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Optimizing Doxycycline delivery through Chitosan Polyelectrolyte Complex films: Development and Characterization

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Abstract:
In this study, doxycyclineloaded chitosan polyelectrolyte complex films were developed using xanthan gum and pectin to achieve controlled drug release for biomedical applications. The polyelectrolyte complexes were characterized through various tests, including physicochemical analysis, moisture content, swelling index, water vapor transmission, drug content, and Fourier Transform Infrared Spectroscopy (FTIR). The *in vitro* release of doxycycline was examined in pH 7.4 buffer at 37°C. The release data were interpreted using various kinetic models, with the Korsmeyer-Peppas model showing the best fit. The doxycyclineloaded PEC films demonstrated good antibacterial activity against*Staphylococcus aureus*. The prepared films were appropriate for slow and sustained release of doxycycline over a 24-hour period, deemed to be ideal for use as antimicrobial wound dressings.
Keywords: Doxycycline, chitosan,polyelectrolyte, drug release.

1. Introduction:

Wound healing is a complex biological process involving tissue repair and regeneration. While acute wounds typically follow a predictable healing path, chronic wounds require extended treatment and specialized care. A wide range of wound dressings, ranging from traditional materials to advanced solutions, has been developed to protect wounds and enhance the healing process [1]. Among these, hydrogel dressings have emerged as a cornerstone of advanced wound care due to their exceptionalcharacteristics. Hydrogels protect wounds, retain moisture, facilitate cell migration and proliferation, promote tissue regeneration, and closely mimic the natural extracellular environment [2]. Hydrogels are cross-linked polymeric, three-dimensionalnetworks that can absorb and retain substantial amounts of water, resulting in soft and elastic materials [3]. They can be synthesized from either natural or synthetic polymers, with the natural ones offering superior biocompatibility and creating an optimized healing environment with reduced immune response risk [4]. Additionally, hydrogels can load several bioactive compounds, enabling the targeted delivery of drugs, antimicrobial agents, and growth factors directly to the wound site [5-7].

Doxycycline, a tetracycline antibiotic, demonstrates antibacterial efficacy against both Gram-positive and Gram-negative bacteria by binding to bacterial ribosomes and inhibiting synthesis of proteins [8]. Beyond its antimicrobial action, doxycycline inhibits matrix metalloproteinases (MMPs) and TNF-α-converting enzyme (TACE), which is crucial for wound healing. Recent research highlights the drug's potential to improve wound healing through low-dose applications, with topical doses up to 10 g/mL showing no toxic effects [9,10].

Polyelectrolyte complexes (PECs) are formed through ionic interactions between oppositely charged polyanions and

polycations, resulting in networks with unique properties distinct from their original components. The stability and formation of PECs depend on several factors, including ionization degree, charge density, mixing ratios, pH, temperature, and ionic strength [11-13].

Chitosan, a natural cationic polysaccharide derived from chitin, is well-known for its biodegradability, biocompatibility, and ability to form PECs through electrostatic interactions. When combined with other polymers or crosslinked, chitosan exhibits enhanced water-retention and physicochemical properties [14]. It interacts with various natural and synthetic polyanions, including alginate, carrageenan, carboxymethyl cellulose, and poly(acrylic acid), forming stable PECs [15]. Xanthan gum, a hydrophilic, natural, anionic polysaccharide derived from bacterial sources, forms PECs with chitosan through strong but reversible ionic interactions. The properties of these complexes can be fine-tuned by modifying formulation parameters and preparation conditions [16]. Similarly, pectin, a low-toxicity anionic polysaccharide extracted from fruits, interacts with chitosan to form PECs. The stability of these complexes depends on environmental factors such as pH, temperature, and ionic strength [17].

This study aims to explore the preparation and evaluation of filmscomposed ofchitosan-based PECs to achieve controlled release of doxycycline, enhancing wound healing and therapeutic outcomes.

2. Materials:

All chemical reagents employed in this study were of analytical grade. Doxycycline hyclate was sourced from Merck Chemicals. Chitosan, xanthan gum, and pectin were acquired from Loba Chemie Pvt. Ltd., Mumbai, India. Throughout the investigation, double-distilled water was used.

