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Research Article

FORMULATION AND EVALUTION OF DOXYCYCLINE *IN-SITU* FILM FOR THE TREATMENT OF PERIDONTITIS

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ABSTRACT

The periodontal disease is confirmed by a group of illnesses affecting the gums and dental support structures. They are caused by certain bacteria found in the bacterial plaque. Doxycycline broad-spectrum antibiotic of the tetracycline class generally prescribed in the treatment of periodontitis for localized therapy. In present work, *in-situ* film of Doxycycline was prepared. The *in-situ* film was formulated using biodegradable polymer like pectin, xanthan gum and acacia. Gels were prepared by dissolving biodegradable polymer in hydro alcoholic solvent along with PEG 400 as plasticizer. Then gel is applied to gums which convert into the film (*in-situ*) by solvent evaporation mechanism. The prepared drug loaded gels and *in-situ* film were subjected for various evaluation parameters like speradability, pH, viscosity, thickness of film, weight uniformity, percent moisture loss, drug content respectively. Experimental result revealed that viscosity of gel 1745.65±0.14 to 1958.18±0.28cps, pH of gel range between 6.56±0.124 to 6.75±0.169, value of spreadability index was in between 17.25±0.28to 19.81±0.17 cm^2, thickness of film were less than 0.2mm, weight uniformity0.052±0.002 to 0.055±0.001gm, percent moisture loss 5.264±1.958 to 5.969±1.845%. From experiment study it was concluded that doxycycline *in-situ* film was provided better retention, hence effective for local delivery of doxycycline which may improved patient compliance and effective management of periodontitis.

Keywords: *In-situ* film; Doxycycline; Solvent evaporation; Periodontitis

1. INTRODUCTION

Periodontal disease is considered as major public health problem throughout the world. Good daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can occur in all age groups, ethnicities, races and genders. Periodontal diseases including gingivitis and periodontitis are serious infections which can lead to tooth loss when left untreated. The word periodontal literally means "around the tooth"

Periodontal diseases are infections of the bone and gums that bound and support the teeth. In its early phase called gingivitis, gums can turn into inflamed and red, and they may bleed. In its more severe form, called periodontitis, the gums can draw away from the tooth, bone can be lost, and the teeth may loosen or even fall out [1].

Controlled release local drug delivery systems offer advantages compared to systemic dosage forms for periodontitis. The objective of this research was to design and evaluate sustained release in-situ film containing a bio-degradable polymer for targeted delivery of drug.

In pharmaceutical field novel and controlled drug delivery system are becoming more popular which capable of improving patient compliance as well as therapeutic efficacy [2].

Doxycycline, a broad-spectrum antibacterial, synthetically derived from oxytetracycline is a prescription antibiotic medication, indicated for the treatment of periodontitis. Doxycycline is conventionally delivered through oral route in a tablet dosage form but it has slow absorption and having first pass metabolism. So its required alternative route of drug delivery. Several scientific works were reported where Doxycycline was delivered via topical route. In topical route, the drug absorption depends upon the drug properties and the gum condition [3].

Film forming system or in-situ film is the innovative approach. The film forming formulations are defined as non-solid dosage form that produces a considerable film in-situ after application on the skin or any other body surface. The solution of polymer is applied to skin in the form of liquid or semisolid and by the help of solvent evaporation method it forms an invisible *in-situ* film. In

situ gelation is a process of gel formation at the site of action after the formulation has been applied at the site [4].

2. MATERIAL AND METHOD

Doxycycline was obtained as gifted sample from Sun Pharmaceticals Ltd., Ponta Sahib, India. Pectine, Carbopol 934, HPMC K4M, PEG 400, PG, Ethanol were obtained from Central Drug House Pvt Ltd India.

2.1. Drug-Excipient compatibility study

FTIR of drug with polymer was taken by using Diamond ATR (model no. 630) technique using Albunt FTIR Spectrophometer in the wavelength region of 100 to 4000 $cm^{-1.}$ The procedure consisted of dispersing a sample (drug alone or mixture of drug and excipient or formulation) in diamond ATR Spectrophotometer. Force was applied to the sample pushing it into the diamond surface. After the spectrum has been collected which should typically take not more than 32 sec the user must return to the 'Preview mode'.

