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Formulation and Development of Oral Dental Films of Doxycycline Loaded Chitosan Films for efficient Treatment of Periodontitis

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ABSTRACT: The present study demonstrates the development of intrapocket dental films of Doxycycline for efficient treatment of periodontitis. The films were developed by solvent casting method using chitosan, TPP, and PEG 400 as polymer, crosslinking agent, and plasticizer respectively. The developed films were characterised by physicochemical properties including drug release, tensile strength, and bactericidal activity against *E. coli* and *Staphylococcus aureus*. One of the major challenges was achieving uniform drug distribution in the chitosan matrix. Doxycycline has poor solubility in water and acidic pH, which are the conditions required for chitosan gelation. To get uniform drug distribution proper mixing of the drug was carried out in polymeric dispersion. All physical parameters of the films were found to be acceptable for dental application. It was observed that the drug release occurred in a sustained manner. The optimised film formulation was found to be stable over 2 months at accelerated conditions. This newly developed film could be used as a potential alternative drug delivery system for peridontitis conditions.

Keywords: Doxycycline, chitosan, TPP, PEG 400, bactericidal.

INTRODUCTION

Periodontitis is an inflammatory disorder of the gum that destructs the alveolar bone, forms periodontal pockets, and causes degeneration of periodontal ligaments that results in the disruption or destruction of the support required for the teeth. Periodontal pockets are formed when the gingiva detaches from the tooth, creating the perfect favourable condition for anaerobic bacterial growth (Joshi *et al*., 2016). All around the world, dental diseases are acknowledged as serious public health issues. According to the WHO, 10 to 15 % of people worldwide have severe periodontitis. In India, severe periodontitis affects between 19-32 % of people (Fisher *et al*., 2018). Severe periodontal disease ranked as the 11th most common disease in the world, following the Global Burden of Disease Study (James *et al.,* 2018). According to reports, the incidence of periodontal disease varies between 20-50 % worldwide (Sanz *et al*., 2010). It is one of the leading causes of tooth loss, which can impair quality of life, mastication, aesthetics, and self-confidence.

Systemic antibiotics have shown some potential for the treatment of periodontitis; however, they are only advised in the case of resistant or quickly developing periodontitis (Genco, 1981). Minimum concentration of antibiotics at the application site, rapid decline in antibiotic concentration, and rapid development of microbial resistance are just a few of the drawbacks associated with multiple systemic doses of antibiotics (Gates *et al*., 1994). Intrapocket delivery of active ingredients has sparked attention considering these obvious drawbacks (Greenstein, 2006). A periodontal pocket is an accessible natural reservoir for the implantation of a drug delivery system. Additionally, gingival crevicular fluid (GCF) acts as a leaching medium for a drug's release from the dosage form and for its distribution throughout the pocket. Together with the fact that periodontal diseases are limited to the pocket's natural environment, these features make the periodontal pocket a perfect area for therapy employing local delivery systems. Intra-pocket drug delivery systems are highly desirable because they may have fewer adverse side effects, are more effective, and have more patient compliance (Jain *et al*., 2008).

Drugs are distributed throughout the polymer in intrapocket oral dental films, which are matrix-type delivery devices. Drug release occurs via diffusion, matrix dissolution, or matrix erosion. This dosage form is beneficial physically for usage within the pocket. Depending on the size of the pocket to be treated, it is simple to adjust the form and size of the films. It may be quickly put into the pocket's base while causing the patient the least amount of discomfort possible (Junmahasathien *et al*., 2018). It has been demonstrated that this novel treatment strategy is more efficient than conventional drug delivery systems. Based on the physicochemical characteristics of the utilized polymers, the best forming method is selected. By mixing different co-polymers, the drug release can be prolonged for a long period (Joshi *et al*., 2016).

The manufacturing of the films involved the use of several natural and synthetic polymers. Chitosan is a natural polymer have the significant benefit of not interfering with the regeneration of periodontal tissue. Chitin, a key component of arthropod shells, is

converted into the hydrophilic biopolymer chitosan by an alkaline deacetylation process. Chitosan is advantageous because of its nontoxicity, biocompatibility, bioadhesive, and biodegradability (Rodrigues *et al*., 2012). Chitosan was investigated for its antibacterial properties against *P. gingivalis* by Ikinci *et al.* (2002) and co-workers. They discovered that chitosan films have antimicrobial action, which was enhanced by increasing the molecular weight of the chitosan and combining it with chlorhexidine (Ikinci *et al*., 2002; Prashanth *et al*., 2022).

