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RESEARCH ARTICLE

Development of Enhanced Site Specific Periodontal Drug Delivery Systems for Minocycline HCl and Ciprofloxacin Films

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ABSTRACT

Ciprofloxacin hydrochloride, fluoroquinone antibiotic and Minocycline hydrochloride, tetracycline antibiotic that could be used in the treatment of periodontitis for localized therapy. The ciprofloxacin film was formulated using biodegradable polymer, gelatin and sodium alginate with poly ethylene glycol (PEG) 400. The film was evaluated for physicochemical properties such as thickness of the film, folding endurance, texture analysis, tensile strength, drug(s) content; *in vitro* drug(s) release studies, stability studies, *in vitro* antibacterial activity and bio-degradability study. The film displayed dose dependent antibacterial activity and was found to be stable. The approach provides an opportunity and potential for development of periodontal film containing both Ciprofloxacin and minocycline HCl for extended release.

Keywords: Films, Ciprofloxacin, Minocycline, Gelatin, Periodontal diseases.

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CONTENTS

1. Introduction

Drug delivery is an application of biochemical engineering with technologies aimed at the improvement of safety and efficacy, better compliance and life extension of products [1]. Parenterals depot systems (PDS) have been subject of intensive research efforts over the past two decades. PDS can be classified into films or micro particles [2]. PDS allow the control and modulation of drug release using biodegradable polymers [3]. These polymers have become increasingly important in the development of controlled release systems. Conventional therapy, based on scaling surgery and the use of antibiotics or antimicrobials has been proposed. But due to bacterial resistance and toxic side effects of the administered antibiotics local delivery system are designed to maintain the antibiotic, in the gingival

crevicular fluid at a concentration higher than that achieved by systemic administration.

Periodontitis is set of inflammatory diseases affecting the periodonitium (the tissues that surround and support the teeth). It is caused by micro-organisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against microorganisms [4]. The presence of periodontal pathogens such as Porphyromonas gingivalis, Prevetella intermedia and Actinobacillus actinomycetemcimitans are responsible for periodontal destruction [5]. The treatment of periodontitis is aimed at controlling the population of microorganisms. High doses of antibacterial agents for a longer period of time required for the treatment of periodontitis. Ciprofloxacin is one of the second generation fluoroquinolone derivative and Minocycline hydrochloride, tetracycline antibiotic anti-infective, exhibiting activity against a wide range of gram-negative and gram-positive facultative bacteria as well as periodontal pathogens. It is reported as more effective in the treatment of periodontitis [6]. Ciprofloxacin and Minocycline is available in the market as a conventional dosage forms such as tablets (extended release), parenterals (intravenous) and suspension for the treatment of bacterial infections but not for the treatment of local infection. Hence it was a challenge to develop implants containing ciprofloxacin with rate controlling polymers which has a prolonged action and shows the antibacterial activity directly at the site of infection without loss of dosage [7].

The present work aims to fabricate biodegradable film of ciprofloxacin and Minocycline for sustained release. The literature review shows that with ciprofloxacin hydroxyl propyl methyl cellulose and poly vinyl alcohol the drug release at the 6^{th} hour was found that 78%. In oder to improve the drug release rate, the fabricated films are studied for various physicochemical parameters like weight variation, thickness, drug content uniformity, drug polymer interaction, *in-vitro* dissolution rate studies are performed on the film.

2. Materials and Methods

2.1. Materials:

Ciprofloxacin (CPX) was obtained as gift sample from seimens Laboratories, Gurgaon, India. Minocycline HCl (MHCl) was obtained as gift sample from Welcure Drugs and Pharmaceuticals, New Delhi, India. Gelatin and Sodium alginate were purchased from S.D fine chemical Ltd., (Mumbai). PEG 400 was purchased from Hi Pure fine chemical industry, (Chennai). Luria Bertani agar was purchased from Qualigens fine chemicals, Mumbai. Other materials used in the study were of analytical reagent grade.

