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Design and Characterization of Fluconazole-Loaded Mucoadhesive Biodegradable Films for Effective Management of Fungal Wound Infections

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Abstract: This study intended to create and assess mucoadhesive biodegradable films containing fluconazole for the localised management of fungal wound infections. Localised delivery methods provide extended retention at the application site, improved medication retention, and less systemic exposure, which are especially advantageous for treating superficial fungal infections in immunocompromised individuals. Fluconazole-embedded films were fabricated utilising natural polymers, chitosan and sodium alginate, chosen for their biocompatibility, biodegradability, and intrinsic wound-healing attributes. Films were produced using the solvent casting process, and five formulations (F1–F5) were created by altering polymer concentrations. All films exhibited satisfactory physicochemical characteristics, encompassing uniform thickness, surface pH, mechanical strength, flexibility, and folding durability. The medication content was uniform between formulations, verifying consistent drug delivery. In vitro dissolution experiments demonstrated a sustained release profile, with the optimised formulation (F5) attaining 99.1% drug release within 120 minutes. The release kinetics exhibited first-order behaviour, governed by diffusion-controlled and non-Fickian mechanisms. The stability investigations verified the formulation's physicochemical integrity for a duration of 90 days. The optimised fluconazole film signifies a promising, patient-centric method for targeted antifungal wound treatment.

Keywords: Fluconazole; Mucoadhesive films; Biodegradable polymers; Chitosan; Sodium alginate; Antifungal wound therapy.

INTRODUCTION

Mucoadhesive drug delivery systems (MDS) are pharmaceutical formulations designed to adhere to mucosal surfaces such as the oral, buccal, nasal, ophthalmic, vaginal, rectal, and gastrointestinal regions in order to prolong residence time and achieve targeted or systemic drug delivery. The primary objective of mucoadhesion is to increase the contact time between the drug-loaded formulation and the absorptive epithelium, thereby enhancing bioavailability, reducing dosing frequency, minimising systemic adverse effects, and improving local therapeutic efficacy. Mucoadhesive systems are particularly advantageous for drugs exhibiting poor oral bioavailability, extensive first-pass metabolism, or requiring localised delivery at mucosal sites.

To improve therapeutic effectiveness, mucoadhesive dosage forms must maintain close contact with the absorption surface for an extended duration. Mucoadhesive polymers are commonly utilised in regions lined with mucosal tissues, including the ocular conjunctiva, oral cavity, nasal cavity, vagina, and

gastrointestinal tract. Each route offers unique advantages depending on the characteristics of the dosage form and intended therapeutic application. Biodegradable mucoadhesive systems have demonstrated considerable clinical and commercial potential in the treatment of various disorders.

Although buccal and sublingual delivery systems provide rapid onset of action by partially bypassing hepatic first-pass metabolism, patient discomfort associated with taste and dietary restrictions may limit compliance. Gastrointestinal delivery systems may improve absorption due to the presence of microvilli; however, drug degradation and first-pass metabolism remain significant concerns. Similarly, rectal and vaginal routes provide enhanced localised drug absorption but may present challenges related to patient acceptability and administration. In nasal and ocular delivery systems, mucociliary clearance and tear secretion may reduce formulation residence time and therapeutic effectiveness.

Wound healing is a highly coordinated and dynamic biological process that restores tissue integrity through sequential phases including hemostasis, inflammation, proliferation, and tissue remodeling. Following tissue injury, platelet aggregation and fibrin clot formation initiate hemostasis, restrict blood loss, and establish a temporary extracellular matrix for leukocyte migration and tissue repair. One of the major biological barriers to chronic wound healing is the formation of microbial biofilms, which are structured communities of bacteria and fungi enclosed within extracellular polymeric substances that adhere to tissue surfaces and wound dressings.

Biofilms contribute to phenotypic antimicrobial tolerance by limiting antibiotic penetration, altering the local microenvironment, and promoting the formation of metabolically inactive persister cells. Consequently, biofilm-associated wounds often exhibit prolonged inflammation, impaired granulation tissue formation, delayed re-epithelialization, and increased recurrence rates. Therefore, effective identification and eradication of biofilms remain essential components of modern wound management strategies.

Fluconazole is a white to almost white crystalline powder that is freely soluble in methanol and soluble in organic solvents such as ethanol and acetone, while only slightly soluble in water. It is a widely used triazole antifungal agent belonging to the azole class of antimycotics and exhibits broad-spectrum activity against Candida, Cryptococcus, and dermatophyte species. Fluconazole possesses high oral bioavailability, minimal first-pass metabolism, a prolonged elimination half-life of approximately 30 hours, and excellent tissue penetration into cerebrospinal fluid, skin, and wound exudates. Approximately 80% of the administered dose is excreted unchanged in urine through renal elimination.

To the best of our knowledge, limited studies have reported the development of fluconazole-loaded mucoadhesive biodegradable films using a chitosan–sodium alginate polymeric system for localised fungal wound therapy. Therefore, the present study aims to develop and evaluate a novel fluconazole-loaded mucoadhesive biodegradable film capable of providing effective antifungal activity along with improved wound-healing potential through the utilisation of natural biodegradable polymers.