3. Methods:

3.1. Preparation of polyelectrolyte complex films:

Films were prepared using the polyelectrolyte complexation followed by solvent casting. Xanthan gum and pectin were separately dissolved in distilled water to prepare 2% w/v solutions. Chitosan was dispersed in 1% v/v acetic acid to makesolutions ranging from1-2% w/v solution. Chitosan solution (100 mL) was added in small amounts to the xanthan gum or pectin solution (100 mL) while stirring continuously. Glycerol (0.3 g/g of total polymer) was then added to the mixtures as a plasticizer.The resulting mixtures were then poured into petri dishes of9 cm diameter and dried at 40-45°C for 48 hours. To ensure sterility, the membranes were subjected to UV light exposure for 10-15 minutes. Table 1 provides the detailed composition of all the film formulations.

Table 1: Composition of PEC films

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6
Chitosan	1	1.5	2	1	1.5	2
Xanthan gum	2	2	2	-	-	-
Pectin	-	-	-	2	2	2

3.2. Preparation of drug loaded PECfilms:

Freshly prepared PEC films were immersed in a solution containing doxycycline at a concentration of 3% w/v in distilled water. The membranes were soaked in this solution for 12 hours maintained at 4°C to achieve equilibrium drug loading.To reduce the burst effect, the drug-loaded films were slightly rinsed with distilled water to eliminate any loosely bound excess drug on the surface [18]. After the loading process, the drug-loaded films were dried to a constant weight and stored at 4°C in air-tightcontainers. Throughout the process, the samples were kept in the dark to prevent degradation.

3.3. Thickness, Weight Variation and Folding endurance:

The thickness of the films was measured with a digital micrometer, taking readings at five different points on each sample. The results are reported as the mean ± standard deviation (SD). For the weight variation test, each 3×3 cm^2 film was individually weighed, with the results presented as the mean ± SD of five measurements. Folding endurance was evaluated by folding the film repeatedly at the same location until it broke or showed visible signs of damage. The number of folds before failure was recorded as the folding endurance value. This test was conducted on five random samples and the results are reported as the mean value [19].

3.4. pH:

To determine the pH, the film samples were added to 10 mL of water, mixed, and allowed to rest for 2 hours. The mixture was then evaluated using a digital pH meter. Each sample was analyzed in triplicate, and the results are presented as mean ± SD.

3.5. Moisture content:

The prepared films were weighed individually (initial weight) and then stored in a desiccator maintained at 37°C for 24 hours, using silica gel as the desiccant. The films were monitored until no further weight change was observed, and the final weight was recorded. The percent moisture content was calculated using Equation 1 [20]:

$$\text{Moisture Content (\%)} = \frac{(\text{Initial Weight} - \text{Final Weight})}{\text{Initial Weight}} \times 100 \quad \textbf{Eq. 1}$$

3.6. Swelling Index:

Films (1cm^2) were dried at 50-60°C in a hot air oven until a persistent weight (W_i) was attained. The dried films were immersed in distilled water at 37°C and weighed every 2 hours until constant weight was achieved (W_f). Before weighing, excess liquid on the samples was gently removed with filter paper. The immersion medium was checked and refreshed to ensure the samples remained fully immersed. The swelling index (SI) was calculated using the following Equation 2[21]:

$$S.I\ (\%) = \frac{(W_f - W_i)}{W_i} \times 100 \quad \textbf{Eq. 2}$$

3.7. Water vapour transmission test:

Each film was mounted on the opening of a test tube containing 10 mL of distilled water. The test tubes with the mounted films were pre-weighed and kept in a desiccator with calcium chloride as a desiccant. After 24 hours, the test tubes were re-weighed. To ensure accuracy, the experiment was conducted in triplicate. The water vapour transmission rate (WVTR) was calculated using Equation 3 [22]:

$$WVTR = \frac{(W_i - W_f)}{A \times t} \quad \textbf{Eq. 3}$$

Where W_i and W_f are the weights of the test tube along with the film before and after being placed in the desiccator, respectively, A^2 is the area of the film (m^2) and t is the time duration (in days).

