

LOCAL DRUG DELIVERY SYSTEM OF CHITOSAN STRIPS CONTAINING SPARFLOXACIN FOR PERIODONTAL DISEASE

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Summary

An objective of periodontal treatment is to suppress or eliminate sub gingival periodontal pathogens. This is generally achieved through sub gingival debridement, resulting in the reduction of the total bacterial load. However, some patients may experience continued periodontal attachment loss, and this may be due to some periodontal pathogens that are inaccessible during mechanical periodontal therapy. Therefore there is a need of a safe and effective low dose treatment, local drug delivery device is highly desirable. Sparfloxacin is a newer antibiotic, shown wide spectrum antibacterial activity against a number of periodontal pathogens. Hence sparfloxacin is selected for site specific delivery ie., into periodontal pocket for the treatment of periodontitis. In the present investigation, chitosan strips containing sparfloxacin (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% sparfloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extended the release. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength and *in-vitro* dissolution. The average weight and thickness of both the crosslinked and uncross-linked strips were uniform. There was a reduction in the tensile strength 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. *In vitro* antibacterial activity was carried out on *Streptococcus mutans*.

Keywords: Periodontitis, Chitosan, sparfloxacin, strips.

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Introduction

Periodontal diseases are a group of inflammatory and localized microbial-induced infections involving the supporting tissues of the teeth, the gingiva, periodontal ligament, and alveolar bone. Inflammation of the gingiva is referred to as gingivitis, whereas extension of inflammation into deeper tissues accompanied with bone loss is termed periodontitis¹. Recently, it has been estimated that 36.8% (43 million) of adult Americans have periodontal disease, making it one of the world's most prevalent chronic diseases².

The presence of periodontal pathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* are responsible for periodontal destruction. Therefore, an objective of periodontal treatment is to suppress or eliminate subgingival periodontal pathogens³.

Antibacterial agents have been used effectively in the management of periodontal infection. The effectiveness of mechanical debridement of plaque and repeated topical and systemic administration of antibacterial agents are limited due to the lack of accessibility to periodontopathic organisms in the periodontal pocket. Systemic administration of drugs leads to therapeutic concentrations at the site of infection, but for short periods of time, required repeated dosing for longer periods. Local delivery of antimicrobials has been investigated to overcome the limitations of conventional therapy. The use of sustained release formulations to deliver antibacterial to the site of infection (periodontal pocket) has gained interest recently. These products provide a long-term, effective treatment at the site of infection at much smaller doses thereby reduces the side effects and frequent dosing.

Several polymers have been adopted in the formulation of such sustained local delivery systems. Those polymers are classified as biodegradable and non-biodegradable polymers. But, biodegradable polymers are extensively employed in periodontal drug delivery devices because of their abundant source, lack of toxicity, and high tissue compatibility. A major advantage of natural polymers is that they do not affect periodontal tissue regeneration⁴.

Amongst various natural polymers, Chitosan, a natural polysaccharide, is being widely used as a pharmaceutical excipient. It is obtained by the partial deacetylation of chitin. It is obtained from the alkaline deacetylation of chitin which is a glucose-based unbranched polysaccharide widely distributed in nature as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi. Chitosan exhibits a variety of physicochemical and biological properties resulting in numerous applications. The absence of toxicity, allergenicity, biocompatibility, biodegradability and antibacterial property, make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields⁵.