2.2. Preparation of film:

Solvent casting technique was used for the preparation of periodontal films using chloroform and dichloromethane in (1:1) mixture. Table No-1 shows composition of periodontal films. Periodontal films were organized by dissolving ethyl cellulose in combination with other polymers such as, hydroxypropyl cellulose (HPC) hydroxyl propyl methylcellulose K4M (HPMC K4M), polymethyl methacrylate (PMMA1, 20,000) in chloroform and dichloromethane (1:1) mixture, dibutylphthalate (10% v/w of that of polymer) and glycerol (20%v/v) as plasticizers. Accordingly film of Eudragit L- 100 was prepared by dissolving Eudragit L-100 into 40:60 mixture of acetone: propane-2-ol and glycerol 10% v/w as plasticizer using magnetic stirrer in a closed beaker [8]. Into this, Ciprofloxacin and Minocycline of essential amount were auxiliary. After the complete intercourse was done, 5 ml solution was poured over the inverted clean petridish covered, by the aluminum foil. After ample evaporation of solvent, cast films were acquired.

2.3. Physicochemical parameters

2.3.1. Thickness of the film

Thickness of the film was leisurely by using calibrated micrometer screw gauge with a least count of 0.01 mm. The typical of three determinations was made. The thickness of each film was measured using screw gauge at different positions of the film and the average thickness was calculated [9].

2.3.2. Folding endurance

Folding endurance of the film was unwavering by constantly folding a small strip of film of 2x2 cm size at the identical place till it cracked at the site of folding. The experiment was conceded out in triplicate. This test was carried out on all the film [10].

2.3.3. Texture Analysis

Texture profile analysis (TPA) expresses the mechanical parameters in terms of tensile strength, mucoadhesiveness, properties that will distress ease product presentation into and maintenance within, periodontal pocket. TPA also consents an estimate of scope of structural restructuring ensuing product tender. Therefore in this honour, TPA is a pertinent practice for the characterization of formulations considered for solicitation to periodontal pocket [11].

2.3.4. Tensile Strength

Tensile strength of the films was eminent on texture analyzer (TAXT plus Express) using a film strips of 4x1cm, which was clinched on vertical clamps and the force prerequisite to break the film was leisurely. Test was carried out in tension mode with a pretest speed of 0.5 mm/sec, applied force of 509.9 gm, return distance of 10 mm, contact time of 20 sec, trigger force of 5.0 gm [12].

2.3.5. Drug(S) Content

Drug(s) content was resolute by extracting a film of size 0.25 cm^2 in 0.1 N HCl. After complete solubilization of drugs, the solution was filtered and rightly diluted with phosphate buffer of pH 7.4, absorbance recorded at 319 nm, 273.8 nm and 291.6 nm and drug content was unwavering by simultaneous equation method as well as Q-Absorbance analysis [13].

2.3.6. *In Vitro* Drug(S) Release Studies

In vitro drug(s) release study was achieved by placing a film of cm^2 in a vial comprising 5 ml of phosphate buffer of pH 7.4. Sampling was done at 24 hours interval and medium was exchanged with phosphate buffer of pH 7.4 from 1^{st} to 7^{th} day. The trials were analyzed spectrophotometrically and the drug(s) release was estimated by Q1 equation method [14].

2.3.7. Stability Studies

The periodontal film of size 0.25 cm^2 was wrapped in aluminium foil and stored at ambient humidity conditions at three altered conditions i.e. room temperature (25°±2°C), oven temperature (45±2°C) and in refrigerator (5-8°C) for a period of 90 days[162]. The physical stability of the illustration was studied and sample was assayed for drug content by extracting a film of size 0.25 cm^2 in 0.1 N HCl, filtered, rightly diluted with phosphate buffer of pH 7.4, absorbance recorded at 319 nm, 273.8 nm and drug(s) content was unwavering by simultaneous equation method after every 30 days [15].