Doxycycline (DOX) is a tetracycline antibiotic used to treat bacterial infections. Due to its low minimum inhibitory concentration, DOX is the most widely used broad-spectrum antibiotic and is effective against most periodontal infections (Chaturvedi *et al*., 2013). DOX is a bacteriostatic drug that suppresses bacterial protein synthesis and is well-recognized for its antibacterial properties (Mahmoud and Samy 2016). Collagenase inhibition, anti-inflammatory effects, and bone resorption inhibition are among the other qualities. It can, however, attach to the solid tissue walls of pockets to create a drug reservoir (Rajeshwari *et al*., 2019). Dinte *et al.* (2023) developed mucoadhesive buccal films of DOX for the efficient treatment of periodontitis. The developed film served as a potential supportive treatment for periodontitis by providing sustained release of the antibiotic after local application (Dinte *et al.*, 2023). Swain *et al.* (2023) developed site-specific and local action dental films loaded with DOX for the treatment of periodontitis. Based on the study it was conducted that the use of DOX-loaded dental films made of HPMC E15 and Eudragit RS 100 was effective in the local treatment of periodontitis. Moreover, the combination of HPMC E15 and Eudragit RS 100 can be utilized in the development of dental films for other drugs, as it has shown promising results in terms of mucoadhesion, drug release, and stability (Swain *et al*., 2023). Ghavami *et al*. (2020) studied the antibacterial activity of polymeric local drug delivery system against pathogens associated with periodontitis. In this research work, they developed polymeric films of the DOX and metronidazole. DOX-loaded polymeric films were more effective on multispecies bacteria, inhibiting both planktonic and biofilm growth at relatively low concentrations (Ghavami *et al*., 2020). Chuenbarn *et al.* (2022) also developed DOX-loaded Eudragit RSPO in situ forming microparticles for the treatment of periodontitis. The obtained microparticles were spherical with a porous structure. Moreover, the formulation exhibited effectiveness against periodontal pathogens, which was appropriate for development into local drug delivery to treat periodontitis (Chuenbarn *et al.,* 2022). Considering all these latest research studies it has been observed that very limited work was performed with DOX-loaded chitosan films with the DOE approach. Chitosan itself has antibacterial activity and that could show a synergistic effect when coupled with DOX. Considering all these current research gaps as well as potential benefits and characteristics DOX was used in the present investigation to develop oral dental films for the efficient treatment of periodontitis.

MATERIALS AND METHODS

Materials: Doxycycline (DOX) was purchased from Cipla Ltd. Mumbai, India, and Chitosan (CS) was obtained from the Indian Sea Food Company (Kochin, India). Polyethylene glycol 400 (PEG 400), Tripolyphosphate (TPP) was purchased from Sigma Aldrich, India.

Methods:

Statistical Design of experiments (DOE): 2^3 full factorial design approach was utilised in the development of DOX-loaded intrapocket films. The concentration of Chitosan (A, mg), TPP (B, mg), and PEG 400 (C, ml) were considered as independent variables which were varied at two levels (-1 and +1) while tensile strength (Y1) and *in vitro* drug release at 12^{th} day (DR) (Y2) were considered dependent variables. Variables and three levels are presented in Table 1**.** The statistical experimental data were analyzed using the Design-Expert® Software.

Development of DOX-loaded chitosan (CS) films: DOX-loaded CS films were developed by using the solvent-casting evaporation method (Khajuria *et al*., 2018). The chitosan was used as a gelling polymer, as well as a film former, TPP as a crosslinking agent, and PEG 400, was used as a plasticizer. The formula composition in coded form is presented in Table 2. The CS was dissolved in acetic acid (3% v/v; 10 ml) under continuous magnetic stirring till a clear and transparent clear solution was obtained. PEG 400 was added slowly under stirring to the chitosan solution and mixed properly for 15-20 minutes. DOX (100 mg) was added to CS-PEG 400 solution and vortex for 10 minutes for even distribution in polymeric dispersion. This viscous dispersion containing DOX was kept aside for 2 hours for the complete removal of air bubbles. TPP already dissolved in distilled water was added dropwise to the viscous dispersion under continuous stirring to form crosslinking between CS and TPP. This dispersion was again stirred for 3-4 hours and kept aside to remove the air bubbles.

Table 1: Variables and levels.

Variable	(-1) Low level	(+1) High level
Independent		
A= Chitosan	15 (mg)	30 (mg)
B= TPP	3 (mg)	6 (mg)
C= PEG 400	2.5 (ml)	5 (ml)
Dependent		
Y1= Tensile strength		
Y2 = % DR at 12^{th} day		

Table 2: Formulation batches of films.

Batch	Factor		
	A	*B*	*C*
F1	+1	+1	+1
F2	+1	-1	+1
F3	+1	-1	-1
F4	-1	+1	+1
F5	-1	+1	-1
F6	+1	+1	-1
F7	-1	-1	+1
F8	-1	-1	-1

The drug-polymer dispersion was cast in a petri dish and dried at 35-40°C for 24 h in a vacuum oven. The films were removed carefully and observed for any physical damage or imperfections and further used for characterization.

Characterization of DOX-loaded CS films:

Thickness: The patch thickness (n=3) was determined using a vernier caliper and the average thickness of the patches was calculated (Ozdogan *et al.,* 2018).

