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Development and evaluation of dental film of doxycycline for the treatment of periodontitis

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***Abstract*---**The study has been designed to formulate intrapocket dental film loaded with doxycycline to be used for insertion into the periodontal pockets and evaluate its clinical efficacy. Doxycycline dental films were prepared by the solvent casting technique using polymers such as gelatin was used as film former. Glycerin as plasticizer and glutaraldehyde as cross linking agent were used. The prepared films (F1-F9) were evaluated for various physicochemical parameters employing established pharmaceutical procedures such as surface pH, folding endurance, tensile strength, swelling index, drug content, *in-vitro* drug release, antibacterial efficacy, stability studies. Experimental parameters of the obtained film exhibited results within the desired limit. Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry studies revealed the formulated film to be stable during drug stability and compatible between drugs and excipients. *In-vitro* dissolution studies showed an initial burst release to achieve an immediate therapeutic level of drug in the periodontal pocket followed by a progressive fall and extended-release of the drug for 12 days. The antimicrobial activity was retained for 168 hours against *Staphylococcus aureus* and *E. coli*. 5% dental film showed sustained drug release by kill kinetics as there is decrease in the colony count and it is compared with pure drug and control both strain of bacteria. The formulations were subjected to stability studies, the results thus obtained showed no significant changes in the formulation. The experimental results suggest that doxycycline dental film can be experimentally identified as a potential drug delivery system. Hence, doxycycline dental film can be used for topical treatment of periodontal diseases and to achieve optimum therapeutic efficacy.

***Keywords*---**doxycycline dental film, site-specific delivery, film, antibacterial activity, periodontitis.

Introduction

The oral cavity provides a diversified environment for a wide spectrum of bacteria to colonize. Gingivitis and periodontitis are two pathological illnesses that fall under the umbrella of periodontal diseases. Periodontitis is a local infection in the gingival crevices caused by a primary bacterial etiology that affects the structural organs around the teeth, such as the periodontal ligament, connective tissue, and bone. In the sub-gingival area, the warm and wet pocket environment promotes the growth of gram-negative, anaerobic bacteria. Adult periodontitis affects around 70 million people worldwide. The goal of dental care is to keep the population of bacteria under control. Controlling bacterial plaque can help to slow or stop the progression of oro-dental infections. Antibiotics administered systemically are effective in reducing sub-gingival flora, but it has been discovered that stopping systemic antibiotic medication results in bacterial recolonization(Mthethwa & Matjila, 2018; Queen et al., 2020). As a result, total eradication of germs necessitates long-term antibiotic therapy. High oral doses are required to achieve effective concentrations in the pocket, but high doses given for lengthy periods of time result in the development of resistant bacteria strains, super infection, and gastrointestinal and central nervous system problems. Recent technological breakthroughs have led to the development of innovative drug delivery systems that allow controlled therapeutic activity by targeting the distribution of a drug to a specific site, overcoming the shortcomings of systemic chemotherapy with antibiotics. When a medicine is focused to a specific place, the drug's distribution to other body organs is reduced. When compared to traditional dosage forms, controlled drug delivery systems provide a number of advantages(Goodson, 1985). We can immediately deposit the medicine in the cavity in the form of films, reducing the antibacterial agent's influence on non-oral body parts.

Periodontal films are a type of matrix delivery device in which the medicine is released through diffusion or matrix dissolution and applied to the periodontal pocket or paradentium to treat periodontal diseases. The pharmaceutical composition, which comes in gel, sheet, film, or bar-like form, distributes a controlled and effective amount of an active substance into the periodontal pocket(Anjana et al., 2021). Periodontal disease treatment films including polymers and active substances have been created. Plaques and irritation beneath the gingival margin are believed to be treated using these films. These can be immediately placed to the lesional location to be treated; allowing the active ingredient to be concentrated to the appropriate site. This new therapeutic approach has been shown to be more effective than traditional medication. The most appropriate forming technique is chosen, among other things, based on the physicochemical properties of the polymers used. The drug release can be extended for a long time by combining various co-polymers with ethyl cellulose(Addy & Renton-Harper, 1996; González-Matheus et al., 2015).

The film or sheet can have a thickness of 0.1-0.5 mm, a width of 0.5-3 mm, and a length of 5-50 mm. Furthermore, the user may cut the composition to a reasonable size based on numerous criteria, including the severity of the disease and the breadth and depth of the locus to be administered(Meher & Dighe, 2019). By insertion, the substance can be applied to the periodontal pocket or

paradentium. When a film is deposited in a periodontal cavity, the polymer swells, expands, and reaches the cavity's narrow fissures and furcations, spreading the active ingredient throughout. This results in the best efficacy at the treatment site(Schwach-Abdellaoui et al., 2000).

Doxycycline is a tetracycline antibiotic that is used to treat bacterial infections. Tetracycline class antibiotic having broad spectrum activity against gram positive and gram negative microorganism. For the treatment of anaerobic infection, 500 mg oral; i. v every 6-8 hours for seven days is advised. Antimicrobial medicines are increasingly being delivered locally because it results in a larger concentration of the antibiotic at the desired site of action with a lower dose and fewer side effects(Dasari et al., 2021; Graber, 2021). The major goal of this study was to create and test controlled release dental films containing Doxycycline for use in periodontitis patients' periodontal pockets for targeted drug administration and to minimize periodontal infections. To find the best formulation, researchers looked at the effects of several formulation variables on the drug release profiles from the films.

Materials and Methods

Doxycycline was obtained from Cipla Ltd. Mumbai, India, Gelatin, glutaraldehyde and glycerin procured from Research lab chemical corporation Mumbai. Other materials used in the study were of analytical grade and UV spectrometer (Shimadzu 2401/PC Japan) was used for analytical purpose.

Preformulation studies

Preformulation study of the drug was carried out to establish its identity and purity which includes λmax of the drug. An absorption maximum of doxycycline was determined using water and methanol solution ranging from 10-50µg/ml were scanned from 200-400 nm using UV spectrophotometer(Rani & Singh, 2018).

FTIR Studies

Compatibility of doxycycline with the excipient was established by Infrared Absorption Spectral Analysis (FTIR). Any changes in the chemical composition after combining with the excipient were investigated with IR spectral analysis(Shaikh et al., 2021).

Differential Scanning Calorimetry (DSC) studies

DSC curves were obtained by Mettler Star SW 9.01**.** Sample 1-4 mg was placed in aluminum pan press sealed with an aluminum cover. An empty sealed in the same way was used as reference. Thermo grams were measured by heating the sample from 35 to 300^0C at the rate of 10^0C /min, under a nitrogen flow of 10 mL /min(Dasari et al., 2021; Shaikh et al., 2021).

Factorial design

A factorial design is used to evaluate two or more factors simultaneously. The treatments are combination of levels of the factors. It is used in experiments of different factors conditions, on experimental results are to be elucidated. Factorial designs are the designs of choice for simultaneous determination of the effect of several factors and their interactions.

3^2Full Factorial Designs

Factorial Design was employed for optimization of dental film formulation to identify the critical factors that affect the process or product. This design was used primarily for screening significant factors, and also used sequentially to model and refine a process. A 3^2 full factorial design was constructed where the concentration of Gelatin (X_1) and Glycerin (X_2) were selected as the independent variables. The dependent variables (levels) percent *In-vitro* drug release and Tensile strength of film were selected on the basis of the preliminary studies carried out before implementing the experimental design. All other formulation and processing variable were kept constant throughout the study.

Variables for Experimental Designs
Independent variable: X_1= Concentration of Gelatin, X_2= Concentration of Glycerin
Dependent variable: Y_1= *In-vitro* drug release, Y_2= Tensile strength of film

Preparation of Doxycycline-Loaded Gelatin Film
Periodontal films were prepared by solvent casting technique. Glass molds were used for casting of the film. Formulations were designed as shown in the Table 1, in which Gelatin was taken as the main biodegradable polymer in combination with different concentrations for each cast films. Films were prepared by dissolving Gelatin in water at 60^{o}C for 15 min. cool it, using Glycerin as plasticizer. Doxycycline was added in to the gelatin solution and mixed homogenously using magnetic stirrer in a closed beaker. The crosslinking reagent was added to the gelatin solution and mixed by magnetic stirrer for 10 min. The viscous dispersion was kept aside for 30 min for complete expulsion of air bubbles. Films were cast by pouring the drug-polymer solution into the center of glass molds and allowed to dry at room temperature. The dry films were cut into strips of (5×5 mm), wrapped in aluminum foil and stored in desiccators at room temperature pending evaluation.

Table 1
It shows formulae used for the development of Doxycycline dental films as per factorial Design Layouts

Batches	Doxycycline (%)	Gelatin(gm)	Glycerin(mL)	Glutaraldehyde (%)
F1	1	4	1	0.5
F2	1	4	1.5	0.5
F3	1	4	2	0.5
F4	1	4.5	1	0.5
F5	1	4.5	1.5	0.5
F6	1	4.5	2	0.5

F7	1	5	1	0.5
F8	1	5	1.5	0.5
F9	1	5	2	0.5

Evaluation of drug loaded Periodontal Films

Thickness of the films

Thickness of the film was measured using screw gauge at different areas of the film and the average was calculated(Umadevi et al., 2012).