2.3.8. *In Vitro* Antibacterial Activity

The nutrient agar media was poured aseptically into sterilized petridish and acceptable to solidify. *In vitro* antibacterial activity was completed by placing the films of altered surface area on nutrient agar plates seeded with periodontal bacteria Bacillus subtilis. After 48 hours of incubation at 37°C, the zone of inhibition was measured [16].

2.3.9. Bio-degradability Study

Film pieces of size 0.25 cm^2 were immersed in 5 ml of three diverse media namely 0.9% w/v NaC1 solution, distilled water and phosphate buffer of pH 7.4 each comprising 0.05% w/v a-amylase enzyme in a glass vial for a period of 3 months. Film degradation was estimated by visual inspection. Degradation was painstaking complete when film was no longer visible to naked eye and only a powdered suspension was evident.

3. Results and Disussion

3.1. Physicochemical parameters:

3.1.1. Thickness:

Drug loaded film was tested for thickness by using screw gauge. The physiochemical evaluation data indicates that the thickness of periodontal film varied from 0.37 mm to 0.41 mm (Table-2), thus suggesting uniform thickness of the formulation designed, ensuring the reproducibility of the method.

3.1.2. Folding endurance: All the formulations unveiled a folding endurance of more than 200 portentous the film to retain good elasticity as well as elasticity (Table 2). Extreme folding endurance was perceived for F3 formulation comprising of ethyl cellulose and Eudragit L-100 encompassing dibutyl phthalate as plasticizer. Minimum folding endurance was of F9 formulation entailing of eudragit L-100, possibly be due to alteration in plasticizer i.e. glycerol instead of dibutyl phthalate.

3.1.3. Texture analysis

For all the formulations, the percentage moisture loss mottled among 0.54% to 12.5% (Table 2) Formulation F6 revealed extreme of moisture loss remaining to extreme water loss. Formulation F1 exhibited minimum percentage moisture loss because of the presence of hydrophobic polymethyl methacrylates. In common films casted with hydrophilic polymers displayed greater moisture loss when associated to those formulated with hydrophobic polymers.

3.1.4. Tensile Strength

Tensile strength of films was initiate to be in the variety of 37.281- 272.369 g/cm2 (Table No. 5.19). The formulation Fl exhibited maximum tensile strength thus will be able to preserve its integrity through its handling and placement in periodontal pocket and will create the alleged clinical effect. Formulation F7 entailing of hydroxy propyl methyl cellulose exhibited minimum tensile strength possibly due to its hydrophilic nature that can engage moisture from the atmosphere thereby initiating reduction in the strength.

3.1.5. Drug(S) Content Determination

Percentage drug(s) content of the prepared periodontal films was estimated. The spectrophotometric analysis is performed in duplicate for all the samples, it was observed that percentage drug content of all the periodontal films ranged between 95.28% to 101.96% for both CPX and MHCl as determined by simultaneous equation method and between 95.86% to 99.87% (Table 3) as determined by Q-absorbance ratio method. The drug(s) content was uniform in all periodontal films and was in good agreement with theoretical drug content.

3.1.6. *In Vitro* Drug(S) Release

In vitro drug(s) release studies were carried out for formulations Fl to F9 in, phosphate buffer of pH 7.40. In vitro drug(s) release performed using phosphate buffer of pH 7.40 showed an initial burst release which is expected to kill most of periodontal pathogen, followed by controlled release for about 7 days, (Figure- 1 and 2) sufficient to inhibit growth of periodontal pathogens.

