces the effectiveness of traditional dosage forms. Consequently, there is a growing need for advanced drug delivery systems capable of providing prolonged therapeutic action and enhanced healing at the ulcer site.

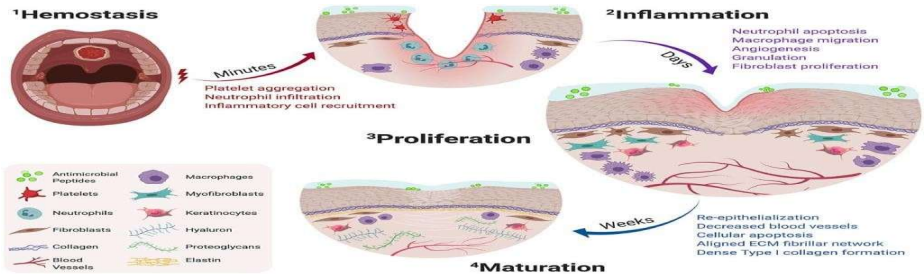

Figure. 2 Pathophysiology of mouth ulcer

Mucoadhesive oral films have gained considerable attention as an innovative approach for the management of oral ulcers. These thin polymeric films adhere to the oral mucosa and facilitate localized, controlled, and sustained delivery of therapeutic agents directly to the affected area. Such systems improve drug residence time, enhance bioavailability, reduce dosing frequency, and provide a protective barrier over the ulcer surface, thereby promoting faster healing and improved patient comfort.

Figure.3 Occurrence of oral ulcer

The present study focuses on the formulation and evaluation of a probiotic oral film infused with quercetin and hyaluronic acid for the treatment of oral ulcers. Quercetin is a naturally occurring flavonoid with potent antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties, while hyaluronic acid contributes to tissue hydration, mucosal regeneration, and pain reduction through its protective and moisturizing effects. The incorporation of probiotics further supports oral health by restoring microbial balance, inhibiting pathogenic microorganisms, and enhancing mucosal immunity. The synergistic action of these bioactive components within a mucoadhesive film offers a promising therapeutic strategy for effective ulcer management, accelerated healing, and improved quality of life for patients suffering from recurrent oral ulcers.

Figure.4 Mouth ulcer films

2. AIM & OBJECTIVES

Aim:
To formulate and evaluate Quercetin and Hyaluronic acid infused probiotic film for the treatment of oral ulcer.

Objective:
To evaluate the physicochemical properties of the prepared oral films, including thickness, folding endurance, surface pH, and moisture content.
To perform the antimicrobial activity of the probiotic film.

PLAN OF WORK

Preformulation study

Calibration curve of Quercetin

Compatibility study using FTIR

Formulation of Quercetin and Hyaluronic acid infused probiotic film.

Evaluation test of oral film

Folding endurance

Drug content uniformity

Drug release kinetic study

Antimicrobial activity

S.no	Name of instruments	Supplier
1	Digital balance	Shimadzu scientific instruments (model: Bl - 220H)
2	FTIR spectrophotometer	Shimadzu, Japan. (Model: FTIR-84005)
3	Dissolution test apparatus	Lab India. (Model: Disso 2000)
4	Magnetic stirrer	Plastocrafts
5	PH meter	Digi sun electronic system

3. MATERIALS AND METHODOLOGY

Materials

Materials used for formulation

Table.1 Materials used for formulation of films

S.NO	Ingredients	Suppliers
1	Quercetin	Aldrich Co
2	Hyaluronic acid	Nalinc pharmaceuticals limited, Mumbai.
3	Glycerol	Aldrich Co
4	Raspberry (flavouring agent)	SDFCL chemical.
5	Ethanol	Aldrich Co
6	*Lactobacillus*(probiotic)	Tata 1MG health care

Table.2 Instrument used for formulating film

Methodology

The methodology involved preformulation studies, formulation development, and evaluation of Quercetin and Hyaluronic acid infused probiotic mouth-dissolving films. Preformulation studies included determination of the melting point of Quercetin using the capillary tube method to confirm its purity and identity. Solubility studies were carried out in various solvents, including distilled water, ethanol, methanol, acetone, and phosphate buffer (pH 7.4), using the shake-flask method. A calibration curve of Quercetin was prepared by measuring the absorbance of standard solutions at 370 nm using a UV–Visible spectrophotometer. Compatibility between Quercetin and the selected excipients, namely Hyaluronic acid and HPMC, was evaluated using Fourier Transform Infrared (FTIR) spectroscopy to identify any possible drug–excipient interactions.

