Development and characterization of target retentive polymeric films of gatifloxacin for periodontal infections

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Abstract

Periodontitis is a family of bacterial infections resulting in destruction of the tissue that supports the tooth. The inflammatory responses which bacterial accumulations elicit in the gingival tissue is ultimately responsible for progressive destruction of collagen supporting and periodontal ligament, which, if unchecked, can cause the tooth to loosen and then to be lost. The toxic side effects at higher dose levels of anti-biotic makes systemic administration unacceptable; therefore a safe and effective low dose drug delivery device is highly desirable. Gatifloxacin is a broad-spectrum antimicrobial agent, which is active against a number of periodontal pathogens, is selected for site specific delivery ie., into periodontal pocket. Gatifloxacin is formulated into strips by using polymers and the prepared strips were evaluated for various properties such as weight variation, tensile strength, moisture loss, folding endurance, stability studies, *in-vitro* release and antibacterial study. The average weight and thickness among the different films was uniform. Tensile strength was maximum for plain films and minimum for films containing drug. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and also shown extended release with more uniformity of release. The *in – vitro* release kinetics of gatifloxacin followed zero order pattern and followed higuchi's diffusion model. The mass balance studies done after the *in-vitro* dissolution, did not deviate by more than 3% from the experimental drug content. The *in vitro* anti bacterial activity showed that Gatifloxacin is very active against *P aerogenosa* when compared to *S aureas* and *E coli*. The stability studies did not show any significant changes with respect to content and appearance.

Key Words: Periodontitis, Gatifloxacin, Strips, Local drug delivery.

1. Introduction

Periodontitis, the severely debilitating disease of the periodontium, is characterized by the loss of bone, collagen support of the affected teeth and accumulation of bacterial pathogens mainly with in the periodontal pockets (Brown, 1996; Yeung, 1983). To eliminate bacterial infections, antibiotics are administered either locally or systemically. The repeated and long term use of systemic antibiotics is associated with potential adverse effects (Kornman, 1993; Slots and Rams, 1990) like resistant strains and superimposed infections. This problem can be overcome by delivering the drug at the desired target site (ie periodontal pocket) to show local therapeutic action. The main advantage of local route of drug delivery is the reduction of drug dose and possibility to increase the concentration of the drug in the periodontal pockets, at the same time keeping comparatively low systemic drug concentration (Kim et al., 2004). By means of controlled local delivery within the periodontal pocket, a single administration of a few milligrams of an antibacterial agent can maintain therapeutic concentration within the crevicular fluid for a longer period of time; this appears to be holding some promise in periodontal therapy.

Gatifloxacin is a broad-spectrum antimicrobial agent, which is active against a number of aerobic, anaerobic, gram positive and gram negative periodontal pathogens. Gatifloxacin is a second generation fluoroquinolone group and is a synthetic broad-spectrum antibacterial agent active against aerobic and anaerobic microorganisms. The antibacterial action of gatifloxacin results from inhibition of DNA gyrase and topoisomerase IV. It is well absorbed after oral administration and peak plasma concentration occurs in 1-2 hours with 96% absolute bioavailability (Patel et al., 2005; Tripathi, 2003).

The objective of this study is to develop and evaluate polymeric strips containing gatifloxacin for the local controlled drug delivery with in the periodontal pocket for the effective treatment of periodontitis.

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2. Materials and methods

Ethyl cellulose, hydroxyl propyl methyl cellulose (K_4M), hydroxy propyl cellulose (HPC) and poly vinyl pyrrolidone (PVP K-30) were obtained from Loba Chemicals Pvt. Ltd., Mumbai. Eudragit RL-100, Eudragit RS-100 from Degussa India Pvt. Ltd., Mumbai. Gatifloxacin as a gift sample from Micro labs, Bangalore. Chloroform, dichloromethane and dibutyl phthalate from Merck Limited Mumbai. All other chemicals used in this study were of analytical grade.