Uniformity of weight of the films: Randomly selected three patches were weighed on analytical balance individually. The average weight of the patches was calculated with standard deviation (Ozdogan *et al*., 2018).

Folding endurance: The films were folded manually at the same point till it breaks down. The value for folding endurance was determined by how many folds the film could withstand without tearing. The test was performed in triplicate and the average value was determined (Ozdogan *et al*., 2018).

Drug Content Uniformity: Films with 5 cm^2 dimensions were randomly obtained from different places. The films were dissolved separately in 3% v/v acetic acid. The samples were filtered and analyzed at 273 λ_{max} using UV spectroscopy and drug content was calculated (Ozdogan *et al.*, 2018).

Moisture content:

The formulations were weighed accurately and stored in desiccators with anhydrous calcium chloride. The films were removed and weighed after 3 days. The following formula was used to calculate moisture content (Ozdogan *et al*., 2018).

$$\text{Moisture Content (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \times 100$$

Tensile strength: Using a tensile strength tester with a 5 g load cell, the tensile strength of the film was assessed. Films having a 5 cm^2 surface area and no physical damage were held between two clamps. The top clamp was pushed during the measurement at a speed of 0.5 mm/s, and the force applied when the film broke was recorded. The outcomes from the film samples that ruptured between the clamps were recorded (Ozdogan *et al*., 2018).

IR spectroscopy: IR spectra were obtained for DOX, CS, and optimized film by the KBr pellet method. The IR spectra were compared and possible drug excipient interaction was determined (Nyavanandi *et al.*, 2023).

Differential Scanning Calorimetry (DSC) Studies:A small amount of sample was accurately balanced in an aluminum pan and heated from 40 °C to 400 °C, with a heating rate of 10 °C/min. DSC thermograms of pure drug, optimised formulations were recorded using DSC (Narala *et al.,* 2022).

***In vitro* drug release study:**DOX release study was performed in pH 6.8 phosphate buffer (release media) which was simulated with gingival fluid. The test was performed under static conditions because, after the application of film in pockets, it will remain immobilized. Six films with a 5 cm^2 area were placed separately in test tubes already filled with 1 ml of release media. The entire setup was kept at 37± 0.5 °C. The sampling of 1 ml was done at a predetermined time interval and replaced with fresh buffer to maintain sink condition. Using a UV/VIS spectrophotometer, the drug's concentration was found at 273 nm. The operation was carried out for 12 days in a row (Nakahara *et al*., 2003).

Zone of inhibition study using cup plate method: The suspension of the microorganisms (*Staphylococcus aureus* ATCC29737 was prepared in the medium at a temperature between 40 ºC and 50 ºC. This inoculated medium was poured into previously sterilized Petri plates to give approximately 3 to 4 mm depth with uniform thickness. These plates were stored properly to ensure that no growth or death of the microorganisms occurred till the agar layer gets solidified. The test and standard antibiotic solution were applied in the cavities at the same volume and concentration. The plates were kept for incubation for 18 hours at 35-37 ˚C. At the end of the experiment, the zone of inhibitions was measured and compared (Khan *et al*., 2021).

***In-Vitro* Kill Kinetics Study:** The test was performed on (*Staphylococcus aureus* ATCC29737 and *E. coli).* Pure DOX, DOX-loaded films, and positive control were added to the broth cultures of 10^6 CFU/mL. A kill kinetics study was performed at twice the concentration of MIC (2MIC) of the antimicrobial agent. Viable counts were performed at 0, 1, 2, 4, 6, 8, and 24, 168 h after the addition of pure DOX, DOX-loaded film, and a positive control following serial dilution in saline solution. The bacterial count was determined at 24 hours and the graph was plotted as a viable count against time (Naik *et al*., 2019).

Stability study. The optimized formulations were kept for stability studies after wrapping them with aluminum foil and butter paper at 40 ± 2°C, 75% ± 6% relative humidity for 2 months. At the end of the stability studies, the samples were analysed and compared with the initial results (Khagga *et al.*, 2019).

RESULTS AND DISCUSSION

Physicochemical properties of the developed films. The developed DOX-loaded films were characterized for various physicochemical properties to check whether the formulation satisfies the requirement of the dental pocket. Table 3 describes various parameters of the DOX-loaded film.

Thickness: The thickness is one of the important parameters to be considered for the formulations to be applied in dental pockets. The thickness of the films was found between 0.288 mm (F7) to 0.300 mm (F3). The variation in film thickness was due to the polymer concentration used in formulations. The films with higher thickness would be difficult to apply in dental pockets. F7 formulation showed very lesser thickness and that can be ideally placed at the site of application. The minimum thickness observed in the F7 formulation was attributed to the lower chitosan concentration used.