3.8. Drug content:

A pre-weighed sample of the film was immersed in 100 mL of phosphate buffer (pH 7.4) and agitated for 5-6 hours to ensure complete drug extraction. The resulting solution was filtered to remove any undissolved particles, and the drug content was quantified using a UV-Visible spectrophotometer at 274 nm.

3.9. *In vitro* drug release studies:

The *in vitro* release of drug from the films was studied using a USP Type II dissolution test apparatus (Paddle type) following a modification of method adapted by Chopra *et al* [23]. Films (2×2 cm^2) were affixed between a stainless-steel mesh screen and a glass slide. The assembly was left at the bottom of the jar, with mesh screen side facing the paddle. 100 mL pH 7.4 phosphate buffer was used as the dissolution medium. The test was conducted at 37 ± 0.5°C with 50 rpm paddle rotation speed. Aliquot samples (2 mL) were collected at predetermined time intervals, and the concentration of the drug released from the film was estimated using a UV-Visible spectrophotometer at 274 nm. After each withdrawal, the dissolution medium was replenished with fresh phosphate buffer to maintain a constant

volume. The experiment was conducted in triplicate, and the results were expressed as mean±SD. The drug release data were analysed using various kinetic models to elucidate the release mechanism:

- Zero-order model: $A_t = A_0 + kt$
- First-order model: $\ln A_t = \ln A_0 - kt$
- Higuchi model: $A_t = kt^{1/2}$
- Hixson Crowell model: $A_r^{1/3} = kt$
- Korsmeyer-Peppas model: $A_t/A = kt^n$

Where A_0 is the initial amount of drug in the formulation, A_t is the amount of drug released at time t, A_r is the remaining amount of drug in the formulation at time t, A is the total amount of drug released, k is the kinetic constant, n is the release exponent in the Korsmeyer-Peppas model [24]

3.10. Fourier-Transform Infrared Spectroscopy (FTIR):

FTIR was used to analyze the interactions between the different components of the films. Dried films were subjected to FTIR analysis using a Bruker IR spectrometer and the spectra were recorded in the range of 4000–650 cm^{-1} at a resolution of 4 cm^{-1}.

3.11. Stability studies:

The stability of doxycycline in the films was assessed under controlled conditions of 4°C and 25°C,75 ± 5% relative humidity for a period of up to 3 months. The film samples were wrapped in aluminium foil to protect them from light and placed in a stability chamber set to the specified conditions. Samples were withdrawn at 1-month intervals (after 1, 2, and 3 months) and evaluated for folding endurance, moisture contentand drug content, to determine the stability and integrity of the films over time.

3.12. Antibacterial Activity:

The antibacterial activity of films was estimated by the disc diffusion assay against a Gram-positive bacterial species (*Staphylococcus aureus*). Sterile agar media was inoculated with the bacterial suspension using the pour plate method. Film samples of 0.5-0.6 cm diameter were placed over the surface of the inoculated agar. Standardized doxycycline discs (50 µg/mL) were used as the positive control and plain paper discs were used as the negative control. The test plates were placed in a BOD incubator and incubatedat 37°C for 24 h. After 24 hours, each sample was carefully evaluated and measured to determine the diameter of the zone of inhibition. This measurement provided insight into the level of antibacterial activity of the films against the tested strain. The assay was conducted in triplicate.

4. Results and discussion:

Using natural polymers as polyelectrolyte complexes in wound dressings results in a synergistic effect that enhances the properties of the material. The type of polymer, its concentration, and the addition of the drug have significantly influenced the characteristics of the films. In this study, chitosan was combined with either xanthan gum or pectin, along with doxycycline. All samples demonstrated a uniform and homogeneous appearance, with the films being dark yellow and opaque in colour.

4.1. Thickness, Weight Variation and Folding endurance:

The prepared films were subjected to various physicochemical tests, as summarized in Table 2. The results demonstrated that thickness ranged from 0.71±0.05 mm to 0.81±0.06 mm while weight variation was found to range between

0.61 ± 0.03 g and 0.75 ± 0.03 g. An increase in the concentration of chitosan was associated with an improvement in the folding endurance, which ranged from 305.24 ± 9.75 to 380.09 ± 12.91 folds.