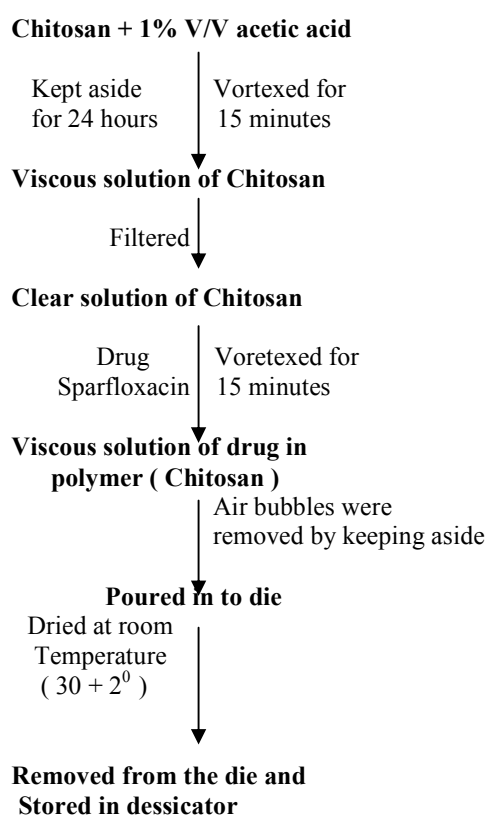
Several classes of antibacterial agents have been adopted in the treatment of Periodontitis⁶. However, fluoroquinolones are the most popularly adopted antibacterials for the purpose. Sparfloxacin is a potent, long-acting, third generation, bactericidal fluoroquinolone derivative. The drug has shown potent antimicrobial activity against a wide range of Gram positive and Gram negative bacteria, including glucose

nonfermentors and anaerobes, which commonly cause periodontitis⁷. Hence in the present study an attempt was made to formulate and evaluate sustained release periodontal strips containing sparfloxacin using chitosan as polymer.

Materials and Methods

A gift sample of Sparfloxacin was obtained from Zydus cadila Mumbai. Chitosan (85% deacetylated with viscosity of 8000-11000 cps) from Central Institute of Fisheries Technology, Kochi and all other chemicals used were of analytical grade.

Preparation of Drug Loaded Chitosan Strips⁸



Preparation of Cross-Linked Chitosan strips: The general procedure was used with little modification⁸. The strips (S-30%) were prepared as described were cross-linked by placing in a chromatographic chamber, which was previously saturated with vapors of 2%v/v glutaraldehyde solution. The strips were exposed to vapors in the chromatographic chamber for 2 hours and 4 hours and then dried. After drying the strips were wrapped in aluminum foil and were placed in desiccators for further study⁹.

Table 1: Composition of different periodontal strips

Uncross-Linked Films	Strip code	% of drug Loaded
	CP	0
	S-10	10
	S-20	20
	S-30	30
Cross-Linked Films (2hr and 4 hr)	S-30	30

CP-Chitosan Plain Strips, S-Sparfloxacin

Evaluation of Chitosan strips:

Characterization of the polymeric strips: Compatibility studies were conducted using Fourier transform infrared (FTIR) spectroscopy of the drug alone, polymer alone and prepared strips. Physicochemical properties such as thickness, content uniformity, weight variation, folding endurance and tensile strength of prepared strips were determined.

- Thickness:** The thickness of polymer strips (4×4cm) was determined by using digital screw gauge (Mitutoyo).⁸
- Weight variation:** Twenty strips of same size (7×2mm) were weighed on electronic balance and average weight was calculated.¹⁰
- Tensile strength:** Tensile strength of the films was determined by Universal strength testing machine. It consists of two load cell grips, the lower one is fixed and upper one is movable. The test film of specific size (4 × 1 cm²) was fixed between these cell grips and force was gradually applied till the film breaks. The tensile strength of the film was taken directly from the dial reading in kilograms.¹¹
- Folding endurance Studies.** This study was determined by repeatedly folding a 2 X 2 cm size strips, at the same place, till it broke¹¹
- Drug content and content uniformity:** The drug loaded strips of known weight of 2X7 mm dimension were dissolved in 10 ml of 1% acetic acid and shaken until it dissolved. The drug solution was suitably diluted with 1% acetic acid and absorbance was measured at 298nm.
- In-vitro drug release pattern by using static dissolution test apparatus:** A static dissolution method reported in the literature was adopted in this method. Sets of six strips of known weight and dimension (7×2 mm) were placed in a small test tube containing 1ml of phosphate buffer, pH.6.6. The tubes were sealed and kept at 37 ° C for 24 hours. The buffer medium was collected and replaced with a fresh 1ml phosphate buffer pH6.6. The concentration of drug in the buffer was measured at 298nm. The same procedure was continued until no more drug release takes place or the film completely disintegrated.
- Stability studies:** The stability of drug loaded strips were studied at different temperature and at ambient humid condition, at room temperature (27±2°c), oven temperature (40±2°c) and in refrigerator (5-8°c) for a period of 10 weeks. The samples were analyzed for physical changes and drug content.

