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Satranidazole Biodegradable Inserts For Local Long Term Treatment of Periodontitis

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ABSTRACT

An attempt has been made in the present research to formulate periodontal inserts of Satranidazole (STZ) to increase residence time and prolong drug release. The periodontal inserts were prepared using chitosan, a natural biodegradable polymer. Chitosan inserts containing Satranidazole (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. Further inserts containing 30% Satranidazole were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water for two different time period of 2 and 4 hours to retard the release of drug. FTIR and DSC studies revealed that there was no interaction between drug and polymers. The inserts were then evaluated for their physicochemical parameters like uniformity of thickness, weight, folding endurance, % moisture loss, tensile strength; drug content and *in vitro* drug release studies. *In vitro* drug release data indicated that the films showed an initial burst release followed by sustained release of the drug. The drug-loaded films that were not cross linked had released the drug up to 10 days and the films which were cross linked for different duration showed a progressive fall in the release of the drug and extended up to 18 days.

Keywords: Satranidazole, cross-linking, chitosan, periodontal insert.

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INTRODUCTION

According to the World Oral Health Report (World Health Organization, 2004), most children show signs of gingivitis (bleeding gums), and among adults, the initial stages of periodontal disease are widespread. Severe periodontitis, which may result in tooth loss, is found in 5–15% of most populations.[1]

The most common diseases of tooth-supporting structures are plaque-induced inflammatory alterations in the gingiva and the periodontum. It has been established that periodontal diseases are inflammatory conditions, affecting the structural organs supporting the teeth. The gingiva becomes detached from the tooth to form periodontal pockets, providing an ideal ecological niche for the proliferation of anaerobic bacteria.[2]

Current periodontal therapy focuses on the removal of the bacterial plaque by mechanical scaling and root planning but it is time consuming, not convenient for patients and technically difficult to perform. As an adjunctive approach, systemic or local administration of antibiotics is used because of the microbial etiology of periodontitis. Antibiotics also aid in pocket elimination with nonsurgical periodontal therapy, where surgery is contraindicated.

However, to obtain an effective concentration of the antimicrobial drug in the periodontal pocket after systemic administration, repeated intakes over a prolonged period of time are required. Furthermore, when broad spectrum antibiotics are used, there is always a risk of inducing bacterial resistance and distortion of commensal flora. Therefore, a more satisfactory approach to administering antimicrobial drugs directly into the pocket involves use of a controlled release device. Using such a device not only sustains an effective dose for the required length of time but also bypass systemic complications and targets localized areas of periodontal destruction.[3]

The use of local delivery of antibacterial agent to the site of infection (periodontal pocket) is becoming more prevalent since, it leads to higher concentration of drug at the site of action using lower dose with an associated reduction in side effects relative to systemic administration.[4]

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 regeneration. Amongst various natural polymers, chitosan, a deacetylated product of chitin is widely used in drug delivery devices. Since it exhibits favourable biological properties such as non-toxicity, biocompatibility, biodegradability and wound healing activity, makes it a suitable film former in the pharmaceutical and biomedical fields.[5]

Satranidazole (STZ) is a 5-nitroimidazole substituted at the 2-position and has been found to be more active against aerobic, microaerophilic, and anaerobic bacteria than Metronidazole (MZ). The MIC90 of Satranidazole (STZ) was found to be fourfold lower than MZ against 50 clinical isolates of anaerobes. The literature review indicates that Satranidazole (STZ), though more effective than Metronidazole (MZ), has not been focused on for the treatment of periodontal disease yet as a inserts.[6] In view of all above reasons, an attempt has been made to formulate and evaluate satranidazole biodegradable inserts for local long term treatment of periodontitis.