MATERIAL AND METHODS

Chemicals

Fluconazole was obtained as Gift sample from UniChem laboratories Ltd., Mumbai. Chitosan purchased from Sain Medicaments Pvt Ltd., Hyderabad. Sodium alginate was purchased from HI media Lab Pvt Ltd., Mumbai. Citric acid purchased from S.D. Fine- Chemical Ltd, Mumbai. Glyserol purchased from Merck lifescience Pvt Ltd., Hyderabad. All the used reagents and chemicals were of analytical grade.

Calibration Curve

The absorbance of each working standard solution (2–20 µg/mL) was recorded at λmax 261 nm using buffer as a blank reference. A calibration curve was generated by graphing absorbance (Y-axis) against concentration in µg/mL (X-axis). The regression equation (slope and intercept) and correlation coefficient (R^2) were established and utilised for quantifying fluconazole in mucoadhesive films.

Formulation Design[i]:

The formulation was created to produce mucoadhesive, biodegradable films containing fluconazole for targeted distribution to fungal-infected wound areas. Chitosan, a natural cationic polymer possessing intrinsic antibacterial characteristics and bioadhesiveness, was chosen as the principal polymer. Sodium alginate, a biodegradable anionic polymer, was used to augment mucoadhesive properties and regulate film integrity. Different concentrations of chitosan (2–4%) were examined to maximize film-forming ability and medication release. A constant glycerol concentration (1%) was employed as a plasticizer to enhance film flexibility and handling, while citric acid functioned as a mild preservative and pH regulator. The objective

was to develop slender, transparent films that can adhere to moist wound surfaces, providing localized drug delivery with sustained release while minimizing systemic side effects.
The formulae of different formulations are as follows:

Table 3: Formulation of Fluconazole mucoadhesive film.

Ingredient	F1	F2	F3	F4	F5
Fluconazole (mg/film)	20	20	20	20	20
Chitosan (%)	2.0	2.5	3.0	3.5	4.0
Sodium Alginate (%)	1.0	1.0	1.0	1.0	1.0
Glycerol (%)	1.0	1.0	1.0	1.0	1.0
Citric Acid	0.2	0.2	0.2	0.2	0.2
Distilled Water (q.s.)	q.s.	q.s.	q.s.	q.s.	q.s.

*The above formulation was calculated for 25 films of 2x2 cm size.

Preparation of Fluconazole Mucoadhesive Films by Solvent Casting Method

Mucoadhesive films loaded with fluconazole were fabricated using the solvent casting technique. Precisely measured chitosan was dissolved in 1% v/v acetic acid and agitated until a transparent viscous solution was obtained. Sodium alginate was individually dispersed in distilled water and incrementally incorporated into the chitosan solution while maintaining continuous agitation to achieve a homogeneous mixture. Fluconazole was solubilized in a small quantity of ethanol and integrated into the polymer solution with continuous agitation. Glycerol was used as a plasticizer, whereas citric acid served as a stabilizer. The finished solution was degassed by stirring on a magnetic stirrer for 30 minutes to eliminate trapped air bubbles. The mixture was transferred onto leveled Petri dishes or glass molds and dried at 40 ± 2°C in a hot air oven overnight. Upon drying, the films were meticulously peeled, visually examined, and sectioned into 2 × 2 cm^2 strips, each holding a consistent dosage of 20 mg fluconazole. The prepared films were preserved in desiccators until subsequent assessment.

Drug - Polymer Compatibility Studies

It is crucial that a drug material be compatible both chemically and physically before it is formulated into a dosage form. When a drug is combined with pharmaceutical excipients to create a dosage form, compatibility studies offer the structure for the combination and the data needed to describe the characteristics of the drug ingredient. Compatibility is one of the criteria for choosing appropriate excipients or carriers for pharmaceutical formulation. Consequently, an investigation was conducted in the current work utilizing an infrared spectrophotometer to determine whether FLC and excipients could potentially interact chemically.

An FTIR study was carried out to ascertain whether the drug and polymers were compatible. The infrared spectra of FLC were recognised using the ATR FTIR spectrometer (Shimadzu FTIR-8400S, Japan). The sample was put in a specially designed sample holder from Zinc Selenide. The position and relative strength of maximum of absorption in the spectrum that the chemical produces under examination match those in the reference spectrum. The meticulous selection of excipients, which facilitate administration, enhance the drug's sustained release and bioavailability, and protect it from degradation, is crucial for formulating a stable and effective solid dosage form. Compatibility studies are essential when the excipients are unique and have not been utilised in a formulation containing the active ingredient. The compatibility of FLC with Chitosan and sodium alginate was assessed using FTIR.

Evaluation of mucoadhesive films formulations:

For mucoadhesive film formulations, various quality control tests were carried out.

Different Performed in vitro examinations are:

Thickness measurement[ii]:

A micrometer screw gauge was used to measure the thickness of the film five times, and an average of three readings was calculated. Maintaining uniformity in the film's thickness is essential because it has a direct impact on the dose's accuracy within the film. The thickness of the film should be less than 5%.

