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Design of Chitosan-Peo Hybrid Films As Wound Dressings: A Study On Sustained Doxycycline Release

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Abstract:
Wound healing is a multifaceted biological process that requires optimal conditions for effective tissue repair and regeneration. This study investigates the design and performance of chitosan-polyethylene oxide (PEO) hybrid films as innovative wound dressings, focusing on the sustained release of doxycycline. Chitosan, exhibits biocompatibility, biodegradability, and antimicrobial properties, making it an ideal candidate for wound management. When combined with polyethylene oxide, the hybrid films showed enhanced flexibility, and controlled drug release profiles. Doxycycline was efficiently incorporated into the chitosan-PEO films using solvent casting, resulting in a prolonged and controlled release pattern. All evaluated physicochemical parameters, including weight variation, thickness, folding endurance, drug content, swelling index, and water vapor transmission rateyielded acceptable results. *In vitro* studies confirmed that the hybrid films were consistently releasing doxycycline over a 48-hour period, ensuring sustained antimicrobial activity. Among all formulations, F4 demonstrated optimal evaluation results, making it the preferred choice for wound dressing applications. FTIR and DSC studies confirmed the absence of drug-excipient interactions in the final formulation. Surface morphology analysis revealed that the films exhibited a smooth and homogeneous surface. Stability studies indicated good drug stability during storage.
Key words: Wound dressing. Chitosan, PEO, Doxycycline

1. Introduction:

The skin is the largest and most important barrier in the human body against external pathogens. However, wounds may arise from localised tissue damage due to external mechanical forces, burns, cuts, chemical exposure, high temperatures, surgical interventions, or disease outcomes[1]. Wounds can be either acute or chronic based on the mechanism and duration of healing. Healing of acute wounds occurs within a predictable time period of 8 to 12 weeks based on the level and extent of the injury[2]. Chronic or non-healing wounds fail to heal for prolonged periods even under treatment conditions and may persist for many months or years. Chronic wounds are generally painful and debilitating, often associated with underlying pathological conditions that contribute to impaired healing. Venous stasis ulcers, pressure ulcers and diabetic foot ulcers are examples of chronic wounds[3]. The most common challenge in the chronic wound healing process is the risk of infection or sepsis, which is mostly preventable by the use of antimicrobial agents[4]. Application of higher doses and cyclic use of antibiotics might cause systemic toxicity, and may favour the development of antibiotic resistance. Hence, control of bioburden also becomes an important aspect of wound management[5].

Topical wound dressings serve as an important therapeutic tool for wound healing. Wound dressings applied over the affected area provide protection against further damage and microbial invasion as well as maintaining optimal conditions

for wound healing with minimised scar formation. Wound dressings must be biodegradable and biocompatible, as they should encourage the native cells to grow. They should be able to withstand mechanical stress during implantation. They should accurately mimic the physical structure of normal skin[6,7]. There are different types of wound dressings which can be used, based on the type of wound such as traditional gauze wound dressings, moist occlusive films or semipermeable membranes, hydrocolloids, hydrogels, alginate dressings, seaweed derived non-woven fibres, foams and collagen products[8-10].

Antimicrobial wound dressings are able to provide sustained release of topical antibiotics or antiseptics at the wound surface and help in providing prolonged antimicrobial action along with maintenance of an ideal healing environment[3]. Doxycycline is one of the broad-spectrum antibiotics of tetracycline class with well-known antimicrobial activity. Its unique property of inhibiting the activity of matrix metalloproteinases and tumour necrosis factor-α converting enzymes is crucial in promoting a favourable environment for wound healing[11]. Topical administration of doxycycline is reported to exhibit favourable results in skin wound healing that are not possibly achieved by oral dosing such as reducing scar thickness[12], modulation of tissue levels of collagen in cutaneous repair [13] and inhibiting the release of tumour necrosis factor-alpha (TNF-α) and activity of matrix metalloproteinases[14].

Biopolymers have gained significant attention in the field of wound healing due to their remarkable properties, providing effective and versatile solutions for various types of wounds[15]. Wound dressings can be produced using natural or synthetic biomaterials in different forms such as hydrogels, nanofibrous mats, sponges, films, foams, etc. Natural polymers include polysaccharides such as alginates, celluloses, chitosan, hyaluronic acid, etc., and polypeptides, such as collagen and gelatin. Synthetic biomaterials include poly(ε-caprolactone) (PCL), poly(ethylene oxide) (PEO), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(vinyl alcohol) (PVA), and poly(vinylpyrrolidone)(PVP)[7]. Hybrid polymers are a combination of natural and synthetic polymers that carry the unique properties of both types of polymers. Chitosan-PVA, chitosan-PCL, collagen-PVA and alginate-PEO are some examples of hybrid materials used in wound dressings. The synergism and compatibility of combinations of natural and synthetic biopolymers are advantageous for biomedical applications[16].