MATERIALS AND METHODS:

A gift sample of Satranidazole was obtained from Alkem Laboratories. 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 inserts:

Chitosan (2% w/v) was soaked in acetic acid (1% v/v in water) for 24 hours to get a clear solution. This dispersion was filtered through a muslin cloth to remove undissolved portion of the polymer (chitin). Required amount of the drug was added (0%, 10%, 20% and 30% w/w of the drugs) to the weight of polymer and vortexed to dissolve the drug in chitosan solution. The viscous dispersion was kept aside for 30 minutes for complete removal of air bubbles. The inserts were casted by pouring the dispersion into the center of leveled glass moulds and allowed to dry at room temperature for 24 hours. After drying, films were cut into inserts of required size (7 x 2 mm), wrapped in aluminium foil separately and stored in desiccators for further use.[7]

Preparation of Cross-Linked Chitosan inserts:

The general procedure was used with little modification. The inserts (STZ 30%) were subjected to cross-linking by keeping in a chromatographic chamber which was previously saturated with vapors of 2%v/v gluteraldehyde solution, and then inserts (STZ 30%) were exposed for 2 and 4 hours, then dried. After drying the inserts were wrapped in aluminum foil and were placed in desiccators for further study.[7]

Table 1: Composition of different periodontal inserts

Uncross-linked inserts	Inserts code	% of drug loaded
	CP	0
	STZ-10	10
	STZ-20	20
	STZ-30	30
Cross-linked inserts (2 hrs and 4 hrs)	STZ-30	30

Compatibility studies:

Compatibility studies were conducted using FTIR and DSC[8].

Fourier Transform Infra-red Spectroscopy (FTIR):

The FTIR absorption spectra of the pure drug, pure chitosan and physical mixture of drug and polymer were recorded in the range of 400 - 4000 cm^{-1} by KBr disc method (2 mg sample in 200 mg KBr) using FTIR spectrophotometer (Shimadzu FT-IR spectrophotometer)

Differential Scanning Calorimeter (DSC):

Differential scanning calorimetry, (DSC) (Perkin-Elmer, USA). Scans of pure drug, pure chitosan and physical mixture of drug and polymer were performed. The analysis was performed with a heating range of 50-480°C and a rate of 10° C min^{-1}.

PHYSICO-CHEMICAL CHARACTERIZATION:

The prepared inserts were subjected to various physicochemical properties such as thickness, weight variation, folding endurance, tensile strength, moisture loss, content uniformity and *in vitro* release study.

Thickness: The thickness of polymer films (4×4 cm) was determined by using digital screw gauge (Mitutoyo).[9]

Weight variation: Twenty films of same size (7×2 mm) were weighed on electronic balance and average weight was calculated.[9]

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 cm^2) 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.[10]

Folding endurance Studies. This study was determined by repeatedly folding the (2 x 2 cm size) films, at same place, till it broke.[10]

Percentage moisture loss: The percentage moisture loss was carried out to check integrity of the film at dry conditions. Implants were weighed and kept in a desiccators containing anhydrous calcium chloride. After three days, the implants were taken out and reweighed; the percentage moisture loss was calculated using the formula.[11]

Formula:- $$\% \text{ moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Drug content:

The drug loaded inserts of known weight (7 x 2 mm) were taken in 10 ml of phosphate buffer

(pH 6.6) and crushed until dissolved. The drug solution was suitably diluted and the amount of drug present was estimated using UV spectrophotometer at 319nm.[9]

***In-vitro* drug release studies:** To simulate the actual physiological conditions prevailing in the buccal cavity phosphate buffer pH.6.6 was used. Sets of six inserts of know weight and dimension (7×2 mm) were placed in a small test tube containing 1ml phosphate buffer (pH.6.6). The tubes were sealed and kept at 37 ^{o} C for 24 hours. The buffer was collected and replaced with a fresh 1ml phosphate buffer (pH.6.6). The concentration of drug in the buffer was measured at 319nm. The procedure was continued until no release of drug takes place or the film completely disintegrated.[9]

Stability studies: The stability of drug loaded films were studied at different temperature and at ambient humid condition, at room temperature (27±2^{o}C), oven temperature (40±2^{o}C) and in refrigerator (5-8^{o}C) for a period of 3 months. The samples were analyzed for physical changes and drug content.[9]

RESULT AND DISCUSSION:

The amount of chitosan was selected on the basis of optimum quantity required for insert preparation, which has been reported in various literatures [7, 9] because at this concentration the inserts were flexible and easily removable from the die. Phosphate buffer pH6.6 was composed of potassium dihydrogen orthophosphate and sodium hydroxide in water and was used to simulate the gingival fluid environment.

