ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

PREPARATION AND EVALUATION OF PERIODONTAL STRIPS OF GATIFLOXACIN FOR PERIODONTAL DISEASES.

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ABSTRACT

Gatifloxacin is a broad-spectrum antimicrobial agent, which is active against a number of aerobic, anaerobic, gram positive and gram negative periodontal pathogens. In the present investigation, chitosan strips containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. Further strips containing 30% gatifloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extended the release. Macroscopical features revealed that drug was dissolved in the polymer matrix rather than dispersing. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength, hardness and *in-vitro* dissolution. The average weight and thickness of both the cross-linked and uncross-linked strips were uniform. There was a reduction in the tensile strength and increase in hardness when the films were cross-linked. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and extended upto 19 days once the strips were cross-linked. Release kinetics of gatifloxacin from chitosan strips followed the higuchi's diffusional model and also showed zero order release profile.

KEYWORDS

Chitosan, Crosslinking, Gatifloxacin, Local drug delivery, Static dissolution, periodontitis.

INTRODUCTION

Periodontitis is an inflammatory response to the overgrowth of anaerobic organisms in the subgingiva and if unchecked, results in the destruction of the bone and soft tissues supporting the tooth, which results in tooth loss [1, 2].

In conventional mode of drug administration, many drugs do not reach target areas in the body in sufficient concentration because of premature inactivation and excretion. The systemic drug administration has been useful in treating periodontitis but the disadvantage is that, drug is diluted several thousand folds before it reaches the site and exposes the rest of the

body to potential side effects. This problem can be overcome by administering the drug directly to the intended site of action with lesser dose[3].

Sustained drug delivery systems are able to provide very precise control over drug release for a prolonged period of time eliminating the need for frequent dosing and minimizing side effects, there by increasing patient compliance and comfort[4]. A site-specific system aims at delivering the therapeutic agent at sufficient levels inside the pocket and at the same time minimizing the side effects associated with systemic drug administration[2]. 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. The advantage of chitosan polymer is that, it has a wound healing property which supplements anti-bacterial therapy. Since the chitosan strips swell and are biodegradable, can be left in-situ. Hence an attempt was made to develop polymeric strips containing gatifloxacin for local drug delivery for the treatment of periodontitis.

MATERIALS AND METHODS

Gatifloxacin a gift sample from Micro labs Bangalore, Chitosan from Central Institute of Fisheries Technology, Kochin. All other chemicals used in this study are of analytical reagent grade.

Preparation of Drug Loaded Chitosan Strips

Chitosan (2% w/v) was soaked in acetic acid (1% v/v in water) for 24 hours to get a clear solution[5, 6]. This dispersion was filtered through a muslin cloth and the required amount of the drug was added and vortexed for 15 minutes to dissolve in chitosan solution[7]. The viscous dispersion was kept aside for 30 minutes for complete expulsion of air bubbles. The films were casted by pouring the drug-polymer dispersion into the center of leveled glass moulds and allowed to dry at room temperature for 24 hours. After drying, films were cut into strips of required size (7 × 2 mm), wrapped in aluminum foil separately and stored in desiccators until further use [8, 9].

Films containing 0%, 10%, 20% and 30% w/w of the drug to the weight of polymer were prepared. Table 1 shows the composition of different dental strips.

Table 1.
Composition of different dental strips

Uncross-Linked Films	Strip code	% of drug Loaded
	CP	0%
	GC10	10%
	GC20	20%
	GC30	30%
Cross-Linked Films	GC30CL	30%

Preparation of Cross-linked Chitosan Strips

The gatifloxacin loaded film (GC 30%) was crosslinked for the duration of 2 hours and 4 hours by keeping the strips in glass chamber which was previously saturated with 2 % v/v gluteraldehyde vapours for 24 hrs[10]. After drying the films were wrapped in aluminum foil and were placed in decicator for further studies.