Weight uniformity: The weight variation in the films was found due to the different chitosan and PEG 400 used in the formulations. The weight of the films was found between 8.38 ±0.15 mg (F7) to 10.02±0.09 mg (F1). The weight variation showed that the weights of the film from various film sections were quite similar.

Folding endurance: The higher folding endurance is quite desirable for dental films which indicates that the film will remain intact and integrated into the periodontal pocket. The folding endurance is indirectly correlated to the strength of the films. F7 formulation showed the highest folding endurance of 314.4 ±0.17 indicating excellent strength and can maintain integrity in the periodontal pocket. The higher folding endurance was found to be dependent on the perfect concentration of CS and PEG 400. The CS played an excellent role as films former and PEG 400 provided the desired plasticity to the film and avoided brittleness (Vieira *et al.,* 2011).

Drug content: The uniformity in the drug content (94.25 ±0.77 to 98.50 ±0.55%) was observed between all the formulated batches of the films. This observation indicated that the drug was evenly distributed in the polymeric dispersion. The uniformity in drug content is quite desirable for any dosage form. The highest drug content was found in the F7 formulation indicating minimum loss during the film manufacturing process.

Moisture content: Moisture content is a very crucial parameter for films. Higher moisture content in the film will promote bacterial growth during its storage at room temperature. The films with lower moisture content are considered ideal formulations for avoiding any bacterial growth (Loke *et al.,* 2000). It has been observed that the moisture content was associated in a linear relationship with the concentration of PEG 400. A similar observation was noted by Swain *et al* in their research work (Swain *et al.,* 2023). The F7 formulation showed minimum moisture content of 2.5±0.08 % due to the minimum PEG 400 concentration.

IR spectroscopy: FTIR spectra of pure DOX showed characteristic peaks at 3481 cm^{-1} corresponding to the (O-H) group, 3250 cm^{-1} corresponding to the (N-H) group, 2997 cm^{-1} corresponding to the (C-H) group, 1650 cm^{-1} corresponding to (C=O) group and at 1498 cm^{-1} corresponding to (CO-NH) group. Similar IR peaks were also observed in one of the studies (Swain *et al.,* 2023). All these characteristic peaks of DOX are also present in the final formulation which indicates the absence of any physical as well as chemical incompatibility between DOX and other excipients. The comparative IR spectra of DOX, chitosan and DOX-loaded films are presented in Fig. 1.

DSC analysis: The DSC analysis of the pure DOX showed an endothermic peak at 206.09 °C corresponding to its melting point while DOX-loaded films showed an endothermic peak at 205.73 °C (Fig. 2). From the DSC thermograms, it was observed that the DOX did not interact with other excipients present in films indicating good compatibility.

Statistical analysis of Tensile strength (Y2): The intrapocket DOX-loaded films were developed using CS as film former, TPP as a crosslinking agent, and PEG 400 as a plasticiser. The tensile strength of the film is also one of the important parameters that need to be considered during the development of the films and hence is considered the dependent variable in this study. The results of the tensile strength of all the formulations are presented in Table 4 along with the coded levels of the dependent variables.

The tensile strength of the developed films ranged between 0.722±0.11(F1) to 2.785±0.11 (F7) Kg/cm^2. The F7 formulation showed the highest tensile strength in comparison to the rest of the formulations. It has been observed that the tensile strength is directly related to the chitosan concentration used in the formulation. The batch (F7) with the highest concentration of Chitosan, PEG 400, and crosslinking agent TPP showed the highest tensile strength. Chitosan concentration had a very tremendous impact on the tensile strength of the films. Higher polymer concentration might have increased the viscosity of the dispersion leading to the improvement in the tensile strength. In addition to it, TPP being used as the crosslinking agent contributed to tensile strength. A perfect crosslinking happened between Chitosan and TPP that gave additional strength to the formulation (Yanat and Schroen 2021). PEG 400 was used as a plasticizer to provide plasticity, and flexibility as well as to prevent the breakage of the films. Also, it prevented the brittleness of the film. PEG 400 also had a positive impact on the tensile strength like chitosan and TPP concentration. Higher levels of DBT increased physical strength, which increased the resistance to breaking the film. The effect of independent variables on tensile strength is presented in Fig. 3.

The polynomial equation for tensile strength (Y1) can be presented below

$$Y1 = + 42.15+4.11A+13.23B+0.39C \quad (1)$$

In the above equation, Y1 is tensile strength, A is Chitosan concentration, B is TPP concentration and C is PEG 400 concentration. The effect of all these independent variables is statistically significant with $p< 0.05$. The model was also found to be statistically significant with F value of 0.0025. The 2FI model was suggested for Y1 as shown in Table 5. The correlation

coefficient (R^2) was found to be 0.9890 for Y1 indicating a good fit model of 2FI.

The model for Y1 was found to be statistically significant based on the *p*-value of 0.0012 The effect of independent variables was also found to be statistically significant based on the *p*-value shown in Table 6.