Uniformity of weight of the films

Film (size of 5x5 mm) was taken from different areas of film. The weight variation of each film was calculated(Umadevi et al., 2012).

Folding endurance

The folding endurance of the films was determined by repeatedly folding the film at the same place till it broke or folded, which is considered satisfactory to reveal good film properties. This test was carried out on all the films(Sakellariou & Rowe, 1995).

Drug content uniformity of films

Film (size of 5x5 mm) was taken from different areas of the film and placed into a 100 ml volumetric flask, in to which 100 ml of water was added and kept aside till the film is completely dissolved. The absorbance of the solution was measured at 273 nm.

Estimation of percentage moisture loss

The films of different concentrations were weighed accurately and then they are kept in desiccators for 3 days and then reweighed and by using the formula % moisture loss was calculated by formula: (Initial wt - Final wt/Initial wt) ×100(Naik et al., 2019).

Tensile strength of the film

Tensile strength was evaluated using a tensile strength tester (Brookfield Engineering Labs, Inc.) with a 5 g load cell. Films of the required dimension and free from air bubbles or physical imperfections were held between two clamps. During measurement, the top clamp, at a rate of 0.5 mm/s, was pulled, and the force was measured when the film broke. Only results from film samples that broke between the clamps were used(Fulzele et al., 2002).

***In-vitro* release studies**

The pH of gingival fluid lies between 6.5-6.8, so phosphate buffer pH 6.8 was used as simulated gingival fluid. Also, since the film should be immobile in the periodontal pocket, a static dissolution model was adopted for the dissolution studies. Sets of six films of known weight and dimension were placed separately in small sealed 1 ml test tubes containing 1.0 ml of phosphate buffer (pH 6.8) and kept at 37 ± 0.5 °C for 24 h. The buffer was then drained off and replaced with a fresh 1.0 ml of buffer. The concentration of the drug was determined by UV/VIS spectrophotometer at 276 nm. The procedure was continued for 12 consecutive days(Nakahara et al., 2003).

Zone of inhibitions of film

Inoculate a previously liquefied medium appropriate to the assay with the requisite quantity of suspension of the microorganism, add the suspension to the medium at a temperature between 40º C and 50º C and immediately pour the inoculated medium into the petri dishes to give a depth of 3 to 4 mm. Ensure that the layers of medium are uniform in thickness, by placing the dishes or plates on a level surface. Store the prepared dishes in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes used and that the surface of the agar layer is dry at the time of use. Apply the solutions to the surface of the solid medium in sterile cylinders or in cavities prepared in the agar. The volume of solution added to cavity must be uniform and sufficient almost to fill the holes when these are used. When Petri dishes are used, arrange the solutions of the Standard Preparation of antibiotic under examination on each dish so that, they alternate around the dish and so that the highest concentrations of standard and the films ($0.5cm^2$) were placed over the medium and plates were incubated for 24 hours at 37°C. Accurately measure the diameters or areas of the circular inhibition zones and calculate the results(S. Khan et al., 2021; S. L. Khan et al., 2020; Shntaif et al., 2021; Siddiqui et al., 2021).

***In-Vitro* Study (Kill Kinetics Study)**

Kill kinetics was performed on *Staphylococcus aureus* and *E. coli.* Doxycycline pure drug, gelatin loaded doxycycline film and a positive control were added to broth cultures of approximately 10^6 cfu/mL. Kill kinetics, was conducted with a final concentration of antimicrobial agent at two times the MIC (2 x MIC). Viable counts were performed at 0, 1, 2, 4, 6, 8 and 24, 168 hr. after the addition of Doxycycline pure drug; film containing Doxycycline and a positive control following serial dilution in saline solution. Bacteria were counted after 24 hr. incubation at 35-37°C and time kill kinetics were plotted as viable count against time(Naik et al., 2019).

Stability Studies

Stability study was carried out on the optimized formulation. The formulation was wrapped in aluminum foil and then placed in a stability chamber. It was stored at 40 ± 2°C, 75% ± 6% relative humidity for 2 months. The samples were analyzed for its physiochemical properties of film after Zero, One, & Two months(Bajaj et al., 2012; Khagga et al., 2019).

Results and Discussion

Result

To confirm the identity, purity and suitability of drug for formulation and to establish a drug profile, Preformulation studies were undertaken.

λmax of the drug

The λmax of the drug was found to be 276 nm and it was in accordance with the official standard.

FTIR study

FTIR spectra of Doxycycline alone and its combination with polymers are shown in fig. 1-3. From the spectra confirmed the absence of any chemical incompatibility between the drug and the polymer.

Differential Scanning Calorimeter (DSC) study

The DSC curves of pure drug and gelatin physical mixture were shown in the fig. 4&5. Doxycycline showed an endothermic peak at 205.73°C corresponding to its melting point. Drug: gelatin (1:1) physical mixture showed two endothermic peaks; at 173.6ºC.The intact DSC peak of drug in the physical mixture indicates that the drug did not interact with the excipient used in the film.