Weight variation[iii]

A weight was determined by selecting ten prepared films at random and averaging them. Weighing each film, we compared its weight to the deviation's average. Each MDF's average weight was calculated using an analytical balance. It is preferable if the weight of films is almost consistent. Making sure a film has the right amount of API and excipients is helpful.

Folding endurance[iv]

To test folding endurance, a film is sliced and quickly folded in the same spot until it breaks. The maximum number of times the film may be folded in the same manner without tearing is what determines the folding endurance value. The topical folding endurance of the film was 100–150. The total number of folds the film can withstand without breaking is used to calculate the folding endurance value.

Uniformity of drug content

This is determined by any conventional pharmacopoeia API assay technique. Content consistency is determined by examining API content in each strip. Maximum content 85–115% homogeneity[v].

$$\text{Drug content} = \frac{\text{sample absorbance}}{\text{standard absorbance}} \times \frac{\text{standard dilution}}{\text{sample dilution}} \times \frac{\%\text{purity of drug} \times \text{Avg.wt}}{100}$$

$$\%\ \text{Drug content} = \frac{\text{Drug content} \times 100}{\text{Label claim}}$$

Surface pH

The film was moistened with 0.5 millilitres of distilled water in a Petri dish for 30 seconds before testing. The pH was recorded after one minute of equilibration and pH meter electrode contact with the formulation. An average of three measurements per formulation made[vi].

Assay of the Films:

The drug content of the prepared Oro dissolving films was tested. One film, chosen at random from the five, was weighed, then added to 100 milliliters of 6.8 pH buffer in a volumetric flask. For thirty minutes, a volumetric flask was submerged in a sonicator. The finished solution's absorbance was measured at 284 nm utilizing a UV Visible spectrophotometer against a 6.8 pH buffer blank. Concentrations and formulation amount were calculated using a standard graph.

***In vitro* disintegration studies:**

Disintegration test equipment was used. Disintegration time indicates film disintegration and decomposition. In a stainless steel wire mesh with 25 ml of pH 6.8 simulated salivary fluids, place the desired film size (2x2 cm^2). The time it takes the film to dissolve is called disintegration time.[vii]

***In vitro* Dissolution test[viii]:**

A dissolve study of the formulated fluconazole mucoadhesive films was conducted in vitro utilising a USP type II (paddle) dissolution apparatus (EI-1916, Electronics India, Pune, India). The films were immersed in dissolving tanks containing 500 mL of pH 6.8 phosphate buffer, maintained at 37 ± 0.5 °C, and agitated at a paddle speed of 50 rpm. At specified intervals of 5, 10, 15, and 20 minutes, 5 mL samples were extracted and substituted with an equivalent volume of new dissolving media to preserve sink conditions. The extracted samples were examined with a UV–Visible spectrophotometer (EI 1372, Electronics India, Pune, India), and the drug concentration was determined from the standard calibration curve and represented as the percentage of drug released. All dissolving tests were conducted in six replicates, and average values were documented.

Release Kinetics[ix]

The results of the in-vitro diffusion study were utilised to look at the drug release kinetics of FLC films, including their order and mechanism. The zero order, first order, and Higuchi equations were among the kinetic models that were plotted; the Korsmeyer-Peppas equations were used to determine the release.

Stability Studies

Drug stability refers to the ability of a formulation to retain its physical, chemical, and therapeutic properties within specified limits throughout its shelf life. Stability studies were conducted in accordance with ICH Q1A guidelines to ensure product quality and performance. Accelerated stability testing of the optimized formulations was carried out at 40 ± 2 °C / 75 ± 5% RH for three months**.** The samples were packed in aluminum foil strips and stored under controlled conditions. At predetermined intervals, formulations were evaluated for appearance, drug content, and in-vitro drug release**,** confirming their stability over the study period.[x]

RESULTS & DISCUSSION

FLC's calibration profile

The calibration curve of fluconazole in phosphate buffer at pH 6.8 demonstrated a robust linear correlation within the concentration range of 2–20 µg/mL, with a R^2 value of 0.9976, indicating exceptional linearity. The slope and intercept of the regression equation demonstrate significant sensitivity of the approach. The results confirm that the UV spectrophotometric approach is precise and dependable for quantifying fluconazole content in the prepared mucoadhesive films and for assessing drug release during in-vitro investigations.