Chitosan (CS), a deacetylated derivative of chitin, is widely used in wound dressings due to its hemostatic, antibacterial, fungistatic, and biocompatible properties[17.] It promotes wound healing by regulating cell differentiation, proliferation, and cytokine production[18]. However, its limitations, such as poor solubility in neutral aqueous media and inadequate mechanical properties, have led researchers to enhance its performance by blending it with polymers like polyethylene oxide (PEO)[19].PEO, awater-soluble polymer with low toxicity and good biocompatibility, has been used in tissue sealants and wound dressings[20,21]. Mixing two different polymers, like chitosan and polyethylene oxide (PEO), to prepare films, will have better physical and mechanical properties than films made from just one polymer. Adding chitosan to PEO makes the PEO films stronger with more consistent properties[22].

Therefore, the present study investigated the development of films composed of chitosan and PEO, incorporating doxycycline as an antimicrobial agent. This formulation offers a promising solution for advanced wound healing applications by combining the beneficial properties of these polymers to enhance functionality and effectiveness.

2. Materials:

Medium molecular weight chitosan with deacetylation degrees (DD) of 75–85% and glacial acetic acid were purchased from Loba ChemiePvt. Ltd., Mumbai, India. Polyethylene oxide (Molecular weight: 3,00,000 Daltons) was purchased from Thermo Fisher Scientific Pvt Ltd, India. Doxycycline was purchased from TCI Chemicals, Chennai, India. All other chemicals were of analytical grade obtained from Loba ChemiePvt. Ltd., Mumbai, India.

3. Methods:

3.1. Preparation of films:

Chitosan solution (2% w/v) was prepared in 1% v/v glacial acetic acid, while 5% w/v PEO was prepared in distilled water at room temperature (27°C) by stirring at 500 rpm for 5-6 hours. Films were fabricated using the solvent casting technique, with 30% w/w glycerol (based on polymer content) as a plasticizer. Appropriate amount of drug was added to the polymeric mixture and the dispersion was stirred under dark conditions at room temperature for 5-6 hours. The dispersions were cast onto 9 cm diameter petri dishes and dried at room temperature under dark conditions for 72 hours. The films were then stored in aluminium foil at 4°±2°C for further evaluation. The composition of the drug loaded films is given in Table 1.

Table 1: Composition of drug loaded films

Ingredients (%w/w to the total polymer content)	F1	F2	F3	F4	F5	F6	F7
Drug	2	2	2	2	2	2	2
Chitosan	20	30	40	50	60	70	80
PEO	80	70	60	50	40	30	20
Glycerol	30	30	30	30	30	30	30

3.2. Characterization of films:

Drug loaded films were characterised by assessment of uniformity of weight, thickness, folding endurance, drug entrapment efficiency, swelling index, water vapour transmission rate, *in vitro* drug release, FTIR, DSC, surface morphology and *invitro* microbiological testing.

3.2.1. Uniformity of Weight:

Films were cut into 1×1 cm² strips, and five strips were randomly selected for evaluation. Each strip was weighed individually, and the average weight (mg) along with the standard deviation was calculated to assess uniformity.

3.2.2. Thickness:

The thickness of the film was measured using a Vernier callipers. Five random locations on the film were chosen, and the average thickness (mm), along with the standard deviation, was determined.

3.2.3. Folding Endurance:

Folding endurance was assessed by folding 2×2 cm² film strips at the same spot until they broke. Five randomly selected samples were tested, and the folding endurance value was reported as the average number of folds ± SD.

3.2.4. Drug entrapment efficiency:

Drug entrapment efficiencywas assessed using five randomly selected film samples. Each sample was crushed with a mortar and pestle and made into a uniform mixture using 50 mL of pH 7.4 phosphate buffer. The mixture was stirred for 2 hours and filtered through a 0.45 µm membrane filter. The amount of drug was calculated by measuring the absorbance of the filtrate at 274 nm using a UV-Visible spectrophotometer. Drug entrapment efficiency (%) was calculated using Equation 1.