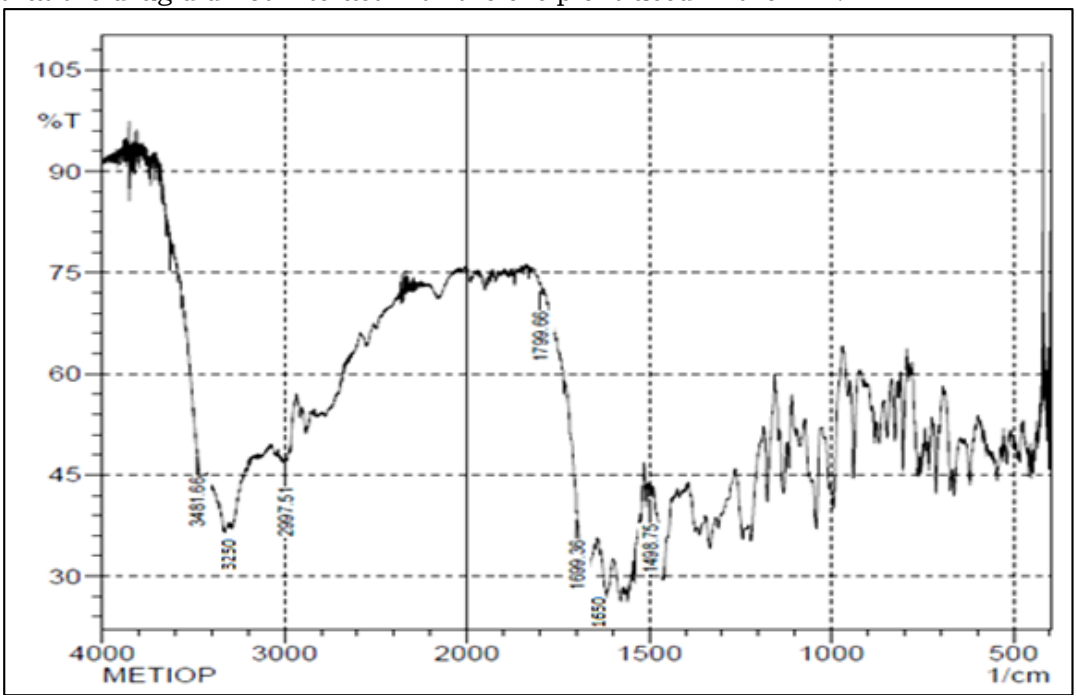


Fig.1: It shows FTIR Spectrum of Doxycycline

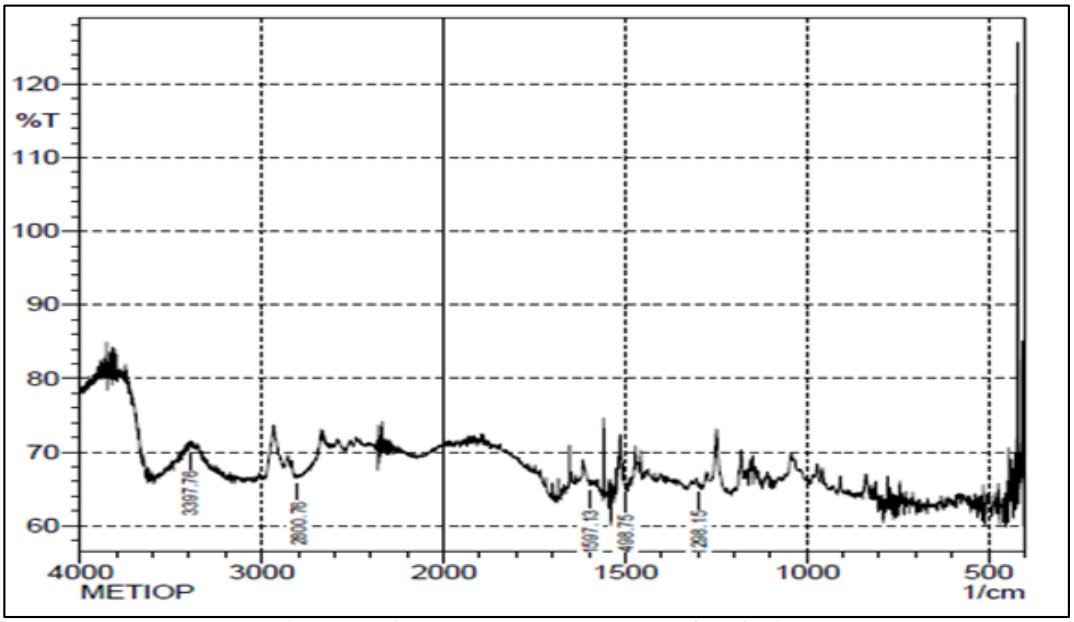


Fig.2: It shows FTIR Spectrum of Gelatin

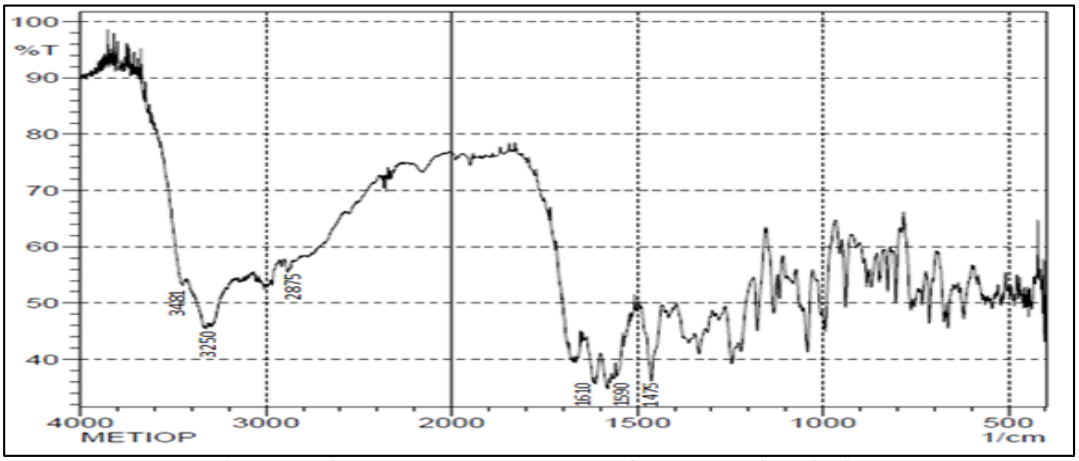


Fig.3: It shows FTIR Spectrum of Drug and Gelatin

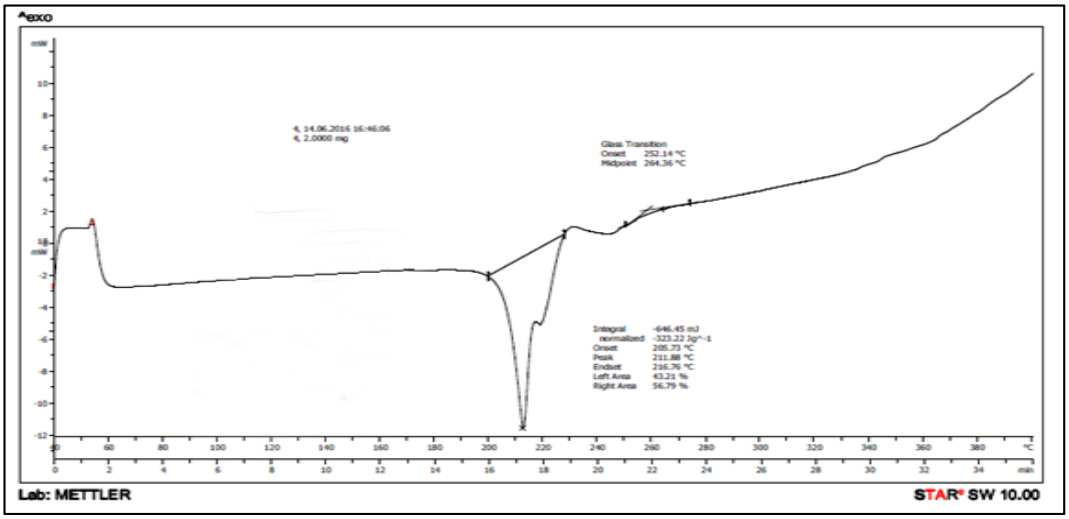


Fig.4: It shows DSC Spectrum of Doxycycline

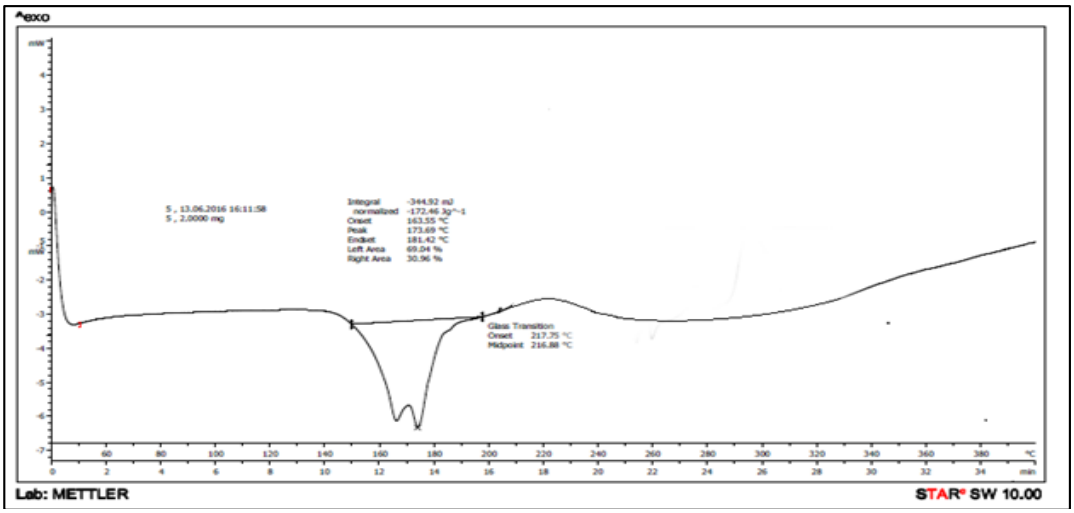


Fig.5: It shows DSC Spectrum of Doxycycline and gelatin

Evaluation of the prepared films
Thickness of the films

Films of bathes F1 to F9 were evaluated for their thickness of area 2cm^2. It was confirmed that the film prepared was of uniform thickness.Results showed in Table 2. There was no statistically significant difference between the all formulated batches that is P>0.05. From this result it was conclude that as the concentration of film forming polymer increases the thickness of film also increases.

Uniformity of Weight of the films

The weight variation indicate that different weights were relatively similar of the film form different areas of film and Films of bathes F1 to F9 were evaluated for their weight was showed in Table 2. From this result it was conclude that as the concentration of film forming polymer increases the weight of film also increases.

Folding endurance

Folding endurance results of dental films were showed in Table 2. It indicates that the film would not break and would maintain their integrity with the periodontal pocket. Folding endurance of the films >250 times indicate that the formulations have good film properties. The table 2 showed that as the concentration of film forming polymer i.e. Gelatin increases the number folding the film was decreases. Concentration of Gelatin increases the brittleness of film.

Table 2
Mechanical properties of gelatin film
Drug content uniformity of films

Formulations	Thickness (mm)	Weight variation (mg)	Folding endurance	Tensile strength (kg/cm^2)
F1	**0.315 ±0.04**	**8.38 ±0.15**	**272.5 ±0.12**	**0.764± 0.12**
F2	0.316 ±0.05	8.45 ±0.12	289.9 ±0.24	0.833±0.23-
F3	0.312 ±0.02	7.68 ±0.04	275.9 ±0.30	0.935±0.10
F4	0.352 ±0.02	8.18 ±0.05	250.8 ±0.35	0.950±0.19
F5	0.368 ±0.07	9.05 ±0.03	236.6 ±0.37	1.158±0.15
F6	0.348 ±0.05	7.74 ± 0.12	249.0 ±0.39	1.298±0.28
F7	0.384 ±0.03	10.06 ±0.03	235.7 ±0.25	1.518±0.13
F8	0.372 ±0.01	10.26 ±0.03	240.4 ±0.44	2.137±0.34
F9	0.384 ±0.04	10.1 ±0.08	254.9 ±0.28	2.543±0.12

Drug content of the films were showed in Table 3. All the films were found to contain an almost uniform quantity of the drug, as per content uniformity studies, indicating reproducibility of technique.

Moisture Loss (%)

The moisture content of the films was showed in Table 3. The moisture content in the formulations helps them to remain stable and from being a completely dried and brittle film.

