RESEARCH PAPER

Development and Optimization of Coaxial Electrospun Bioadhesive Muco-Invasive Nanofibrous Films Loaded with 5-Fluorouracil for Localized Mucosal Cancer Therapy Using Box–Behnken Design

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ABSTRACT

Background: Mucosal cancers require localized drug delivery systems capable of maintaining therapeutic drug concentrations at the disease site while minimizing systemic toxicity. Conventional administration of 5-Fluorouracil (5-FU) is associated with rapid drug clearance and significant systemic adverse effects, limiting its therapeutic efficacy.

Aim: The present study aimed to develop and optimize coaxial electrospun bioadhesive muco-invasive nanofibrous films loaded with 5-Fluorouracil for localized mucosal cancer therapy.

Methods: FTIR analysis was performed to evaluate drug–polymer compatibility. A UV–Visible spectrophotometric method was developed at 266 nm (2–12 µg/mL, R^2 = 0.9998). Nanofibrous films were prepared using coaxial electrospinning and optimized using Box–Behnken Design with PCL concentration, chitosan concentration, and core flow rate as independent variables. The optimized formulation was evaluated for morphology, swelling behavior, entrapment efficiency, mucoadhesion strength, in-vitro drug release, ex-vivo permeation, and stability.

Results: The optimized formulation containing 9.46% PCL, 1.85% chitosan, and 0.51 mL/h core flow rate produced uniform nanofibers with an average diameter of ~435 nm, entrapment efficiency of ~84%, and mucoadhesion strength of ~33 g. Swelling studies showed high hydration capacity (~95%), while in-vitro drug release demonstrated controlled release (~76% in 8 h). Drug release followed the Higuchi model with non-Fickian diffusion. Ex-vivo permeation studies using goat buccal mucosa confirmed sustained drug permeation. Stability studies indicated good physical and chemical stability.

Conclusion: The developed coaxial electrospun bioadhesive muco-invasive nanofibrous films offer a promising localized drug delivery approach for effective mucosal cancer therapy.

Keywords: Coaxial electrospinning, Nanofibrous films, 5-Fluorouracil, Mucoadhesion, Localized drug delivery, Box–Behnken Design, Mucosal cancer therapy.

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INTRODUCTION

Mucosal cancers, including oral, buccal, nasal, and gastrointestinal malignancies, represent a significant global health burden due to challenges in achieving effective localized drug delivery and minimizing systemic toxicity. Conventional chemotherapy often leads to non-specific drug distribution, resulting in reduced therapeutic efficacy and severe adverse effects. Among anticancer agents, **5-Fluorouracil (5-FU)** is widely used for the treatment of mucosal and epithelial cancers; however, its clinical effectiveness is limited by rapid drug clearance, short half-life, and systemic toxicity. Therefore, the development of localized drug delivery systems capable of maintaining therapeutic drug concentrations at the mucosal site while minimizing systemic exposure has gained considerable attention.[1,2]

Electrospinning has emerged as a versatile and promising technique for fabricating nanofibrous drug delivery systems due to its ability to produce fibers with high surface area, tunable porosity, and excellent drug-loading capacity. These electrospun nanofibers mimic the

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extracellular matrix and provide improved drug retention and controlled release, making them suitable for localized cancer therapy. Furthermore, electrospun nanofibers have shown significant potential for delivering chemotherapeutic agents directly to tumor sites, thereby enhancing therapeutic efficacy and reducing systemic side effects.[3,4]

Among various electrospinning techniques, **coaxial electrospinning** has gained significant interest due to its ability to produce core–shell nanofibers capable of encapsulating drugs within the core while maintaining structural integrity through the shell layer. This approach enables sustained drug release, reduces burst release, and enhances therapeutic performance in cancer treatment. Coaxial electrospinning also protects sensitive drugs and allows co-loading of multiple therapeutic agents for improved therapeutic outcomes.[5]

Mucoadhesive nanofibrous films represent an advanced strategy for mucosal drug delivery by enhancing residence time at mucosal surfaces and improving drug absorption. Chitosan is widely used as a mucoadhesive polymer due to its biocompatibility, biodegradability, and inherent bioadhesive properties. Additionally, polycaprolactone (PCL) is commonly used to provide mechanical strength and sustained drug release behavior. Chitosan-based electrospun nanofibers have demonstrated promising applications in transmucosal drug delivery systems.

Furthermore, statistical optimization techniques such as **Box–Behnken Design (BBD)** are widely employed in pharmaceutical formulation development to optimize process variables and improve formulation performance. BBD allows systematic evaluation of formulation parameters and their interactions while minimizing the number of experimental trials. This design has been successfully applied to optimize electrospinning parameters and improve nanofiber characteristics such as fiber diameter and drug release behavior.[6,7]

Based on these considerations, the present study focuses on the **development and optimization of coaxial electrospun bioadhesive muco-invasive nanofibrous films loaded with 5-Fluorouracil for localized mucosal cancer therapy using Box–Behnken Design**. The developed system is expected to provide sustained drug release, improved mucoadhesion, enhanced mucosal penetration, and reduced systemic toxicity, thereby offering an effective strategy for localized mucosal cancer therapy.