Statistical analysis of drug release on the 12th day (Y2): The *in vitro* DOX release was performed in phosphate buffer pH 6.8 for 12 days to check for the sustained release behaviour of the films. At the end of 12 days, the drug release was found to be in the range of 72.51(F7) to 99.55 % (F8). The comparative drug release profile of all the formulations is presented in Fig. 4.

The concentration of the Chitosan played a very significant role in the retardation of DOX release from the films. The effect of Chitosan was found to be concentration-dependent. The films with a higher concentration of Chitosan showed retarded drug release from the formulation. Similarly, the TPP has also shown a positive effect on the retardation of the drug release from the formulations. It has also been observed that an increase in plasticizer PEG 400 concentration in formulations also helped in the retardation of drug release. Chitosan being hydrophilic, sustained-release polymer, along with TPP, formed a great complex and sustained the drug release efficiently (Fig. 5).

The polynomial equation for DR (Y2) can be presented below

Y2= +92.12+3.14A+2.21B+1.12C (2)

In the above equation, Y2 is tensile strength, A is Chitosan concentration, B is TPP concentration and C is PEG 400 concentration. The effect of all these independent variables is statistically significant with $p< 0.05$. The model was also found to be statistically significant with F value of 0.023. The 2FI model was suggested for Y2 as shown in Table 5. The correlation coefficient (R^2) was found to be 0.9798 for Y2 indicating a good fit model of 2FI. The model for Y2 was found to be statistically significant based on the *p*-value of 0.0395 The effect of independent variables was also found to be statistically significant based on the *p*-value shown in Table 6.

Zone of inhibition (ZOI)study. The ZOI study of different concentrations of pure DOX and films manufactured with concentrations were studied against *Staphylococcus Aureus*. It has been observed that DOX-loaded chitosan films showed greater zone oh inhibition in comparison to the pure DOX. The comparative graphical representation of the ZOI is presented in Fig. 6. The actual antibacterial effect in the form of ZOI of pure DOX and DOX-loaded films is presented in Fig. 7. Chitosan itself has some sort of antibacterial activity (Eldin *et al.,* 2008) and hence the films manufactured from Chitosan displayed greater potency against *Staphylococcus Aureus.* In one of the studies DOX loaded films showed MIC of 4, 0.25, and 4 µg/mL, against *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* respectively (Ghavami *et al.,* 2020). The results of this study are comparable with our study showing excellent antibacterial activity.

Time–Kill Kinetics on *Staphylococcus aureus* and *E. Coli*: A kill kinetic study was performed on *Staphylococcus Aureus* and *E. Coli* using optimised film formulation (F7). The film was found to be successful in killing *Staphylococcus Aureus* and *E. Coli* because there was a reduction in the colony count treated with chitosan film and pure drug when compared to the control, and the results were repeatable (Fig. 8). The fundamental idea behind the Time-Kill Kinetic study is to determine the rate at which a microorganism is killed by a dosage form as a function of survival data collected at enough exposure time points to enable the construction of a graph that models the population's decline over time until it reaches extinction (Levin and Udekwu 2010). The study confirmed the bactericidal activity against the selected microbial strength proving efficiency in periodontitis disease.

Stability studies. The optimised formulation was kept for stability studies at 40 ºC ± 2 ºC/75% ± 5 % RH for 2 months. The physicochemical parameters tested after 2 months did not show any significant change in storage. The comparative parameters are presented in Table 7. The results demonstrated excellent stability of the developed films.

Table 3: Physicochemical properties of the DOX-loaded dental films.

Batch	Thickness (mm)	Weight Uniformity (mg)	Folding endurance	Drug content (%)	Moisture content (%)
F1	0.298	10.02±0.09	250.1 ±0.22	95.11 ±0.45	3.9±0.11
F2	0.297	8.40 ±0.11	248.3 ±0.17	96.17 ±0.65	4.1±0.14
F3	0.300	8.59 ±0.13	262.5 ±0.27	97.24 ±0.70	3.8±0.12
F4	0.298	9.12 ±0.21	259.4 ±0.35	95.87 ±0.52	3.9±0.17
F5	0.296	9.17 ±0.05	265.8 ±0.42	96.18 ±0.64	4.0±0.10
F6	0.299	9.59 ±0.17	287.1 ±0.12	95.24 ±0.11	3.9±0.10
F7	0.288	8.38 ±0.15	314.4 ±0.17	98.50 ±0.55	2.5±0.08
F8	0.921	9.87 ±0.12	272.1 ±0.21	94.25 ±0.77	4.1±0.07

Table 4: DOX loaded films with coded form and their responses.

Batch	Factor			Response	
	A	B	C	Y1 (kg/cm^2)	Y2 (%)
F1	+1	-1	+1	0.722±0.11	87.24
F2	-1	-1	+1	0.883±0.12	96.51
F3	+1	-1	-1	0.983±0.16	89.12
F4	-1	+1	+1	1.114±0.24	90.21
F5	-1	+1	-1	0.945±0.09	94.67
F6	+1	+1	-1	0.834±0.10	82.51
F7	+1	+1	+1	2.785±0.11	72.51
F8	-1	-1	-1	0.612±0.10	99.55

Table 5: Statistical analysis of responses Y1 and Y2.