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Measured amount of drug in the film}}{\text{Theoretical amount of drug added}} \times 100 \quad \text{Eq. 1}$$

3.2.5. Swelling index[23]:

The swelling properties of the prepared films were assessed in a pH 7.4 phosphate buffer solution. Initially, three randomly selected film strips were weighed, and their initial weights were noted. The strips were then suspended in 100 mL of the pH 7.4 phosphate buffer at room temperature. After 8 hours, the swollen samples were removed, excess buffer was blotted off using filter paper, and the strips were reweighed to determine their final weights. The swelling index (%) was calculated using Equation 2.

$$\text{Swelling index (\%)} = \frac{(\text{Weight of wet film} - \text{Weight of dry film})}{\text{Weight of dry film}} \times 100 \qquad \text{Eq.2}$$

3.2.6. Water vapour transmission rate (WVTR)[24,25]:

To assess the moisture barrier properties of films at room temperature, samples with appropriate dimensions were used to seal test tubes containing 10 mL of distilled water using adhesive tape. The initial weight of the test tube (W_1) and the area of the opening of the test tube (A) in square meters were recorded. The test tubes were then placed in a desiccator containing silica gel (maintained at approximately 20% relative humidity) for 1 day period (t), after which they were re-weighed (W_2). WVTR of the films in g/m^2/day was calculated using Equation 3.

$$\text{WVTR} = \frac{(W_1 - W_2)}{A \times t} \qquad \text{Eq. 3}$$

3.2.7. *In vitro* drug release studies and determination of release kinetics:

The *in vitro* drug release profile of drug-loaded films was evaluated in pH 7.4 phosphate buffer. Afilm strip (1×1 cm^2) was clamped between the donor and receptor compartments of a Franz diffusion cell containing 10 mL of pH 7.4 phosphate buffer. The system was incubated at 37°C in an orbital shaking incubator at 50 rpm under dark conditions. At predetermined intervals, 1 mL of the receptor solution was withdrawn, replenished with fresh buffer, and further incubated. The collected samples were diluted with pH 7.4 phosphate buffer, filtered through a 0.45 µm membrane filter, and analysed using a UV-Visible spectrophotometer at 274 nm to determine the absorbance. The cumulative percentage release of doxycycline was calculated. All drug release experiments were conducted in triplicate. The release kinetics and mechanisms were evaluated by fitting the release data to mathematical models such as zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer–Peppas. These models provided insights into the drug release mechanism and helped identify the most suitable kinetic model for the release profile.

3.2.8. Fourier Transform Infrared Spectrophotometry (FTIR):

The FTIR spectra of the drug-loaded films were obtained using an FTIR spectrometer in the range of 4000–650 cm^{-1}, employing the press pellet technique with FTIR grade potassium bromide discs.

3.2.9. Differential Scanning Calorimetry (DSC):

Thermal properties were characterised to study the drug excipient compatibility using a differential scanning calorimeter. Thermograms were recorded with the temperature range set from 10°C to 250-300°Cat a heating rate of 10°C/min and nitrogen flow rate of 15 mL/min.

3.2.10. Surface Morphology:

The surface morphology of the optimized film was studied using scanning electron microscopy. Small piece of the dried film was mounted and pasted on a metallic stage, sputter coated with thin layer of gold and then analysed using a scanning electron microscope at 15kV and at 500X, 1000Xmagnifications.

3.2.11. *In vitro* microbiological testing:

The optimized film was evaluated for *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by disc diffusion method. Doxycycline loaded filter paper discs (100 µg/mL) were used as positive control and blank filter paper discs were used as negative control for the study. After test samples were placed over the inoculated media, the petri dishes were incubated at 37°C in a BOD incubator for 24 hours. The antibacterial activity of the formulations was evaluated by measuring the diameter of the zone of inhibition around the test sample discs (n=2).

4. Results and discussion:

Drug-loaded films were prepared using the solvent casting technique. These films remained stable without any noticeable physical changes when stored at 4°±2°C. However, a visual colour change was observed when they were stored at room temperature. Therefore, the films were kept in aluminium foil at 4°±2°C until further evaluation studies could be carried out.

4.1. Uniformity of weight, thickness, folding endurance:

The films were characterized for uniformity of weight, thickness and folding endurance. The results are within the

acceptable limits as shown in Table 2. The weight of the films ranged from 95.4±0.89 to 97.4±1.67 mg, while their thickness ranged from 0.95±0.018 to 0.97±0.023 mm. Films, F1 to F4 demonstrated acceptable folding endurance, exceeding 300, whereas F5 to F7 had folding endurance below 300. The results indicate that the mechanical properties of the films were influenced by the concentrations of chitosan and PEO. As the concentration of chitosan in the films increased, their folding endurance decreased, whereas the presence of a higher amount of PEO in films, F1 to F4 contributed to their better mechanical properties.