Tensile strength of the films

The tensile strength of films was showed in Table 2. Which show that the film shows good tensile strength indication the film wound not break and will remain intact in the periodontal pocket. From this study it was concluded that as the concentration of films forming polymer gelatin increases, the tensile strength of films was also increases

Table 3
Physicochemical properties of gelatin film
In-vitro release studies

Formulations	Drug content (%)	% Moisture loss	Drug release (%)
F1	**92.37 ±0.42**	**4.01 ±0.04**	**95.92±0.65**
F2	93.56 ±0.44	3.2 ±0.03	92.39±0.74
F3	90.66 ±0.56	4.1 ±0.06	91.10±0.23
F4	89.00 ±0.54	2.6 ±0.03	89.76±0.61
F5	88.65 ±0.66	5.3 ±0.04	88.26±0.37
F6	91.87 ±0.36	4.04 ±0.06	87.69±0.39
F7	90.78 ±0.74	3.3 ±0.05	87.24±0.34
F8	87.96 ±0.65	4.5 ±0.02	84.45±0.35
F9	85.54 ±0.59	2.3 ±0.06	83.74±0.50

The release time profile for different concentration of polymer films were shown in fig.6.The *in-vitro drug release studies* showed % drug release of 10.61% to 95.92% up to 12 days and ranked in the order F1>F2>F3>F4>F5>F6>F7>F8>F9. The release profile showed that, show and sustained release was seen up to 12th days. At the end of 12th days the amount of drug release were showed in Table 3&4.

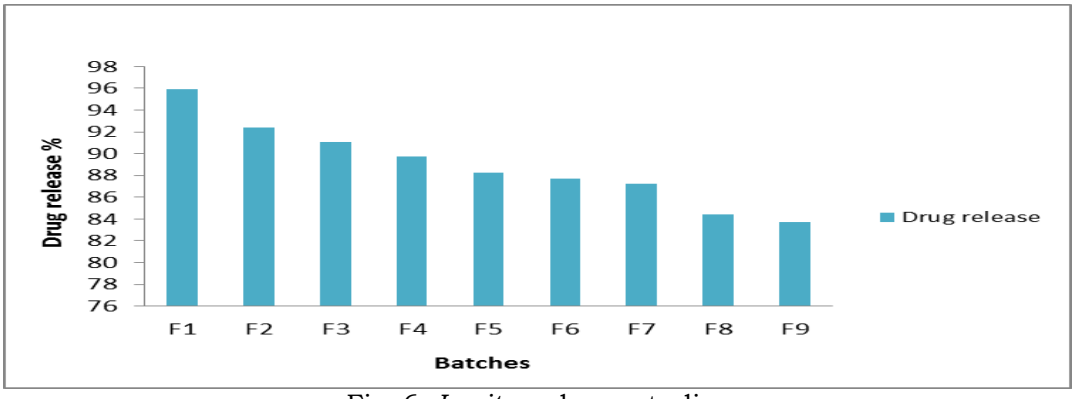


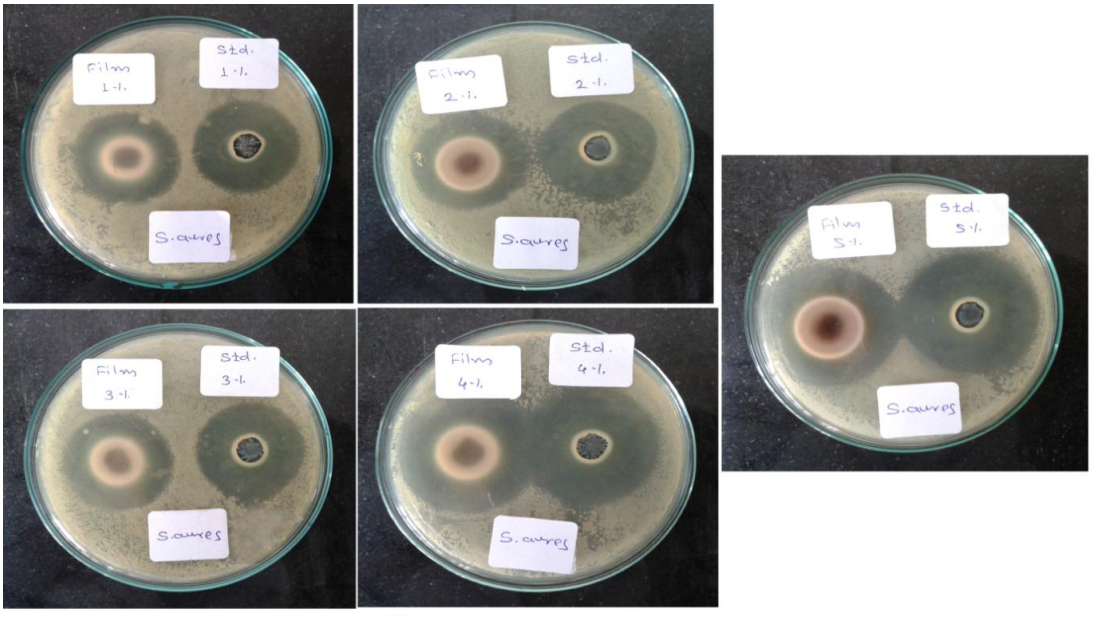
Fig. 6: *In-vitro* release studies

Table 4
In vitro Release Studies of films

Formulation / Days	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	10.61± 0.32	10.33 ±0.56	10.22 ±0.97	10.16± 0.39	9.65±0 .22	9.76±0 .62	9.99±0 .73	9.42± 0.19	9.65± 0.90
2	20.12± 0.87	19.55 ±0.87	19.30 ±0.14	19.21± 0.63	18.62± 0.69	18.76± 0.80	18.96± 0.20	17.99 ±0.38	18.16 ±0.31
3	29.15± 0.53	28.28 ±0.45	27.90 ±0.35	27.70± 0.54	26.99± 0.45	26.99± 0.17	26.90± 0.45	26.05 ±0.79	26.02 ±0.88
4	37.39± 0.44	36.25 ±0.23	35.76 ±0.74	35.46± 0.20	34.62± 0.65	34.56± 0.44	34.40± 0.50	33.39 ±0.81	33.28 ±0.60

5	45.41± 0.10	44.00 ±0.78	43.38 ±0.22	42.98± 0.48	42.04± 0.78	41.91± 0.69	41.65± 0.96	40.48 ±0.47	40.30 ±0.36
6	53.23± 0.62	51.53 ±0.81	50.80 ±0.34	50.31± 0.69	49.27± 0.90	49.08± 0.49	48.72± 0.47	47.38 ±0.89	47.15 ±0.13
7	60.88± 0.79	58.90 ±0.22	58.04 ±0.65	57.46± 0.36	56.32± 0.21	56.05± 0.78	55.61± 0.78	54.09 ±0.64	53.82 ±0.73
8	68.34± 0.22	66.05 ±0.41	65.07 ±0.87	64.40± 0.84	63.17± 0.78	62.83± 0.12	62.31± 0.63	60.59 ±0.15	60.28 ±0.81
9	75.57± 0.31	72.98 ±0.36	71.88 ±0.30	71.10± 0.45	69.79± 0.60	69.37± 0.54	68.78± 0.74	66.87 ±0.73	66.51 ±0.62
10	82.59± 0.80	79.70 ±0.90	78.47 ±0.67	77.57± 0.78	76.19± 0.12	75.69± 0.81	75.03± 0.83	72.93 ±0.68	72.51 ±0.78
11	89.38± 0.78	86.17 ±0.49	84.82 ±0.91	83.79± 0.34	82.35± 0.67	81.77± 0.36	81.06± 0.18	78.75 ±0.31	78.26 ±0.36
12	95.92± 0.65	92.39 ±0.74	91.10 ±0.23	89.76± 0.61	88.26± 0.37	87.59± 0.39	87.24± 0.34	84.45 ±0.35	83.74 ±0.50