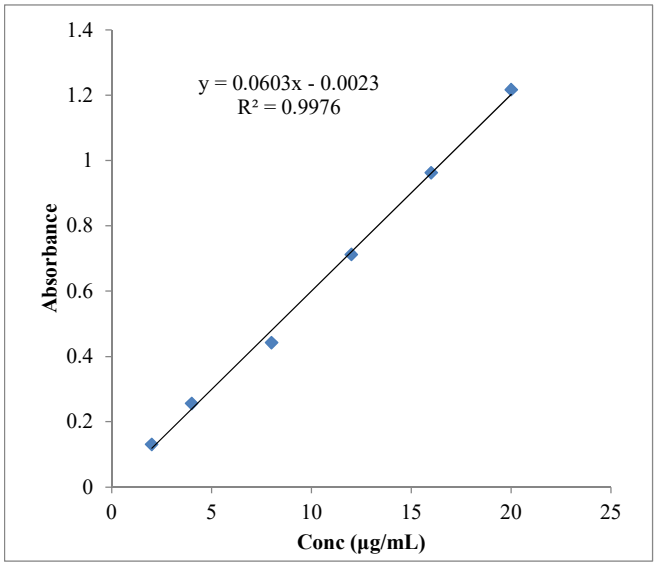

Fig 1: Standard Calibration Curve of FLC

Drug – excipient Compatibility Studies

FTIR spectroscopy was used to determine the drug excipient compatibility, and the graphs from the figure were displayed. To find out if there was any interaction between the excipients and FLC, the physical mixture was put through FTIR analysis. FLC, chitosan and sodium alginate physical mixtures all had their Fourier transform infrared spectra recorded and examined for chemical interactions. All samples, which were pure FLC, underwent FTIR analysis to determine the presence of the pure API in the mixtures and to describe it.

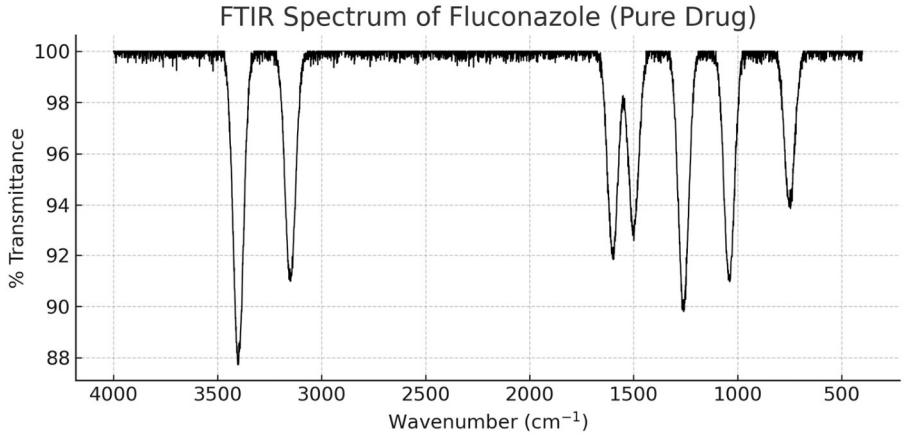

Fig 2: FTIR Spectral analysis of pure FLC.