2.2. Formulation of *in-situ* film

The in-situ film was formed by the solvent evaporation techniques. In this method, Doxycycline film was prepared using different polymer. The Carbopol 934 (1.5%w/v), HPMC (2.5%w/v) and pectin (3% w/v) was dissolved in water and ethanol. The water and ethanolic solution of HPMC was slowly added to the Carbopol water and ethanolic solution and then to pectin water and ethanolic solution. The drug was added to polymeric solution of the Carbopol, HPMC and pectin with continuous stirring for 20 min followed by addition of PEG 400 and glycerol.

Table 1: Composition table of Doxycycline *in-situ* film

S.No	Ingredients	Formulation code				
		FF1	FF2	FF3	FF4	FF5
1.	Doxycycline (%)	0.5	0.5	0.5	0.5	0.5
2.	Pectin (%w/v)	3	3	3	3	3
3.	HPMC(K4M)(%w/v)	2.5	2.5	2.5	2.5	2.5
3.	Carbopol 934(%w/v)	2	2	2	2	2
4.	PEG 400(%v/v)	10	10	10	10	10
5.	Glycerine(%v/v)	0.4	0.4	0.4	0.4	0.4
6.	Ethanol(%v/v)	40	40	40	40	40
7.	Water(%v/v)	60	60	60	60	60
8.	Propylene glycol PG(%v/v)	5	7	10	12	15
9.	PEG 400 (%v/v)	5	5	5	5	5

2.3. Evaluation parameters

2.3.1. Evaluation parameters of gel

2.3.1.1. Spreadability studies

Spreadability of the doxycycline gel was determined by spreading the 500mg of gel on a glass plate and then another glass plate was cited on it. 500g of weight was put on the upper glass plate. After 5min the increase in diameter was noted and spreadability of gel was calculated by given formula [5]:

$$A = \pi r^2$$

2.3.1.2. Viscosity estimation

The viscosity estimation of doxycycline gel was executed on brookfield viscometer. The spindles were rotated at different rpm [6].

2.3.1.3. pH determination

By digital pH meter, the pH of different gel formulations was determined [7].

2.3.2. Evaluation parameter of film

2.3.2.1. Thickness of film

The thickness of prepared *in-situ* film was determined by using digital micrometer screw gauge. The average thickness was determined and reported [8].

2.3.2.2. Weight uniformity

Weight uniformity of film was determined by taking a weight of specific area of a film from different parts of the film by using digital balance. The average weight was then calculated from individual weights [9].

2.3.2.3. Folding Endurance

Folding endurance was determined by repeatedly folding the film at the same place till it broke or folded up to 300 times, which is considered as adequate to reveal good film properties. The patch was folded number of times at the same place without breaking gave the folding

endurance. The test was done on all the films for five times [10].

2.3.2.4. Percentage Moisture Loss

Films were weighed individually and kept in a desiccator at room temperature containing calcium chloride. The films were weighed repeatedly until they showed a constant weight. The percentage moisture loss was calculated using the following formula [11]:

$$\% \text{ moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

2.3.2.5. Drug content

1cm^2 area of film was dissolved in a methanol in specific volume. The solution was shaken and sonicated for 15minutes. The solution was filtered and the drug content was analyzed spectrophotometrically at 360nm wavelength [12].

2.3.2.6. Bio adhesion force

By using a modified method the bio PARK- Robinson adhesion strength of prepared *in-situ* film was measured [13].

2.4. *In-vitro* permeation study

The *in-vitro* permeation of doxycycline from the prepared *in-situ* film was performed by using Franz diffusion cell. The formulation was applied on the dialysis membrane which was placed between the donor and receptor compartment. The donor compartment was filled with the phosphate buffer pH 7.4 as a diffusion media. The assembly was kept on magnetic stirrer and solution was stir continuously. The sample was withdrawn at the predetermine time interval and the sink condition was maintain throughout the process. The sample was analysed by the UV spectrophotometry at 273nm and then cumulative drug release was determined [14].

