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Research Article

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Formulation and evaluation of ciprofloxacin dental films for periodontitis

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ABSTRACT

Ciprofloxacin hydrochloride, fluoroquinone 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 weight uniformity, thickness, content uniformity, percentage moisture loss, surface pH and IR. The result of weight uniformity, content uniformity and surface pH was uniform for all the formulations. The thickness and percentage moisture loss was different for the formulation from batch to batch. From the result of IR, there was no interaction between the drug and polymer. Optimized formulation F2 gelatin and sodium alginate1:1 ratio was found to release 98.97% of drug at the end of 6th hour and considered as a best formulation. The release mechanism for invitro release was studied by using various mathematical models. The 'n' value for the koresmeyer-peppas equation was in the range of 0.87-0.98 indicating the anamolous behaviour (non-fickian release). Invitro antibacterial activity was carried out in Staphylococcus aureus and Enterobacter aerogen had an inhibitory effect after 24hours of incubation. The stability studies were carried out at 25±2°C/60±5% RH and 40±2°C/75±5% RH which does not shows any significant change after 3 month.

Keywords: Films, Ciprofloxacin, Gelatin, Sodium alginate, Periodontal diseases.

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 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 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 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%. Inoder 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, *invitro* dissolution rate studies are performed on the film.

EXPERIMENTAL SECTION

2.1. Materials:

Ciprofloxacin was obtained as gift sample from Pharmafabricon Ltd, (Madurai). 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 Himedia, (Mumbai). Other materials used in the study were of analytical reagent grade.

2.2. Preparation of film:

Weighed quantity of polymer, gelatin was sprinkled on the surface of water and kept aside for 30 minutes to hydrate. Sodium alginate was added and PEG 400 as a plasticizing agent with continuous stirring and the solution was heated on a water bath at 60ºC until gelatin was dissolved. 1% w/v ciprofloxacin was dissolved separately in small quantity of water and added to gelatin and sodium alginate solution. The solution was poured in a glass petridish and placed on ice bath for 30 minutes to become gel and they were dried for 3 days at room temperature. After drying, the films were cut into appropriate dimension 2×2 cm^2 by stainless steel cutter [8].

Table (1). Formulation chart for ciprofloxacin film

Ingredients	Formulations						
	F1	F2	F3	F4	F5	F6	F7
Drug(mg)	100	100	100	100	100	100	100
Gelatin (mg)	300	350	450	525	600	675	-
Sodium alginate(mg)	450	350	300	225	150	75	-
PEG 400(ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Distilled water(ml)	25	25	25	25	25	25	25

2.3. Physicochemical parameters

2.3.1. Weight uniformity test:

10 patches were weighed and they were cut into different pieces. The individual weights were determined by using the electronic balance and the average weight was calculated [9, 10].

2.3.2. Folding endurance:

Folding endurance of the film was determined by repeatedly folding the film at the same place up to 300 times till it broke or folded, which is considered satisfactory to reveal good film properties. This test was carried out on all the film [11].

2.3.3. Percentage moisture loss:
The percentage moisture loss was carried out to check integrity of the film at dry conditions. Films were weighed and kept in a desiccator containing anhydrous calcium chloride. After 3 days, the films were taken out and reweighed; the percentage moisture loss was calculated using the formula [12].
% moisture loss =initial weight-final weight/initial weight

2.3.4. Thickness of the film:
The thickness of each film was measured using screw gauge at different positions of the film and the average thickness was calculated [13].

2.3.5. Drug content uniformity:
Implants containing ciprofloxacin was dissolved in 100 ml of distilled water and kept aside for overnight. Then the solution was filtered with the Whatmann filter paper. From the filtrate 5 ml was taken and diluted with distilled water in 100 ml standard flask. The absorbance of the solution was measured at 278 nm using a UV-spectrophotometer [14]**.**

2.3.6. Surface pH:
Periodontal films were left to swell for 1 hour on the surface of the agar plate, prepared by dissolving 2% w/v agar in warmed distilled water by stirring. The solution was poured into the petridish to gelling/solidify at room temperature. The surface pH was measured by means of pH paper placed on the surface of the swollen film. The mean of three readings was recorded [15].