In the present investigation, chitosan inserts containing satranidazole with three different concentrations, 10, 20, and 30% to the weight of the polymer, were prepared using the solvent casting method. The prepared inserts containing satranidazole 30% were cross linked with 2% gluteraldehyde for two 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 of the film were observed.

Drug-Excipient compatibility study:

Fourier transport infrared spectroscopy (FTIR):

Interaction studies were carried out to ascertain any kind of interaction of the drug with the excipients used in the formulations of periodontal inserts. The FT-IR and DSC spectra of the formulations exhibited absorption peaks similar to those of the pure drug sample. The IR spectra of the pure drug showed characteristic peaks at wave number at 3450, 2999.73, 2150, 1521.56, 1301.72 and 1150 cm^{-1} which were similar to those of the physical mixture as shown in figure 1,

2, 3 and 4.

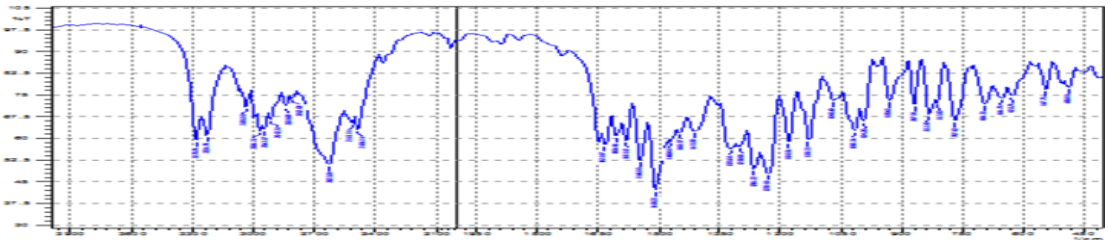

Figure.1: Fourier Transform Infrared Spectroscopy (FT-IR) of satranidazole drug

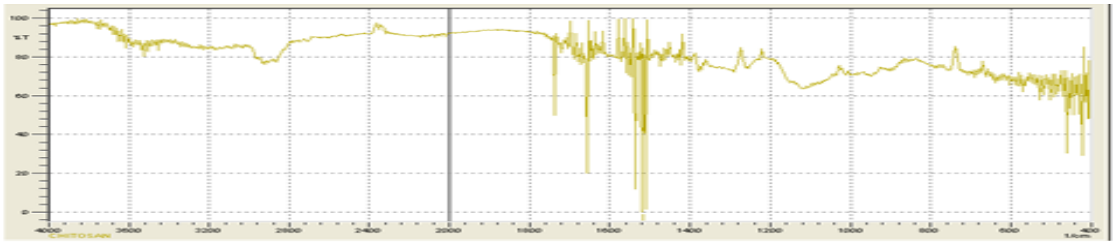

Figure 2: Fourier Transform Infrared Spectroscopy (FT-IR) of chitosan polymer

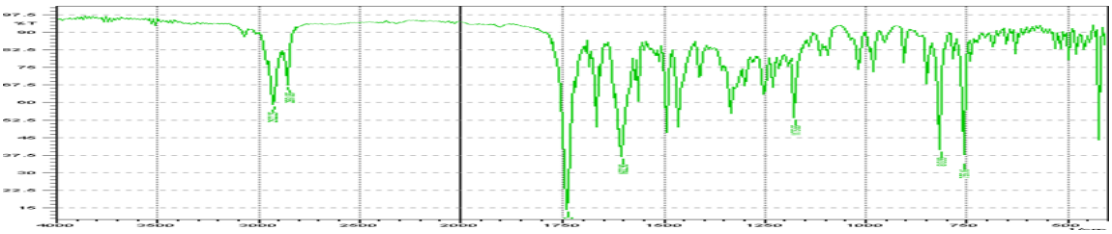

Figure 3: Fourier Transform Infrared Spectroscopy (FT-IR) of chitosan uncross linked inserts