Zone of inhibitions of film



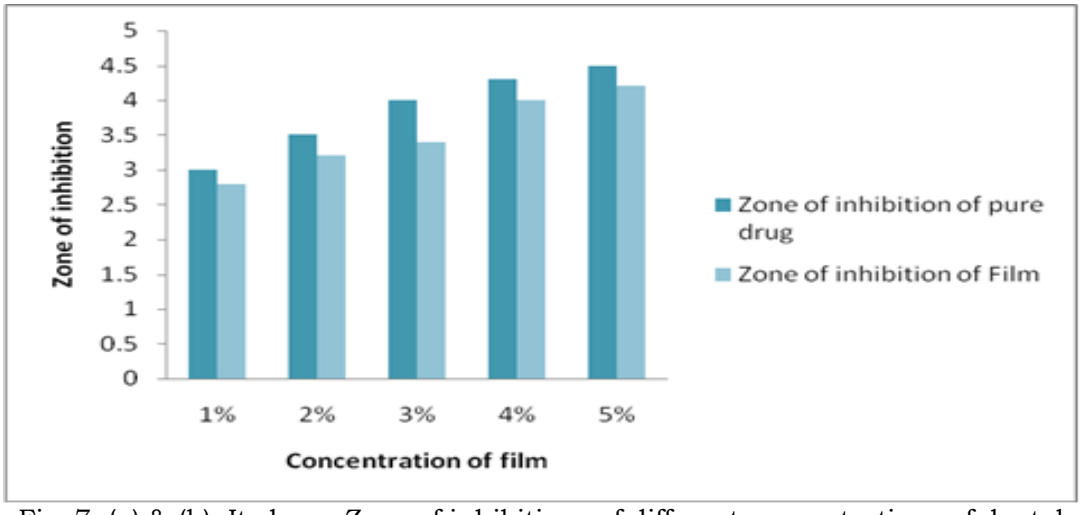
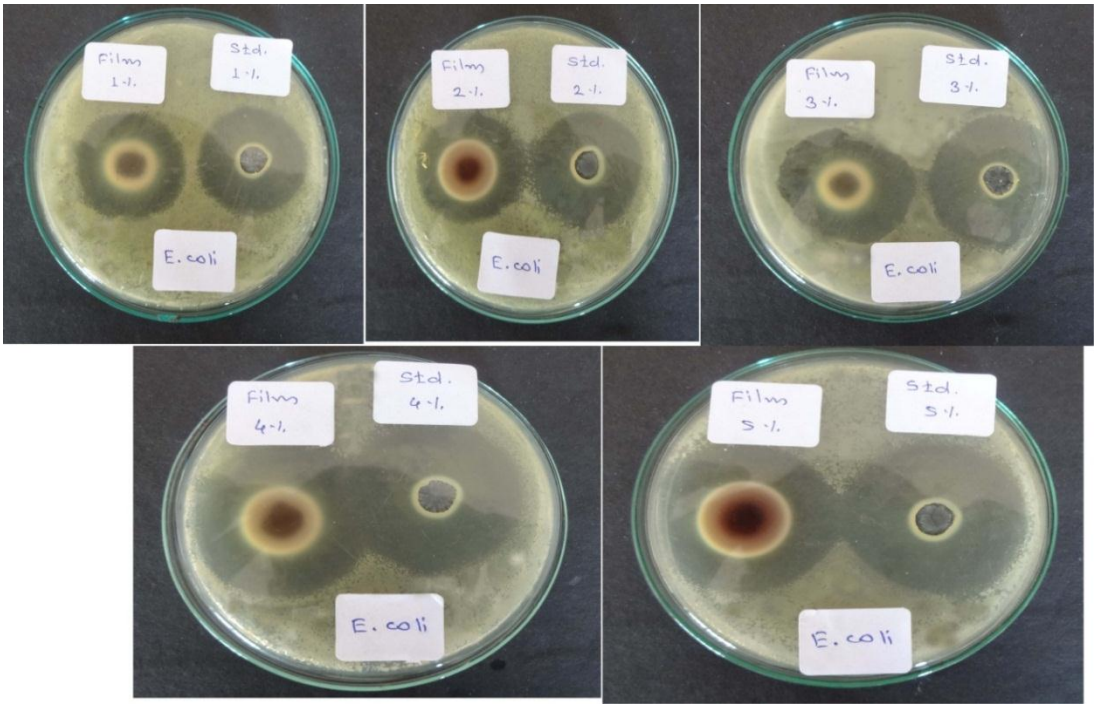


Fig. 7. (a) & (b): It shows Zone of inhibitions of different concentrations of dental films and pure drug against *S.aureus*



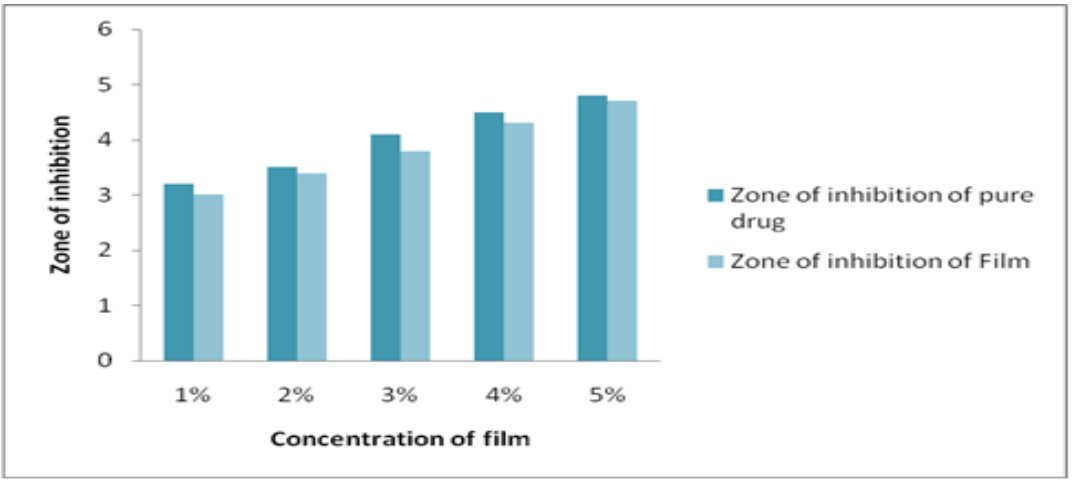


Fig. 8. (a) & (b): It shows Zone of inhibitions of different concentrations of dental films and pure drug against *E.coli*

Experimental design and data analysis
Tensile Strength

The goal of this study was to develop optimum formulations by analyzing the impact of several key elements and their interactions on the physiochemical properties of gelatin films during the process production. Meanwhile, the gelatin film was being developed, and the impact of various elements was being assessed by varying their quantities. Finally, the independent variables were chosen to be two of the most significant factors. Following that, different formulations were created to determine the low and high values of each element, and the results are listed in Table 5. An experimental matrix was created using a three level two factorial design using these two variables, and nine experiments were carried out as shown in Table 5.The Tensile strength and drug release was mentioned 9 formulations were obtained given in the Table 5.

Table 5
Experimental design of the optimization step

Formulations	Factor 1 Gelatin (gm)	Factor 2 Glycerine (mL)	Response I Tensile strength (kg/cm^2)	Response II Drug release (%)
F1	4	1	0.764	95.92
F2	4	1.5	0.833	92.39
F3	4	2	0.935	91.10
F4	4.5	1	0.950	89.76
F5	4.5	1.5	1.158	88.26
F6	4.5	2	1.298	87.69
F7	5	1	1.518	87.24
F8	5	1.5	2.137	84.45
F9	5	2	2.543	83.74

Analysis of Variance and Model Equations for Tensile strength

According to applied 3 levels 2 factorial experimental design including 9 experiments were performed to optimize the formulation of Doxycycline containing film to get minimum disintegration time and moderate tensile strength in terms of responses. The obtained results were entered in design expert® software 7.1 and a model equation were obtained to get the fit result for disintegration time and tensile strength. The final models in terms of coded and actual factors are,

Table 7
Analysis of Variance for Tensile strength

Source	Sum of Squares	DF	Mean Square	F Value	p-value Prob > F	Inference
Model	2.85	4	0.71	13.66	0.0133	significant
A-gelatin	2.44	2	1.22	23.47	0.0062	
B-glycerin	0.40	2	0.20	3.85	0.1169	
Residual	0.21	4	0.052			

The Model F-value of 13.66 implies the model is significant. There is only a 1.33% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 8
Model Summary Statistics

Std. Dev.	0.23	R-Squared	0.9318
Mean	1.35	Adj R-Squared	0.8636
C.V. %	16.92	Pred R-Squared	0.6547
PRESS	1.05	Adeq Precision	10.211

The "Pred R-Squared" of 0.6547 is not as close to the "Adj R-Squared" of 0.8636 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 10.211 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors

Tensile strength = + 0.57289 + 0.87156*gelatin + 1.08756*glycerin + 0.86422 + 1.16289*glycerin*gelatin + 1.37889.....(1)

From the equation (I) it was concluded that gelatin (factor A), glycerin (factor B) having a individual as well as combined effect on the increasing in tensile strength.

Effect of gelatin, glycerinand both interactions on tensile strength

The graph shows that when the concentration of gelatin increases, the tensile strength increases as well. The concentration of gelatin may have an individual effect on the Tensile strength, as shown in Fig. 9 (a). The graph shows that when the concentration of glycerin increases, the tensile strength increases as well. The concentration of glycerin may have an individual effect on the Tensile strength, as shown in Fig. 9 (b). Figure 9 (c) depicts the interaction between gelatin with glycerin. Both factors A and B have large influence on their own. The plot indicates that increasing the level of gelatin and glycerin at the prediction point has a considerable impact on tensile strength.