2.3.7. Drug-polymer compatilibility:
IR spectra of pure ciprofloxacin hydrochloride and physical mixture of drug and excipients were recorded on Shimadzu Corporation, (Tokyo, Japan). Potassium bromide pellet method was employed and background spectrum was collected under identical situation. Each spectrum was derived from single average scans collected in the region 400-4000 cm^{-1} at spectral resolution of 2 cm^{-2} and ratio against background interferogram. Spectra were analyzed by software supplied by Schimadzu [16].

2.4. *Invitro* drug release:
Film of known weight and dimensions were taken separately into small test tubes containing 10 ml of pH 7.4 phosphate buffer. The test tubes were sealed with the aluminium foil and kept at room temperature [17]. The sample was withdrawn and replaced with fresh 1 ml of pH 7.4 for every 1 hour up to 6 hours. The concentration of drug in the buffer was measured at 278 nm by using a UV-spectrophotometer.

2.4.1. *Invitro* drug release kinetic studies:
To analyze the *invitro* release data various kinetic models were used to describe the release kinetics [17-20].The zero order rate Equation no: 1 describes the systems where the drug release rate is independent of its concentration. The first order Equation no: (2) describe the release from the system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on the Fickian diffusion Equation no: (3). The Hixson-Crowell root law Equation no: (4) describes the release from the systems where there is a change in surface area and diameter of particles. The Koresmeyer-peppas Equation no: (5) describes the mode of release of drug from swellable matrices.

$C = K_0t$ ---------- (1)
Where, K_0 is zero order rate constant expressed in units of concentration/time and t is the time.

$\text{Log } C = \text{Log } C_0 - Kt/2.303$ ---------- (2)
Where, C_0 is the initial concentration of drug and K is first order rate constant.

$= Kt^{1/2} - Q$ ---------- (3)
Where, K is the constant reflecting the design variables of the system.

$Q_0^{1/3} - Q_t^{1/3} = K_{HC}t$ ---------- (4)
Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug and K_{HC} is the rate constant for Hixson-Crowell rate equation.

$M_t/M = Kt^n$ ----------(5)

Where, M_t/M is fraction of drug release at time t, K is constant incorporating the structural and geometrical characteristics of the drug/ polymer system, n is diffusion exponent related to the mechanism of the release

The following plots were made:

1. Cumulative % drug released versus time (Zero order kinetic model)
2. Log cumulative % drug remaining versus time (First order kinetic model)
3. Cumulative % drug release versus square root of time (Higuchi plot)
4. Log cumulative % drug release versus log T (Koresmeyer-peppas model)
5. Cube root of drug % remaining versus time (Hixson-Crowell model)

2.5. Stability studies:

Stability studies were carried out for optimized formulation. The film was stored at 25±2°C/60±5%RH and 40±2°C/75±5%RH for duration of 3 months and stability data were analyzed.

2.6. Antimicrobial activity:

2.6.1. Disc-diffusion method:

Luria Bertani agar medium was prepared and the test micro-organisms were inoculated by the spread plate method. Filter paper disc were soaked with different concentration of 2, 4 and 6 µg/ml of the ciprofloxacin implant and placed in the prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly and the distance between each disc was maintain at 24 mm. The agar plates were then incubated at 37°C. After 16 to 18 hours of incubation, each plate was examined. The diameter of the zone of complete inhibition was measured [21].

2.6.2. Agar well diffusion method:

Petriplates 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 ciprofloxacin 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 [22].

RESULTS AND DISCUSSION

Physicochemical parameters of the films are represented in Table (2).

3.1. Physicochemical parameters:

3.1.1. Weight uniformity test:

Drug loaded films were tested for uniformity of weight. The weight was found to be uniform in the prepared batches.

3.1.2. Folding endurance:

Film did not show any cracks even after folding for more than 250 times. Hence it was taken as the end point. Folding endurance did not vary when the comparison was made between dummy film and drug-loaded film.

3.1.3. Percentage moisture loss:

From the result it was inferred that F2 has highest moisture loss whereas F7 has lowest moisture loss. If gelatine concentration increases moisture loss decreases, since gelatine has low moisture content.