MATERIALS AND METHODS

Materials

5-Fluorouracil (5-FU) was used as a model anticancer drug and was procured from Yarrow Chem Products Pvt. Ltd., Mumbai, India. Polycaprolactone (PCL) and chitosan were obtained from SRL Pvt. Ltd., Mumbai, India All chemicals and solvents used were of analytical grade and used without further purification.

METHODS

2.1 Experimental Design Using Box–Behnken Design (BBD)

Box–Behnken Design (BBD) was employed to optimize the formulation of coaxial electrospun bioadhesive muco-invasive nanofibrous films. BBD is an efficient response surface methodology used to evaluate the effect of formulation variables and their interactions with a reduced number of experimental runs. Three independent variables were selected based on preliminary trials and literature reports, including polycaprolactone (PCL) concentration, chitosan concentration, and core solution flow rate. These variables significantly influence fiber diameter, drug loading, mucoadhesion, and drug release behavior. The dependent variables selected for optimization were fiber diameter, entrapment efficiency, mucoadhesion strength, and percentage drug release at 8 h.[8]

2.1.1 Independent Variables

The independent variables and their levels used in the Box–Behnken Design are presented in Table 1.

Table 1. Independent variables and levels used in Box–Behnken Design

Factor	Variable	Code	Low (-1)	Medium (0)	High (+1)
PCL concentration (% w/v)	Core polymer concentration	X1	8	10	12
Chitosan concentration (% w/v)	Shell polymer concentration	X2	1.0	1.5	2.0
Core solution flow rate (mL/h)	Flow rate	X3	0.4	0.6	0.8

2.1.2 Dependent Variables and Optimization Goals

The dependent variables and optimization goals are shown in Table 2.

Table 2. Dependent variables and optimization goals

Response	Parameter	Code	Optimization Goal
Fiber diameter (nm)	Fiber size	Y1	Minimize
Entrapment efficiency (%)	Drug loading	Y2	Maximize
Mucoadhesion strength (g)	Adhesion property	Y3	Maximize
% Drug release at 8 h	Release profile	Y4	Target (60–80%)

2.1.3 Design Matrix Generation

A three-factor, three-level Box–Behnken Design was used to generate 17 experimental runs, including five center points to estimate experimental error. The experimental runs were randomized to minimize bias. The generated formulations were prepared and evaluated for selected responses.

2.1.4 Statistical Analysis

Design-Expert® software (Version 13, Stat-Ease Inc., USA) was used to generate the experimental design, analyze the data, and optimize the formulation. Analysis of variance (ANOVA) was performed to determine the significance of model terms. Response surface plots and contour plots were generated to evaluate the effect of independent variables on the responses. The optimized formulation was selected based on desirability function.

2.2 Fourier Transform Infrared (FTIR) Analysis

Drug–polymer compatibility studies were carried out using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of pure 5-Fluorouracil, PCL, chitosan, and their physical mixtures were recorded using an FTIR spectrophotometer (Shimadzu, Japan). Samples were prepared using the potassium bromide (KBr) pellet method and scanned in the range of 4000–400 cm^{-1}. The obtained spectra were analyzed for characteristic peaks and any possible interactions between the drug and polymers. The absence of significant peak shifts or disappearance of characteristic peaks indicated compatibility between drug and excipients.

2.3 Preparation of Polymer Solutions [9]

2.3.1 Preparation of Core Solution

Polycaprolactone (PCL) was dissolved in a solvent mixture of dichloromethane (DCM) and N,N-dimethylformamide (DMF) in a ratio of 7:3 under continuous magnetic stirring. The required amount of 5-Fluorouracil was added to the polymer solution and stirred for 4–6 h until a clear and homogeneous solution was obtained. The prepared solution was used as the core solution for coaxial electrospinning.

2.3.2 Preparation of Shell Solution

Chitosan was dissolved in 1% v/v acetic acid solution with continuous stirring until complete dissolution was achieved. The solution was stirred further to obtain a uniform viscous polymer solution suitable for electrospinning. The prepared solution was used as the shell polymer solution.

2.4 Preparation of Coaxial Electrospun Nanofibrous Films

Electrospinning Setup

The core and shell polymer solutions were loaded separately into two syringes connected to a coaxial needle assembly. The syringes were mounted on a dual syringe pump to control flow rates independently. A high-voltage power supply was connected between the needle and the collector. Aluminum foil-covered rotating collector was used for nanofiber collection.

Electrospinning Parameters

Electrospinning was performed under optimized conditions. A voltage of 15–20 kV was applied, and the distance between the needle tip and collector was maintained at 12–15 cm. The flow rates of core and shell solutions were adjusted according to the Box–Behnken Design. The electrospinning process was continued until uniform nanofibrous films were obtained.

Film Collection and Drying

The electrospun nanofibers were collected on aluminum foil and carefully removed from the collector. The collected films were dried in a desiccator for 24 h to remove residual solvents and stored in airtight containers for further characterization studies.

