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Research Article

Formulation and Evaluation of Norfloxacin Periodontal Film for Local Delivery

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ABSTRACT

Periodontal diseases are recognized as the major public health problem throughout the world. Periodontal diseases, including gingivitis and periodontitis, are serious infections that, left untreated, can lead to tooth loss. A novel periodontal film for the treatment of periodontitis was developed in the present work, for local delivery of Norfloxacin against infecting microorganisms in the periodontal pocket. Calibration curves for Norfloxacin was developed in pH 6.6 phosphate buffers at 273.8 nm respectively. *FT-IR* studies revealed that no interaction between the selected drug and polymers. Periodontal films were prepared by solvent casting technique using ethyl cellulose and other co-polymers in different solvents with dibutyl phthalate and polyethylene glycol as plasticizers. The formulated periodontal films were evaluated for their folding endurance, percent moisture loss, surface pH, viscosity, thickness, uniformity of weight, tensile strength, content uniformity, *in-vitro* release and Stability studies. Data of *In-vitro* release from the formulated periodontal films were fit to different equations and kinetic models to explain release kinetics. Kinetic models used were zero and first-order equations and Higuchi models. Formulation F6 released 99.74% of drug at the end of tenth day was considered as the optimized formulations F6. There were no significant changes in formulation F6 during stability study. Periodontal films might be a potential formulation for the treatment of periodontitis.

INTRODUCTION

Periodontal diseases are recognized as the major public health problem throughout the world. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can do occur in all age groups, ethnicities, races, genders and socioeconomic levels. Periodontal diseases, including gingivitis and periodontitis, are serious infections that, left untreated can lead to tooth loss. Periodontal disease can affect one tooth or many teeth. It begins when the bacteria in plaque causes the gums to become inflamed. Periodontal diseases range from simple gum inflammation to serious disease those results in major damage to the soft tissue and bone that support the teeth. In the worst cases, teeth are lost [1].

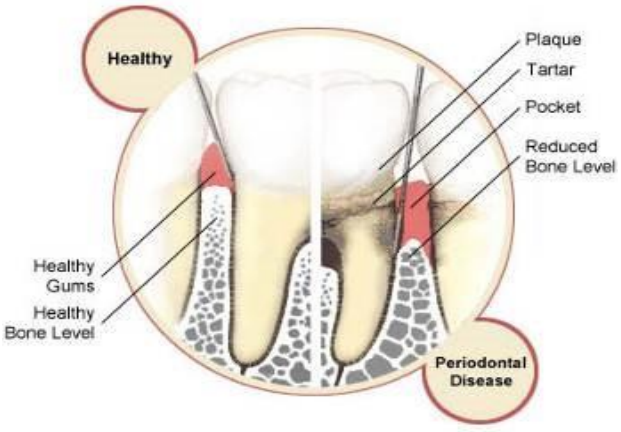

Figure 1: Comparison of Healthy gum and Periodontitis.

Periodontitis is a set of inflammatory diseases affecting the periodontium that is the tissues that surround and support the teeth. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against

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these microorganisms [2]. The main cause of periodontal disease is bacteria plaque, a sticky, colorless film that constantly forms on teeth. However, factors like smoking/ tobacco use, genetics, pregnancy and puberty, stress, medication, clenching or grinding teeth, diabetes and poor nutrition also lead to periodontal diseases. Types of periodontitis are:

- Gingivitis (Fig. 2)
- Moderate Periodontitis (Fig. 3)
- Advanced Periodontitis (Fig. 4)
- Refractory Periodontitis (Fig. 5)