4.2. pH: The pH of the films ranged from 3.38±0.054 to 4.17±0.067. The values of pH are presented in Table 2. Table 2: Physico chemical evaluation of drug loaded PEC films

Formulation Code	Thickness (mm)	Weight Variation (g)	Folding endurance	pH
F1	0.75±0.07	0.66±0.08	331.97±6.93	4.17±0.067
F2	0.77±0.23	0.61±0.03	350.61±10.28	3.71±0.059
F3	0.71±0.05	0.68±0.1	380.09±12.91	3.38±0.054
F4	0.81±0.06	0.69±0.05	305.24±9.75	3.84±0.142
F5	0.74±0.09	0.62±0.02	337.53±4.04	3.66±0.135
F6	0.78±0.08	0.75±0.03	358.92±23.47	3.41±0.054

4.3. Moisture content:

The moisture content of the films was observed to increase with higher chitosan concentration, with values ranging from 28.96±1.4 to 37.73±2.38%. The moisture content values are detailed in Table 3and Figure 1.

4.4. Swelling index:

The swelling index, which measures the fluid uptake capacity of hydrogel films, a key factor in their wound healing properties, was evaluated. It was observed that the swelling index increased as the concentration of chitosan in the hydrogel films was raised. The swelling index values ranged from99.17±2.07 to979.34±15.29%. The results of the swelling index values for different formulations are presented in Table 3 and Figure 1.

4.5. Water vapour transmission test:

Water Vapor Transmission Rate (WVTR) is a measure of the moisture penetration through the film. Table 3 and Figure 1 presents the WVTR analyses for different batches of hydrogel films. An increase in WVTR was observed with an increase in chitosan content from F1 to F3 and from F4 to F6. The WVTR values ranged from 560.299±21.49 to 661.296±24.94 g/m²/day.

4.6. Drug content:

The drugloaded films were prepared using the equilibration method. When PEC films were immersed in an aqueous solution of doxycycline, the drug penetrated the PEC matrix along with water molecules until equilibrium was established between the solution and the swollen film matrix. The drug content of prepared films ranged from 34.169±1.468 to 47.268±2.033 mg/gram of film. The results are presented in Table 3 and Figure 1.

Table 3: Characterization of films

Formulation Code	Moisture Content (%)	Swelling index (%)	Water vapour transmission rate (g/m²/day)	Drug content (mg/g of film
F1	30.91±2.32	764.04±7.81	567.136±23.92	34.675±1.264
F2	34.28±2.29	864.87±12.19	640.689±10.83	39.749±1.969
F3	37.73±2.38	979.34±15.29	661.296±24.94	47.268±2.033
F4	28.96±1.4	99.17±2.07	560.299±21.49	34.169±1.468
F5	32.74±0.68	110.96±2.31	625.749±5.99	36.913±2.711
F6	35.72±0.7	115.23±1.78	631.005±13.57	42.203±2.466