2.5 Characterization of Nanofibrous Films

2.5.1 Physical Appearance

The prepared nanofibrous films were visually inspected for color, uniformity, flexibility, and presence of defects such as cracks or air bubbles. Films were observed under normal daylight against black and white backgrounds to assess surface uniformity and smoothness. [10]

2.5.2 Thickness Measurement

The thickness of nanofibrous films was measured using a digital vernier caliper (Mitutoyo, Japan). Measurements were taken at three different locations of each film, and the average thickness along with standard deviation was calculated.

2.5.3 Weight Uniformity

Nanofibrous films of uniform size (1 cm × 1 cm) were cut from different regions of the nanofiber mat. Each film was weighed individually using an analytical balance (Shimadzu, Japan), and the average weight and standard deviation were calculated. [11]

2.6 Drug Content Determination

A film of known dimensions was dissolved in a suitable solvent (methanol or phosphate buffer pH 6.8) and stirred to ensure complete drug extraction. The solution was filtered, diluted appropriately, and analyzed using a UV–Visible spectrophotometer at 266 nm. Drug content was calculated using the calibration curve. [12]

2.7 Morphological Characterization

2.7.1 Scanning Electron Microscopy (SEM)

Surface morphology of nanofibrous films was examined using scanning electron microscopy (SEM). Samples were mounted on aluminum stubs using double-sided adhesive tape and coated with a thin layer of gold using a sputter coater to improve conductivity. The samples were then examined at suitable magnifications. [13]

2.7.2 Fiber Diameter Analysis

The fiber diameter was measured from SEM images using ImageJ software. Approximately 50–100 fibers were

randomly selected, and the average fiber diameter and standard deviation were calculated.[11]

Functional Evaluation

2.8 Mucoadhesion Strength

Mucoadhesion strength was determined using a modified physical balance method. Fresh goat buccal mucosa was fixed to a glass slide, and the nanofibrous film was attached to another slide. The film was brought into contact with the mucosal tissue, and a specific preload was applied for 2 min. The weight required to detach the film from the mucosal surface was recorded as mucoadhesion strength.

2.9 Swelling Study

The swelling behavior of nanofibrous films was determined by placing pre-weighed films (W1) in phosphate buffer pH 6.8. At predetermined intervals, the films were removed, blotted to remove excess moisture, and weighed (W2). The swelling index was calculated using the following equation:

Swelling Index (%) = (W2 − W1) / W1 × 100

2.10 *In-vitro* Drug Release Study

In-vitro drug release studies were performed using USP dissolution apparatus II (paddle method). Phosphate buffer pH 6.8 (900 mL) was used as dissolution medium maintained at 37 ± 0.5°C with a stirring speed of 50 rpm. Nanofibrous films were placed in the dissolution medium and samples were withdrawn at predetermined intervals. The withdrawn samples were analyzed using a UV–Visible spectrophotometer at 266 nm.

2.11 Drug Release Kinetics

The release data obtained from in-vitro drug release studies were fitted into different kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine the drug release mechanism. The model with the highest correlation coefficient (R^2) was considered the best-fit model. [14]

***Ex-vivo* Studies[15]**

2.12 *Ex-vivo* Mucoadhesion Study

Fresh goat buccal mucosa was obtained from a local slaughterhouse and washed with phosphate buffer pH 6.8. The mucosal membrane was fixed on a glass slide, and the nanofibrous film was placed on the mucosal surface. The system was immersed in buffer solution maintained at 37 ± 0.5°C. The time required for film detachment was recorded as mucoadhesion time.

2.13 Ex-Vivo Permeation Study

Ex-vivo permeation studies were performed using Franz diffusion cells. Goat buccal mucosa was mounted between donor and receptor compartments. Nanofibrous films were placed in the donor compartment, and phosphate buffer pH 6.8 was used as receptor medium. Samples were withdrawn at predetermined intervals and analyzed at 266 nm using a UV spectrophotometer. Flux and permeability coefficient were calculated.

Stability Study

2.14 Stability Studies (ICH Guidelines)[16]

Stability studies were conducted according to ICH guidelines. Optimized formulations were stored under the following conditions:

- 25 ± 2°C / 60 ± 5% RH
- 40 ± 2°C / 75 ± 5% RH

Samples were evaluated at 0, 1, 2, and 3 months for physical appearance, drug content, and in-vitro drug release.

Statistical Analysis

2.15 Statistical Analysis and Optimization

ANOVA

Analysis of variance (ANOVA) was performed to determine the statistical significance of the model and formulation variables.

Response Surface Plots

3D response surface plots and contour plots were generated to evaluate the effect of independent variables on responses.

Optimization Criteria

The optimized formulation was selected based on desirability function by minimizing fiber diameter and maximizing entrapment efficiency, mucoadhesion strength, and controlled drug release.