Characterization of the Polymer Strips

Compatibility studies were conducted using IR spectroscopy of drug alone, and with polymer. Various physico-chemical properties such as size, thickness, content uniformity, weight variation, folding endurance, hardness, tensile strength and percentage moisture loss was determined on prepared strips. Strips of size 7 × 2 mm were cut out from the films with a sharp surgical blade and were selected for the various studies. Thickness of six polymer strips was determined by using

micrometer screw gauge[11]. Individual weights of 20 strips were determined by using an electronic single pan balance. The percentage moisture loss was determined by keeping the weighed strips in a desiccator containing anhydrous calcium chloride. After 3 days, the strips were taken out and re-weighed; the percentage moisture loss was calculated using formula (initial weight-final weight/ initial weight) x 100 [12].Folding endurance of the strips were determined repeatedly folding a strip at the same place till it broke[12]. Tensile strength and hardness were measured by the instrument designed in our lab as per the literature[10]. Drug content estimation was done by dissolving the strips in 10 ml of acetic acid 1 % v/v and the solution was suitably diluted, then the absorbance was measured at 290 nm and concentrations was estimated.

In-Vitro Release Studies

The *in vitro* release was performed by static dissolution method. Set of six strips of known weight and dimension (7 × 2 mm) were placed separately into small test tubes containing 1 ml phosphate buffer, pH 6.6. The tubes were sealed and kept at 37^{o} C for 24 hours. The buffer was then drained off and replaced with a fresh 1 ml phosphate buffer[9]. The amount of the drug release at different time interval was determined using jasco uv/vis spectrophotometer at 290 nm.

Mass Balance Study

Following the *in-vitro* release studies, the test strips were further analyzed for the drug content left in the strip. Each strip was dissolved in acetic acid 1% v/v and diluted suitably. The absorbance was measured at 290 nm. The amount of drug released into the dissolution medium and the residual content in the films were accounted and compared for the actual drug content[9].

RESULTS AND DISCUSSION

Chitosan 2% w/w was used for the preparation of strips because at this concentration the strips were flexible and easily removable from the mould. An optimum concentration of the drug to be loaded was found to be less than 30% w/w to the polymer. At higher drug concentration the strips were stiff and brittle. The cross-linking of these films where done using 2 % v/v glutaraldehyde. Time duration for cross-linking was selected as 2 hours and 4 hours. Increasing the concentration of glutaraldehyde or increase in duration caused these films to be brittle and hard. The physico- chemical evaluation data presented in table 2 indicates that the thickness of the strips ranges from 0.566 – 1.12 mm. The strips loaded with gatifloxacin (GC 30%) showed higher thickness compared to GC 10% and GC 20%. All the strips exhibited uniform thickness with low standard deviation values, ensuring uniformity of the films prepared by solvent casting method. From the point of insertion into periodontal pockets, the thickness of the strips is satisfactory. The individual weights of each strip are quite uniform and cross-linking did not show any change in weight. The tensile strength of plain chitosan strips was much higher than the drug loaded strips, the presence of drug in strips had decreased the tensile strength of chitosan strips. The cross-linking of strips had reduced the tensile strength significantly. Incorporation of drug had substantially increased the hardness of the strips. Gatifloxacin loaded strips (GC 30%) exhibited higher hardness than GC 20% and GC 10% strips. Cross-linked strips showed higher hardness then uncross-linked strips.

Table 2.
Physical characteristics of strips with and without cross-linking*.

Strip Code	Tensile strength (gm/sq.mm) Before C.L		Hardness (gm) Before C.L		Weight variation (mg) Before C.L		Thickness (mm) Before C.L	
CP	61.52 ± 3.09		245 ± 6.65		1.20 ± 0.03		0.57 ± 0.052	
GC 10	31.09 ± 4.15		340 ± 5.30		1.25 ± 0.02		0.87 ± 0.051	
GC 20	23.79 ± 4.59		441 ± 2.29		1.30 ± 0.02		0.92 ± 0.092	
GC 30	21.49 ± 5.15		540 ± 5.49		1.40 ± 0.03		1.05 ± 0.175	
GC 30	**After C.L**		**After C.L**		**After C.L**		**After C.L**	
	2 hr	**4 hr**	**2 hr**	**4 hr**	**2 hr**	**4 hr**	**2 hr**	**4 hr**
	9.05 ±1.91	9.73 ± 0.78	668.33 ±6.67	800.00 ± 6.29	1.42 ± 0.03	1.43 ± 0.02	1.00 ±0.13	1.12 ± 0.18

C.L –Crosslinking, **C.P**-Chitosan Plain Strips, **G.C**-Gatifloxacin and Chitosan
*Each value is a mean and standard deviation of six determinations.