3.1.4. Thickness:

Drug loaded film was tested for thickness by using screw gauge. From the result it was inferred that F1 has least thickness whereas the F6 has a highest thickness since it contain highest concentration of polymer in the ratio 9:1, gelatin and sodium alginate respectively.

3.1.5. Content uniformity:

The results of content uniformity indicated that the drug was uniformly dispersed. Recovery was possible to the tune of 90 to 95 % for formulations F1 to F7. The drug content analysis of the prepared formulations had shown that the

process employed to prepare the film in this study was capable of giving film with a uniform drug content and minimum batch variability.

3.1.6. Surface pH:

The surface pH of all formulations was within 6-7 i.e. close to the neutral pH and hence no irritation was expected.

Table (2). Various parameters for the evaluation of ciprofloxacin film

S.no	Formulation	Weight of films (mg)	Thickness (mm)	Drug content (%)
1	F1	0.029±0.001	0.114±0.015	91.612
2	F2	0.033±0.003	0.131±0.007	92.067
3	F3	0.031±0.008	0.133±0.011	92.219
4	F4	0.037±0.009	0.126±0.004	95.023
5	F5	0.042±0.004	0.11±0.01	94.230
6	F6	0.036±0.003	0.116±0.01	92.328
7	F7	0.024±0.001	0.08±0.001	94.257

3.1.7. Drug-polymer compatibility:

IR spectra of ciprofloxacin hydrochloride and formulation were determined using IR and are presented in Figure (1 and 2). Pure ciprofloxacin spectra showed sharp characteristic peaks at 3437.78 cm^{-1}, 3088.13 cm^{-1}, 2929.96 cm^{-1} 1635.44 cm^{-1} ,1134.05 cm^{-1} ,774.62 cm^{-1} and 565.70 cm^{-1} . IR spectra of ciprofloxacin and its formulation are exactly same and there are no shifts of peaks or disappearance of principle peaks or modification of the principle peaks indicating that there is no interaction between the drug and excipients.

3.4. *In-vitro* release studies:

The *invitro* result of all formulations was carried out in phosphate buffer pH 7.4. The results are shown in the table (3). From the obtained results, the F1, F2, F3 formulations release the drug 96.70, 98.87, 92.15 respectively at 6^{th} hour due to the presence of low polymer concentration. In the F4, F5, F6 formulation the release of the drug was 89.50, 83.70, and 82.69 respectively at 6^{th} hour since the gelatine concentration is increased when compared with the F1-F3 formulations because it was increasing the viscosity of the formulation. In F7 the release rate was 95.84 at 4^{th} hour due to the absence of the polymer. The concentration of gelatin was increased in the formulation F6, since the high concentration of gelatin with the low concentration sodium alginate sustain the release due to the thickening property of the sodium alginate.

Table 3. *In-vitro* release studies of Ciprofloxacin film

Time (hr)	F1	F2	F3	F4	F5	F6	F7
1	58.50	46.50	41.30	38.32	38.52	24.20	33.26
2	69.61	61.54	58.41	52.46	43.38	38.52	60.67
3	77.78	70.63	67.58	61.03	51.45	53.46	76.96
4	87.13	78.68	73.64	71.53	64.56	62.63	95.84
5	96.70	86.75	81.68	80.86	73.63	71.53	-
6	-	98.87	92.15	89.50	83.70	82.69	-

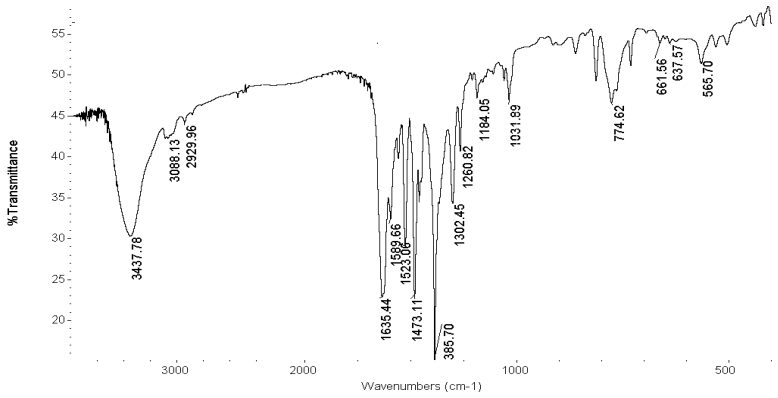
Table (4). *Invitro* release kinetics of the formulation F1 to F7