3 RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was performed to evaluate the compatibility between 5-Fluorouracil and selected excipients. The FTIR spectrum of pure 5-Fluorouracil showed characteristic peaks corresponding to N–H stretching (3130–3400 cm^{-1}), C=O stretching (1650–1700 cm^{-1}), C–N stretching (1240–1320 cm^{-1}), and C–F stretching (1000–1100 cm^{-1}).

In the drug–excipient mixture, these characteristic peaks were retained with slight shifts, indicating possible hydrogen bonding interactions without any chemical incompatibility. A broad peak observed in the range of 3200–3500 cm^{-1} confirmed the presence of hydrophilic polymeric excipients. Overall, the FTIR results indicated good compatibility between 5-Fluorouracil and selected polymers, supporting their suitability for the development of coaxial electrospun bioadhesive muco-invasive nanofibrous films.

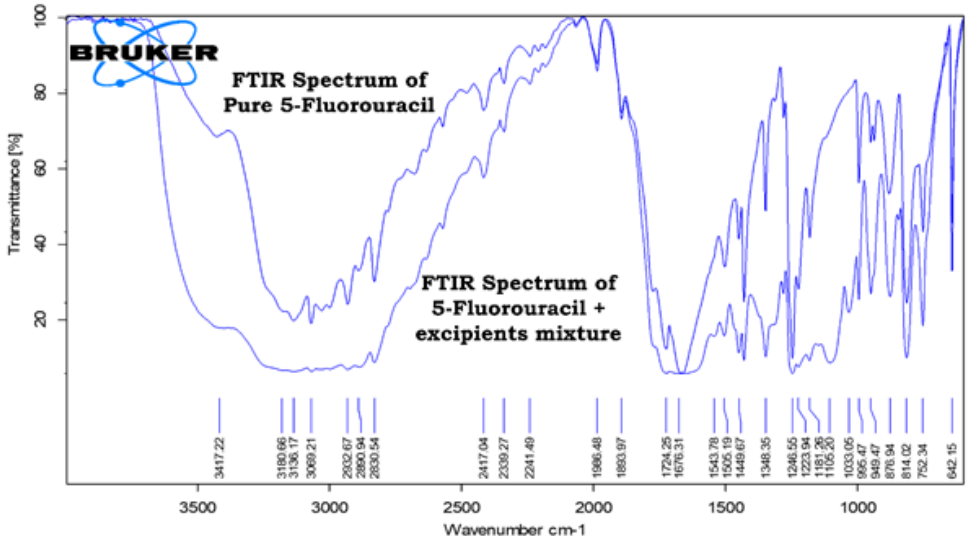

Figure 1: FTIR spectra of pure 5-Fluorouracil and drug–excipient mixture

3.2 Box–Behnken Design Optimization

3.2.1 Statistical Model Fitting

A three-factor, three-level Box–Behnken design was employed to optimize coaxial electrospun bioadhesive muco-invasive nanofibrous films. The independent variables included PCL concentration (A), chitosan concentration (B), and core flow rate (C), while fiber diameter (Y1), entrapment efficiency (Y2), mucoadhesion strength (Y3), and drug release at 8 h (Y4) were selected as dependent variables. The experimental runs and observed responses are presented in **Table 3.**

The obtained data were fitted to a quadratic polynomial model, and regression equations were generated. The model showed good agreement between predicted and experimental values, indicating suitability of the Box–Behnken design for optimization.

Table 3. Box–Behnken design matrix with experimental responses

Run	PCL (%)	Chitosan (%)	Core flow (mL/h)	Y1 (nm)	Y2 (%)	Y3 (g)	Y4 (%)
1	8	1.5	0.4	340	76.2	25	90
2	10	1.5	0.6	450	85	30	78
3	10	2	0.4	460	83.5	35	76
4	10	1.5	0.6	452	84.7	31	77
5	8	1.5	0.8	430	80.1	23	82
6	10	1.5	0.6	448	85.3	30	79
7	12	1.5	0.8	620	89	28	68
8	10	1.5	0.6	451	85.1	30	78
9	8	1	0.6	360	74.8	18	89.5
10	10	1.5	0.6	455	84.9	31	77
11	12	2	0.6	600	88.4	36	65
12	12	1	0.6	520	82.1	20	74
13	10	1	0.4	380	78	19	86
14	12	1.5	0.4	510	84.2	26	74
15	8	2	0.6	410	79.3	33	80
16	10	2	0.8	540	87	34	70
17	10	1	0.8	440	81	21	81

3.2.2 Effect of Independent Variables on Fiber Diameter

The fiber diameter ranged from 340 to 620 nm. Increasing PCL concentration significantly increased fiber diameter due to increased solution viscosity. Similarly, increasing core flow rate also increased fiber diameter. Chitosan concentration showed moderate influence on fiber diameter.

3.2.3 Effect of Independent Variables on Entrapment Efficiency

Entrapment efficiency ranged from 74.8% to 89%. Increasing PCL and chitosan concentration improved entrapment efficiency due to increased polymer matrix formation and drug encapsulation.