4.2. Drug entrapment efficiency:

All the films exhibited suitable drug entrapment efficiency, with values ranging from 94.95±1.11% to 96.98±1.03%, as shown in Table 2.

4.3. Swelling index:

The films were characterized for swelling index, with values ranging from 497.35±35.4% to 603.46±26.0%, as shown in Table 2 and Figure 1. The swelling index values were influenced by the concentrations of chitosan and PEO. As PEO is a hydrophilic polymer, its presence enhanced the swelling index values up to a CS/PEO concentration of 50/50. However, beyond this concentration, the presence of PEO did not further enhance the swelling index due to the higher concentration of chitosan compared to PEO. The highest swelling index was observed in the F4 film, which had a CS/PEO concentration of 50/50.

4.4. Water vapour transmission rate:

Managing and maintaining wound moisture is a crucial aspect of promoting healing. An effective wound dressing should balance moisture levels to prevent both accumulation of wound exudate and dehydration. High WVTR values are essential for this purpose[26]. The ideal WVTR for wound dressings typically ranges from 2000 to 2500 g/m²/day[27].The WVTR for the films ranged between 539.96±12.7 and 3180.73±19.9 g/m²/dayas given in Table 2 and Figure 1.The WVTR was highlyinfluenced by the proportion of polymers used. With the addition of PEO, the WVTR of the chitosan/PEO blended films increased. Higher concentrations of chitosan and lower concentrations of PEO resulted in reduced water vapor permeation. Conversely, increasing the concentration of PEO made the films wetter, thereby reducing their barrier properties. Formulations F1 to F4 showed acceptable WVTR values, making them suitable for wound dressings.

Table 2: Characterization of drug loaded films

Formulation code	Uniformity of weight (mg)	Thickness (mm)	Folding endurance	Swelling index (%)	Water vapour transmission rate (g/m^2/day)	Drug entrapment efficiency (%)
F1	97.4±1.67	0.952±0.02	385.80±1.3	497.35±35.4	3180.73±19.9	96.67±0.39
F2	95.6±1.14	0.95±0.018	355.99±1.2	531.99±36.3	2859.32±13.2	96.98±1.03
F3	96.2±1.92	0.962±0.016	339.18±0.7	573.62±25.7	2537.92±8.3	94.95±1.11
F4	96.2±1.48	0.958±0.019	321.25±0.8	603.46±26.0	2380.79±8.2	95.08±1.12
F5	95.8±2.39	0.956±0.015	283.91±1.1	594.19±32.5	1769.88±11.5	96.77±0.55
F6	96.4±1.67	0.964±0.03	249.60±0.8	545.12±26.7	1070.59±9.6	96.40±0.57
F7	95.4±0.89	0.97±0.023	207.15±0.8	529.35±21.4	539.96±12.7	96.33±0.44

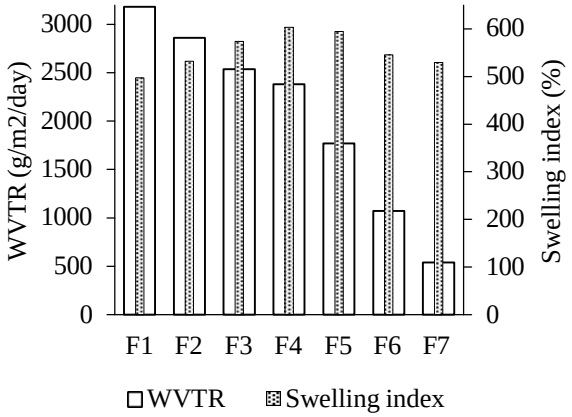


Figure 1: WVTR and Swelling index of films

4.5.*In vitro* drug release studies and drug release kinetics:

The release profiles of the prepared doxycycline-loaded films varied among the different formulations, with sustained release observed for up to 48 hours as shown in Figure 2. Higher chitosan concentrations resulted in more sustained drug release, whereas in formulations with higher PEO levels, there was an initial burst release of doxycycline, with the drug release reaching 50% in less than 6 hours for F1 and F2, and less than 8 hours for F3. The film, F1 sustained release up to 24 hours, while F2 and F3 released the drug completely within 36 hours. Formulation F4, with a 50/50 CS/PEO concentration, achieved optimal release for up to 48 hours. Formulations F5, F6, and F7 extended the release beyond 48 hours. The combination of chitosan and PEO produced a controlled release pattern, with the release rate influenced by the polymer ratios.