Formulation code	Zero Order	First order	Higuchi	Koresmeyer-peppas		Hixson-Crowell
	R^2	R^2	R^2	R^2	N	R^2
F1	0.973	0.014	0.965	0.648	0.829	0.015
F2	0.968	0.030	0.974	0.644	0.841	0.033
F3	0.959	0.057	0.979	0.640	0.855	0.056
F4	0.812	0.098	0.915	0.668	0.836	0.100
F5	0.801	0.142	0.923	0.641	0.876	0.125
F6	0.789	0.178	0.929	0.519	0.991	0.143
F7	0.725	0.005	0.932	0.636	0.883	0.022

3.5.1. *Invitro* drug release kinetics:

From the data shown in the table (4), the *invitro* dissolution data were fit into the different kinetic model, the Koresmeyer-peppas giving linear relationship. In zero order plot the r^2 value obtained is 0.789 and first order gave 0.178 describes the drug release rate relationship with concentration of drug. The best linearity was found in

higuchi's equation plot the r^2 value obtained is 0.929 indicating the release of drug from matrix as a square root of time independent process because the value is more than 0.5. In koresmeyer-peppas model the r^2 value obtained is 0.519 and n-value is 0.991 which undergo non-fickian or anamolous transport. In Hixson-crowell cube root law the r^2 value obtained is 0.143 indicating there is a change in surface area and diameter with the function of time.



Figure(1). IR spectrum of pure ciprofloxacin

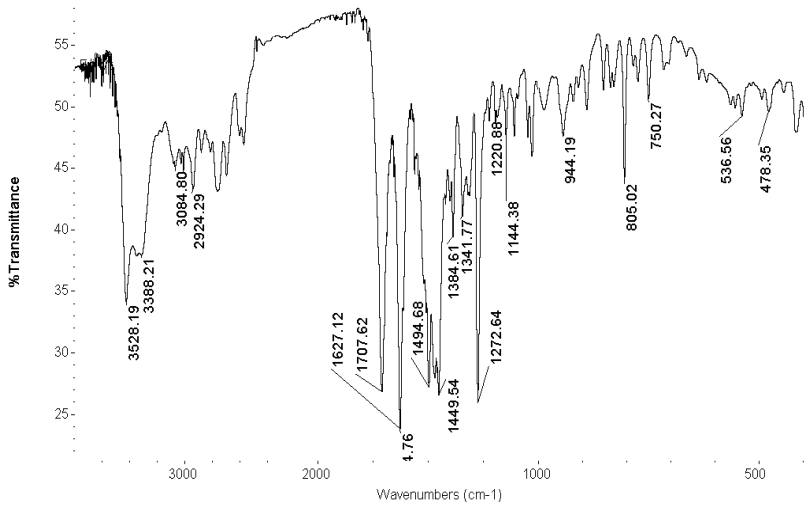


Figure (2). IR spectrum of optimized formulation of ciprofloxacin film

3.7. Stability studies:

The optimized formulation F2 was charged on accelerated stability and monitored for physicochemical and drug release study at 1, 2 and 3 month. The stability study reveals no significant variation in physicochemical parameter and *invitro* release study up to 3 months. Stability studies for F2 formulation at different temperature were shown in the table (5).