3.2.4 Effect of Independent Variables on Mucoadhesion Strength

Mucoadhesion strength ranged from 18 to 36 g. Chitosan concentration significantly increased mucoadhesion strength due to its bioadhesive nature.

3.2.5 Effect of Independent Variables on Drug Release

Drug release ranged from 65% to 90%. Increasing polymer concentration reduced drug release due to dense matrix formation.

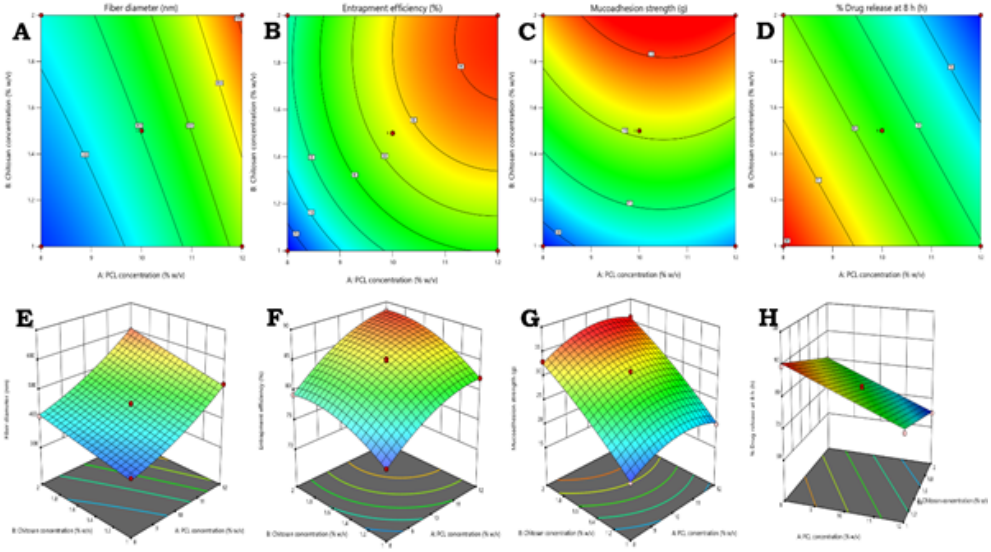

Figure 2. Contour plots (A–D) and three-dimensional response surface plots (E–H) showing the effect of formulation variables on (A, E) fiber diameter, (B, F) entrapment efficiency, (C, G) mucoadhesion strength, and (D, H) drug release at 8 h.

3.2.6 ANOVA Analysis

ANOVA results showed that the developed models were statistically significant ($p < 0.0001$).

Table 4. ANOVA results for response surface models

Source	Sum of Squares	df	Mean Square	F-value	p-value	
ANOVA Results for Fiber diameter (nm)						
Model	91787.73	9	10198.64	89.04	< 0.0001	significant
A-PCL concentration	63012.50	1	63012.50	550.12	< 0.0001	
B-Chitosan concentration	12012.50	1	12012.50	104.87	< 0.0001	
C-Core flow rate	14450.00	1	14450.00	126.15	< 0.0001	
AB	225.00	1	225.00	1.96	0.2038	
AC	100.00	1	100.00	0.8730	0.3812	
BC	100.00	1	100.00	0.8730	0.3812	
A^2	1795.46	1	1795.46	15.68	0.0055	
B^2	1.78	1	1.78	0.0155	0.9043	
C^2	41.78	1	41.78	0.3647	0.5649	
ANOVA Results for Entrapment Efficiency (%)						
Model	267.20	9	29.69	231.56	< 0.0001	significant
A-PCL concentration	138.61	1	138.61	1081.09	< 0.0001	
B-Chitosan concentration	62.16	1	62.16	484.82	< 0.0001	
C-Core flow rate	28.88	1	28.88	225.25	< 0.0001	
AB	0.8100	1	0.8100	6.32	0.0402	
AC	0.2025	1	0.2025	1.58	0.2492	
BC	0.0625	1	0.0625	0.4875	0.5076	

A^2	15.60	1	15.60	121.69	< 0.0001	
B^2	15.60	1	15.60	121.69	< 0.0001	
C^2	2.06	1	2.06	16.09	0.0051	
ANOVA Results for Mucoadhesion strength (g)						
Model	531.93	9	59.10	212.17	< 0.0001	significant
A-PCL concentration	15.13	1	15.13	54.29	0.0002	
B-Chitosan concentration	450.00	1	450.00	1615.38	< 0.0001	
C-Core flow rate	0.1250	1	0.1250	0.4487	0.5244	
AB	0.2500	1	0.2500	0.8974	0.3750	
AC	4.00	1	4.00	14.36	0.0068	
BC	2.25	1	2.25	8.08	0.0250	
A^2	30.69	1	30.69	110.19	< 0.0001	
B^2	3.80	1	3.80	13.64	0.0077	
C^2	20.38	1	20.38	73.16	< 0.0001	
ANOVA Results for % Drug release						
Model	730.69	3	243.56	303.57	< 0.0001	significant
A-PCL concentration	457.53	1	457.53	570.26	< 0.0001	
B-Chitosan concentration	195.03	1	195.03	243.08	< 0.0001	
C-Core flow rate	78.13	1	78.13	97.37	< 0.0001	

3.2.8 Optimization and Desirability Function

The optimized formulation was obtained using desirability function with desirability value of 0.786.