The drug release data of the films were analysed using zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas models. The investigation revealed that the drug release from the films followed first-order kinetics. Films F1, F2, and F5 best fit the Korsmeyer-Peppas model, indicating a Fickian diffusion mechanism as their "n" value is below 0.5. Films F3 and F4 were best described by the Hixon-Crowell model, suggesting drug release by erosion. Films F6 and F7 followed the Higuchi model for release kinetics. Table 3 presents the release kinetics data.

Considering the results of folding endurance and WVTR values, films F1 to F4 were identified as suitable formulations. Among these, F4 demonstrated a higher swelling index and optimal drug release sustained for up to 48 hours. Therefore, F4 is considered the optimized filmfor use as a wound dressing.

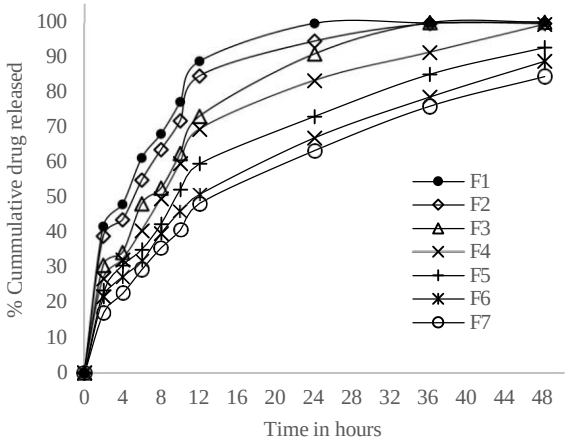


Figure 2: *In vitro* drug release profile of films

Table 3: Release kinetics of films

Model		F1	F2	F3	F4	F5	F6	F7
Zero order	K_0	1.545	1.610	1.790	1.756	1.662	1.595	1.579
	R^2	0.588	0.648	0.763	0.795	0.840	0.877	0.892
First order	K_1	0.170	0.122	0.157	0.089	0.051	0.042	0.036
	R^2	0.967	0.967	0.960	0.940	0.990	0.989	0.996
Higuchi	R^2	0.840	0.880	0.944	0.959	0.979	0.993	0.993
Hixon-Crowell	R^2	0.877	0.915	0.968	0.970	0.965	0.973	0.972
Korsmeyer-Peppas	R^2	0.911	0.929	0.955	0.962	0.980	0.990	0.990
	n	0.301	0.330	0.415	0.441	0.452	0.463	0.521

4.6. FTIR spectroscopy:

The FTIR spectrum of optimized film (F4) revealed characteristic bands similar to the pure drug. Peaks related to OH and NH stretching (3275 cm^{-1}), aromatic C=O stretching(1734 cm^{-1}), amide I (1640 cm^{-1}), aromatic N-H bending of amide II (1610 cm^{-1}), C-N stretching (1220 cm^{-1}) and C-O-C stretching (1031 cm^{-1}) were observed in the film which are very closer to the bands observed in pure spectra. The analysis confirmed no chemical interaction between doxycycline, PEO and chitosan, ensuring drug stability in the optimized formulation. The FTIR spectra of doxycycline, chitosan, PEO and F4 film are represented in Figure 3.

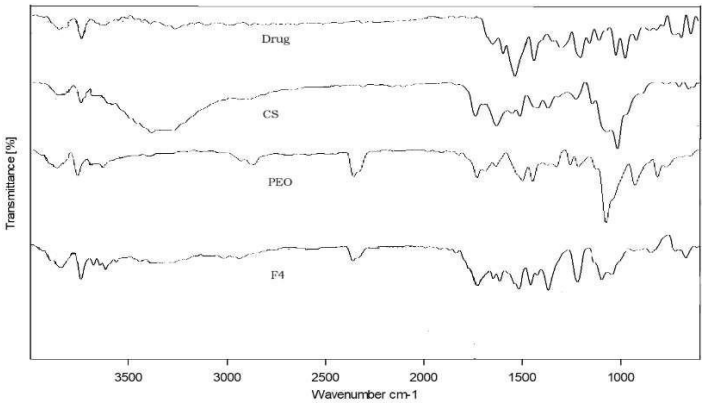


Figure 3: FTIR spectra of F4

4.7. Differential scanning calorimetry:

The analysis of the DSC thermogram for the F4 film revealed an endothermic melting peak at 173°C, which matches the endothermic melting peak of doxycycline observed at 172.8°C. This alignment indicates that there is no chemical interaction between the drug and the excipients used in the formulation. The absence of any additional peaks or shifts in the thermogram suggests that the drug remains in its pure form within the film matrix, maintaining its stability and integrity. The DSC thermograms for both doxycycline and the F4 film are shown in Figure 4.