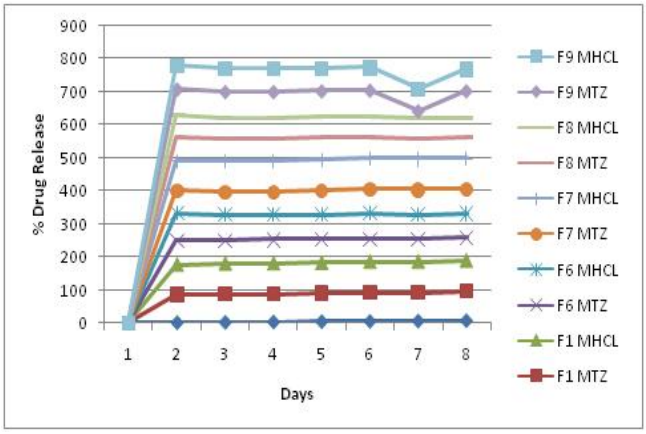

Figure 1: Percent drug(s) release of periodontal films in phosphate buffer ph 7.40 formulations (f1, f6-f9)

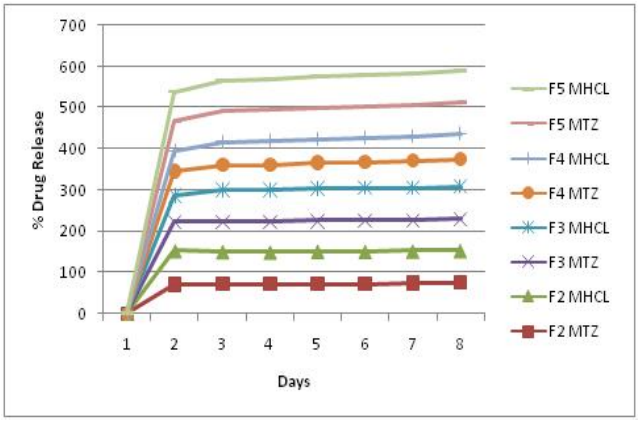

Figure 2: Percent drug(s) release of periodontal films in phosphate buffer ph 7.40 formulations (f2-f5)

In F8 formulation, presence of matrix forming agent ethyl cellulose at the levels of 10%, resulted in the diffusion controlled release with a linear (zero order) release profile. Such a release profile is desirable in controlled release applications, as it allows for constant dose per time delivery. As ethyl cellulose dissolved in an organic solvent can be used on its own to produce water insoluble, stronger and more durable films.

F8 formulation consists of ethyl cellulose released 65-69 % of drug(s), it may be due to its solubility by the addition of plasticizer i.e. dibutyl phthalate. Drug release from F6 formulation consisting of hydroxy propyl methyl cellulose is 69-75% and from F7 formulation consisting of hydroxy propyl cellulose is 70-85% as hydroxy propyl methyl cellulose and hydroxl propyl cellulose are biodegradable polymers and acts as resorbable carriers, they dissolve readily during in vitro drug release. Hydroxy propyl methyl cellulose is used as film former and rate controlling polymer for sustained release and hydroxy propyl cellulose is primarily used as extended release matrix former.

Formulation Fl consists of polymethyl methacrylates releases 85% to 93% of both the drug(s) as it swells in water, insoluble in water and give rise to pH independent drug release because of presence of ammonium groups as salts. The mechanism of drug release would probably be through direct dissolution of partially embedded drug followed by diffusion of embedded drug via the matrix pores. Formulation F8 consists of ethyl cellulose releases 65-69 % of drug(s), whereas F5 formulation consists of ethyl cellulose in combination with hydroxy propyl methyl cellulose releases 76% of drug(s), increase in release may be due to solubility modulation by hydroxy propyl methyl cellulose and due to formation of more pores and channels due to solubilization of hydroxyl propyl methyl cellulose in the medium.

F4 formulation consists of ethyl cellulose in combination with hydroxyl propyl cellulose releases 66% of drug(s) as drug release through ethyl cellulose coated dosage forms is controlled by diffusion. Formulation F2 consists of combination of ethyl cellulose and polymethyl methacrylates releases 70-80% of drug(s) and the drug(s) release of polyrnethyl methacrylates significantly decreased when formulated in combination with ethyl cellulose, so formulation Fl was selected as better formulation in contrast with F2.