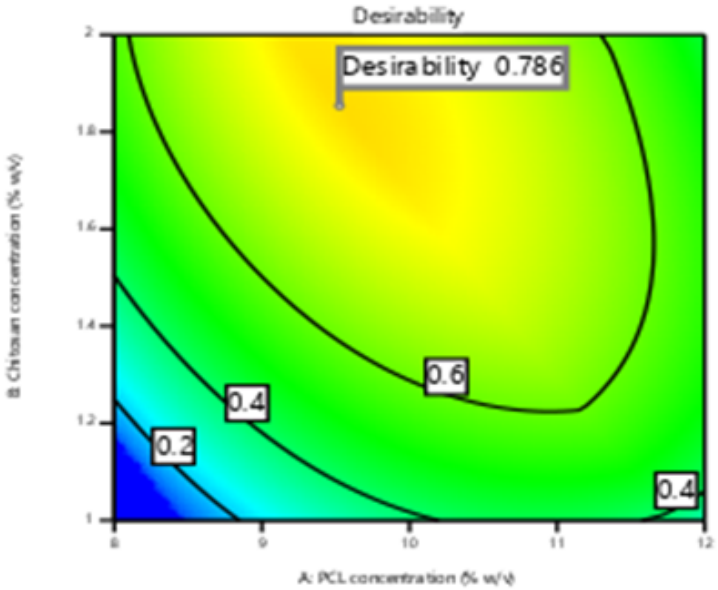

Figure 3. Desirability plot of optimized formulation

3.2.9 Validation of Optimized Formulation

Table 5. Predicted vs Experimental values

Response	Predicted	Experimental
Fiber diameter (nm)	435.74	434.50
Entrapment efficiency (%)	83.69	84.12
Mucoadhesion strength (g)	34.40	33.28
Drug release (%)	77.89	76.28

The experimental values were close to predicted values, confirming model validity.

3.3 Characterization of Nanofibrous Films

3.3.1 Physical Characterization

The prepared coaxial electrospun nanofibrous films were smooth, uniform, flexible, and free from visible defects such as cracks or air bubbles. The films appeared white to slightly translucent and showed uniform fiber distribution, indicating successful fabrication.

Table 6. Physical characterization of optimized nanofibrous films

Formulation	Thickness (mm)	Weight (mg)	Drug Content (%)
F1	0.20 ± 0.02	19.6 ± 0.8	97.5 ± 1.2
F2	0.21 ± 0.01	20.1 ± 0.7	99.1 ± 1.0
F3	0.22 ± 0.02	20.4 ± 0.6	98.6 ± 0.9

The prepared nanofibrous films exhibited smooth surface morphology and flexibility, indicating appropriate electrospinning conditions and polymer compatibility. The thickness of the films ranged from 0.20 ± 0.02 to 0.22 ± 0.02 mm, demonstrating uniform nanofiber deposition. Similarly, minimal weight variation (19.6 ± 0.8 to 20.4 ± 0.6 mg) indicated uniform polymer distribution within the nanofibrous matrix.

The drug content ranged from 97.5 ± 1.2% to 99.1 ± 1.0%, confirming efficient drug incorporation and minimal drug loss during electrospinning. These results suggest uniform formulation characteristics and consistent drug distribution, which are essential for controlled drug release and reproducible therapeutic performance. Overall, the findings confirmed successful fabrication of coaxial electrospun bioadhesive muco-invasive nanofibrous films suitable for localized mucosal drug delivery.

3.4 Morphological Characterization

3.4.1 Scanning Electron Microscopy (SEM)

SEM analysis revealed smooth, continuous, and randomly oriented nanofibers forming a uniform fibrous network. The fibers were bead-free with an average diameter of approximately **450 ± 25 nm**, indicating stable electrospinning conditions and successful formation of nanoscale fibers.

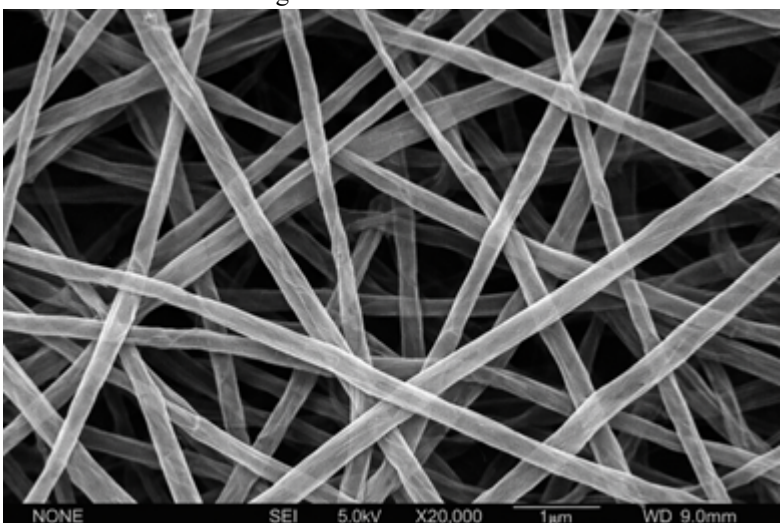

Figure 4. SEM micrographs of optimized coaxial electrospun 5-Fluorouracil-loaded nanofibrous films.