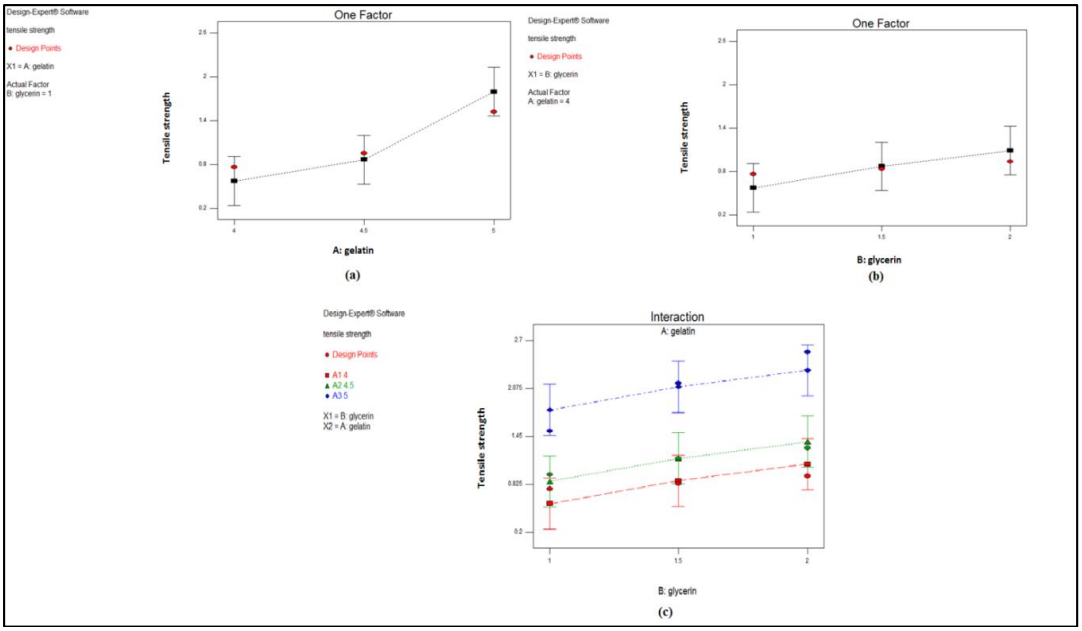


Fig 9 (a): Effect of gelatin on tensile strength, (b) Effect of glycerine on tensile strength,
(c) Effect of gelatin and glycerine interaction on tensile strength

Drug release
Analysis of Variance for Experimental Matrix (ANOVA)

According to applied 3^2 experimental factorial designs including 9 experiments was performed to optimize the Drug release. The obtained results were entered in design expert® software and a model equation were obtained to get the fit result for drug release.

Table 9
Analysis of Variance for Drug release

Source	Sum of Squares	DF	MeanSquare	F value	P value Prob> F	Inference
Model	3.11	4	0.7775	15.62	0.0159	significant
A-gelatin	2.93	2	1.465	29.04	0.0096	
B-glycerin	0.59	2	0.295	4.15	0.147	
Residual	118.07	4	14.76			

The Model F-value of 15.62 implies the model is significant. There is only a 1.59% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 10
Model Summary Statistics

Std. Dev.	3.84	R-Squared	1.6584
Mean	88.95	Adj R-Squared	4.875
C.V. %	4.32	Pred R-Squared	2.675
PRESS	149.44	Adeq Precision	63.231

A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model.

Final Equation in Terms of Actual Factors

Drug release = 88.95000 + 0.88623*gelatine + 1.06325*glycerine + 0.88423 + 1.14235*glycerine*gelatine + 1.36954……(II)
From the equation (II) it was concluded that gelatin (factor A), glycerin (factor B) having an individual as well as combined effect on the increasing in Drug release.

Effect of gelatin, glycerin and both interaction on drug release

The graph shows that as the amount of gelatin in the solution increases, the drug release decreases. The concentration of gelatin might have an individual effect on drug release, according to Fig. 10 (a). It is clear from the graph that as the concentration of glycerin rises, the drug release decreases. The concentration of glycerin might have an individual effect on drug release, according to Fig. 10 (b). Figure 10 (c) depicts the interaction between gelatin with glycerin. Both factors A and B have large influence on their own. The plot demonstrates that the decreases in gelatin and glycerin at the prediction point had a major impact on tensile drug release.

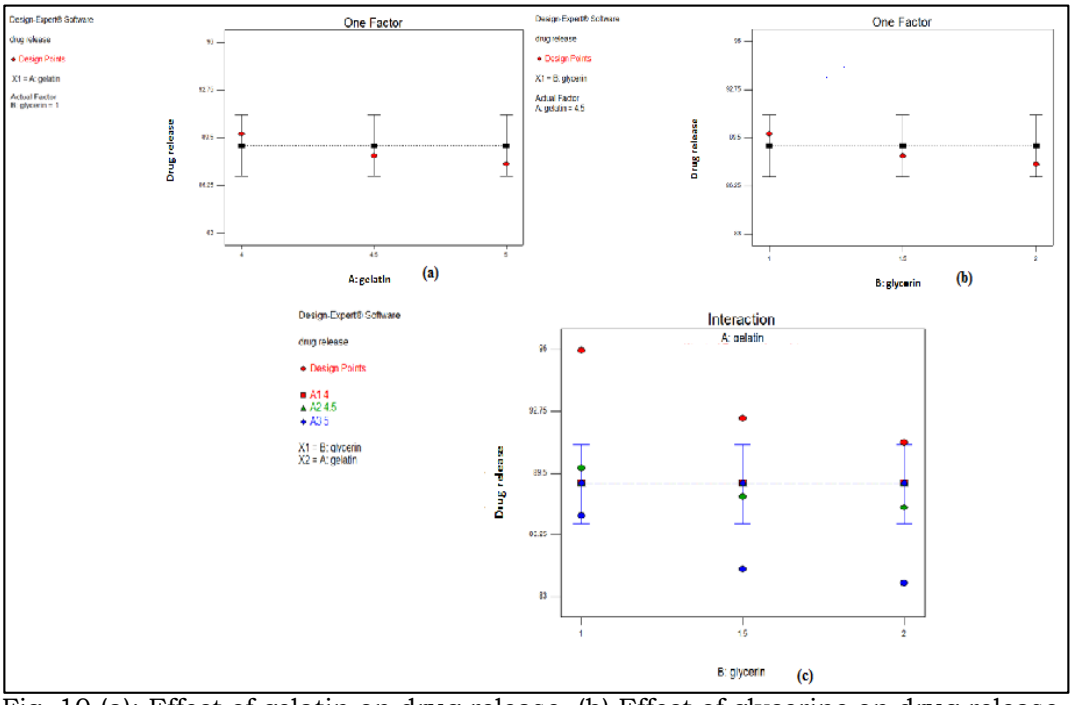


Fig. 10 (a): Effect of gelatin on drug release, (b) Effect of glycerine on drug release, (c) Effect of gelatin and glycerine interaction on drug release

Selective Formulation for Optimized Batch

Table 11
Low and High Level for the generation of Optimized Batch

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Gelatin	Minimize	4	5	1	1	3
B: Glycerin	Minimize	1	2	1	1	3
Tensile strength	is equal to	0.764	2.543	1	1	3
Drug release	Maximum	83.74	95.92	1	1	3

Table 12
No. of Solutions Obtained by DE 7.1

Solution Number	Gelatin (gm)	Glycerin (mL)	Tensile strength (kg/cm^2)	Drug release (%)	Desirability
1	4	1	0.764	95.92	1.000

The optimized solution obtained from the model was formulated and the results are performed in the triplicates for determination disintegration time and Tensile strength. The solution was found to comply all specifications hence considered optimized.

Table 13
Composition of the Optimized Formulation (F1)

Ingredients (mg)	Optimized formulation
Doxycycline (%)	1
Gelatin (gm)	4
Glycerin (mL)	1
Glutaraldehyde (%)	1
Water (mL)	100

Evaluation of Optimized Formulation

The optimized formulation was evaluated for its physical and mechanical parameters

Table 14
Evaluation of Optimized Formulation

Sr. No.	Parameters	Result
1.	Thickness	0.315±0.04mm
2.	Weight variation	8.38±0.15mg
3.	Folding endurance	272.5±0.12
4.	Tensile strength	0.764±0.12kg/cm^2
5.	Drug content	92.37±0.42%
6.	% moisture loss	4.01±0.04%
7.	Drug release	95.92±0.65%

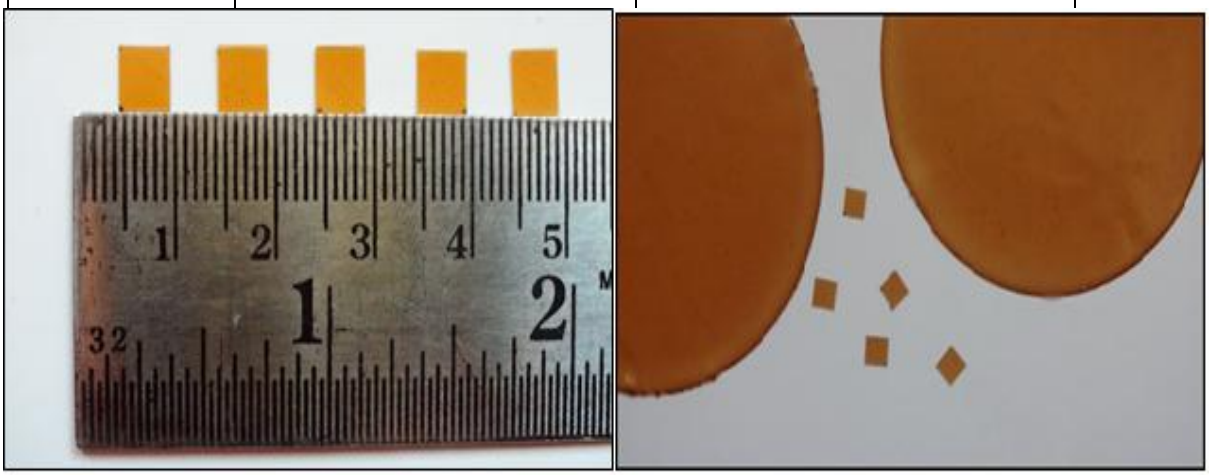


Fig. 11: Casted gelatin film of optimized batch (F1)