Model	R^2	Adjusted R^2	Predicted R^2	Std. Dev	Press	Remarks
Response Y1						
Linear	0.7825	0.7942	0.6312	6.15	412.25	……
2FI	**0.9890**	**0.9521**	**0.8215**	**4.44**	**575.51**	**Suggested**
Quadratic	0.9122	0.8951	0.7642	0.39	450.11	
Cubic	0.8745	0.8559	0.6517	0.35	397.14	……
Response Y2						
Linear	0.5213	0.5535	0.2914	4.24	102.11	……
2FI	**0.9798**	**0.8212**	**0.5314**	**1.25**	**189.14**	**Suggested**
Quadratic	0.8956	0.8632	0.8771	5.89	135.51	……
Cubic	0.9235	0.8911	0.8799	7.13	155.45	……
Regression equations of the fitted models						
Y1 = + 42.15+4.11A+13.23B+0.39C						
Y2 = +92.12+3.14A+2.21B+1.12C						

Table 6: ANOVA of models for Y1 and Y2.

Source	DF	Sum of squares	Mean Square	F Value	P value
Model for Y1	3	2215	405.12	18.22	**0.0012**
A	1	72.15	81.21	1.00	0.0123
B	1	1850.22	1897.21	71.75	0.0221
C	1	1.5	1.89	0.015	0.0011
Model for Y2	4	271.89	67.97	10.85	**0.0395**
A	1	19.59	19.59	3.13	0.0351
B	1	216.53	216.53	34.56	0.0098
C	1	18.24	18.24	2.91	0.02865

Table 7: Comparative physicochemical parameters of film stored at 2 M (40 ºC ± 2 ºC/75% ± 5 %).

Sr. No.	Parameter	Initial	2 M (40 ºC ± 2 ºC/75% ± 5 %)
1.	Thickness (mm)	0.288	0.298
2.	Weight Uniformity (mg)	8.38 ±0.15	9.01 ±0.20
3.	Folding endurance	314.4 ±0.17	302.1 ±0.11
4.	Moisture content (%)	2.5±0.08	2.9±0.12
5.	Drug content (%)	98.50 ±0.55	98.90 ±0.95
6.	Drug release (%) at 12 days	72.51	75.12

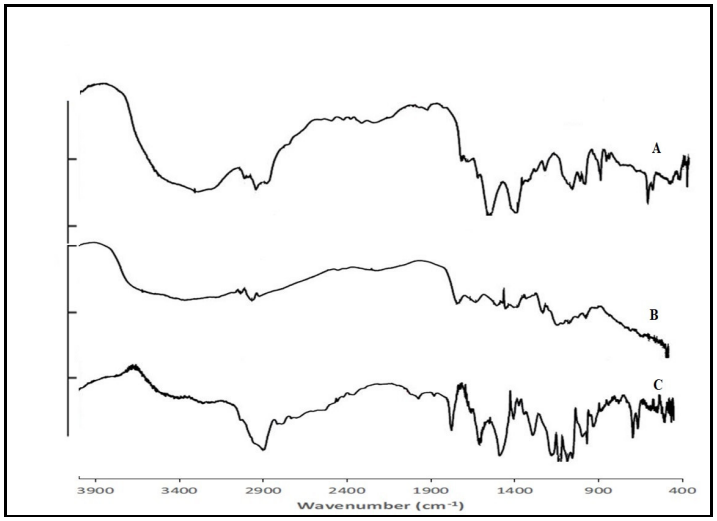

Fig. 1. FTIR spectra of (A): Pure DOX; (B): Chitosan; (C): DOX loaded films.

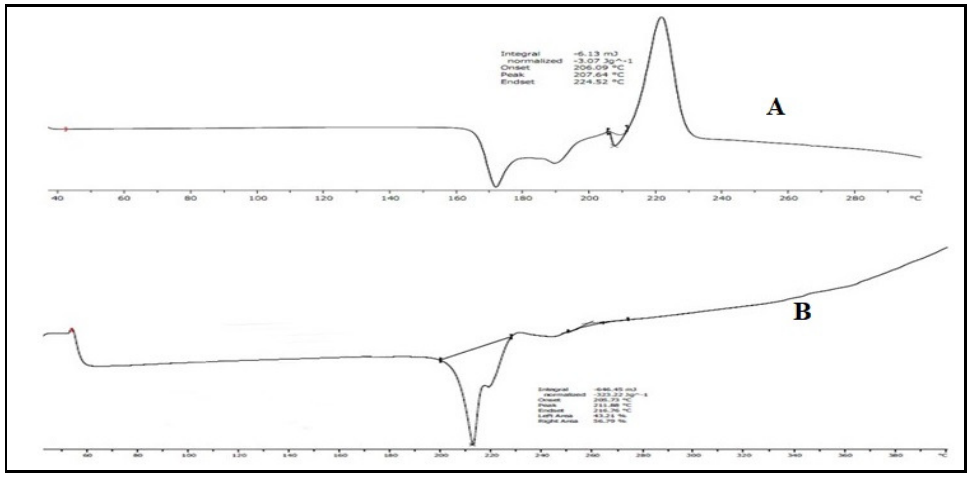

Fig. 2. DSC analysis of (A): Pure DOX and (B): Dox loaded films.