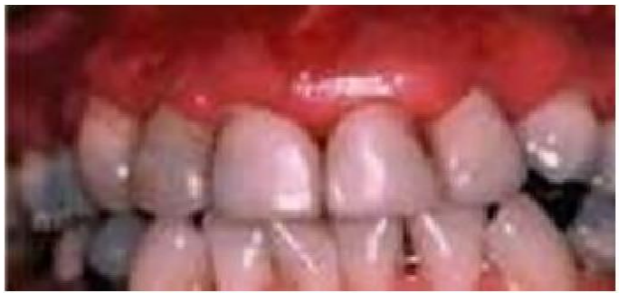

Figure 2: Gingivitis

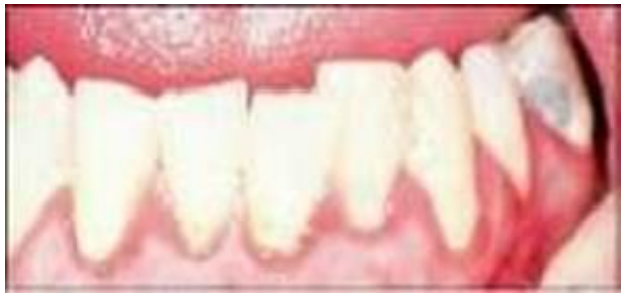

Figure 3: Moderate Periodontitis

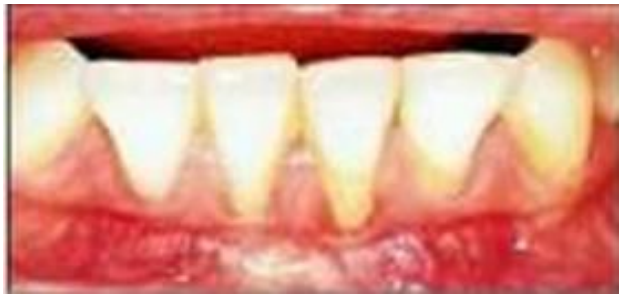

Figure 4: Advanced Periodontitis

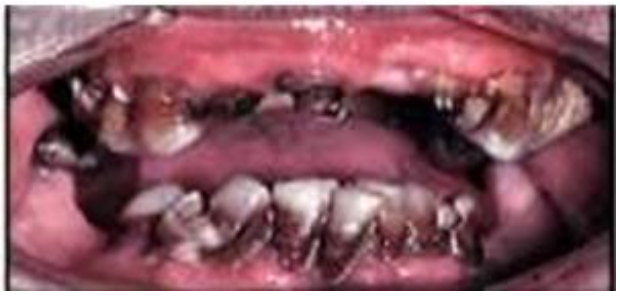

Figure 5: Refractory Periodontitis

Systemic Antibiotic Therapy

An ideal antibiotic for use in prevention and treatment of periodontal diseases should be specific for periodontal pathogens, allogenic and nontoxic, substantive and inexpensive. Combination of antibiotics may be necessary to eliminate all putative pathogens from some periodontal pockets.

Norfloxacin is a synthetic antibiotic belonging to the fluoroquinolones drug considered to be a first generation fluoroquinolones. Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting gyarase, a type II, topoisomeras and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division [3-5].

Local delivery consists of a drug reservoir and limiting elements that controls the rate of drug release. The goal is to maintain effective drug concentrations of therapeutic agent at site of action for longer periods despite drug loss from GCF or periodontal clearance.

Films appear to be a suitable dosage form to deliver drugs into periodontal pocket, because the anatomic construction of the pocket allows for relatively easy insertion of such a delivery device.

MATERIALS AND METHODS

Materials

Norfloxacin was obtained as a gift sample from KAPL Bangalore. Ethyl cellulose was obtained from Ozone International, Mumbai. Eudragit-RL100 was obtained from Evonik Industries, Mumbai. HPMC K4M was obtained from Loba Chemie Pvt. Ltd., Mumbai. Polyethylene glycol (PEG 400) was obtained from S D fine-chem Ltd., Mumbai. Di butyl phthalate was obtained from Merck Ltd, Mumbai. Chloroform was obtained from S D fine-chem Ltd., Mumbai. Dichloro methane obtained from S D fine-chem Ltd., Mumbai. Ethanol obtained from Loba Chemie Pvt. Ltd, Mumbai. Sodium chloride was obtained from Karnataka fine chem, Bangalore. Potassium dihydrogenphosphate was obtained from Karnataka fine chem, Bangalore. Disodium hydrogen Phosphate was obtained from Karnataka fine chem, Bangalore.