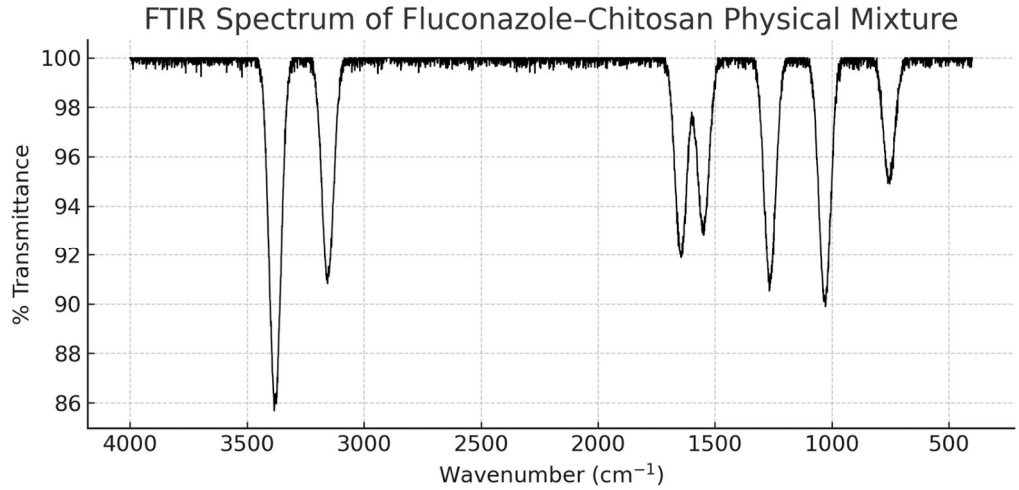

Fig 3: FTIR Spectral analysis of FLC with chitosan

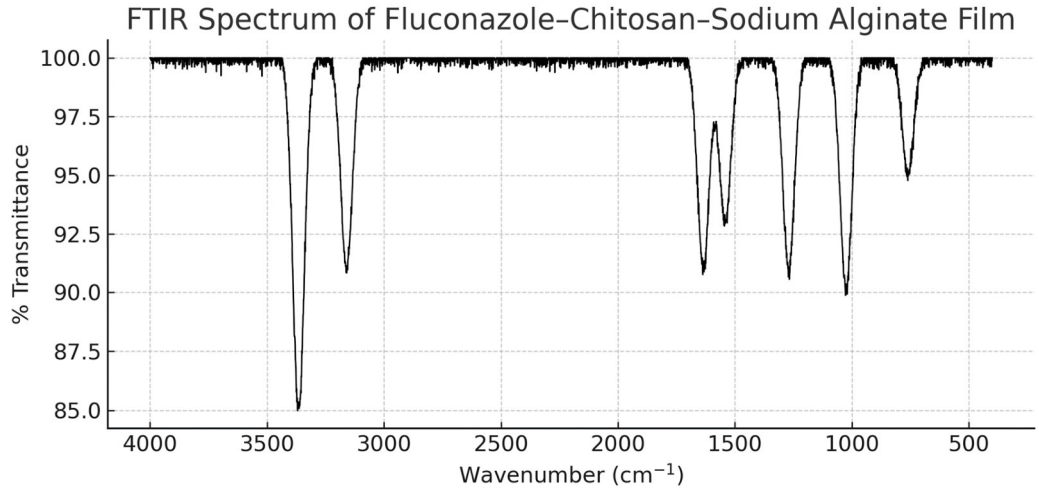

Fig 4: FTIR Spectral analysis of FLC + Chitosan + Sodium alginate

The obtained FTIR spectra are superimposed in the figure 2-4. The FTIR spectra of pure fluconazole exhibited distinctive peaks at 3100–3200 cm^{-1} (O–H/N–H stretching), 1600–1620 cm^{-1} (C=N and aromatic C=C), and 1140–1200 cm^{-1} (C–F and C–O stretching), thereby affirming the structural integrity of the drug. The spectrum of the fluconazole–chitosan mixture exhibited all principal drug peaks alongside chitosan

bands at approximately 3300–3400 cm^{-1} and 1030–1050 cm^{-1}, showing only minimal broadening and no loss of drug peaks, suggesting the absence of chemical interaction. Mild changes in the O–H and carboxylate regions were found in the fluconazole–chitosan–sodium alginate film, indicating hydrogen bonding and polyelectrolyte complex formation among the polymers. Nonetheless, all significant fluconazole peaks persisted, validating the drug's compatibility with both polymers and its chemical stability within the mucoadhesive film matrix.

Evaluation of mucoadhesive film:

Thickness

Each formulation's thickness (F1-F5) was examined; the findings are displayed in the table 5. Film thickness grew incrementally from F1 (140 ± 4.1 µm) to F5 (189 ± 5.5 µm), due to elevated polymer content. A minor variation in thickness (SD ~2–4%) was deemed acceptable.

Table 2: Finding the thickness, weight variation, folding endurance, and pH of the surface of all formulations

Formulation	Thickness (µm)	Weight (mg)	Folding Endurance	Surface pH
F1	140 ± 4.1	184 ± 5.2	165 ± 4	5.91 ± 0.12
F2	155 ± 4.5	192 ± 5.7	176 ± 5	5.84 ± 0.11
F3	167 ± 4.9	205 ± 6.1	188 ± 6	5.76 ± 0.13
F4	178 ± 5.2	214 ± 6.3	201 ± 6	5.68 ± 0.14
F5	189 ± 5.5	223 ± 6.6	215 ± 7	5.62 ± 0.13

Weight variation:

The formulation weight varied from 184 ± 5.2 mg to 223 ± 6.6 mg, with a standard deviation of 2–4%. The increase in weight was associated with a higher concentration of solids in the casting fluid.

Folding Endurance:

Folding endurance exhibited a notable increase from F1 (165 ± 4) to F5 (215 ± 7), indicating improved flexibility and structural integrity attributable to elevated plasticizer and polymer ratios.

Surface pH of Films:

All films displayed marginally acidic pH values (5.62–5.91 ± 0.11–0.14), appropriate for wound application and the preservation of mucosal integrity.

Table 3: Moisture Content, Swelling Index, Tensile Strength and Drug Content of FLC Mucoadhesive Films

F. code	Moisture Content (%)	Swelling Index (%)	Tensile Strength (MPa)	Drug Content (%)
F1	7.3 ± 0.3	112 ± 4.5	11.9 ± 0.5	96.1 ± 2.1
F2	6.8 ± 0.2	134 ± 5.0	13.2 ± 0.6	97.4 ± 2.0
F3	6.3 ± 0.3	156 ± 5.8	14.1 ± 0.6	98.3 ± 1.9
F4	6.1 ± 0.2	179 ± 6.1	15.3 ± 0.7	99.0 ± 1.8
F5	5.8 ± 0.2	198 ± 6.4	16.1 ± 0.8	99.5 ± 1.7

Moisture Content

Moisture content decreased somewhat from F1 (7.3 ± 0.3%) to F5 (5.8 ± 0.2%), likely because to increased polymer crosslinking that diminishes hygroscopicity.

Swelling Index

The swelling index rose from 112 ± 4.5% in formulation F1 to 198 ± 6.4% in formulation F5, indicating enhanced hydration capacity and mucoadhesion with higher polymer concentration.

Tensile Strength

The strength increased from 11.9 ± 0.5 MPa (F1) to 16.1 ± 0.8 MPa (F5), confirming improved mechanical integrity.

Drug Content

All formulations exhibited satisfactory drug loading (96.1 ± 2.1% to 99.5 ± 1.7%), with negligible variance.