Formulation F7 consists of hydroxyl propyl cellulose releases 68-73% of CPX and 89-93% MHCl as the drug release of CPX is less in contrast with formulation Fl which releases 85-92 % of CPX, so formulation Fl was selected as better in comparison with formulation F7. Three formulations Fl, F2 and F7 were selected (Figure-3) and study was done in triplicate and among these formulations F1 formulation was selected as an optimized formulation because it is able to maintain the sustained release of both CPX as well as MHCl upto 85% to 93% for 7 days.

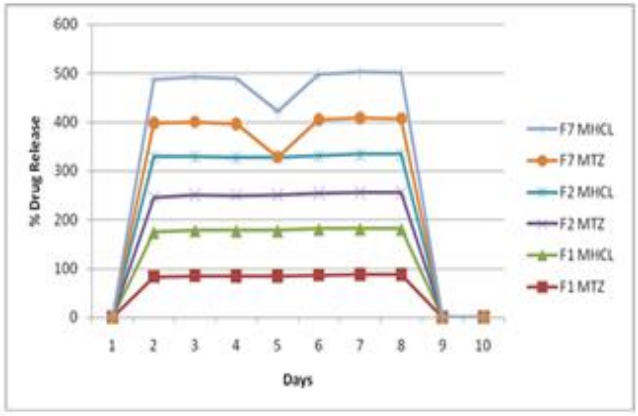

Figure 3: Percent Drug(S) Release of Mean Of Selected Formulations (N=3)

3.1.7. Stability Study

The result showed in table -4 revealed that the optimized F1 formulation was stable when stored at room temperature ($25^{o}\pm2^{o}C$), oven temperature ($45\pm2^{o}C$) and in refrigerator ($5\text{-}8^{o}C$) for a period of 90 days as the drug(s) content was found determined to be greater than 95% percent till the end of three months. In terms of appearance the film texture and color remained unchanged. Thus proving the stability of periodontal film.

3.1.8. *In Vitro* Antibacterial Activity

In vitro antibacterial activity were carried out by placing the periodontal films loaded with different concentrations of drug(s) on nutrient agar plates seeded with periodontal bacteria Bacillus subtilis (Table 5).

As the drug(s) concentration in the film was increased the zone of inhibition also increased. The microbiological studies at one end revealed that the drug released was able to inhibit the growth of microbes, as the regression value i.e. r^2 is 0.9848 it indicates that there is linearity in graph of zone of inhibition and diameter of film (Figure-4), it indicates that if there is severe periodontal infection then dose of drug(s) can be increased by increasing the surface area of the film. This is an advantage of periodontal film over discs like devices.