2.1. Preparation of Drug Loaded Polymeric Films

Method used for the preparation of strips was by solvent casting technique using chloroform and dichloromethane (1:1) mixture. A total of six formulations were designed shown in Table 1, in which ethyl cellulose was taken as the prime polymer alone and in combination with different co-polymers and coating agent for each cast strips. The preparation of films was done by dissolving ethyl cellulose alone and with any one of these co-polymer HPC or HPMC K_4M or PVP or Eudragit RL-100 or RS-100 in the chloroform: dichloromethane (1:1) mixture and dibutyl- phthalate (1% w/w of that of polymer) as a plasticizer. These mixtures are mixed thoroughly by using magnetic stirrer in a closed beaker to get uniform distribution of polymer(s) into the solvent mixture. To the above mixture, required concentration of gatifloxacin (3 % w/w to the weight of polymer(s)) was added and mixed well to disperse the drug. After complete mixing 10 ml solution was poured into the clean-leveled glass moulds of 25 sq. cm. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug closed in the stem of the funnel at room temperature for 24 hours. After complete evaporation of solvent, cast strips were obtained, which were then cut into pieces of 7×2 mm (containing 60ìg of drug/strip) wrapped in an aluminum foil and stored in desiccator at relative humidity at room temperature in a dark place until for further evaluation study (Matiholimath et al., 2006; Udupa, 1999).

2.2. Evaluation of polymeric films

The compatibility studies were conducted by using FT IR spectroscopy of drug alone, and individual polymers along with drug. Various physicochemical properties such as size, thickness, content, weight variation, folding endurance, tensile strength and percentage moisture loss was determined on prepared films. Thickness of the three films (1×1 cm) was determined at six different places using film thickness tester (Mitutoyo 4026F,Japan) (Anuradha et al., 2007). Individual weights of twenty films of the equivalent size were weighed on an electronic balance and the average weight was calculated. Percentage moisture loss was determined by keeping the film of known weight in a dessicator containing anhydrous calcium chloride. After three days, the films were taken out and re-weighed; the percentage moisture loss was calculated using formula (Initial wt – Final wt/Initial wt) ×100. Folding endurance of the film was determined by repeatedly folding a small strip of film of 2×2 cm size at the same place till it broke (Matiholimath et al., 2006).

2.3. Measurement of tensile strength and percentage elongation

Tensile strength was evaluated using Instron Universal testing instrument (Model 4206, Instron Ltd., Japan) with a 2 kg load cell. Films in special dimension and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. During measurement, the top clamp at rate of 100 mm/min pulled, the force (Tensile strength) and elongation were measured when the film broke by applying the following equations (Mashru et al ., 2005). The film samples, which broke at clamps were not included in the calculations.

$$\text{Tensile strength} = \frac{\text{Force at break (N)}}{\text{Initial cross sectional area of the sample (mm}^2\text{)}}$$

$$\text{Percent Elongation} = \frac{\text{Increase in length}}{\text{Original length}} \text{ X100}$$

2.4. Estimation of content uniformity

The drug-loaded films of known weight (7 × 2 mm) were dissolved in 10 ml of aqueous acetic acid, suitably diluted and the amount of drug(s) present was estimated by spectrophotometer (Venkateshwari et al., 1995).

2.5. In vitro drug(s) release studies.

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 model was adopted for the dissolution studies. Sets of six films of known weight and dimension were placed separately into small sealed test tubes containing 1.0 ml of phosphate buffer. The tubes were 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 drug(s) was determined and this procedure was continued for 9 consecutive days. Following the in-vitro release studies, the test films were further analyzed for the drug content left in each film by dissolving the films in aqueous acetic acid and suitably diluted. The amount of drug released into the dissolution medium plus residual drug content in the films were accounted and compared for the actual drug content (Venkateshwari et al., 1995).

2.6. Scanning Electron Microscopy

The morphology and surface topography of the films were examined by SEM (Model JSM-840 A, Jeol, Japan). The samples to be examined are cut into round shape of dimension 5 mm^2 and were mounted on the SEM sample stab using a double sided sticking tape. The samples mounted were coated with gold (200 A^0) under reduced pressure (0.001 torr) for 2 minutes using an ion sputtering device (model JFC-1100 E, jeol, japan). The gold coated samples were observed under the SEM and photomicrographs of suitable magnifications were obtained (Shivakumar, 2007).