The correlation studies between tensile strength and hardness showed that as the tensile strength increased the hardness of the film decreased this might be due to the incorporation of drug decreased the tensile strength of chitosan strips and linear structural feature of the polymer chains, figure-3.

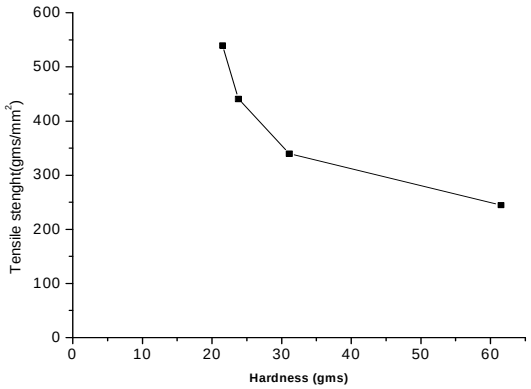

Figure 3.
Correlation study of tensile strength and hardness.

The drug loading was found to be 94.28, 163.34 and 209.15 µg for GC 10, GC 20 and GC 30 respectively. The percent drug loading varies from 86 – 74%. The films showed decrease drug content, the data were shown in table 3.

Table 3.
Drug content studies of strips containing gatifloxacin.

Strip Code	Theoretical Drug Loading (µg)			Drug Content* (µg)			% Drug Loading		
	Before C.L	After C.L		Before C.L	After C.L		Before C.L	After C.L	
		2 hr	4 hr		2 hr	4 hr		2 hr	4 hr
CP	-	-	-	-	-	-	-	-	-
GC10	108.0	-	-	94.28	-	-	87.10	-	-
GC20	189.0	-	-	163.34	-	-	86.42	-	-
GC30	283.5	283.5	283.5	209.15	197.28	167.33	73.77	69.59	59.03

*Each value is a mean and standard deviation of six determinations

The percentage moisture loss varied in the range 4 to 9.5%. The strips GC10 showed maximum amount of moisture loss, whereas GC30 showed minimum amount of moisture loss. All chitosan strips exhibited more than 95 folding endurance. The data is given in table-4.

Table 4.
Moisture loss and folding endurance studies.

Formulation	Percent moisture loss ± SD*	Folding endurance
CP	7.23 ± 1.341	108±8.33
GC10	6.889 ± 1.141	97 ±9.147
GC20	6.721 ± 0.945	69 ±7.457
GC30	5.218 ± 1.451	57 ±8.871

**Each value is a mean and standard deviation of six determinations*

The release of drug is prominent diffusion controlled mechanisms across the membrane or the matrix, the pH of the gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid for the dissolution studies. Since the film remains immobile in the periodontal pocket, a static dissolution model was adopted in this work. The release study were conducted for nine days since the strips were not cross-linked, whereas for uncrossed linked the release studies were conducted for 19 days. There was a rapid initial release of gatifloxacin on day one (GC 10 = 63.22 %, GC 20= 61.48 % and for GC 30= 42.30 %) and day two the cumulative amount of drug released for GC 10, GC 20 and GC 30 was 72.60 %, 71.60 % and 66.91 % respectively. From day three onwards, the release of gatifloxacin was more uniform and constant respectively per day. After nine days the strips lost integrity and hence were not fit for the release study. The release profile of gatifloxacin chitosan strips was given in Figure 1.

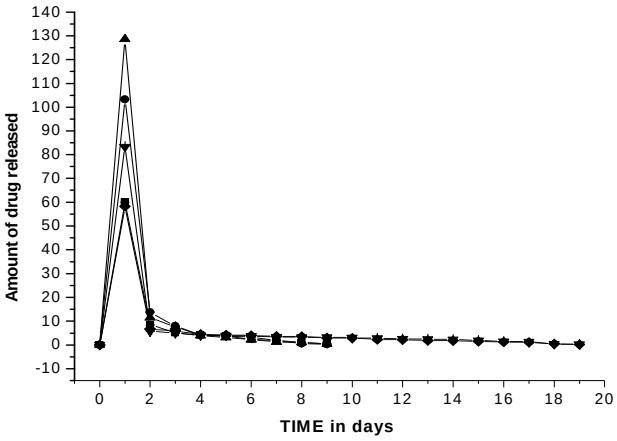

Figure 2.
Release profile of chitosan strips containing different concentration of gatifloxacin.