2.5. *In-vitro* kinetic study

To understand the drug release kinetics and mechanism of drug release, all the *in-vitro* release data were fitted to different kinetic equation such as zero order cumulative percent release vs. time, first order log percent drug remaining vs. time, higuchi percent cumulative drug release vs. square root of time [11].

2.6. Antimicrobial activity

2.6.1. Agar well diffusion method

Petri plates containing 20 ml Luria Bertani agar medium were seeded with the fresh culture of bacterial strains. Wells were cut and 2.5, 25, 50, 100 and 200 µg/ml of the liquid doxycycline film were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. [14]

3. RESULT AND DISCUSSION

pH of all formulation were found in the range of 6.56±0.124 to 6.75±0.169 (**table 2**) which match with the physiological pH of mucus membrane (pH 6.2-7.6) that indicate it does not cause any irritation on application. The spreadibility of gel ranged from 17.25±0.28to 19.81±0.17 cm^2 (**table 2**) which indicate that prepared gel is easily spreadable by small amount of shear. From the result 1745.65±0.14 to 1958.18±0.28 cps (**table 2 and figure 4**) it was concluded that all the formulations follow non- Newtonian pseudoplastic flow, a shear thinning behavior, as with increase in the shear rate the apparent viscosity of gel was reduced.

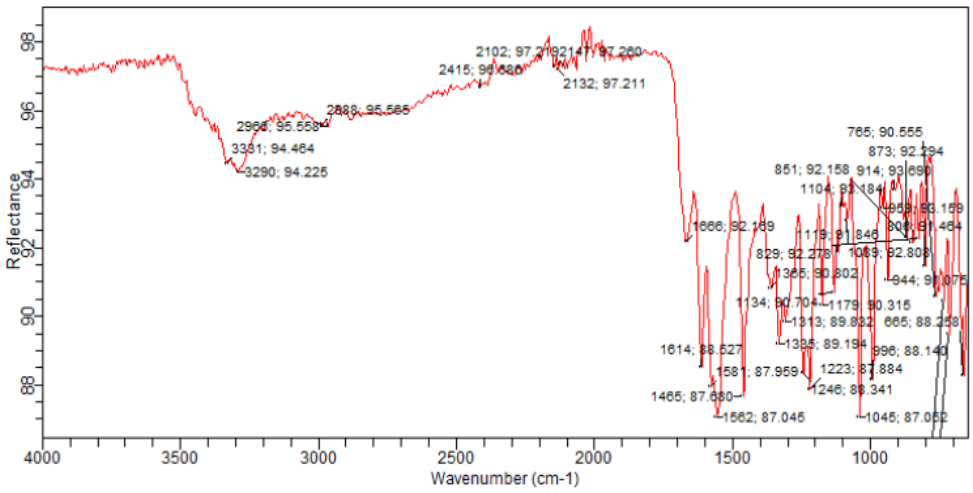

Fig. 1: FTIR spectrum of Doxycycline (pure drug)