2.7. In vitro antibacterial activity

In-vitro antibacterial activity was performed on all formulations against E-coli, P aerogenosa and S.aureas. The agar medium was prepared, to study mean antibacterial activity for each formulation. The antibacterial activity was studied by placing the strips and pure drug sample on agar plates seeded with the above mentioned bacteria's and kept for 48 hours incubation at 37°C. After 48 hours of inhibition, the films were transferred onto freshly seeded agar plates for an additional 48 hours for incubation. This procedure was repeated until no inhibition of bacterial growth was detected on the agar plate. The zone of inhibition area on the agar plate was measured (Steinberg et al., 1990).

2.8. Stability studies

The films of size (7×2 mm) were weighed in three sets (12 strips in each set). The films were wrapped individually in aluminium foil and butter paper and placed in glass containers. These containers were stored at ambient humid conditions i.e., Refrigeration Temperature (4-8^{0}C), Room Temperature (25 ± 2^{0}C) and oven (45 ± 2^{0}C). The samples were analyzed for physical changes such as color and texture. The drug content was estimated at regular intervals and the degradation rate constant k values are determined (Nagaraju and Udupa, 1998).

3. Results and discussion

The compatibility studies conducted by FT IR spectroscopy showed no interaction of drug with individual polymer(s). Macroscopical features revealed that drug was dispersed uniformly in the polymer matrix. The physicochemical evaluation data given in Table 2 showed that the average weight of the films ranges from 1.42 to 1.77 mg and the maximum weight was observed for films F2 and F3. The thickness of the films ranges from 0.139 to 0.180 mm and film F6 showed maximum thickness may be due to the co polymer HPC. There was no significant difference in the thickness among the different films.

The tensile strength of the strips ranges from 365 to 682 gm/sq mm, tensile strength was maximum for plain films (708gm/sq mm) and minimum for F6 films. For all the films, the percentage moisture loss varied between 7.2 ± 2.29 and 10.8 ± 2.04. Film F2 showed maximum amount of moisture loss and F1 showed minimum moisture loss due to hydrophilic and lipophilic nature respectively. All the films contains more than 95% drug as per the content uniformity studies and exhibited more than 140 folding endurance.

The release time profile of gatifloxacin from various formulations showed that there was rapid initial release of drug on day one ie., 22.34%, 31.84%, 29.50%, 40.50%, 17.02% and 37.43% for F1,F2,F3,F4,F5 and F6 films respectively. There was

a marked reduction in the release from day 3 onwards and release was controlled and extended up to 11 days for all the films. A perusal of Figure 1 indicated that the initial rapid release must be due to burst effect and it is because of elution of the drug from the outer surface and cut edges of the polymer matrix. Once the burst effect was completed (2 days) drug was more sustained up to 11 days. At the end of 10th day the release of drug from all the formulations was found to be more than 91%. Mass balance studies done after in vitro release study showed that the drug content did not differ from the experimental drug content by more than 3 %.

The in-vitro release profile of drug from all the films could be best expressed Highuchi's equation, as the plots showed high linearity (R^2: 0.9019 to 0.9896). Thus, the cumulative percentages of drug release per square mm area versus square root of time in days were revealed that there is a near linear relationship from 3rd day to 10th day. Further it is confirmed by Korsmeyer et al's equation showed good linearity (R^2: 0.9124 to 0.9675), with slope (n) values ranging from 0.167 to 0.289, indicating that zero order diffusion is the prime mechanism of drug release. The comparative kinetic values of all the plots were given in Table 3.

The in-vitro antibacterial activity showed the gatifloxacin is more active against P aerogenosa when compared to S.aureas and E-coli from all the formulations. Scanning electron microscopy showed the upper surface of plain films were found to be smooth, where as the surface of films containing gatifloxacin was found to be rough due to the drug dispersing in the polymer matrix. The films are found to be stable when stored at refrigerator and even at room temperature after stability studies. Further it was observed that the films stored in aluminium foil shows negligible degradation when compared to films stored in butter paper.