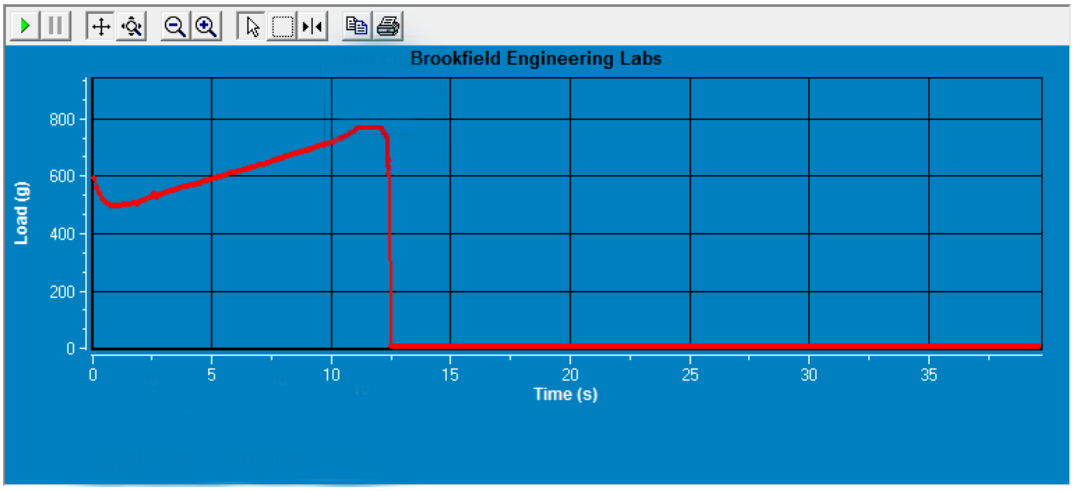


Fig. 12: Time (min) Vs Load (gm) curve of optimized batch F1

***In-Vitro* Study of Doxycycline Film (Time–Kill Kinetics on *Staphylococcus Aureus*)**

The basic concept of the Time-Kill Kinetic study is establishment of the rate at which a microorganism is killed by a product as a function of survival data recorded at enough exposure time points such that a graph can be constructed modelling the decline in population over time to a point of extinction as shown in table 15. Time–kill curves are pharmacodynamics examples of bactericidal activity expressed as rate of killing by a fixed concentration of antimicrobial. Time-kill kinetics gives a good overview of how fast an antimicrobial can kill certain bacteria and prevent their regrowth.

Kill kinetics for 5 % dental film on *Staphylococcus aureus*

Table 15
Kill kinetics for 5 % Dental film on *Staphylococcus aureus*

Dental film (5% drug concentration)			
Hrs	Pure drug (cfu/ml)	5% dental film (cfu/ml)	Control(cfu/ml)
0	$2.9*10^5$	$2.6*10^5$	$3.1*10^5$
1	$2.1*10^5$	$3.4*10^5$	$3.9*10^5$
2	$1.5*10^5$	$4.1*10^5$	$4.7*10^5$
4	$1.1*10^5$	$4.5*10^5$	$5.6*10^5$
6	$0.7*10^5$	$3.4*10^5$	$6.9*10^5$
8	$0.3*10^5$	$1.5*10^5$	$8.2*10^5$
24	$0.1*10^5$	$0.4*10^5$	$9.1*10^5$
168	$0.8*10^5$	$0.3*10^5$	$10.3*10^5$

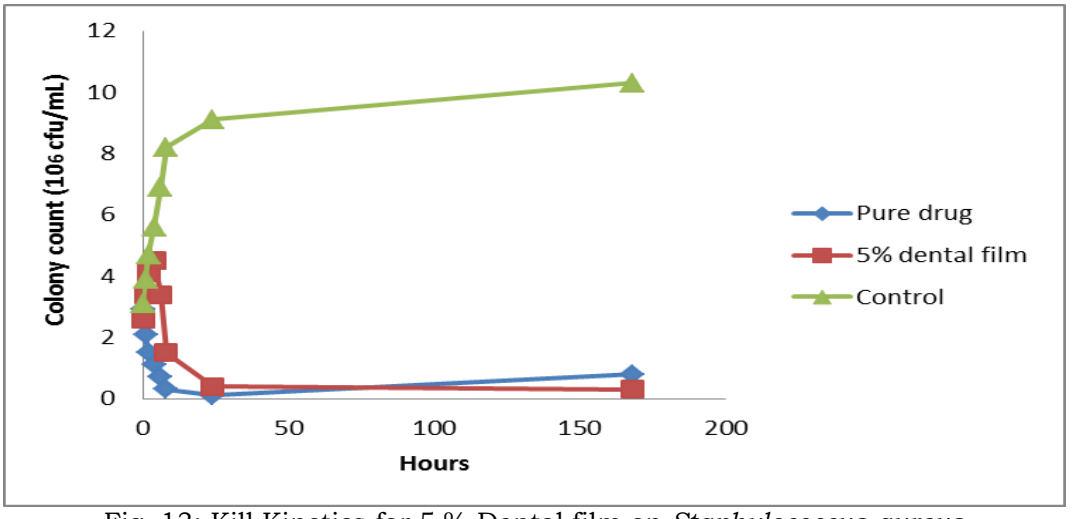


Fig. 13: Kill Kinetics for 5 % Dental film on *Staphylococcus aureus*

From the Table 15 and fig. 13, it was observed that there was decrease in colony count of Gelatin film and pure drug as compared to control along with reproducible results.

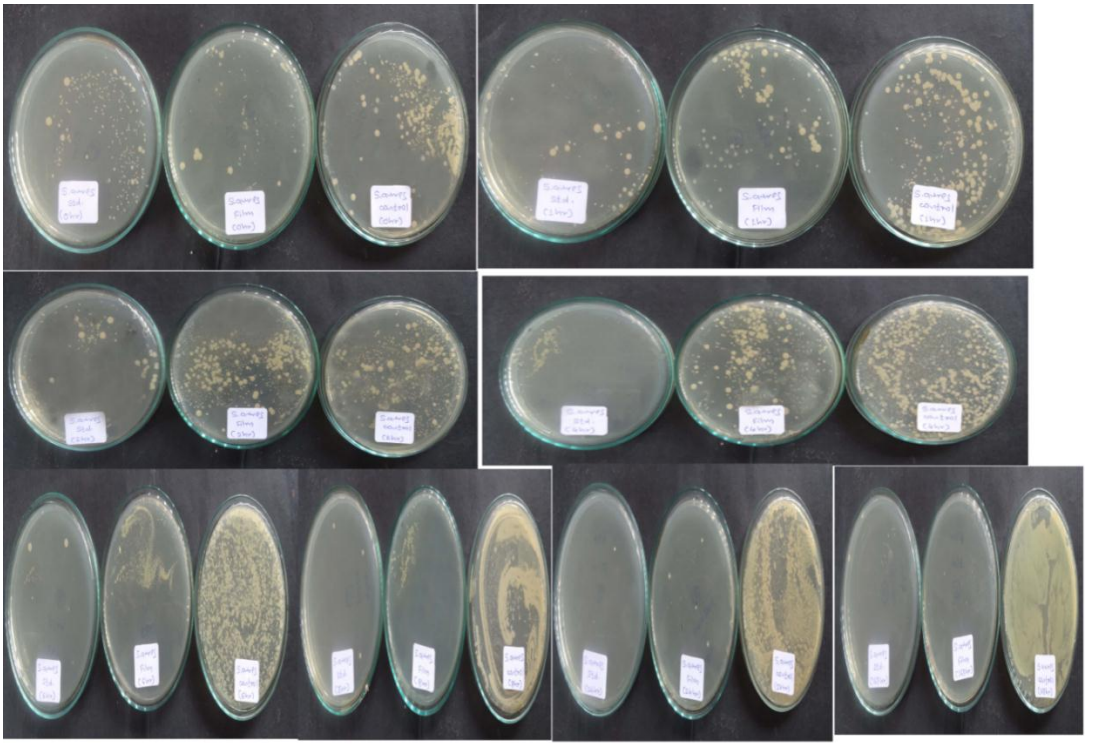


Fig. 14: It shows Kill kinetics (*Staphylococcus aureus)* of 5% dental film from 0 to 168 hrs.