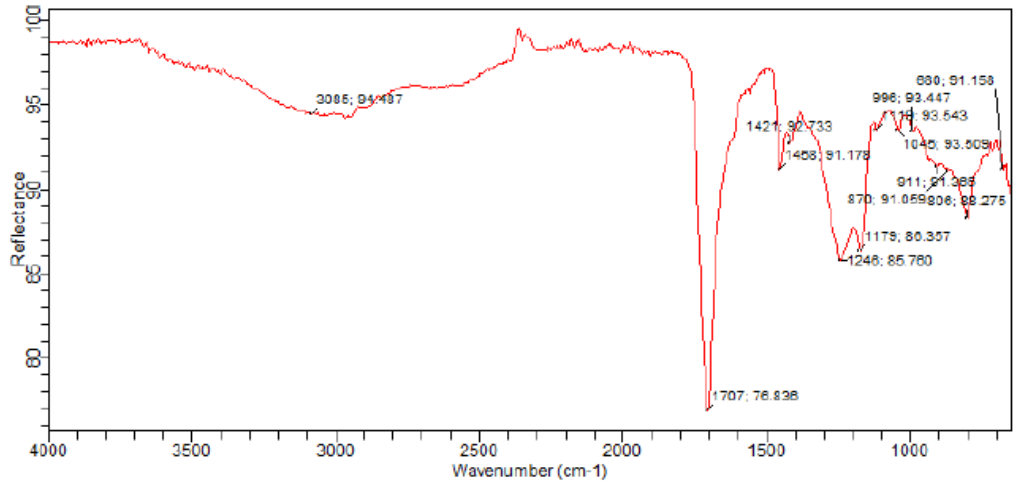

Fig 2: FTIR of Doxycycline and Carbopol

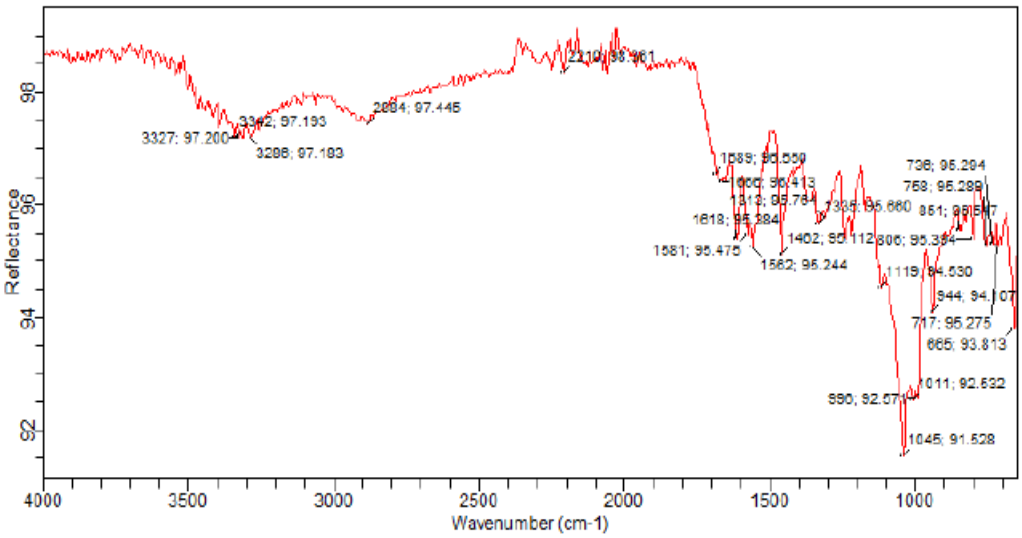

Fig 3: FTIR of Doxycycline and HPMC

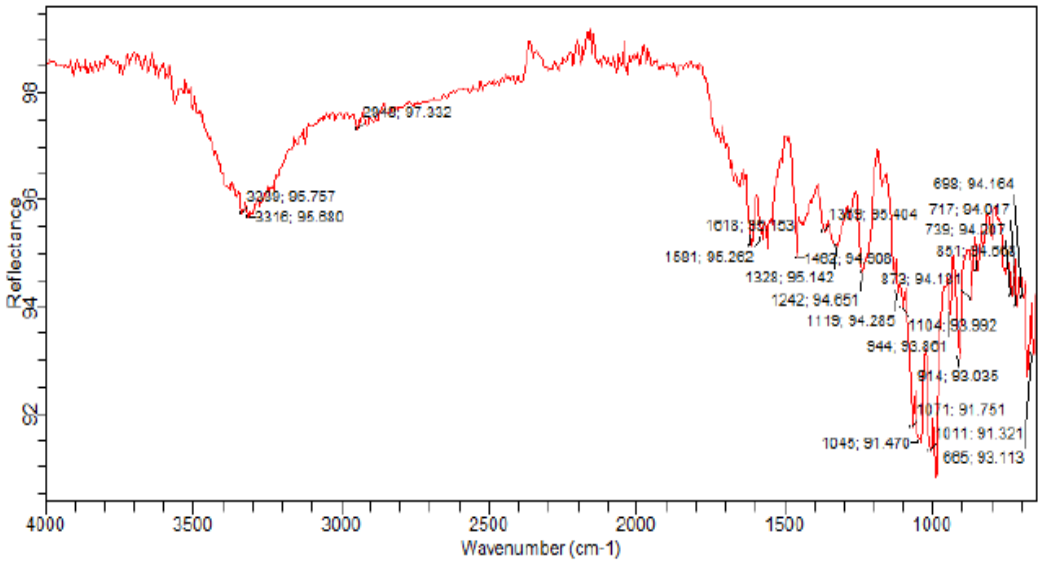

Fig 4: FTIR of Doxycycline and Pectin

Table 2: Physical parameters of doxycycline gels

Formulation code	pH value	Spreadability(cm^2)	Viscosity (cps)
F1	6.66±0.169	18.21±0.37	1745.65±0.14
F2	6.65±0.128	18.56±0.24	1755.65±0.19
F3	6.56±0.124	17.25±0.28	1865.85±0.16
F4	6.75±0.169	17.95±0.41	1950.36±0.24
F5	6.62±0.047	19.81±0.17	1958.18±0.28

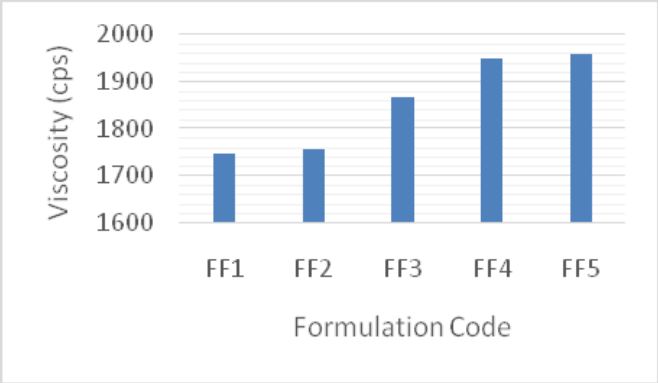

Fig 5: Viscosity of gel and formulation FF1-FF

3.1. Thickness of film

The thickness of all the formulations of *in-situ* film was less than 0.2mm.

Table 3: Thickness of in-situ film of FF1-FF5

Formulation code	Thickness (mm)
FF1	0.20
FF2	0.18
FF3	0.18
FF4	0.17
FF5	0.19

3.2. Weight Uniformity

The result indicate that as the polymer concentration remain fixed there is no significant difference between the weights of the prepared films, it was ranged between the 0.052±0.002 to 0.055±0.001gm (**table no 4**).

Table 4: Weight Uniformity of *in-situ* film of FF1-FF5

Formulation code	Weight uniformity (gm) (Mean±S.D)
FF1	0.054±0.002
FF2	0.052±0.002
FF3	0.053±0.002
FF4	0.054±0.002
FF5	0.055±0.001

3.3. Folding Endurance

The folding endurance of the films was > 250 times. It means all the formulations have good folding endurance.

3.4. Percent moisture Loss

Moisture loss studies were carried out on all the prepared Doxycycline formulations and are shown in **(table 5).** It was observed that as the concentrations of Pectin, HPMC and Carbopol polymers increased the percentage moisture loss also increased. This may be due to the more water vapour permeability of these polymers. It was observed that Formulation F1 showed maximum of moisture loss 5.969±1.845% under dry condition because of more concentration of hydroxyl propyl methyl cellulose and formulation F1 showed minimum moisture loss 5.264±1.958 % due to hydrophobic Pectin.