Quercetin and Hyaluronic acid infused probiotic films were prepared by the solvent casting technique. HPMC and PVP were dissolved in purified water, followed by the addition of Hyaluronic acid. Quercetin dissolved in ethanol was incorporated into the polymeric solution, and glycerol was added as a plasticizer. Sodium benzoate and raspberry flavour were included as a preservative and flavouring agent, respectively. After cooling, Lactobacillus probiotic was added with gentle stirring to preserve its viability. The final solution was cast onto a suitable surface, dried under controlled conditions, and cut into uniform 2 × 2 cm films. Eight formulations (F1–F8) were prepared by varying the concentrations of HPMC and PVP while maintaining constant quantities of other ingredients.
The prepared films were evaluated for weight uniformity, thickness, folding endurance, drug content uniformity, surface pH, in-vitro disintegration time, and in-vitro dissolution behaviour. Drug release kinetics were analyzed using mathematical models such as Zero-order, First-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas models to determine the mechanism of release. In addition, thermal stability studies were conducted under controlled storage conditions to assess the physical and chemical stability of the optimized formulations. These evaluations ensured the development of stable, effective, and patient-friendly mouth-dissolving probiotic films for oral drug delivery.

4. RESULTS AND DISCUSSIONS

Calibration curve of Quercetin

The Quercetin calibration curve establishes a linear relationship between absorbance measurements and known concentrations of pure standard dissolved in ethanol or methanol as a suitable solvent. The data confirms the Beer-Lambert Law because the characteristic wavelength measurement at 372nm for pure Quercetin and 415nm for a total flavonoid assay falls within the selected range.

Linear Regression Equation: y = 0.0625x + 0.0066

The high R^2 value which remains close to 1.0 confirms that Quercetin concentration exhibits excellent linearity with its absorbance which is essential for accurate Quercetin quantification in natural extracts and pharmaceutical formulations.

Table. 3 Calibration curve of Quercetin

Concentration	Absorbance(A)
0	0.000
2	0.130

4	0.258
6	0.385
8	0.510

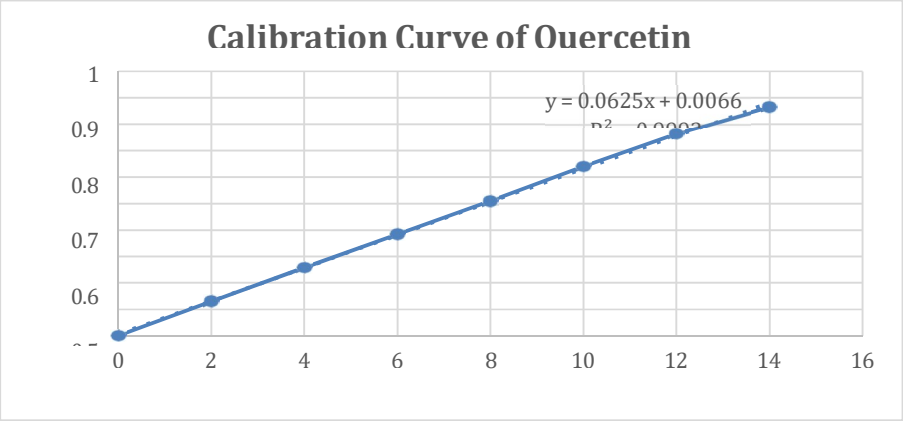

Figure. 5 Calibration curve of Quercetin

Compatibility study using (FTIR)

FTIR analysis was performed to assess the compatibility of Quercetin with Hyaluronic acid, HPMC K4M, and Carbopol. Quercetin exhibited characteristic O–H, C=O, and aromatic C=C peaks. HA, HPMC K4M, and Carbopol showed their respective functional group peaks. The physical mixture displayed all characteristic peaks of the individual components. The absence of significant peak shifts or disappearance confirmed the compatibility of Quercetin with the selected excipients.
confirm compatibility