The smooth and bead-free morphology confirms appropriate polymer concentration and optimized electrospinning parameters. The nanoscale fiber diameter and interconnected structure are favorable for efficient drug loading and controlled drug release, supporting the suitability of the developed nanofibrous films for localized mucosal drug delivery.

3.5 Functional Evaluation

3.5.1 Swelling Index

The swelling behavior of the optimized nanofibrous films was evaluated in phosphate buffer (pH 6.8). The swelling index increased progressively with time and reached approximately 95% within 60 min, indicating good hydration capacity of the nanofibrous films. This swelling behavior may be attributed to the presence of hydrophilic polymers such as chitosan, which facilitate water absorption and polymer relaxation.

The enhanced swelling behavior is advantageous for improving mucoadhesion and facilitating drug diffusion. The hydrated polymer network promotes stronger interaction with the mucosal surface, thereby increasing residence time. These results suggest that the developed nanofibrous films possess suitable swelling properties for localized mucosal drug delivery.

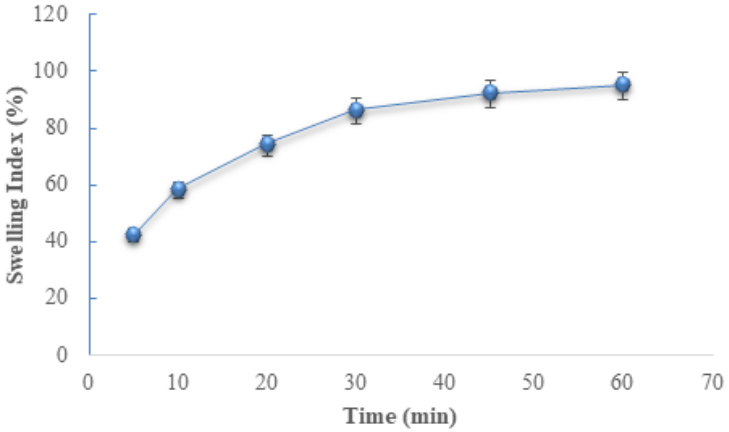

Figure 5. Swelling profile of optimized coaxial electrospun nanofibrous films in phosphate buffer (pH 6.8).

3.6 Drug Release Kinetics

The in-vitro drug release data were fitted to various kinetic models to determine the drug release mechanism. Among the models evaluated, the Higuchi model showed the highest correlation coefficient ($R^2 = 0.987$), indicating that drug release primarily followed a diffusion-controlled mechanism. The Korsmeyer–Peppas model showed a release exponent (n = 0.56), suggesting non-Fickian diffusion, where drug release occurs through a combination of polymer swelling and diffusion. These findings confirm that the optimized nanofibrous films provided controlled and sustained drug release suitable for localized mucosal drug delivery.

3.7 Ex-vivo Studies

3.7.1 *Ex-vivo* Mucoadhesion Study

The *ex-vivo* mucoadhesion study was performed using fresh goat buccal mucosa to evaluate the adhesion ability of the optimized nanofibrous films. The optimized formulation exhibited a mucoadhesion residence time of **4.5 ± 0.20 h**, indicating strong adhesion to the mucosal surface. The enhanced mucoadhesion may be attributed to the presence of chitosan, which promotes electrostatic interaction with mucin, along with the large surface area of the nanofibrous structure. These results suggest that the developed nanofibrous films possess adequate residence time for effective localized mucosal drug delivery.

3.7.2 *Ex-vivo* Permeation Study

The *ex-vivo* permeation study was conducted using Franz diffusion cells with goat buccal mucosa. The optimized nanofibrous films showed gradual and sustained drug permeation, reaching **76.8 ± 2.7%** within 8 h. The steady-state flux and permeability coefficient were found to be **0.82 mg/cm²/h** and **0.041 cm/h**, respectively.

The sustained permeation profile may be attributed to the nanofibrous structure providing high surface area and the presence of chitosan acting as a permeation enhancer. These findings indicate effective drug diffusion across the mucosal membrane, supporting the suitability of the developed formulation for localized mucosal delivery.