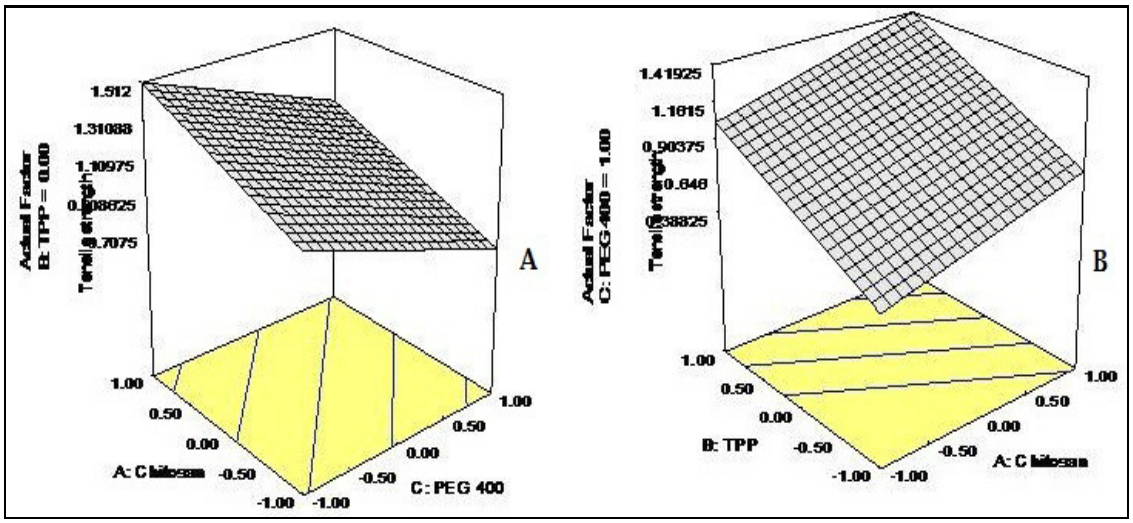

Fig. 3. 3D surface responses of chitosan, TPP and PEG 400 on tensile strength.

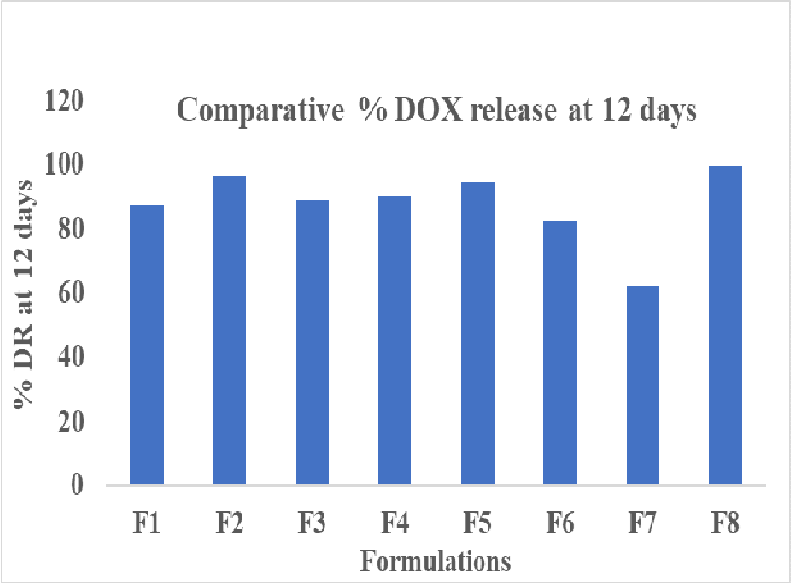

Fig. 4. Comparative % DOX release at 12 days in phosphate buffer pH 6.8.