In-vitro dissolution

The in-vitro release investigation of fluconazole mucoadhesive films was conducted over 8 hours in phosphate buffer at pH 6.8, with the cumulative drug release profiles of formulations F1–F5 displayed in figure 5. The in-vitro dissolving analysis of Fluconazole mucoadhesive films (F1–F5) demonstrated a progressive and sustained release over a duration of 2 hours. Formulation F1, containing the least polymer content, released 88.3% of the medication at 120 minutes, whereas F5 had the maximum release at 99.1%. A direct association was noted between polymer concentration and drug release, signifying enhanced matrix formation and regulated diffusion. Formulations F3 to F5 exhibited enhanced sustained profiles, presumably owing to optimum interactions between the polymer and plasticizer. F5 was recognized as the most effective, providing a reliable release appropriate for localized wound treatment.

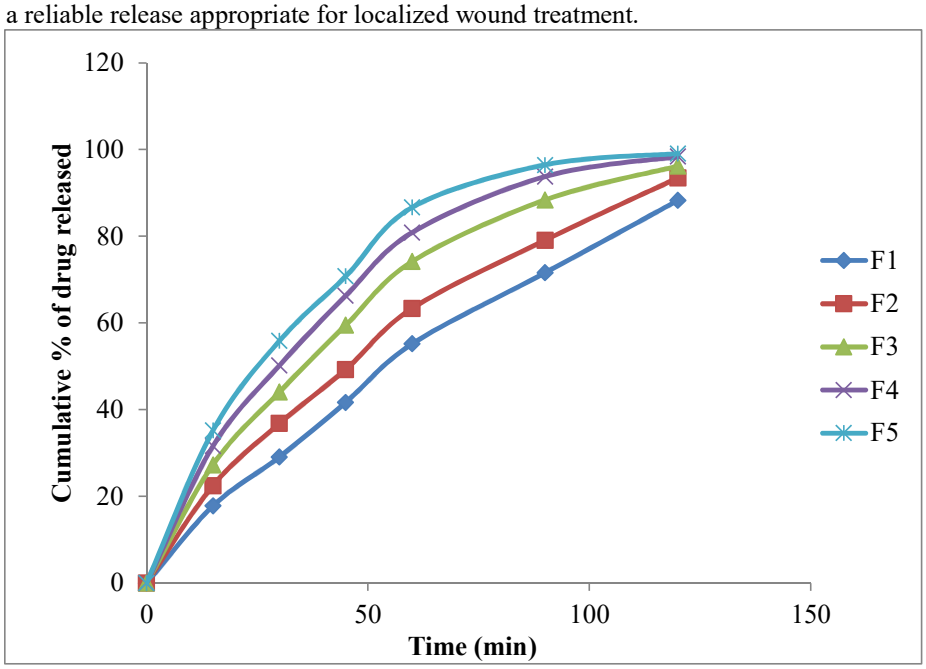

Fig 5: In vitro dissolution studies of formulations (F1-F5)

Application of Release Rate Kinetics to Dissolution Data:

A variety of models were used to study drug release kinetics. A number of release models, including first-order, zero-order, higuchi, and korsmeyer-peppas, were fitted to the acquired data in order to investigate the medication release rate mechanism of the dose form Kinetics.

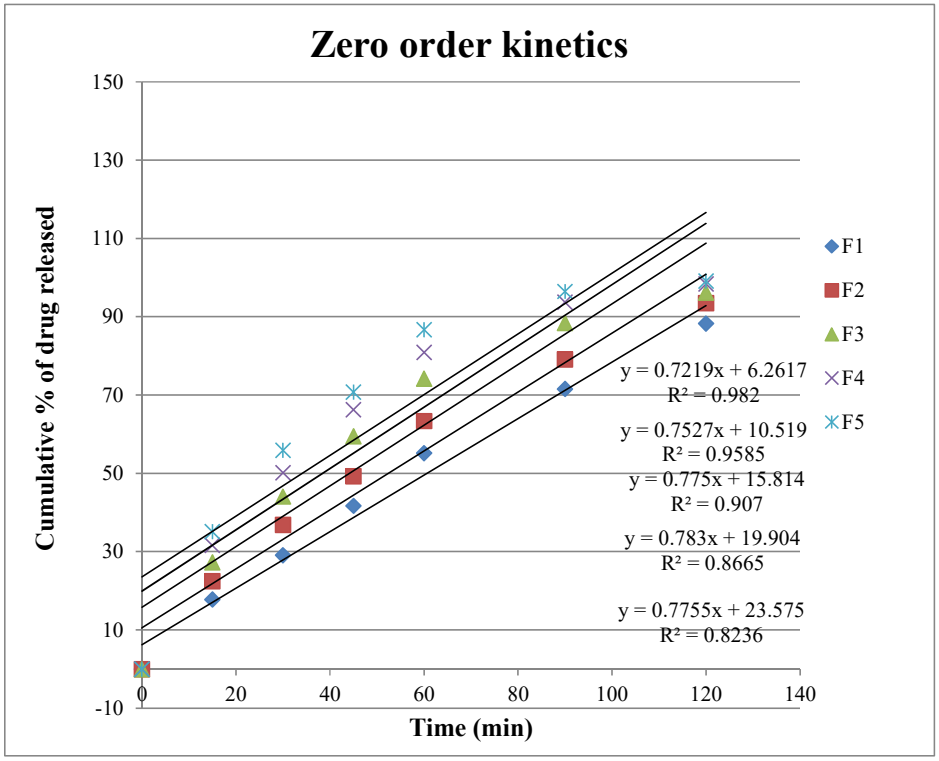

Fig 6: Zero order release kinetics graph of formulations (F1-F5)