Table (5). Stability studies for the optimized formulation F6

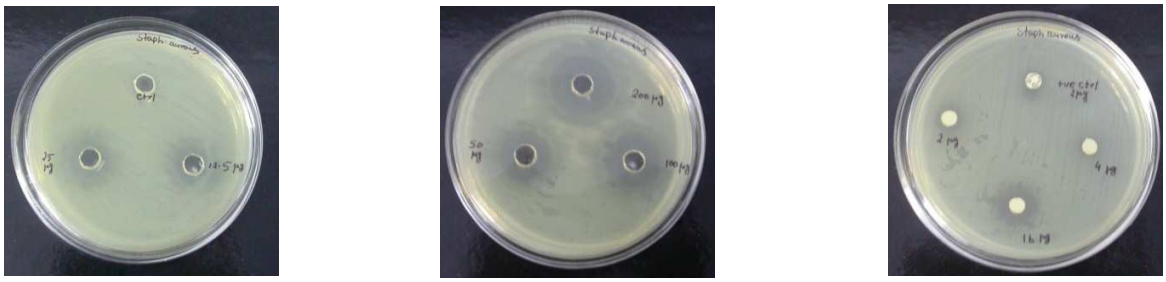
Parameters	0^{th} day	1 month	2 month	3 month
Weight uniformity	0.032	0.028	0.026	0.025
Thickness	0.165	0.166	0.152	0.150
% moisture loss	16.6%	19.01%	18.21%	17.65%
Surface pH	7	6	7	6
Drug content	11.62	11.48	11.29	11.05
Drug release	85.96%	85.69%	85.60%	84.97%

3.8.Antimicrobial activity

After 24hours of incubation, the diameter of zone of inhibition was measured and the value was shown in the table 6. It shows better antibacterial activity over those micro-organisms.The zone of inhibition of clindamycin films were shown in the figure 3 and 4.

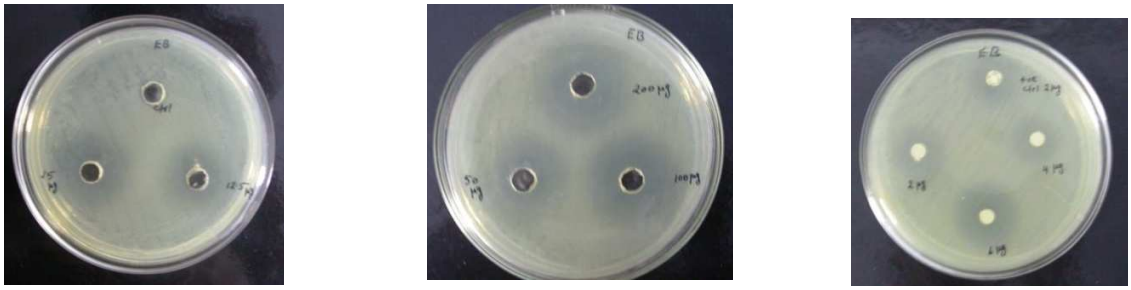
Table (6). Zone of Inhibition of the ciprofloxacin film

ORGANISM NAME	AGAR WELL DIFFUSION METHOD (ZONE SIZE) mm					DISC DIFFUSION METHOD (ZONE SIZE) mm			
	12.5µg	25µg	50µg	100µg	200µg	+ive Control (2 µg)	2 µg	4 µg	16µg
Staphylococcus aureus	4	7	9	11	15	4	5	6	10
Enterobacter aerogens	2	4	5	9	14	1.5	2	4	6



(A) **(B)** **(C)**

Figure (3). (A) & (B) Agar Well Diffusion Zone for *Staphylococcus aureus* using liquid Ciprofloxacin (C) Disc diffusion Zone for *Staphylococcus aureus* using Ciprofloxacin film



(D) **(E)** **(F)**

Figure (4). (D) & (E) Agar Well Diffusion Zone for *Enterobacter aerogens* using liquid Ciprofloxacin (F) Disc diffusion Zone for *Enterobacter aerogens* using Ciprofloxacin film

CONCULSION

The ciprofloxacin films was 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. From the result of *invitro* release studies of the formulation F1 to F7, the formulation F2 release the drug 98.97% 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 *Enterobacter aerogens*, the zone of inhibition was observed by agar disc diffusion and agar well diffusion method and the optimized implant shows better activity over those micro-organisms. The result of stability studies carried out on optimized formulation at 25±2°C/60±5% RH and 40±2°C/75±5% RH which does not shows any significant change after 3 month.

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