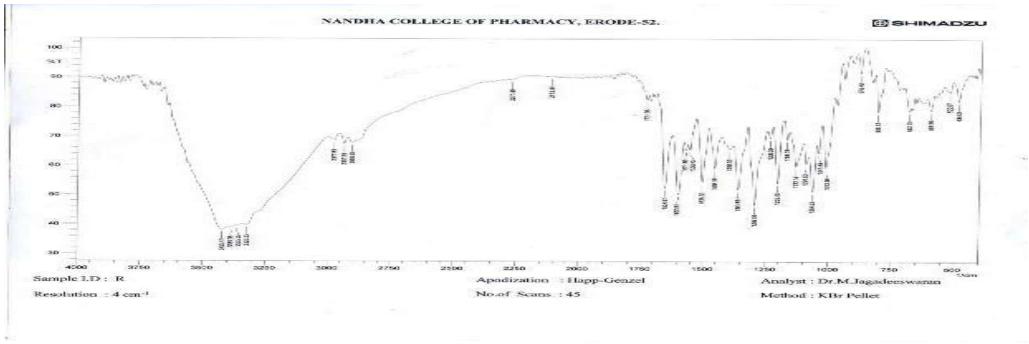

Figure. 6 FTIR of Quercetin

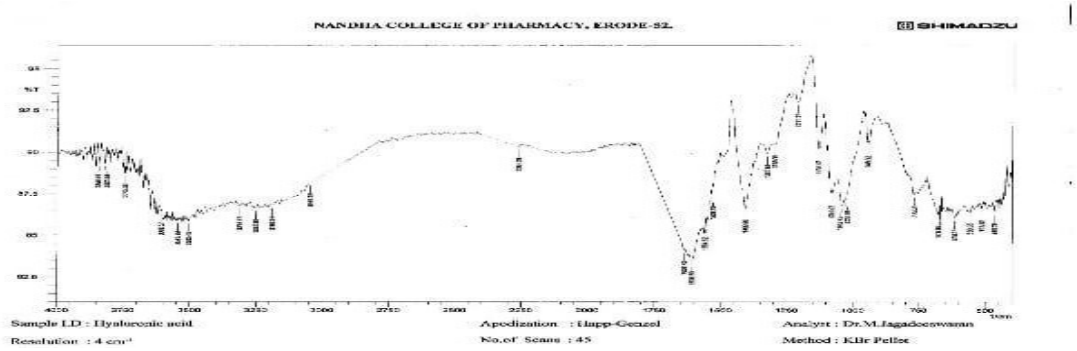

Figure. 7 FTIR of Hyaluronic acid

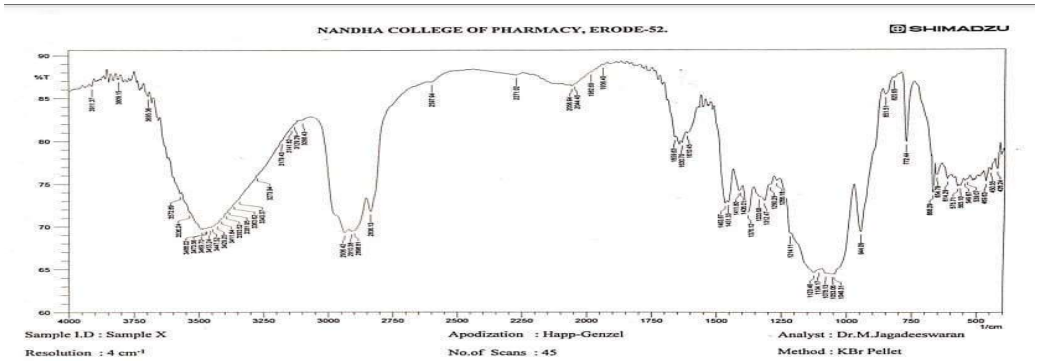

Figure.8 FTIR of HPMC

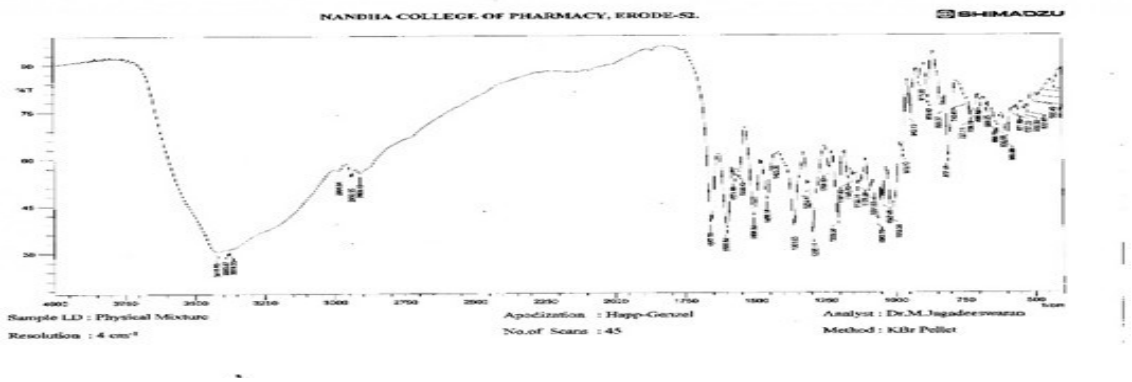

Figure.9 FTIR of physical mixture Table.

FUCTIONAL GROUP	**QUERCETIN**	**HPMC**	**HYALURONIC ACID ACID**	**PHYSICAL MIXTURE**
O-H stretching Phenolic	3400-3200	3350-3410	3200-3400	3300-3400
C=O stretching	1655-1685	-	1600-1650	16500-1680

C=C Aromatic stretching	1500-1600	-	-	1500-1600
C-O-C ether stretching	1200-1270	1150	1040-1100	1100-1250
C-O phenolic stretching	1315-1350	-	-	1300-1350
C-H Aliphatic Stretching	2920-2950	2880-2920	2900-2930	2890-2950
Aromatic C-H Bending	1450-1500	1456	-	1450-1500

Table:4

C-H Bending	1375-1385	1370-1380	-	1370-1385
O-H Bending	1360-1380	1320-1380	1410-1440	1350-1400
C-O carboxyl Stretching	-	-	1400-1450	1400-1450
Amide C=O Stretching	-	-	1600-1650	1600-1650
Amide N-H bending	-	-	1540-1560	1540-1560
Aryl ether C-O-C	1250-1275	1320-1330	-	1250-1330
C=C Plane bending	820-800	-	-	820-800
Pyranose ring	-	-	1020-1080	1020-1080
Amide C=O Stretching	-	-	1600-1650	1600-1650

Table: 5

Antimicrobial activity