From the above studies it can be concluded that the drug release from all the formulations was controlled. Further, studies are in progress to evaluate the clinical efficacy, patient acceptability and compatibility of the designed polymeric films for the effective treatment of periodontitis.

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Table 1: COMPOSITION OF STRIPS

Ingredients	Composition (percentage)					
	F1	F2	F3	F4	F5	F6
Ethylcellulose	3	3	3	3	3	3
Hydroxy propyl methyl cellulose(HPMC K4M)	-	0.5	-	-	-	-
Poly vinyl pyrrolidone(PVP K-30)	-	-	0.5	-	-	-
Eudragit RL-100	-	-	-	0.5	-	-
Eudragit RS-100	-	-	-	-	0.5	
Hydroxy propyl cellulose(HPC)	-	-	-	-	-	0.5
Dibutyl phthalate(%w/w)	1	1	1	1	1	1
Gatifloxacin	3	3	3	3	3	3

In all the formulations 10 ml of chloroform: dichloromethane (1:1) mixture was used.

Table: 2. Physicochemical Evaluation Datas of Gatifloxacin Films.

Strip Code	Weight (mg)	Thickness (mm)	Percent moisture loss	Tensile strength (gm/sq.mm)
F1	1.42 ± 0.03	0.14 ± 0.04	7.2 ± 2.29	477.9 ± 4.22
F2	1.66 ± 0.01	0.19 ± 0.01	10.8 ± 2.04.	383.45 ± 2.97
F3	1.77 ±0.03	0.14 ± 0.01	8.7 ± 3.87	682.04 ± 4.15
F4	1.48 ±0.04	0.15 ± 0.02	9.5 ± 3.12	394.73 ± 4.16
F5	1.54 ± 0.06	0.16 ± 0.02	9.2 ± 2.74	417.31 ± 3.68
F6	1.57 ± 0.03	0.18 ± 0.03	7.8 ± 3.46	365.07 ± 4.99

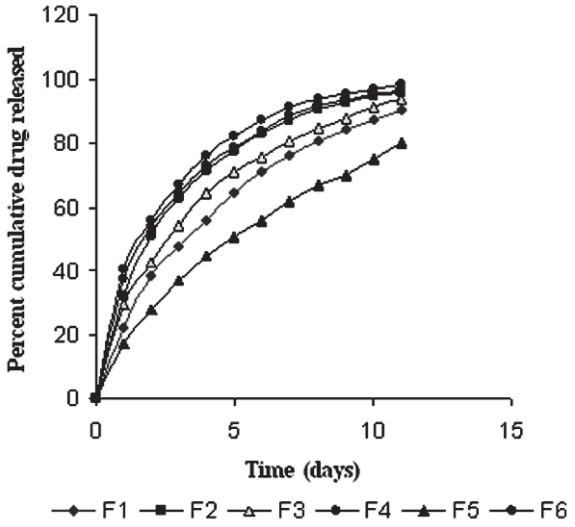
Table 3. Kinetic values obtained from different plots of drug loaded films.

Film type	Zero order plots	Higuchi's Plots	Korsmeyer et al's Plots	
	Regression Coefficient (R^2)	Regression Coefficient (R^2)	Slope (n)	Regression Coefficient (R^2)
F1	0.9241	0.9124	0.289	0.9675
F2	0.9238	0.9049	0.278	0.9568
F3	0.9454	0.9787	0.178	0.9127
F4	0.9568	0.9453	0.256	0.9124
F5	0.9896	0.9016	0.223	0.9238
F6	0.9019	0.9346	0.167	0.9237

Table 4: Stability data for gatifloxacin and drug loaded films

Pure drug Film type	Rate constant values after three months ($k \times 10^{-3}$ days $^{-1}$)		
	At refrigerator (4 - 8^{0}C)	At room temperature (25 ± 2^{0}C)	At oven temperature (45±2^{0}C)
Gatifloxacin	1.126	2.456	4.012
F1	0.674	0.812	0.986
F2	0.567	0.729	0.901
F3	0.457	0.596	0.820
F4	0.564	0.718	0.937
F5	0.458	0.619	0.875
F6	0.493	0.528	0.782

Figure 1. Cumulative percentage of drug release from various films.



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