- h) **Mass Balance Study:** Following the *in-vitro* release studies (static), the test strips were further analyzed for the drug content left in the strips. Each strip was dissolved in acetic acid 1% (v/v) and diluted suitably. The absorbance was measured at 298nm. The amount of drug released into the dissolution medium and residual drug content in the strips were accounted and compared for the actual drug content.
- i) **In-vitro antibacterial activity:** In-vitro antibacterial activity was performed on all formulations by placing the strips, cut into 0.5 X 0.5 sq cm, on agar plates seeded with oral bacteria, *streptococcus mutans*. After 48 hours of incubation at 37° C, the strips 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 growth inhibition area on the agar plate was measured¹².

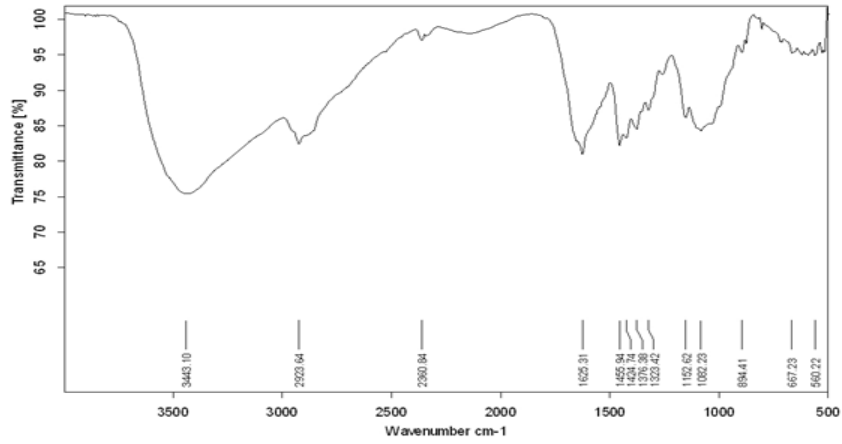
Results

The amount of drug added to the polymer solution altered the film characteristics. The optimum drug loading was less than 30% w/w of the polymer. At higher drug loadings (i.e., > 30%), the films were stiff and brittle. The FTIR studies from the spectra Fig 1, 2 and 3) confirmed the absence of any chemical incompatibility between the drug and the polymer. The results of the physicochemical evaluations are presented table I. The thickness of the strips ranges from 0.088 ± 0.04 to 0.145 ± 0.05 mm. The strips loaded with sparfloxacin (S-30%) crosslinked for 2 hours and 4 hours showed higher thickness compared to S-10%, S-20% and S-30% uncross linked. The films of all the batches were found to be of uniform weight, ranging from 1.01 ± 0.02 mg to 1.46 ± 0.05 mg. ($n = 5$). Folding endurance of the films was > 100 times indicated that the formulations have good film properties.