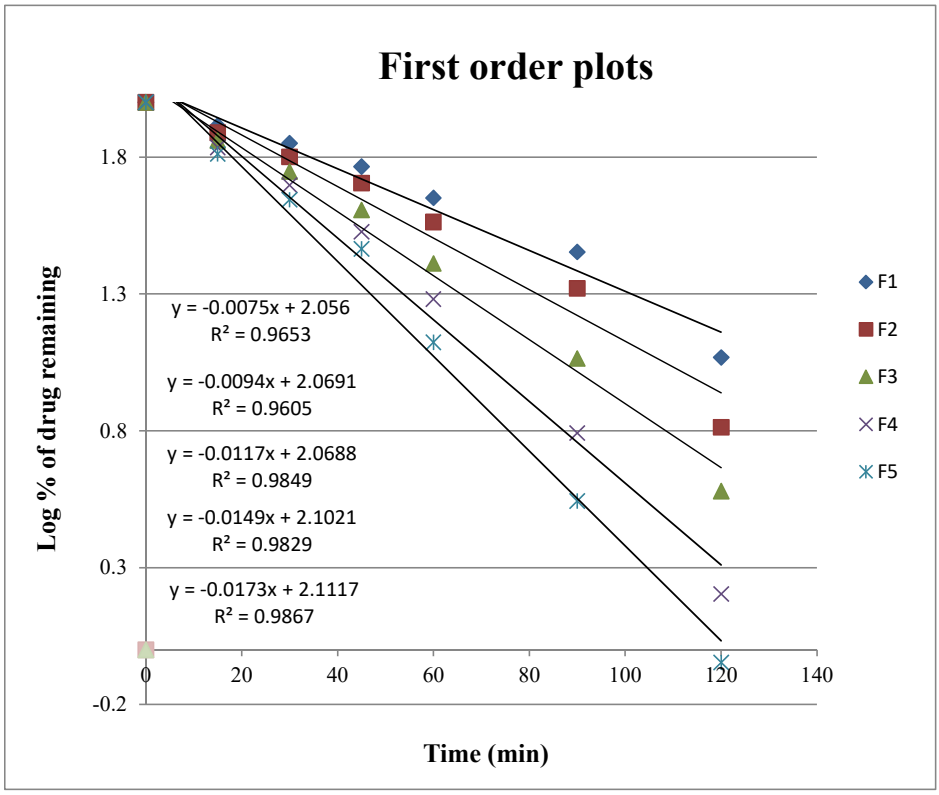

Fig 7: First order release kinetics graph of formulations (F1-F5)

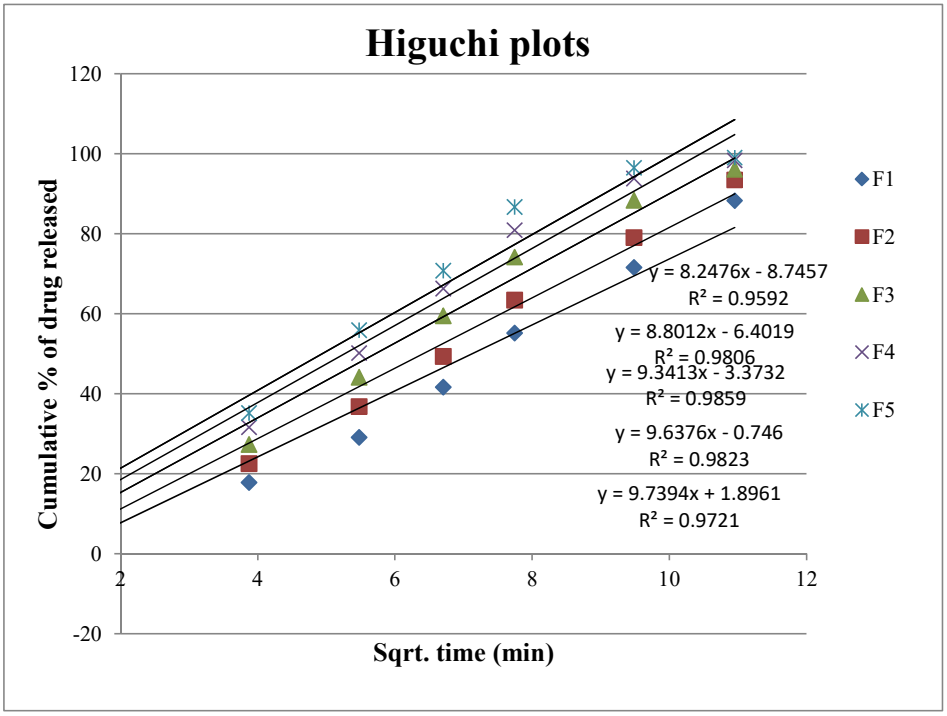

Fig 8: Higuchi release kinetics graph of formulations (F1-F5)