Table 5: Percent moisture loss of in-situ film of FF1-FF5

Formulation code	%moisture loss (Mean±S.D)
FF1	5.562±1.745
FF2	5.264±1.958
FF3	5.862±1.458
FF4	5.659±1.456
FF5	5.969±1.845

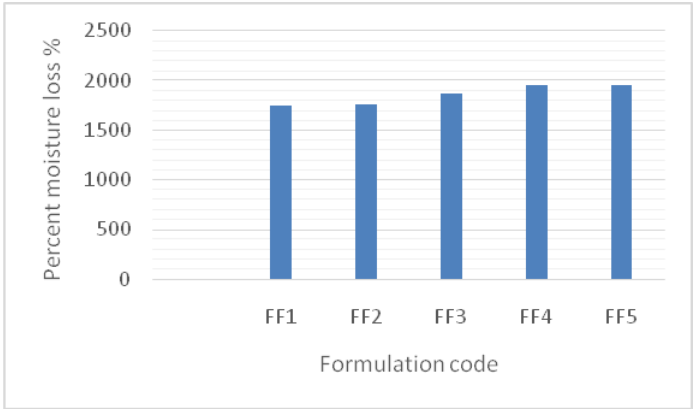

Fig 6: Percent Moisture Loss of gel and formulation FF1-FF5

3.5. Drug content

The drug content estimation was done and the absorbance was measured by UV spectrophotometer drug content was calculated **(Table 6)**. Drug content of all formulations was found between 92.5±0.18 to 98.54±0.13%. From the results drug content, it is quite evident that all the formulations (F1 to F5) have RSD values less than 3% which indicates uniform distribution of the API *i.e* doxycycline throughout the *in-situ* film.

Table 6: Drug content of in-situ film of FF1-FF5

Formulation code	Drug content (Mean±S.D)
FF1	97.26±0.15
FF2	92.5±0.18
FF3	94.56±0.15
FF4	93.62±0.11
FF5	98.54±0.13

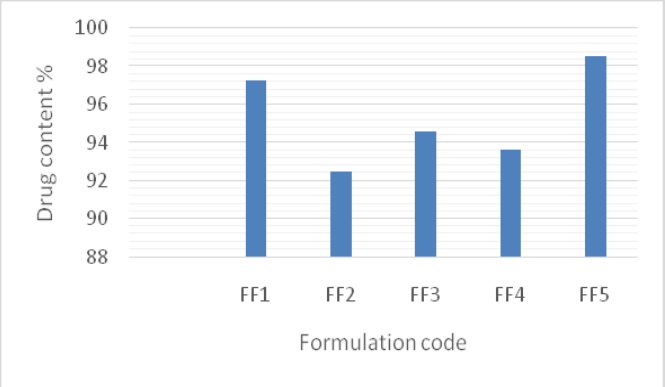

Fig 7: Drug Content of gel and formulation FF1-FF5

3.6. Bio adhesion force

The bio adhesion force range from 53.5±1.5 to 56.4±1.2 dynes/cm^2. The bioadhesive force was significantly increased as the concentration of mucoadhesive polymer increased in the range of 0.6-3.5%.

Table 7: Bio adhesion Force of in-situ film of FF1-FF5

Formulation code	BioadhesiveForce (dynes/cm^2)(Mean±S.D)
FF1	55.6±4.2
FF2	54.5±5.3
FF3	53.5±1.5
FF4	54.6±0.7
FF5	56.4±1.2

3.7. *In-vitro* permeation study

The *in vitro* permeation profile of Doxycycline from the gels containing different concentration of Pectine, carbopol and HPMC is shown in **(fig. 8)** Formulation F1 showed least drug release (95.91%) and formulation F5 showed maximum drug release (98.91%). For the first 6 hours of study, initial burst release was higher in *in-situ* gel formulations. From the *in-vitro* release studies we came to know that release rate was maximum for the formulation F5, but when the concentration of gel was further increased the release rate of the drug was decreased. From the experimental results we can say that release pattern was depends up on the concentration of polymer used.