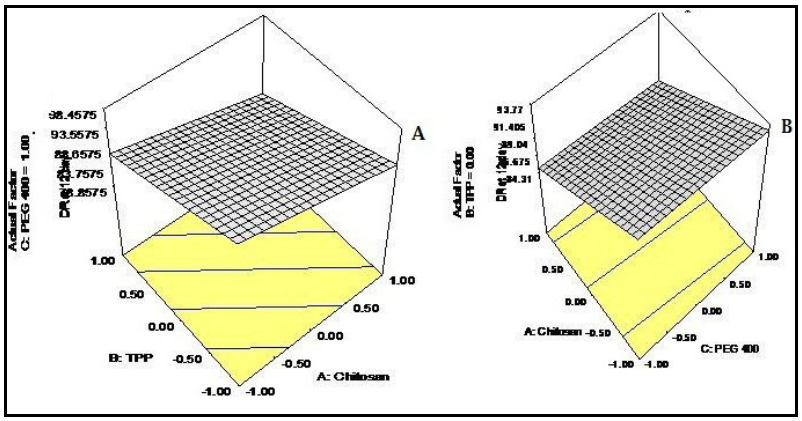

Fig. 5. 3D surface responses of chitosan, TPP and PEG 400 on % DR.

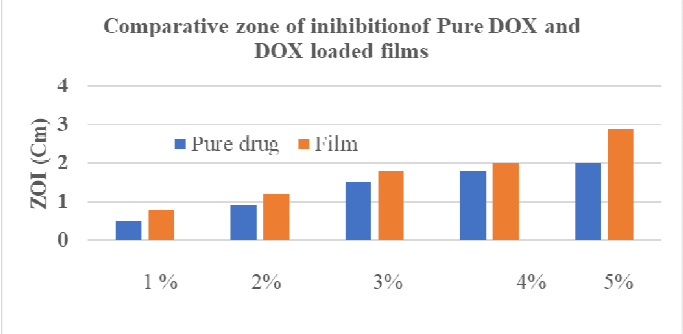

Fig. 6. Comparative ZOI of pure DOX and DOX loaded chitosan films.

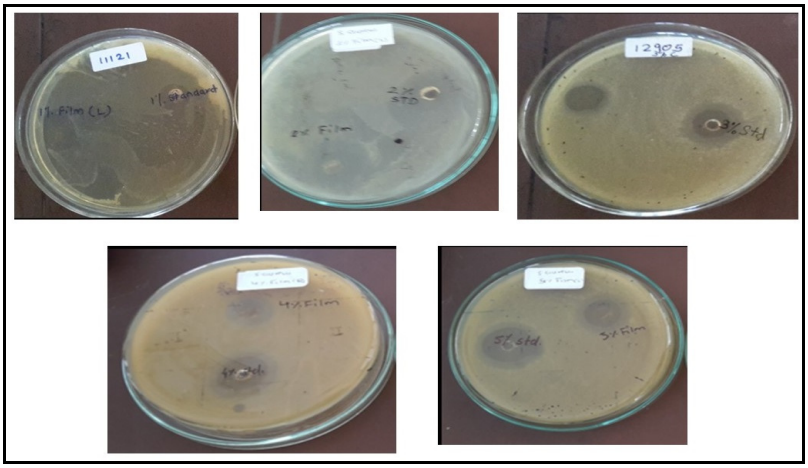

Fig. 7. Zone of inhibition of study of 1 to 5% film against *Staphylococcus aureus.*

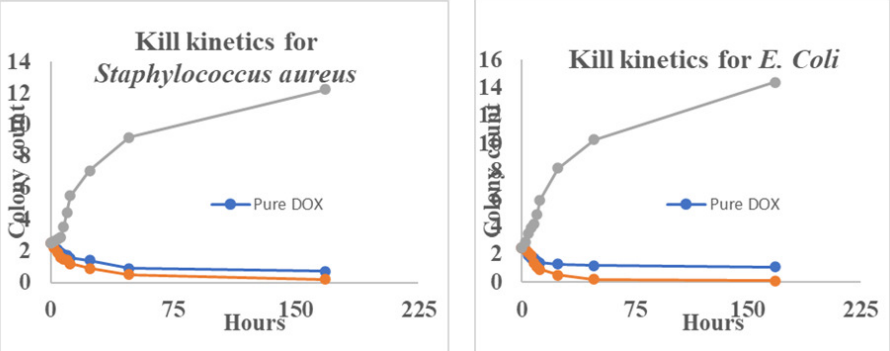

Fig. 8. Kill kinetics study against *Staphylococcus Aureus* and *E. coli*.

CONCLUSIONS

The aforementioned results justify the use of Doxycycline in films developed with chitosan, TPP, and PEG 400 as polymer, crosslinking agent, and plasticizer respectively. The films' physicochemical characteristics were deemed to be acceptable. It was discovered that the drug release occurred in a sustained manner. The DOX-loaded films demonstrated good bactericidal efficacy against *E. coli* and *Staphylococcus aureus*. The drug did not interact chemically with the excipients while being stored at accelerated stability conditions. This newly developed film could be used for future medication delivery applications in peridontitis conditions.

FUTURE SCOPE

The developed Doxycycline dental films can be evaluated preclinically in a suitable animal model to establish their safety. Furthermore, scale-up batches can also be planned to check the process feasibility.

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Conflict of Interest. None.

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