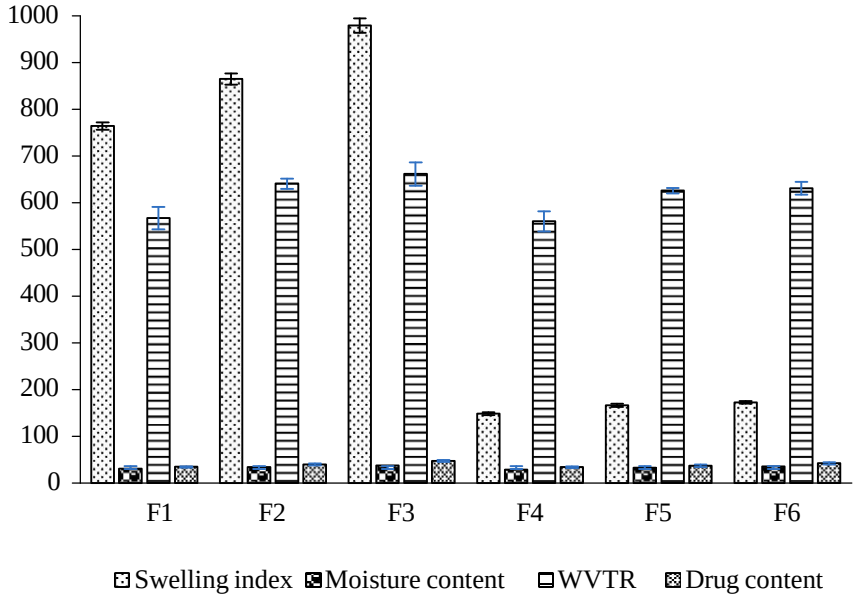


Figure 1: Evaluation of drug loaded PEC films

4.7. *In vitro*drug release studies:

The *in vitro* drug release from various batches of the formulated films indicated a controlled release of doxycycline over an extended period of 24 hours, which is crucial for its effective wound healing properties. Increasing the concentration of chitosan from F1 to F3 and from F4 to F6 hydrogel film batches significantly slowed the release of the drug. The intermolecular networking between chitosan and xanthan gum or pectin formed a strong matrix, which further retarded drug release. The drug release data is presented in Figure 2.The *in vitro* drug release data were fitted to several release models, including zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer–Peppas models (Table 4). The drug release from all the films was observed to follow first order kinetics. The regression coefficients (R^2) for all formulations indicated that the Korsmeyer–Peppas model was the best fit. For all formulations, the n value was found to be below 0.5, suggesting Fickian diffusion as the mechanism of drug release.

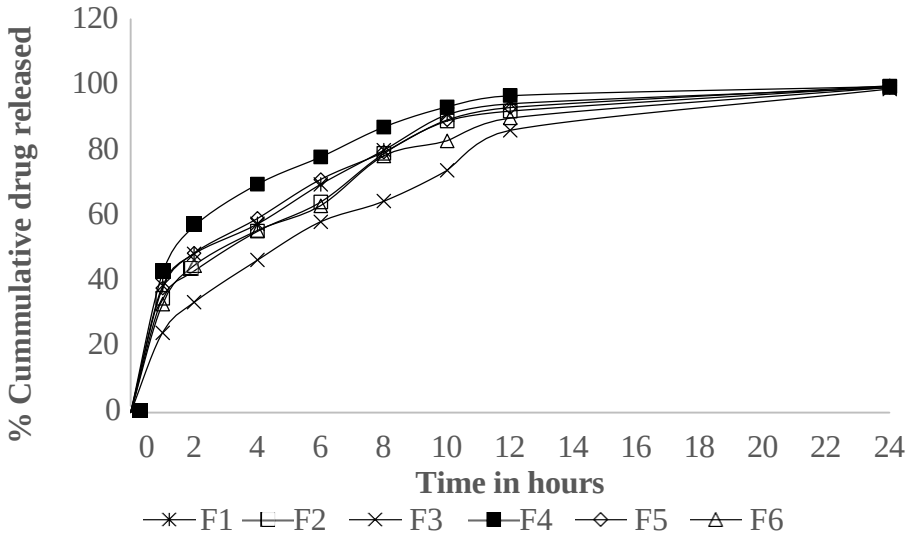


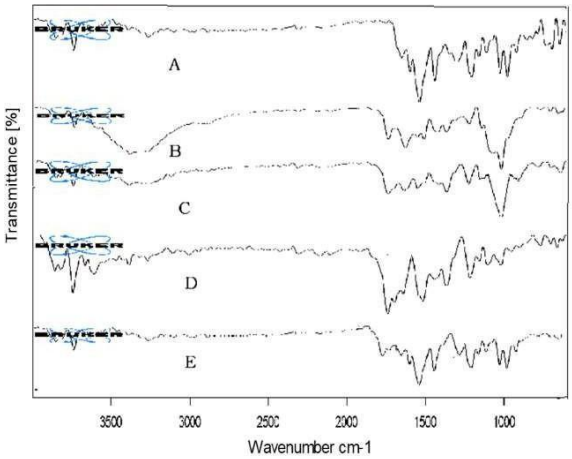
Figure 2: *In vitro* drug release profile of drug loaded PEC films