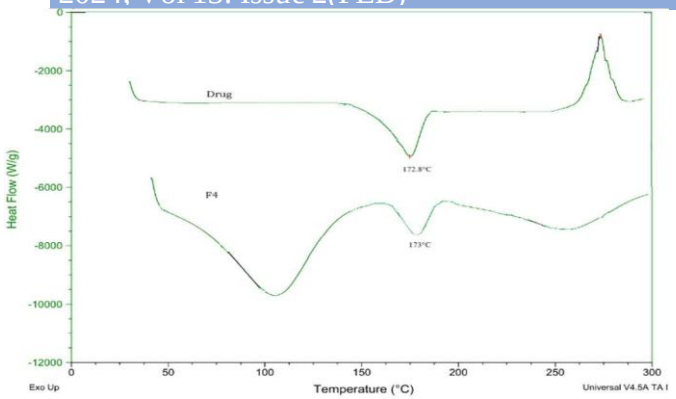
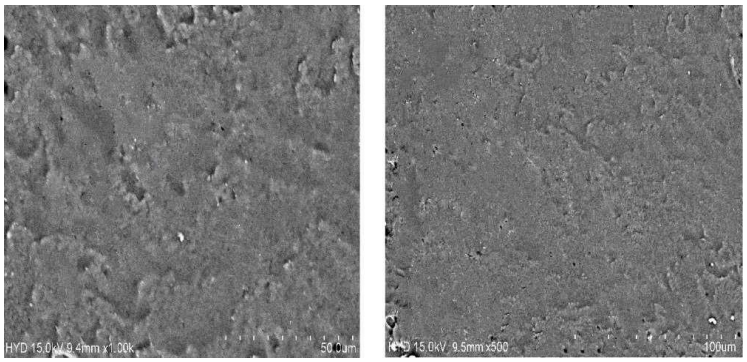


Figure 4: DSC thermogram of F4

4.8. Surface morphology:

The SEM image of the F4 film observed at 500X and 1000X magnification, as illustrated in Figure 5, displays a homogeneous and smooth surface, signifying a uniform distribution of the film's components. This uniformity is important for maintaining consistent drug release and effective moisture management as observed in the F4 film, ensuring controlled and sustained drug delivery.

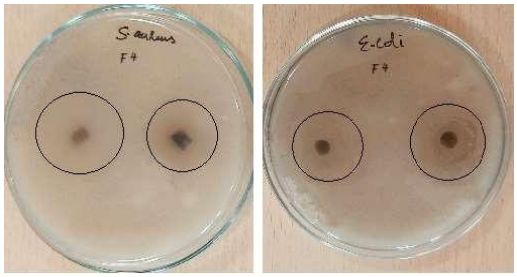


1000 X magnification 500X magnification

Figure 5: SEM image of F4

4.9. *In vitro* microbiological testing:

The antibacterial activity of the optimized film was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The efficacy was assessed by measuring the diameter of the inhibition zones around the test sample discs. The results demonstrated significant antibacterial activity, with clear inhibition zones observed for both bacterial strains, indicating the film's effectiveness in combating bacterial infections, as shown in Figure 6. The diameters of the zones of inhibition were 21.7±0.6 mm for *S. aureus* and 20±1.7 mm for *E. coli*. It was observed that F4 film exhibited slightly better activity against *S. aureus* compared to *E. coli*.



S. aureus *E. coli*

Figure 6: Antibacterial activity of F4

5. Conclusion:

This study thoroughly analysed the physicochemical properties, swelling and water barrier characteristics, drug release kinetics, FTIR, DSC, surface morphology, and microbiological testing of doxycycline loaded chitosan-PEO hybrid films. The results suggest that these advanced wound dressings, particularly the film (F4) loaded with doxycycline and composed of a 50/50 blend of chitosan and PEO, present a promising solution for chronic wound care. By potentially reducing the frequency of dressing changes and minimizing the risk of infection, these films offer significant benefits in wound management. Future research will focus on *in vivo* evaluations to further confirm the clinical applicability of this innovative wound dressing system.

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