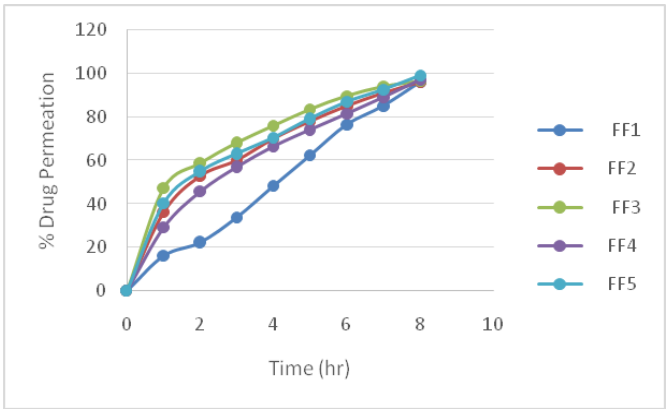

Fig 8: % drug permeation profile of formulation FF1-FF5

3.8. Model Fitting Analysis

Data obtained from *in vitro* release studies of various formulations (F1 to F5) of doxycycline in-situ films were fitted to various kinetic equations such as zero order, first order, Higuchi model, Korsmeyer-Peppas model. The results are presented in table 8. Formulation FF1 and FF4 follow zero order release kinetics, formulation FF2 and FF3 follow first order release kinetics and only formulation FF5 follow higuchi release kinetics.Comparing the coefficient of regression (r^2) value and n value of all the kinetic equations. Formulation FF5 was selected as optimized formulation for the *in-situ* film with (pH 6.62±0.047, viscosity 1958.18±0.28 cps, spreadability 19.81±0.17 cm^2, thickness 0.19 mm, weight uniformity 0.055±0.001gm,, folding endurance> 250 times, percent moisture loss 5.969±1.845 %, drug content 98.54±0.13 %, bio adhesion force 56.4±1.2, *in vitro* permeation study 98.91% . On the basis of experimental result formulation FF5 was selected as optimized formulation and subjected for the antibacterial activity.

Table 8: Model Fitting Analysis of Doxycycline *in-situ* film

Formulation Code	r^2			n	Best Fit Model	Mechanism of Action
	Zero Order	First Order	Higuchi Kinetics			
FF1	0.0.9952	0.8528	0.9126	0.9189	Zero Order	Anamalous Transport
FF2	0.8923	0.9578	0.9262	0.4649	First Order	Fickian Diffusion
FF3	0.8200	0.9805	0.9397	0.3560	First Order	Fickian Diffusion
FF4	0.9351	0.8983	0.9191	0.5655	Zero Order	Anamalous Transport
FF5	0.8818	0.8552	0.9277	0.4273	Higuchi Kinetics	Fickian Diffusion

3.9. *In-vitro* Antibacterial Activity

After 24 hours of incubation, the diameter of zone of inhibition was measured and the value was shown in the **(table 9)**. It shows better antibacterial activity over those microorganisms.

Table 9: Antibacterial activity of Doxycycline *in-situ* films

Organism	Agar Well Diffusion Method (Zone Size mm)	
	Major Axis (mm)	Minor Axis (mm)
S. Aureus	21.91	18.21
S. Typhi	24.25	19.53
Bacillus	21.65	18.38

4. CONCULSION

The Doxycycline in-situ films were formulated by using the biodegradable polymer pectin, HPMC (K4M) and Carbopol, PEG400 as a plasticizer. The physicochemical parameters shows uniform results for all the formulations. On the basis of experimental result formulation FF5 was selected as optimized formulation for the *in-situ* film with (pH 6.62±0.047, viscosity 1958.18±0.28 cps, spreadability 19.81±0.17 cm^2, thickness 0.19 mm, weight uniformity 0.055±0.001gm,, folding endurance> 250 times, percent moisture loss 5.969±1.845 %, drug content 98.54±0.13 %, bio adhesion force 56.4±1.2, From the result of *in-vitro* release studies of the formulation F1 to F5, the formulation F5 release the drug 98.91% and considered as a best formulation. The 'n' values of various mathematical model fittings suggest that all the films exhibit anamolous transport, so the prepared undergoes diffusion mechanism. The antimicrobial activities were performed on *Staphylococcus aureus* and *Staphylococcus Typhi and Bacillus*, the zone of inhibition was observed by agar well diffusion method and the optimized implant shows better activity over those micro-organisms.

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