Methods

Preparation of Periodontal Films by Solvent Casting Method

Seven formulations were designed for Norfloxacin as shown in the Table 1. Ethyl

cellulose was used as the non biodegradable polymer in combination of different co-polymers grades of water-soluble and coating agent for each cast films. Films were prepared by dissolving Ethyl cellulose alone or it is combined with co-polymer HPCM K4M or Eudragit RL100 or PVP K-30 with the Chloroform and Alcohol (1:1) solution, Dibutyl- phthalate and PEG-400 are used as plasticizers in a closed beaker by using magnetic stirrer to get uniform distribution of polymers into the films. Norfloxacin was added into the above polymeric solution. 10 ml of drug polymeric solution was poured into 15 sq.cm leveled petriplate after complete mixing for costing. The solvent was allowed to evaporate slowly at room temperature for 24 hours. Films were obtained after evaporation of solvent completely, which were then wrapped in an aluminum foil and stored in a desiccators at room temperature in a dark place until used for further evaluation studies.

Table 1: Formulations of Periodontal Films

S.No	Ingredients	Formulations						
		F1	F2	F3	F4	F5	F6	F7
1.	Norfloxacin (mg)	20	20	20	20	20	20	20
2.	Ethyl cellulose(mg)	590	-	-	-	500	500	500
3.	HPMC K 4 M(mg)	-	590	-	-	90	-	-
4.	Eudragit RL- 100(mg)	-	-	590	-	-	90	-
5.	Polyvinylpyrrolidine K-30(mg)	-	-	-	590	-	-	90
6.	Dibutyl phthalate (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
7.	PEG - 400(ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
8.	Chloroform (ml)	10	10	10	10	10	10	10
9.	Ethyl alcohol (ml)	10	10	10	10	10	10	10

Evaluation of the Prepared Films

Physical Characteristics Study

Folding Endurance

Folding endurance was determined by repeatedly folding the patch 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 [6].

Percentage Moisture Loss

Films were weighed individually and kept in desiccators 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 [7].

Percentage moisture loss = [Initial weight - Final weight /Initial weight] ×100

Surface pH

Periodontal films were kept to swell for 1 hour on the surface of the agar plate, prepared by dissolving 2% (w/v) agar in warmed double distilled water under stirring and then pouring the solution into the petridish for gelling / solidify at room temperature. Surface pH was measured by using pH paper placed on the surface of the swollen patch. The recorded values were the mean of five determinations [8].

Viscosity

Solutions containing drug, polymers and plasticizer with the same concentrations as that of formulated films were prepared. These solutions were subjected for viscosity determination by Brookfield viscometer attached to the helipath spindle number 18. The viscosity was measured at 100 rpm at room temperature. The recorded values were the mean of five determinations.

Thickness

The thickness of the prepared films were measured at different points (n = 10) using the vernier calipers. Each reading was an average of seven determinations [9].

Uniformity of Weight

Patches (size of 7x2 mm^2) were taken from different areas of film and the weight variation for each patch was calculated, mean and standard deviation were assessed for each film.

Each reading was an average of five determinations.

Tensile Strength

Tensile strength was determined using universal testing machine. It consists of two load cell jaws the upper one is movable and lower one is fixed. The prepared films were cut into rectangular patches of size 2x0.8cm^2 then the patch was fixed between the cell grips. The upper jaw was moved at a speed of 100 mm/min (ISI Standard speed) and force was applied gradually till the patch was broken. The tensile strength of three patches for each formulation were taken directly from the dial reading in kilograms. Average and standard deviation of the values were also calculated. Each value was an average of three determinations [10].

Content Uniformity

Films (7x2 mm^2) were taken from different areas of the film and placed into a 10 ml volumetric flask containing 10 ml of ethyl alcohol. The volumetric flask was kept aside till the patch gets completely dissolved. Withdraw 1 ml of solution and diluted to 10 ml with pH 6.6 phosphate buffers. The absorbance of the drug solutions were measured at 273.8 nm. The polymer solution without drug serves as a blank. In case of HPMC films combination of water and alcohol is used to dissolve the patches.

***In-Vitro* Drug Release Study**

Since the pH of the gingival fluid lies between 6.5-6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid and the film remains immobile in the periodontal pocket, a Static dissolution method was adopted for *In-vitro* drug release studies. Patches of known weight and dimension (7x2 mm^2) were placed separately into small test tubes containing 1 ml of pH 6.6 phosphate buffer. The tubes were sealed and kept at 37° C for 24 hours. The buffer was then drained off and replaced with fresh 1 ml of pH 6.6 phosphate buffer after 24 hours. Concentrations of drugs in buffer were diluted and measured at 273.8nm. The study was carried out for 10 days [11].