Table 4: Release kinetics of drug loaded PEC films

Formulation Code	Zero-order		First-order		Higuchi	Hixson Crowell	Korsmeyer-Peppas	
	k	R^2	k	R^2	R^2	R^2	R^2	N
F1	3.472	0.652	0.198	0.983	0.906	0.904	0.963	0.329
F2	3.60	0.692	0.22	0.99	0.927	0.947	0.966	0.367
F3	3.77	0.804	0.176	0.977	0.976	0.983	0.988	0.464
F4	3.255	0.564	0.218	0.978	0.854	0.863	0.95	0.285
F5	3.463	0.656	0.236	0.988	0.911	0.947	0.973	0.33
F6	3.545	0.706	0.195	0.989	0.938	0.958	0.977	0.365

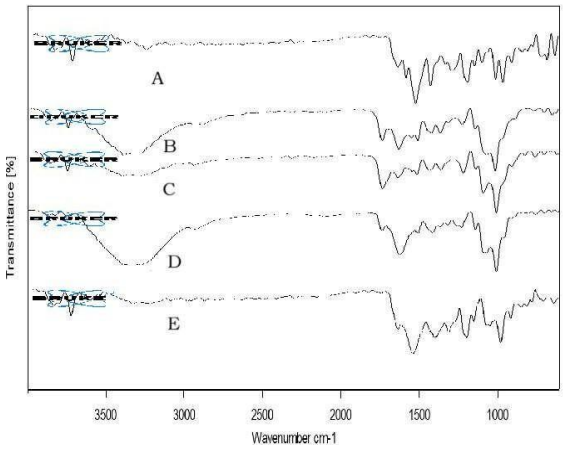
4.8. FTIR:

FTIR analysis of the drug, chitosan, xanthan gum, pectin, and drug-loaded PEC films, as shown in Figure 3 and 4, revealed characteristic absorption peaks. Doxycycline displayed absorption peaks at 3003 cm^{-1} (C-H stretching), 1760 cm^{-1} (C=O stretching), and 1609 cm^{-1} (N-H bending). Chitosan exhibited main absorption peaks at 1630 cm^{-1} (C=O amide) and 1555 cm^{-1} (N-H bending). Xanthan gum had absorption peaks at 1740 cm^{-1} (C=O stretching) and 1640 cm^{-1} (–COO– stretching), while pectin showed peaks at 1735 cm^{-1} (C=O stretching) and 1635 cm^{-1} (–COO– stretching). In the FTIR spectrum of the F3 formulation, shifted peaks were observed at 1517 cm^{-1} (N-H bending) and 1635 cm^{-1} (– COO– stretching). Similarly, in the F6 formulation, shifted peaks appeared at 1507 cm^{-1} (N-H bending) and 1620 cm^{-1} (–COO– stretching). These shifts are likely due to the interaction of NH_3 and COOH groups, indicating the formation of a PEC between chitosan and xanthan gum or pectin. Additionally, the FTIR spectra of F3 and F6 showed no significant shifts or reduction in the intensity of doxycycline-related bands, confirming their compatibility.



(A) Drug (B) Chitosan (C) Xanthan gum (D) Chitosan-Xanthan gum PEC film (E) Drug loaded PEC film F3