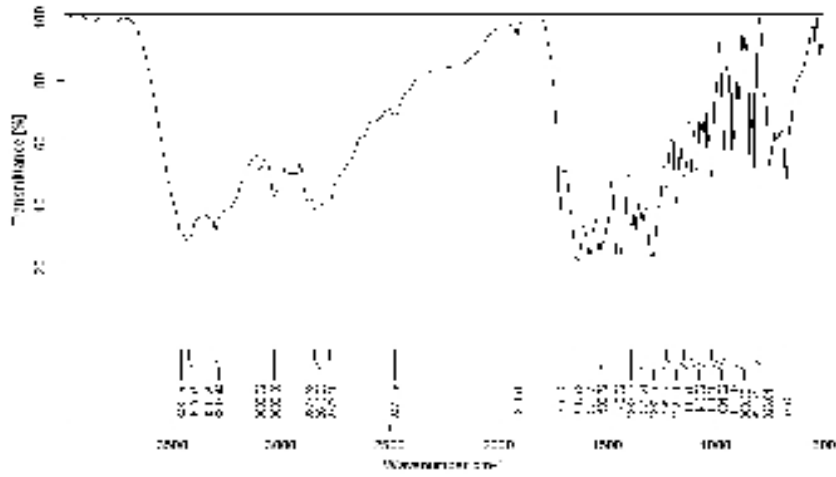
Tensile strength was lowest for uncross linked films and highest for crosslinked films and was in the range, 1.45 ± 0.054 kg to 2.86 ± 0.068 kg. The drug loading was found to be 121.77 ± 1.21 , 205.34 ± 7.05 and 261.73 ± 7.01 µg for uncross linked S-10%, S-20% and S-30% and (S-30%) crosslinked for 2 hours and 4 hours were 250.86 ± 12.11 and 242.93 ± 11.12 respectively. The percent drug loading varies from 56 – 85%.

Drug release data for both uncross linked and crosslinked films are illustrated in Fig 2. The results showed an initial burst release followed by controlled drug release for up to 9 days for uncross linked films (93-95%) and 19 days for crosslinked films (73-86 %).

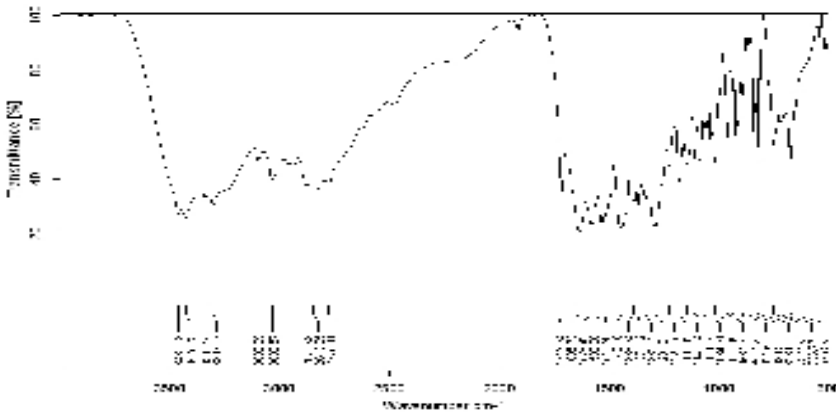
The *in vitro* antibacterial activity demonstrated a significant antibacterial profile of all the films, as shown in Fig 2. Films without the drug were also tested and it was found that the films were not effective against the microorganisms. The stability studies carried out for a period of 10 weeks showed that there were no significant physical changes and the drug content did not deviate by more than 5% from the initial drug content.



Chitosan (a)



Sparfloxacin (b)



Chitosan sparfloxacin film (c)

Fig.1 : (a) FT-IR of plain chitosan film (b) FT-IR of pure sample of sparfloxacin (c) FT-IR of chitosan film containing sparfloxacin

Table 2: Physical characteristics of sparfloxacin loaded chitosan strips with and without corsslinking.

Strip code	Tensile strength (kg) Before C.L		Folding endurance Before C.L		Weight (mg) Before C.L		Thickness(mm) Before C.L		Drug content (µg) Before C.L	
	2 hr	2 hr	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr	2 hr	4 hr
CP	1.45± 0.054		150± 2.85		1.01± 0.02		0.088± 0.04		-	
S-10	1.60± 0.051		141± 3.21		1.11± 0.07		0.098± 0.04		121.77± 1.21	
S-20	1.88± 0.040		128± 6.02		1.21± 0.04		0.106± 0.08		205.34± 7.05	
S-30	2.06± 0.051		115± 3.00		1.35± 0.05		0.116± 0.08		261.73± 7.01	
S-30	After C.L		After C.L		After C.L		After C.L		After C.L	
	2.41± 0.068	2.86± 0.068	107± 6.02	102± 4.50	1.43± 0.05	1.46± 0.05	0.141± 0.04	0.145± 0.05	250.86± 12.11	242.93± 11.12