The antimicrobial efficacy of the sustained-release formulation was assessed against *Escherichia coli (E. coli)* for its antibacterial properties and *Candida albicans* for its antifungal properties by measuring the zone of inhibition (mm). The findings reveal that all formulations displayed antimicrobial characteristics, with varying levels of inhibition based

on their composition. Formulation F5 exhibited the highest antibacterial (18.8 mm) and antifungal (16.2 mm) activities, indicating its superior efficacy attributed to optimized drug release and possible synergistic effects of the excipients. Formulations F3–F6 presented significant inhibition zones, demonstrating strong antimicrobial potential, while F1, F2, and F8 showed relatively lower activity, likely due to variations in drug diffusion and bioavailability. This affirms that sustained- release formulations can deliver effective antimicrobial action, thereby supporting their application in managing oral ulcers by mitigating bacterial and fungal infections while ensuring extended therapeutic effects.

Table. 5: Zone of inhibition

S. No	**Formulation code**	**Zone of Inhibition (mm)- *E.coli (Antibacterial*)**	**Zone of Inhibition (mm)- *Candida albicans (Antifungal*)**
1	F1	12.5	11.2
2	F2	13.8	12.0
3	F3	15.1	13.2
4	F4	16.5	14.5
5	F5	18.8	16.2
6	F6	17.3	15.1
7	F7	14.9	12.8
8	F8	13.5	11.9