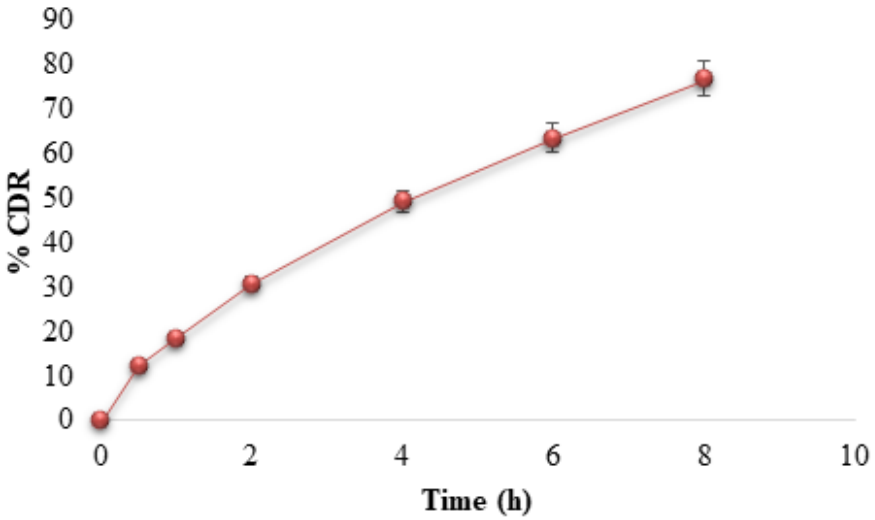

Figure 6. *Ex-vivo* permeation profile of 5-Fluorouracil from optimized coaxial electrospun nanofibrous films across goat buccal mucosa.

3.8 Stability Studies

Stability studies of the optimized nanofibrous films were conducted according to ICH Q1A (R2) guidelines under accelerated (40 ± 2°C / 75 ± 5% RH) and long-term (25 ± 2°C / 60 ± 5% RH) conditions for three months. The samples were evaluated for physical appearance, drug content, and in-vitro drug release at predetermined intervals.

Table 7. Stability study results of optimized nanofibrous films under accelerated and long-term conditions

Storage Condition	Time (Months)	Physical Appearance	Drug Content (%)	Drug Release at 8 h (%)
Accelerated	0	Smooth and uniform	99.1 ± 1.0	78.2 ± 2.1
	1	No change	98.7 ± 1.2	77.6 ± 2.3
	2	No change	98.3 ± 1.4	77.1 ± 2.5
	3	No change	97.9 ± 1.6	76.8 ± 2.6
Long-term	0	Smooth and uniform	99.1 ± 1.0	78.2 ± 2.1
	1	No change	99.0 ± 1.1	78.0 ± 2.2
	2	No change	98.8 ± 1.2	77.8 ± 2.3
	3	No change	98.5 ± 1.3	77.6 ± 2.4

The optimized nanofibrous films remained physically stable with no significant changes in appearance under both storage conditions. Drug content showed minimal variation over three months, indicating no significant degradation of 5-Fluorouracil. Similarly, the drug release profile remained consistent, confirming the stability of the polymeric nanofiber matrix. These results demonstrate that the optimized nanofibrous films possess adequate physical and chemical stability for storage and pharmaceutical application.

4 CONCLUSION

The present investigation successfully developed and optimized coaxial electrospun bioadhesive muco-invasive nanofibrous films containing 5-Fluorouracil for localized mucosal cancer therapy. The drug exhibited pH-dependent solubility and hydrophilic characteristics, which facilitated uniform distribution within hydrophilic polymer matrices.

A reliable UV–Visible spectrophotometric method was developed for drug estimation, and compatibility studies confirmed that no chemical interaction occurred between the drug and selected polymers. The formulation variables were systematically optimized using Box–Behnken Design to obtain nanofibrous films with desirable characteristics.

The optimized formulation containing 9.46% PCL, 1.85% chitosan, and a core flow rate of 0.51 mL/h produced nanofibers with an average diameter of approximately 435 nm, high entrapment efficiency (~84%), strong mucoadhesion (~33 g), and controlled drug release (~76% within 8 hours). Morphological analysis confirmed the formation of smooth, bead-free nanofibers with uniform structure. Swelling studies demonstrated excellent hydration capacity, which enhances mucoadhesion and facilitates drug diffusion.

Drug release kinetic analysis indicated diffusion-controlled release following the Higuchi model with non-Fickian transport behavior. *Ex-vivo* permeation studies demonstrated sustained drug permeation across buccal mucosa, confirming the ability of the nanofibrous system to deliver the drug effectively at the mucosal site. Stability studies conducted under ICH conditions confirmed the physical and chemical stability of the optimized formulation.

Overall, the developed coaxial electrospun nanofibrous films represent a promising localized drug delivery system for mucosal cancer therapy, offering improved drug retention, controlled release, and enhanced mucosal permeation, which may contribute to improved therapeutic efficacy and reduced systemic toxicity.

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7 CONFLICT OF INTEREST

The authors declare no conflict of interest.

8 AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, data analysis, and writing—original draft preparation were performed by the primary author. Supervision, review, and editing were carried out by the corresponding author. All authors read and approved the final manuscript.

9 DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

10 ETHICAL APPROVAL

Goat buccal mucosa was obtained from a local slaughterhouse, and no live animal experimentation was conducted.

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