C.L –Cross linking, CP-Chitosan Plain Strips, S-Sparfloxacin and Chitosan

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

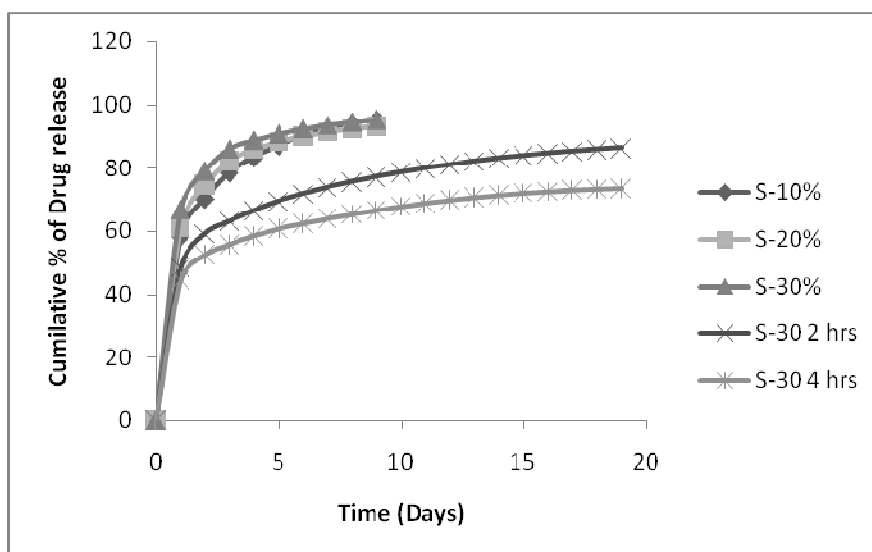


Fig 2: Drug release profile of chitosan films containing sparfloxacin (uncross linked and crosslinked).

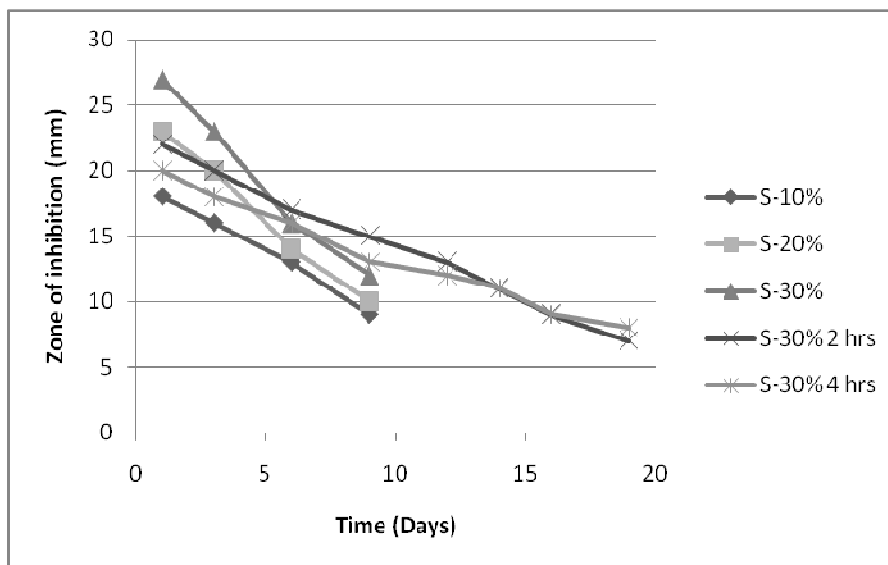


Fig 3: *In-vitro* antimicrobial activity of chitosan films containing sparfloxacin (uncross linked and crosslinked).