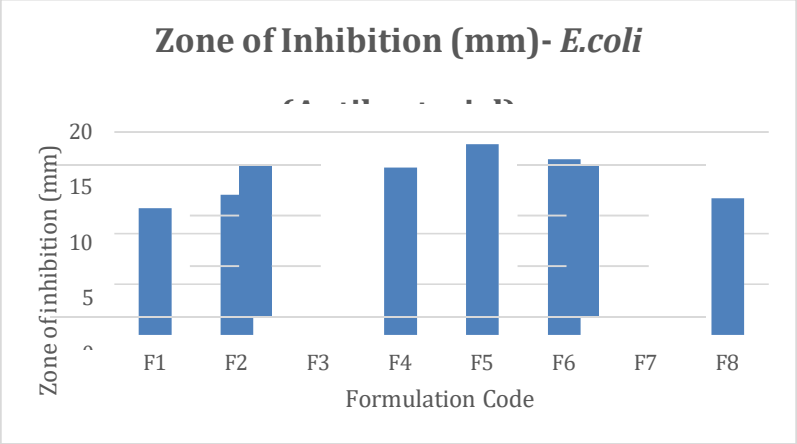

Figure.10: Antibacterial activity

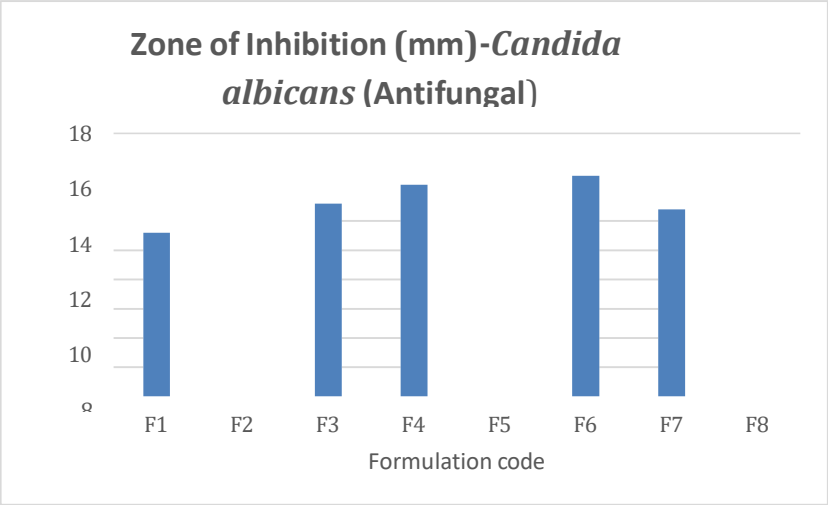

Figure.11: Antibacterial activity

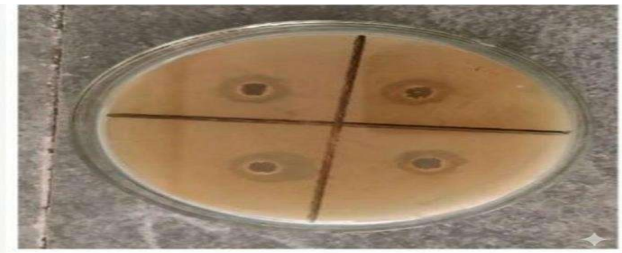

Figure.12 Zone of inhibition

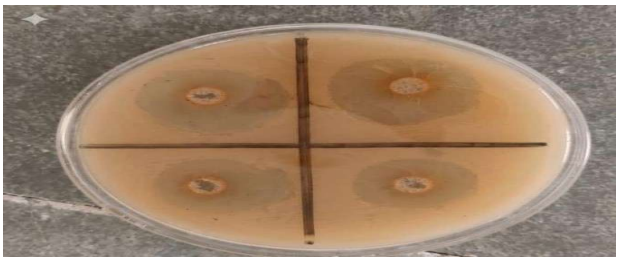

Figure.13 Zone of inhibition

5. Conclusion

The FTIR analysis confirmed the compatibility of Quercetin, Hyaluronic Acid, HPMC K4M, and Carbopol used in the formulation. The characteristic peaks of the individual components were retained in the optimized formulation without significant shifts or disappearance, indicating the absence of chemical interactions between the drug and excipients. This demonstrates that the selected polymers and active ingredients are physically and chemically compatible, ensuring the stability and integrity of the formulation.

The antimicrobial activity study demonstrated that all formulations possessed antibacterial and antifungal properties against *Escherichia coli* and *Candida albicans*. Among the tested formulations, F5 exhibited the highest antimicrobial efficacy, producing inhibition zones of 18.8 mm against *E. coli* and 16.2 mm against *Candida albicans*. The enhanced antimicrobial activity of F5 may be attributed to its optimized composition and improved release characteristics, which facilitated effective diffusion of the active constituents. The results confirm that the developed formulation has significant potential to inhibit microbial growth associated with oral ulcer infections.

Overall, the FTIR and antimicrobial studies established that the developed formulation is compatible, stable, and capable of providing effective antimicrobial action. The superior performance of formulation F5 highlights its potential as a promising oral ulcer treatment system by combining physicochemical stability with enhanced antibacterial and antifungal activity.

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