Kill kinetics for 5% Dental filmon*Escherichia coli*

Table 16
Kill kinetics for 5% Dental filmon*Escherichia coli*

Dental film (5% drug concentration)			
Hrs	Pure drug (cfu/ml)	5% dental film (cfu/ml)	Control (cfu/ml)
0	$3.8*10^5$	$3.5*10^5$	$3.9*10^5$
1	$2.7*10^5$	$4.3*10^5$	$4.8*10^5$
2	$1.6*10^5$	$4.8*10^5$	$5.9*10^5$
4	$1.0*10^5$	$5.4*10^5$	$6.6*10^5$
6	$0.6*10^5$	$3.0*10^5$	$8.4*10^5$
8	$0.2*10^5$	$1.2*10^5$	$10.9*10^5$
24	$0.3*10^5$	$0.4*10^5$	$12.8*10^5$
168	$0.7*10^5$	$0.1*10^5$	$14.5*10^5$

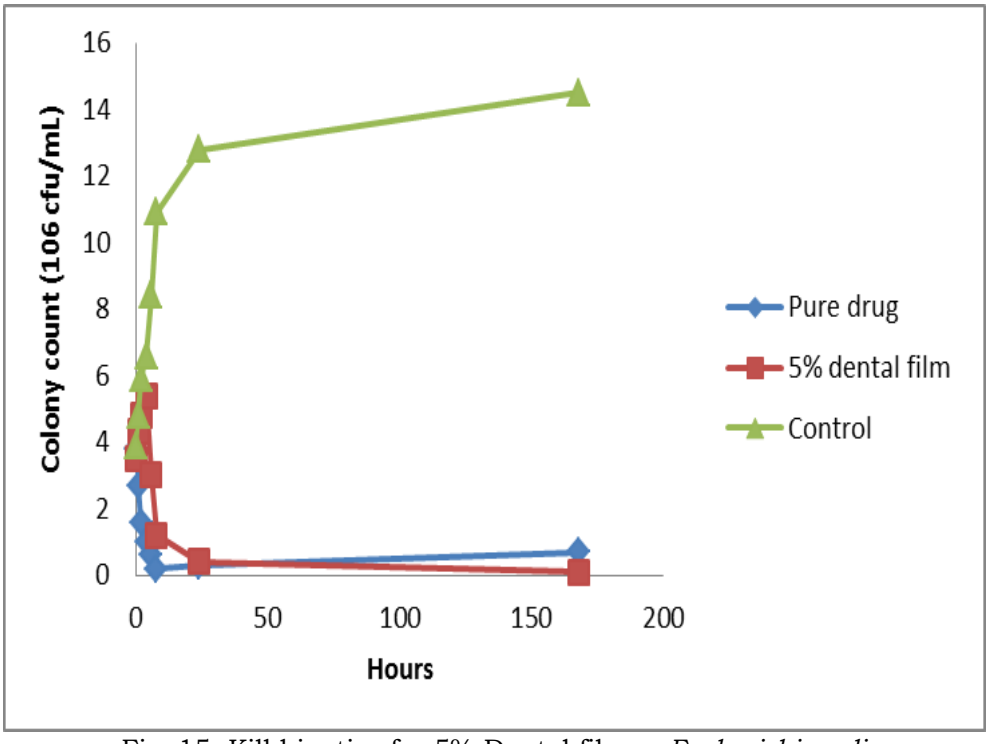


Fig. 15: Kill kinetics for 5% Dental filmon *Escherichia coli*

From the table 15, 5% dental film showed sustained drug release by kill kinetics as there is decrease in the colony count and it is compared with pure drug and control.

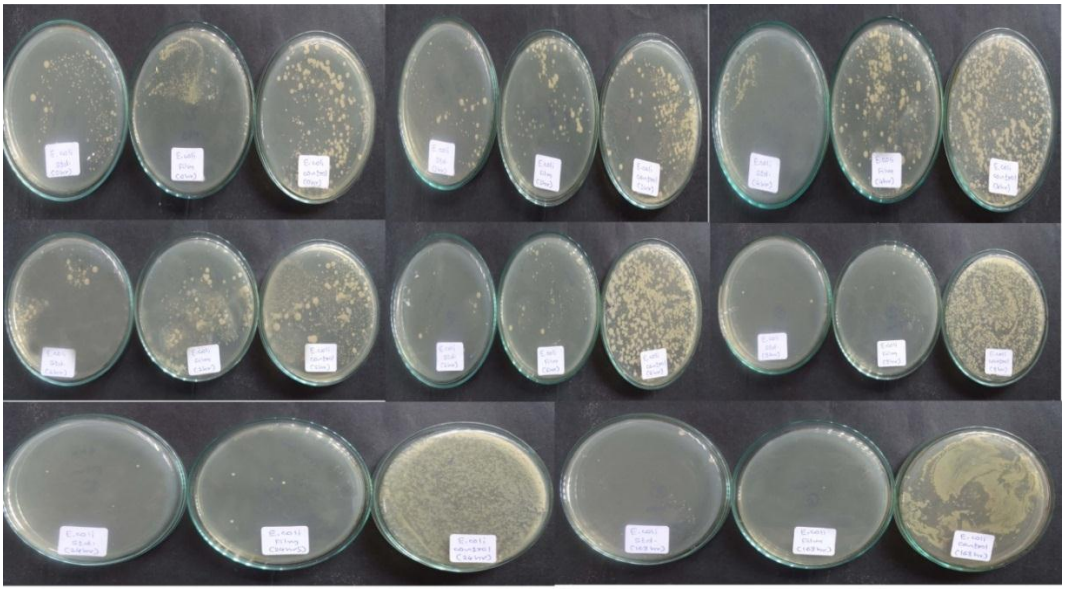


Fig. 16: It shows Kill kinetics (*E. coli)* of 5% dental film from 0 to 168 hrs.

Stability Studies

In order to determine the change in physicochemical parameter on storage, stability study was carried out. Effect of temperature and humidity was studied at 40°C ± 2 °C/75% ± 5 % RH maintained in environmental stability chamber for two month. An evaluation was done after 0, 1, and 2 month. The physicochemical parameter of the optimized formulation as given in Table 17 indicates that there was no significant change on storage gelatin film and showed very less strength and was brittle. The result indicates that the gelatin film formulation was stable on the required storage condition.

Table 17
Physicochemical properties of Dental film (stability studies)

Film type	Thickness (mm)	Weight Uniformity (mg)	Folding endurance	Moisture Loss (%)	Drug content (%)	Drug release (%)
F1	0.312 ±0.01	8.72 ±0.17	296.8 ±0.12	3.12 ±0.04	93.96 ±0.42	93.62±0.65

Discussion

Although a variety of delivery techniques have been studied for use in periodontal disease, an optimum targeted delivery system has yet to be discovered. The benefits of local intra-pocket administration of the periodontal film versus systemic delivery, as well as the need for a lower medication dose to obtain effective therapeutic concentration at the site of action. Dental film may be a better option for local medicine delivery with a longer duration of action and fewer adverse effects. Thickness, consistency of weight of the films, homogeneity of drug content of the films, moisture loss, tensile strength of the film, and in-vitro release

studies are some of the evaluation factors. In all of the insert formulations, the zone of inhibitions of film indicated that the inserts were physico-chemically and mechanically stable. The films were silky and uniform, Non-sticky and flexible (Brahmankar and Jaiswal, 1995).

The dental films containing doxycycline were made using a solvent casting approach using gelatine as the principal polymer and several copolymers in varying ratios. The absence of chemical interaction between the medication and the polymers was confirmed by FTIR investigations. All of the films have the same thickness and weight, according to the physicochemical evaluation data. Because of its hydrophobic nature, the percentage moisture loss in films created with gelatin is relatively low. The folding endurance was found to be larger than 250, indicating that good films were formed.

The drug content studies revealed that the drug was distributed uniformly and consistently throughout the videos. In vitro drug release experiments of F1 revealed that the drug was released slowly and consistently above the MIC level for 12 days. A static dissolving model was used in this study because the film remains immobile in the periodontal pocket. The initial burst effect caused by elution of the pharmaceuticals from the matrix's outer surface and cut edges resulted in a rapid initial release of the medication on day one. F1 film had a slow and controlled release for up to 12 days after the burst impact had worn off, and the percentage cumulative drug release was likewise higher in F1 formulations. According to mass balance studies, the drug content was not significantly different from the experimental drug content. The antibacterial activity of Film F1 against *E. coli* and *S. aureus* was proven. As a result, it has a high drug loading capacity, and studies have shown that it can release drugs. Film F1 was found to be stable at various temperatures during stability tests.

Conclusion

The above findings support the use of Doxycycline in the form of films made of gelatin as the main non-biodegradable polymer and glycerin as the plasticizer in an optimized concentration for controlling the rate of drug release above the MIC for 12 days in the treatment of periodontitis. During storage, the medication remained intact and stable in the periodontal films, with no chemical interactions with the excipients. All of the films had a burst discharge that was then regulated and extended for the duration of the study. The work demonstrates first-order drug release via a diffusion process. The antibacterial activity of the produced formulations was tested using *staphylococcus aureus* and *E. coli* strains, and the antimicrobial activity was found to last for 168 hours. Stability tests were performed on the formulations, and the findings revealed no major changes in the formulation.

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