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

Film type	First order plots	Higuchi's Plots	Korsmeyer et al's Plots	
	Regression Coefficient (R^2)	Regression Coefficient (R^2)	Slope (n)	Regression Coefficient (R^2)
S – I	0.962	0.966	0.224	0.986
S – II	0.876	0.906	0.184	0.947
S – III	0.874	0.897	0.155	0.946
S – III2 CL	0.866	0.953	0.186	0.989
S – III4 CL	0.753	0.953	0.165	0.991

S-I: Sparfloxacin 10%; S-II: Sparfloxacin 20%; S-III: Sparfloxacin 30%; 2CL: 2 hour crosslinking; 4CL: 4 hour crosslinking.

Discussion

As per the basic requirement of easy to insert and Flexible films were prepared for periodontitis treatment easy for insertion into periodontal pockets. The optimum concentration of chitosan used for the preparation of strips was found to be 2% w/w, because at this concentration the strips were flexible and easily removable from the die. An optimum concentration of drug to be loaded was found to be 30% w/w to the polymer or less than that. For the present investigation, chitosan strips containing sparfloxacin with three different concentrations, i.e. 10, 20, and 30% to the weight of the polymer, were prepared using the solvent casting method. The prepared strips containing sparfloxacin 30% were cross linked with 2% gluteraldehyde for two-hour and four-hour duration, in order to extend the drug release. As the concentration of gluteraldehyde or time of cross-linking was increased, changes in the basic properties such as brittleness, tensile strength of the film were altered.

It was confirmed from the FTIR studies that the chemical interaction between the drug and the polymer did not occurred.

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, indicating that the films had become more brittle after loading drug. Thus the drugs might have disrupted the linear structures of the polymer chains. Tensile strength was lower for uncross linked films than for crosslinked films, probably due to the increased toughness and rigidity of the polymeric film following crosslinking. All the formulations were found to contain almost uniform quantity of drug as per content uniformity studies, indicating reproducibility of the technique.

Drug release time profile of sparfloxacin from different concentration chitosan films are shown in Fig 3. The release profile exhibited rapid initial release of the drug on day one, due to initial burst effect, because of elution of the drugs from the outer surface and cut edges of the matrix. Once the burst effect was completed, slow and sustained release was seen up to nine days for 10, 20, and 30% of the drug-loaded films, respectively. Similarly, the cross-linked films also showed a burst effect initially followed by sustained release of the drug up to 19 days, with more uniformity of drug release per day.

In vitro drug release kinetic analysis showed that release mechanism for all the films fitted best to the Higuchi model, as the plots showed high linearity ($R^2 = 0.897$ to 0.966). The plot of cumulative drug release per unit area versus square root of time in days showed a near linear relationship from 3rd to 9th day and 3rd to 18th day for uncrosslinked and crosslinked films, respectively. This was confirmed by data based on Korsmeyer *et al.*'s equation, which showed good linearity ($R^2 = 0.946$ to 0.991) and with slope (n) values ranging from 0.155 to 0.224, indicating that zero order diffusion is the prime mechanism of drug release. Mass balance studies indicated that, the drug content did not differ from the experimental drug content by more than 3%. Findings in respect of *in vitro* antibacterial activity showed that crosslinked films exhibited antibacterial activity for a longer period (20 days) than uncross linked films (9 days).

The stability results indicated that the films were relatively stable when stored in a refrigerator and at room temperature, compared to those stored in oven temperature conditions.

Conclusion

The advantages of intra-pocket delivery over systemic delivery in periodontitis are that administration is less time-consuming than mechanical debridement and a lower dose of drug would be required to achieve effective therapeutic concentration at the site of action. Thus chitosan films loaded with sparfloxacin, particularly those crosslinked with gluteraldehyde, may be useful in the treatment of Periodontitis. Further studies are on to evaluate the efficacy of periodontal strips in clinical conditions.

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