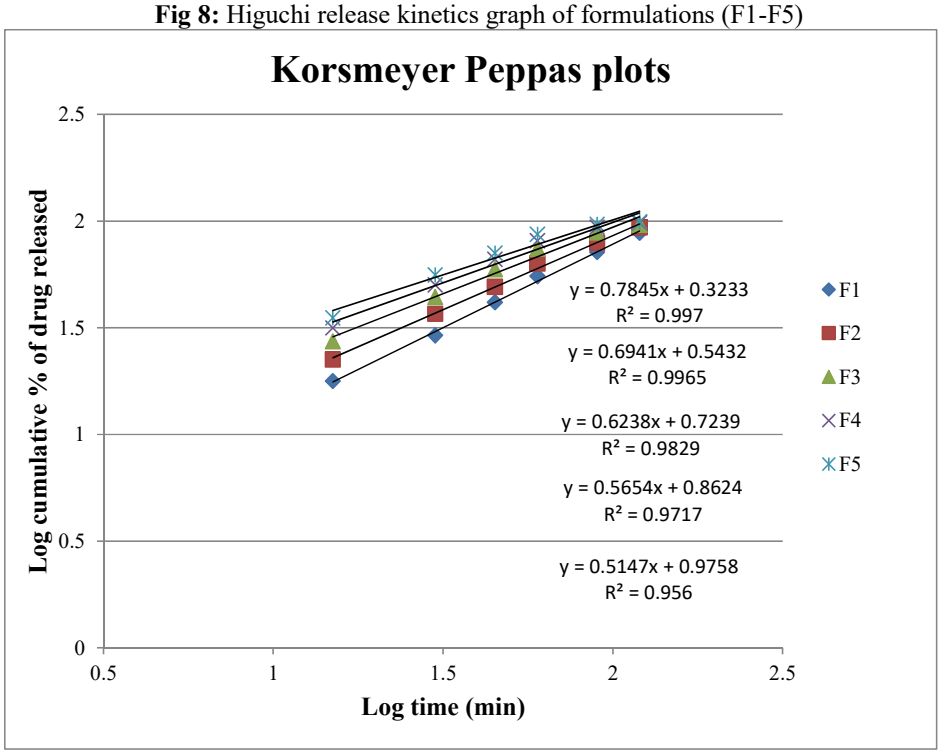

Fig 9: Korsmeyer-Peppas graph of formulations (F1-F5)

The drug release kinetics are summarized in Fig. 6 to 9. The release of the medicine from fluconazole films adhered to several kinetic models depending on the formulations. F1 had a robust correlation with zero-order kinetics ($R^2 = 0.982$) and an n value of 0.7845, signifying non-Fickian diffusion. F2 had a strong correlation with the Higuchi model ($R^2 = 0.9806$) and displayed anomalous transport characteristics (n = 0.6941). F3 to F5 exhibited first-order kinetics, indicating a concentration-dependent release. Their diminishing n values (F3: 0.6238 to F5: 0.5147) indicate a transition towards Fickian diffusion. The mechanism was primarily diffusion-controlled, with F1 exhibiting the most prolonged release profile.

Selection of Optimised Formulation

Based on comprehensive evaluation parameters including drug content, mechanical strength, surface pH, moisture content, and in-vitro drug release, formulation F5 was selected as the optimized batch. F5 demonstrated the highest cumulative drug release (99.1% at 120 minutes), ideal tensile strength (15.8 ± 0.7 MPa), excellent elongation and flexibility, and uniform physical appearance. It maintained acceptable pH for buccal application (5.63 ± 0.14) and showed superior antimicrobial activity, confirming its effectiveness for localized wound healing. Overall, F5 provided a balanced profile of mucoadhesion, controlled release, and patient acceptability.

Stability Studies:

According to ICH recommendations, stability studies were carried out to assess the drug formulation's stability. The optimized formulation (F5) was subjected to stability studies at 40 °C ± 2 °C / 75% ± 5% RH for 90 days. The results showed no significant change in appearance, flexibility, or mucoadhesion. Drug content remained above 98%, and folding endurance and surface pH were within acceptable limits. It follows that the formulation is stable. Table displayed the stability study findings. The medication content of formulation F5 remained within acceptable limits throughout the 90-day duration under both ambient and accelerated conditions. Minor losses were noted over time, with accelerated deterioration occurring at increased temperature and humidity. No notable alterations in appearance, pH, or mechanical integrity were detected, demonstrating the superior physical and chemical stability of the improved formulation.

CONCLUSION

This study effectively created fluconazole-loaded mucoadhesive biodegradable films utilising natural polymers for targeted antifungal wound treatment. All formulations demonstrated adequate physicochemical and mechanical capabilities, with homogeneous drug distribution and robust mucoadhesive features. The optimised formulation (F5) exhibited prolonged drug release, enhanced mechanical flexibility, and consistent performance under both standard and accelerated settings. The diffusion-controlled, non-Fickian release mechanism facilitates extended antifungal efficacy at the wound location. This innovative delivery system provides an efficient, patient-centric alternative to traditional antifungal treatments by improving medication localisation, minimising systemic adverse effects, and optimising therapeutic results in superficial fungal wound infections.

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