Stability studies

Optimized formulation F6 was stored at 40° ± 2°C/75% RH ± 5% RH in stability chamber for 3 months. The optimized formulation stored in the sealed aluminum foil. The optimized formulation was analyzed after 3 months for physical appearance, drug content uniformity, folding endurance.

RESULTS AND DISCUSSION

Drug Estimation

A calibration curve of Norfloxacin in phosphate buffer pH 6.6 was constructed at a λ max of 273.8 nm with UV-VIS spectrophotometer, Beer's law obeyed to construct the calibration curve in concentration range of 2- 10 µg/ml. Analysis was done in triplicate.

Drug and Excipients Compatibility Studies by *FT- IR* Spectroscopy

FT-IR studies were carried out for pure Norfloxacin alone and along with polymers such as Ethyl cellulose, Eudragit RL 100, PVP K 30 and HPMC K4M. 3 mg of pure drug/combination of drug-polymer were triturated with 97 mg of potassium bromide in a mortar to obtain mixtures. These mixtures were then placed in the sample holder of the instrument and scanned in IR spectroscopy between 400 and 4000cm^{-1}. The obtained spectrums were investigated for any possible interactions between Norfloxacin and polymers used in the formulations.

FTIR Spectrum of Drug and Polymers

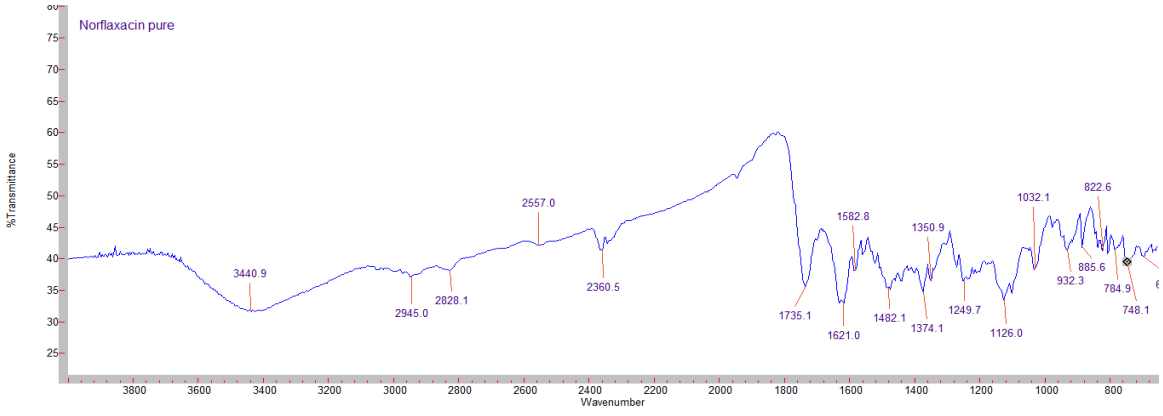

Figure 6: Norfloxacin pure

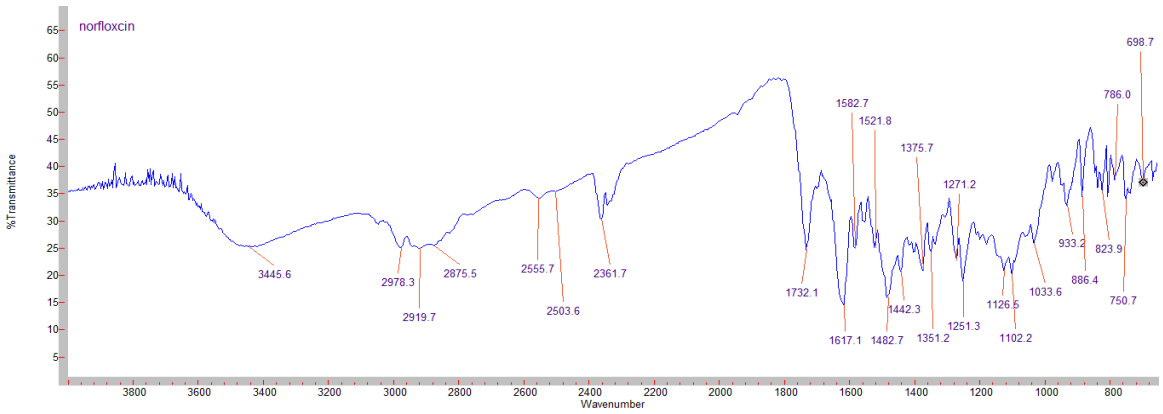

Figure 7: Norfloxacin + Ethyl cellulose

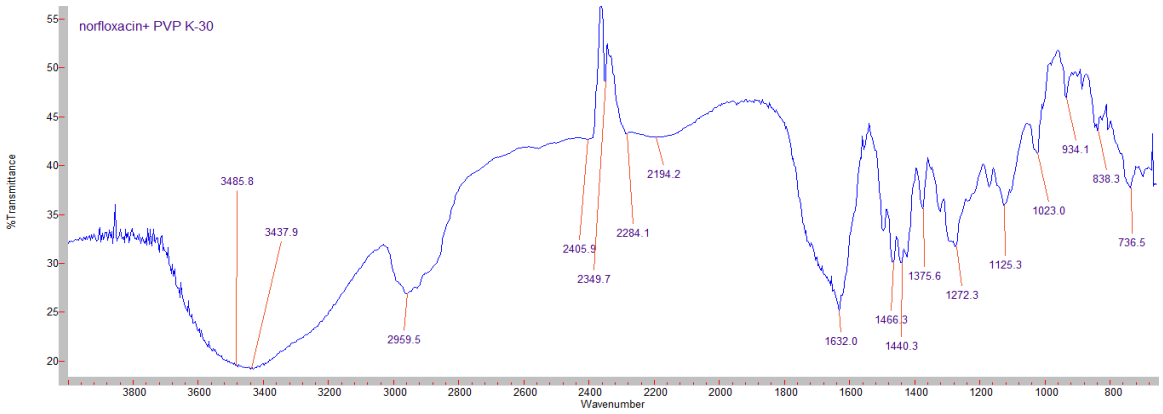

Figure 8: Norfloxacin + PVP and K-30

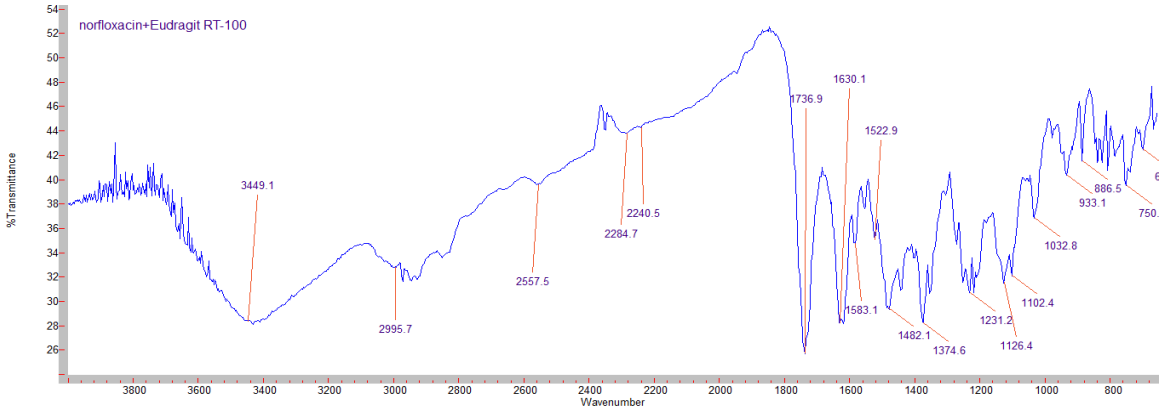

Figure 9: Eudragit RL-100

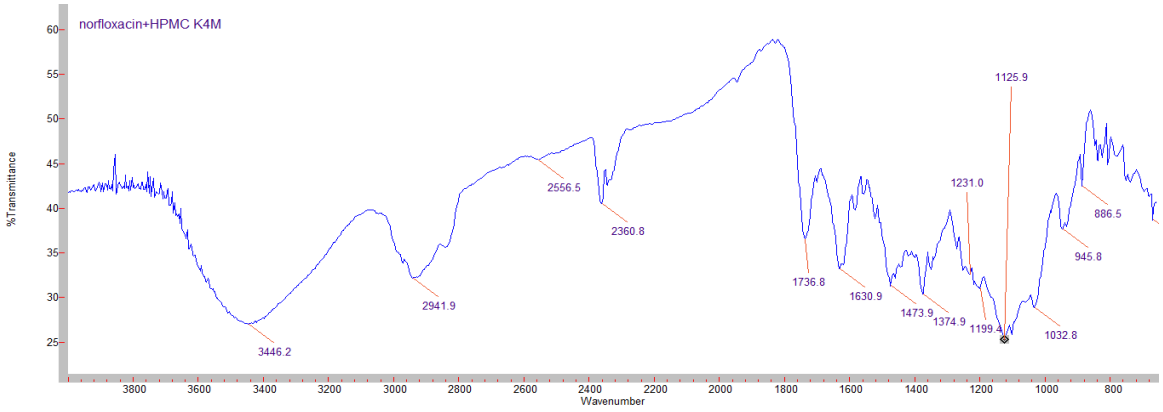

Figure 10: Norfloxacin+HPMC K4M

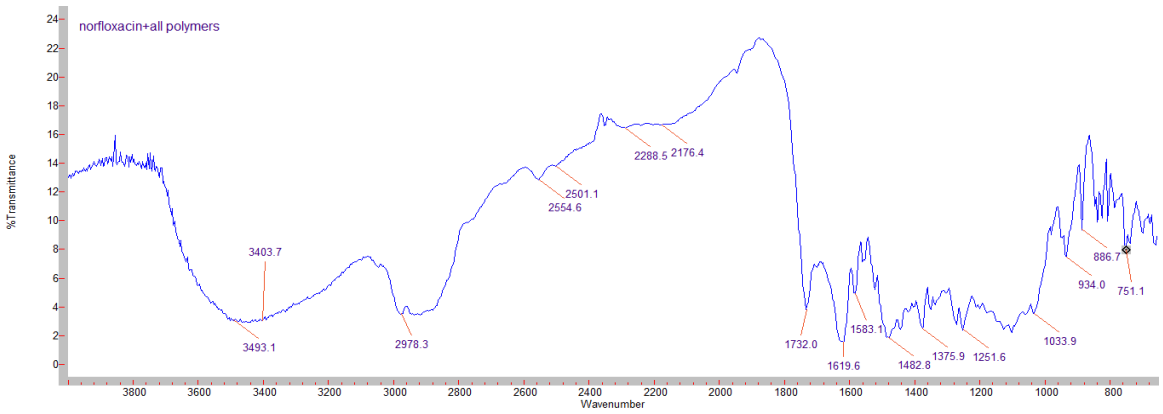

Figure 11: Norfloxacin+ E.C+PVP k30+Eudragit RL-100+HPMC K4M.

EVALUATION

1. Physical Characteristics of Films

Films were translucent, with good strength and visually smooth surfaced. The drug and polymer distribution was uniform all throughout the film. Results are in the Table 2.

2. Folding Endurance

The folding endurance of the films was more than 250 times. It means all the formulations have good folding endurance. Results are in the Table 2.

3. Percentage moisture loss

Moisture loss studies were carried out on all the prepared Norfloxacin formulations. It was observed that the percentage moisture loss increased as the concentrations of Eudragit RL 100, HPMC K4M and PVP- K30 increased. This may be due to the more water vapor permeability of these polymers. It was observed that formulation F5 showed maximum of moisture loss (9.2138 ±2.1137 %) under dry condition because of more concentration of hydroxyl propyl methyl cellulose and formulation F1 showed minimum moisture loss (9.2138 ±2.1137 %) due to hydrophobic ethyl cellulose [12]. Results are in the Table 2.

4. Surface pH

Surface pH of all Norfloxacin films were evaluated by pH paper on agar plate and were found to be approximately pH 7. The surface pH of all films was found to be neutral and hence no periodontal pocket irritation were expected. Results are in the Table 2.