A cumulative percent drug release profile shown in Figure 2 indicates an initial rapid release due to burst effect. Once the burst effect was completed, the release of drug was more uniform and extended upto 9 days.

After cross-linking the burst effect was reduced by more than 40% on day one (GC 30 % 2 hours 42.30 % and GC 30 % 4 hours 34.83 %). From day three onwards, the release of gatifloxacin was more uniform and constant (about GC 30 % 2 hours 2.622 % and GC 30 % 4 hours 2.580 % respectively per day). After 19 days the strips lost integrity and hence not fit for the release study.

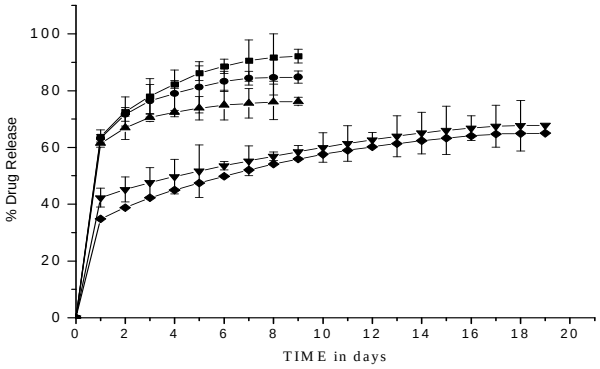

Figure1.
Comparative percentage release profile of various chitosan strips containing gatifloxacin

The release kinetics of gatifloxacin from chitosan strips followed zero order (R^2 = 0.8956, 0.8869 and 0.8843 for GC 10, GC 20 and GC 30 respectively). Similarly the regression values for crosslinked films showed, R^2 = 0.9600 and 0.9294 for G30 CL_2 and G30 CL_4 respectively. The data was given Table 5.

Table 5.
Comparison of orders of in-vitro release of gatifloxacin loaded strips.

Formulation	Regression equations	
	Zero order	First order
G10	y = 3.3774t + 65.9723 R^2 = 0.8956	Log y = 0.01847t + 1.8231 R^2 = 0.8649
G20	y = 2.425t + 66.6139 R^2 = 0.8269	Log y = 0.0139t + 1.8247 R^2 = 0.7978
G30	y = 1.6448t + 63.7558 R^2 = 0.8243	Log y = 0.0102t + 1.8051 R^2 = 0.8034
$G30CL_2$	y = 1.4184t + 44.2212 R^2 = 0.9600	Log y = 0.0109t + 1.6528 R^2 = 0.9344
$G30CL_4$	y = 1.6221t + 38.6651 R^2 = 0.9294	Log y = 0.0136t + 1.5960 R^2 = 0.8867

$G30CL_2$ – Gatifloxacin 30% crossed linked for 2 hrs.
$G30CL_4$ – Gatifloxacin 30% crossed linked for 4 hrs

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The residual drug content in the strip after 19 days was determined and it did not differ from the experimental drug content by more than 3 %. The stability studies of drug loaded chitosan strips were done at 3 different temperatures and relative humidity and the samples kept for stability studies did not show any signs of degradation.

CONCLUSIONS

Gatifloxacin was incorporated in chitosan polymer strips in three different concentrations (10, 20 and 30 % w/w) with respect to the weight of the polymer. The strips containing highest drug content (30% w/w) were further cross-linked with gluteraldehyde 2 % which was aimed to extend the drug release. The drug loaded chitosan strips were flexible and possess good tensile strength and hardness. As the chitosan strips were cross-linked with gluteraldehyde, the tensile strength of the drug loaded polymer strips was reduced but hardness was increased. It is observed that cross-linking has a definite influence on the release rate of drug. *In-vitro* dissolution rate studies of gatifloxacin loaded uncross-linked strips showed release of the drug for nine days and whereas in cross-linked strips the release was extended up to 19 days with more uniformity of drug release per day. The release kinetics of drug was found to be zero order. Highuchi's diffusion model gave a better fit of release data indicating diffusion dominating. Throughout the release study, the strips remain intact without any disintegration, the average release (from day 3 onwards) is 3 µg per day for both cross-linked and uncross-linked strips, which was above the minimum inhibitory concentration of gatifloxacin. Based on the *in-vitro* release studies, it can be concluded the cross linking of these polymer is essential for the management of adult periodontitis.

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