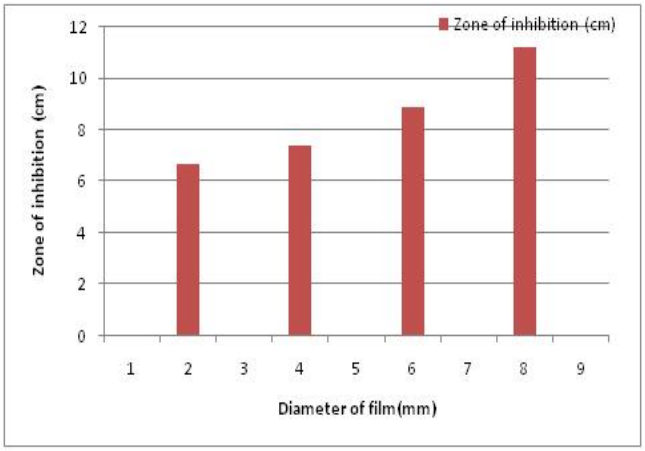

Figure 4: Graph between diameter of film and zone of inhibition

3.1.9. Biodegradability Study

Degradation behavior of various films depended on film structure and on degradation media used. Table No.5.21 shows the degradation time, determined by visual inspection, of all batches of CPX and MHCl loaded periodontal films. Result shows that presence of ethyl cellulose, polymethyl methacrylate and Eudragit L-100 greatly influences degradation time. Eudragit L-100 showed longer degradation times than hydroxyl propyl methyl cellulose and hydroxy propyl cellulose in all the media tested (Table-6). Formulation F8 containing ethyl cellulose showed slower degradation in all media as compared to films made of hydroxy propyl methyl cellulose and hydroxy propyl cellulose. In particular, formulation F1 maintained its integrity in all media tested. The presence of α-amylase increases the degradation of biodegradable polymers as because of its antiproteolytic activity. The biodegradative effect was more evident for all batches of films when soaked in phosphate buffer pH 7.4, than when soaked in distilled water.

Table 1: Formulation design of periodontal films prepared by solvent casting technique

Formulation code	Ciprofloxacin (mg)	Minocycline (mg)	Plasticizer (W/V)	Ethyl cellulose (mg)	Hydroxy propyl cellulose (mg)	Hydroxy propyl methyl cellulose K4M (mg)	Eudragit -L 100 (mg)	PMMA 120,000 (mg)
F1	3	3.5	DBA	-	-	-	-	450
F2	3	3.5	DBA	400	-	-	-	250
F3	3	3.5	DBA	400	-	-	125	-
F4	3	3.5	DBA	400	100	-	-	-
F5	3	3.5	DBA	400	-	100	-	-
F6	3	3.5	DBA	-	-	450	-	-
F7	3	3.5	DBA	-	450	-	-	-
F8	3	3.5	DBA	450	-	-	-	-
F9	3	3.5	Glycerol (20%v/v)	-	-	-	250	-

Table-2: Physical properties of periodontal film prepared by solvent casting technique

Film composition	Thickness (mm)	Folding endurance	Weight variation	Swelling index	Mucoadhesion (g/cm^2)	Tensile strength (g/cm^2)	Percent moisture loss
F1	0.38 ± 0.02	300 ± 2.05	13.9 ± 0.124	0.20	95.756	272.369	0.54 ± 0.09
F2	0.37 ± 0.03	226 ± 3.09	10.0 ± 0.081	0.067	46.560	108.128	2.78 ± 0.44
F3	0.41 ± 0.08	359 ± 3.39	10.0 ± 0.081	0.102	59.811	59.811	0.27 ± 0.16
F4	0.40 ± 0.01	222 ± 6.16	14.1 ± 0.04	0.08	64.862	54.101	6.00 ± 0.10
F5	0.37 ± 0.01	306 ± 3.74	6.00 ± 0.081	0.22	21.816	126.064	2.22 ± 0.09
F6	0.40 ± 0.02	339 ± 4.10	12.1 ± 0.047	Soluble	5.051	37.775	12.5 ± 0.55
F7	0.41 ± 0.01	256 ± 4.32	11.1 ± 0.081	Soluble	26.208	37.281	0.63 ± 0.13
F8	0.39 ± 0.03	240 ± 4.08	13.3 ± 0.047	0.064	28.624	34.042	1.40 ± 0.23
F9	0.38 ± 0.02	201 ± 4.98	13.3 ± 0.047	0.032	76.314	75.916	2.34 ± 0.09

Table 3: Drug(S) Content determined by simultaneous equation method and q-absorbance ratio method

Periodontal Films	Simultaneous Equation Method	
	CPX	MHCl
F1	96.28 ±1.01	98.36 ± 1.46
F2	98.16 ± 2.22	98.21 ± 2.03
F3	98.65 ± 2.44	99.44 ± 1.57
F4	98.15 ± 3.25	98.27 ± 0.132
F5	97.33 ± 3.45	99.59 ± 0.315
F6	96.74 ± 2.47	97.83 ± 0.37
F7	99.08 ± 1.01	99.88 ± 0.74
F8	98.45 ± 2.1	99.26 ± 0.16
F9	95.25 ± 3.7	99.87 ± 0.76