5. Viscosity

Viscosity of film F6 was (44.26 ± 0.1516 cps) high when compared to other films. This could be because of complete solubility of polymers in Chloroform and Alcohol (1:1) mixture, whereas viscosity is least in film F5 due to dispersion of polymer in the solvent mixture. Results are in the Table 2.

6. Thickness

Thicknesses of drug-loaded films were measured with the help of digital screw gauge. Mean values are shown in the table no.2. The values were almost uniform and found to be varying between 0.3880 ± 0.0008 mm to 0.4547 ± 0.0033. Formulation F6 showed maximum thickness due to copolymer Eudragit-RL100. Results are in the Table 2.

7. Uniformity of Weight

Five individual drug loaded formulations F1 to F7 were weighed, mean and standard deviation were calculated for each film and the results are given in table no.2. The weights were quite uniform and the cross-linking has no effect on the weight and values were found to be varying from 4.5752 ± 0.00164 mg to 4.8336 ± 0.00152 mg. Results are in the Table 2.

8. Tensile Strength

Tensile strength was determined using Universal strength testing machine for the drug loaded films. Data are given in the table no.2. Tensile strengths of drug loaded films were in the order of formulation F6 > F3 > F4 > F1>F7> F2>F5. It is an evident from the above data that effective cross linking was produced on addition of Eudragit RL 100 as a co-polymer, which also showed higher tensile strength compared to all other formulations. Cross linking is more with Ethyl cellulose when compared to HPMC K4M and PVP K 30 and there was a clear indication that inclusion of drug into the films reduces the tensile strength due to disruption of the linear structure of the polymer chain [13]. Results are in the Table 2.

9. Content Uniformity

In order to prove uniform dispersion of drug in the films, drug content uniformity was carried out. The drug content was analyzed at 273.8 nm. Corresponding blanks were used for the estimation of drug concentration. All the formulations showed more than 90 % of the drug loading indicating much of the drug is not lost and uniformly dispersed [14]. Content uniformity of formulation F6 showed maximum drug content of 99.285 ± 1.7329% and film F7 showed the least drug content of 78.962 ± 1.3594%. Results are in the Table 2.

10. *In-vitro* Drug release studies

In-vitro drug release studies of Norfloxacin periodontal films were carried out in pH 6.6 phosphate buffer for 10 days and showed that there was an abrupt release observed in first three days, and there after the release of drug was found to be controlled. An average amount of drug released per day after fourth day is found to be the above the minimum inhibitory concentration of Norfloxacin. The Formulation F6 showed the maximum drug release of 99.74 % on tenth day was considered as optimum. The release data of Norfloxacin films, formulations F_1 to F_7 were given in Table 3.

Table 2: Physicochemical characteristics of formulation F1-F7

Formulation	Moisture loss (%)	pH	Viscosity (m^2/s)	Thickness (mm)	Weight uniformity (mg)	Tensile strength (kg/cm^2)	Content uniformity (%)
F1	4.494	6.63	41.22	0.3880	4.6264	1.3494	88.095
F2	9.230	6.66	16.52	0.4253	4.8336	1.9410	89.285
F3	6.259	6.75	38.48	0.4357	4.7862	1.1598	90.550
F4	8.045	6.74	31.94	0.3913	4.6506	1.2815	89.761
F5	7.401	6.83	13.66	0.4213	4.5752	1.1861	89.603
F6	6.258	6.70	44.26	0.4547	4.5970	1.9410	99.285
F7	7.600	6.80	35.14	0.3913	4.6506	1.2727	78.962

Table 3: Cumulative % drug release of formulation F1-F7

Time (Days)	Cumulative % drug release						
	F_1	F_2	F_3	F_4	F_5	F6	F_7
1	36.9	38.48	35.76	36.8	38.1	36.11	34.5
2	55.49	59.14	54.13	57.74	57.16	54.08	55.15
3	69.32	72.34	66.16	71.61	71.53	67.19	68.30
4	78.29	81.79	75.33	81.08	80.62	76.25	76.12
5	85.71	89.49	81.59	89.01	87.16	82.77	85.23
6	91.56	93.64	88.05	93.51	91.14	88.05	89.15
7	94.4	97.24	90.72	97.16	94.17	92.98	97.50
8	97	99.87	93.39	99.74	96.99	95.65	98.13
9	99.39	_	95.7	_	99.53	98.06	_
10	_	_	97.32	_	_	99.74	_