Figure 3: FTIR spectra of F3

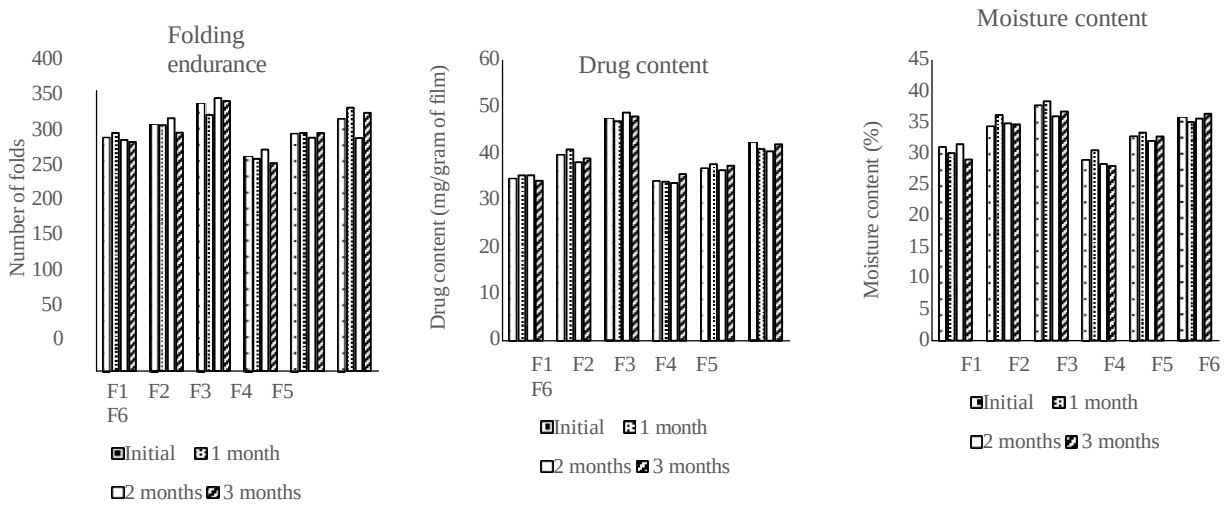


(A) Drug (B) Chitosan (C) Pectin (D) Chitosan-Pectin gum PEC film (E) Drug loaded PEC film F6

Figure 4: FTIR spectra of F6

4.9. Stability studies:

The stability of the F3 and F6 films was evaluated during storage at 4°C and 25°C with 75 ± 5% relative humidity. Over a period of three months, folding endurance, moisture content, and drug content were measured monthly. The comparative results of stability data of folding endurance (A), drug content (B)and moisture content (C) are presented in Figures 5. The results indicated no major changes in these parameters compared to the initial values.



(A) (B) (C)

Figure 5: Comparative stability data

4.10. Antibacterial activity:

The antibacterial activity of the hydrogel films against *Staphylococcus aureus* was evaluated. Figure 6 shows the zone of inhibition for formulations F3, F6. The hydrogel film (F3) demonstrated good antimicrobial activity. The diameter of the zone of inhibition for the F3 and F6 hydrogel films against *Staphylococcus aureus* measured 19.27 ± 0.21 mm and
17.37 ± 0.15 mm, respectively.

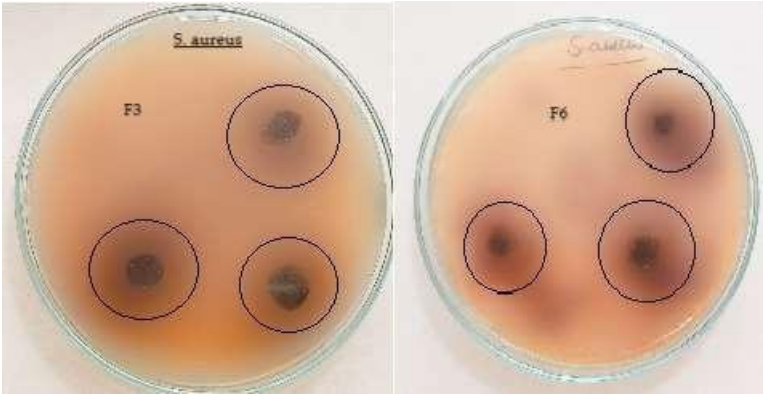


Figure 6: *In vitro* antimicrobial activity of F3 and F6against *Staphylococcus aureus*

5. Conclusion:

Chitosan-based polyelectrolyte complex hydrogel films loaded with doxycycline were successfully formulated for potential wound healing applications. These films demonstrated ideal properties for wound dressings, including excellent swelling, moisture uptake, and mechanical strength. FTIR studies were conducted to examine the drug-polymer interactions and the formation of PECs in the hydrogel films. The *in vitro* release of doxycycline from the films showed a sustained release profile. The developed films exhibited good antibacterial activity, indicating their effectiveness in wound healing. The results suggest that the formulation could be an efficient and controllable drug delivery system for wound healing applications.

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