Table 4: Stability study of periodontal films

S.No.	Time (months)	% Drug(s) content					
		Room temperature (25^o±2^oC)		Oven temperature (45±2^oC)		Refrigerator (5-8^oC)	
		CPX	MHCL	CPX	MHCL	CPX	MHCL
1	1	98.51	98.05	96.83	97.25	99.30	98.55
2	2	97.83	97.05	96.83	97.55	98.58	98.58

3	3	99.01	98.25	86.72	97.52	97.81	98.02
Appearance		Smooth, yellow		Smooth, yellow		Smooth, yellow	

Table 5: Showing effect of increasing drug(s) concentration on zone of inhibition

Formulation	Surface area (cm^2)	Diameter of film (mm)	Zone of inhibition (cm) (mean±s.d.)
F1	0.25	5.64	6.7 ± 0.14
F1	0.41	7.22	7.4 ± 0.10
F1	0.83	10.3	8.9 ± 0.07
F1	1.16	12.18	11.25 ± 0.09

Table 6A: Film degradation study in 0.9% w/v NaCl solution, distilled water and phosphate buffer pH 7.40 each containing 0.05% w/v α-amylase enzyme

Film Degradation Study In Three Different Medium For 60 Days							
S.No.	Periodontal films	MEDIUM I (0.9% w/v NaCl solution and 0.05% w/v α-amylase enzyme)					
		10th day	20th day	30th day	40th day	50th day	60th day
1.	F1	N	N	N	N	N	N
2.	F2	N	S	S	S	S	S
3.	F3	N	N	S	S	S	S
4.	F4	N	N	S	S	S	S
5.	F5	N	N	S	S	S	S
6.	F6	T	T	T	T	T	T
7.	F7	T	T	T	T	T	T
8.	F8	N	N	N	N	N	N
9.	F9	S	T	T	T	T	T

Table 6B: Film degradation study in 0.9% w/v NaCl solution, distilled water and phosphate buffer pH 7.40 each containing 0.05% w/v α-amylase enzyme

Film Degradation Study In Three Different Medium For 60 Days							
S.No.	Periodontal films	MEDIUM II (Distilled water containing 0.05% w/v α-amylase enzyme)					
		10th day	20th day	30th day	40th day	50th day	60th day
1.	F1	N	N	N	N	N	N
2.	F2	N	S	S	S	S	S
3.	F3	S	S	S	S	S	S
4.	F4	N	N	N	S	S	S
5.	F5	N	N	N	T	T	T
6.	F6	T	T	T	T	T	T
7.	F7	N	T	T	T	T	T
8.	F8	N	N	N	N	N	N
9.	F9	S	T	T	T	T	T

Table 6C: Film degradation study in 0.9% w/v NaCl solution, distilled water and phosphate buffer pH 7.40 each containing 0.05% w/v α-amylase enzyme

Film Degradation Study In Three Different Medium For 60 Days							
S.No.	Periodontal films	MEDIUM III (Phosphate buffer pH 7.4 containing 0.05% w/v α-amylase enzyme)					
		10th day	20th day	30th day	40th day	50th day	60th day

1.	F1	N	N	N	N	N	N
2.	F2	N	S	S	S	S	S
3.	F3	S	S	S	S	S	S
4.	F4	N	N	S	S	S	S
5.	F5	N	N	T	T	T	T
6.	F6	T	T	T	T	T	T
7.	F7	N	T	T	T	T	T
8.	F8	N	N	N	N	N	N
9.	F9	S	T	T	T	T	T

4. Conclusion

The ciprofloxacin and minocycline HCl films were formulated by using the biodegradable polymer gelatin and sodium alginate with PEG 400 as a plasticizer. The physicochemical parameters shows uniform results for all the formulations. A bioadhesive periodontal film for simultaneous extended delivery of Ciprofloxacin and minocycline HCl into periodontal pocket was formulated. The film displayed dose dependent antibacterial activity and was found to be stable. The approach provides an opportunity and potential for development of periodontal film containing both Ciprofloxacin and minocycline HCl for extended release.

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