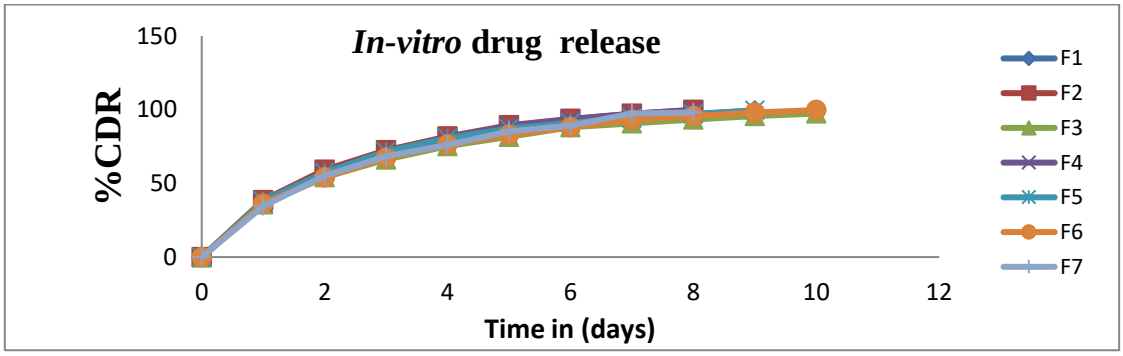

Figure 12: *In vitro* –release profile of Norfloxacin from F1 to F7

11. Stability Studies

The selected formulation F6 was stored at 40±0.5^{0}C and 70% RH in stability chamber, over a period of three months. At the end of three months films were examined visually for physical appearance, folding endurance, drug content.

Table 4: Physical appearance after stability study at 40◦C ± 2◦C/ 75% RH ± 5% RH

Formulation code	physical appearance after 3 months
F6	No change

Table 5: Folding endurance data after stability study at 40◦C ± 2◦C/75% RH ± 5% RH

Formulation Code	Folding Endurance			
	0th DAY	30th DAY	60th DAY	90th DAY
F6	286 ± 2.081	285 ± 1.632	285 ± 1.634	284 ± 0.942

*Average value of three readings

Table 6: Drug content data after stability study at 40°C ± 2°C/ 75% RH ± 5% RH

Formulation Code	% Drug Content			
	0th DAY	30th DAY	60thDAY	90th DAY
F6	99.15± 0.009	99.15± 0.219	99.07 ± 0.046	99.03 ± 0.008

Average value of three readings

CONCLUSION

From the present research work that is preparation and evaluation of periodontal film containing Norfloxacin for local delivery, the following can be concluded.

Suitable analytical methods based on UV- Vis spectrophotometer were developed for Norfloxacin. The IR spectral data indicates that there was no interaction between drug and polymer. Periodontal films of Norfloxacin containing 20 mg of drug were prepared successfully by using ethyl cellulose, eudragitRL-100, H.P.M.C.K4M, PVP K-30 polymers in different combinations and additives such as Dibutyl phthalate, Polyethylene Glycol (plasticizer) in the formulations. The films exhibited satisfactory characteristics regarding to integrity, flexibility, dispersion of drug, and other parameters. Evaluation parameters like folding endurance, percent moisture loss, surface pH, viscosity, thickness, and tensile strength indicates that films were mechanically stable, whereas percentage weight variation and content uniformity were found to be uniform in all the formulations. The Surface pH was found to be in the range of 6.63 to 6.83 in all the formulations which indicate that all the formulations were compatible with the buccal surface. The drug content was found to be uniform in all the formulations. Among the prepared periodontal films, formulation F6 showed 99.74% drug release on 10th day. The prepared periodontal films followed first order release kinetics. Drug release mechanism r^2 values are higher for Higuchi's model compared to Hixson Crowell for Norfloxacin films which indicates that these films followed diffusion rate controlled mechanism. Stability study shows that, there were no significant changes in the formulation F6. Therefore, it can be concluded that the prepared periodontal films containing Norfloxacin for local delivery, might be a potiential formulation for the treatment of periodontitis.

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