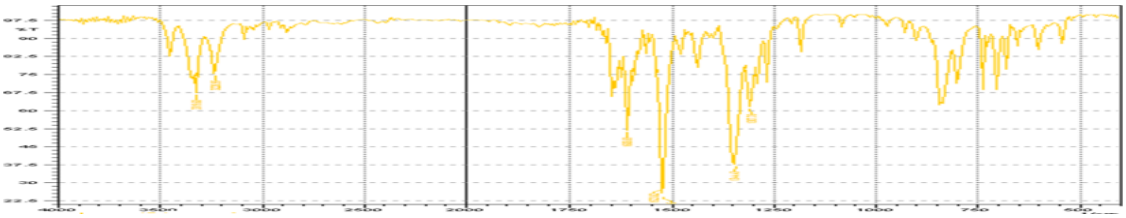

Figure 4: Fourier Transform Infrared Spectroscopy (FT-IR) of chitosan cross linked inserts

Differential scanning calorimeter (DSC):

The DSC curve display in figure no. 5, 6 and 7. The DSC thermogram of plain satranidazole showed a single endothermic peak at 185.66 ^{o}C which describe the drug melting. Whereas the endothermic peak of chitosan at 95-105 ^{o}C. The drug peak was shortened in case of formulation but no characteristics peaks were observed around the melting point of drug in case of formulation spectra, this may be due drug incorporated in polymer matrix.It indicates the absence of any interaction. This observation further supports the IR spectroscopy results, which indicated the absence of any interaction between drug and excipient used in the formulation.

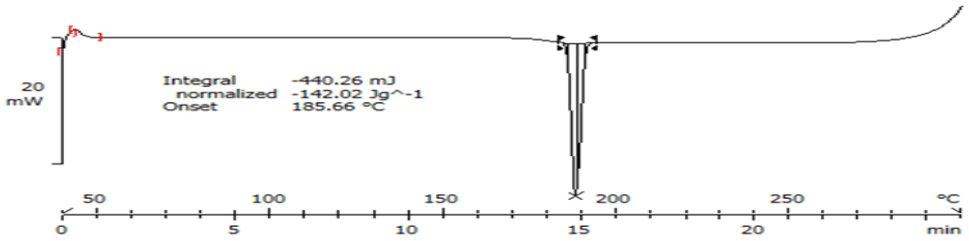

Figure .5: Differential Scanning Calorimetric (DSC) thermogram of satranidazole

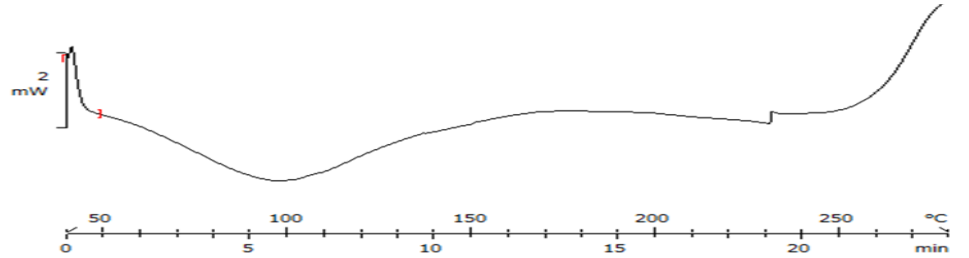

Figure 6: Differential Scanning Calorimetric (DSC) thermogram of chitosan polymer

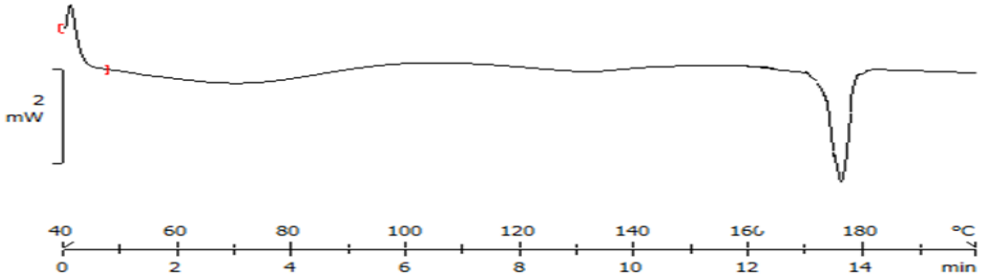

Figure 7: Differential Scanning Calorimetric (DSC) thermogram of inserts

Physico-chemical characterization:

Uniformity of thickness:

The thickness of the prepared inserts was in the range of 0.223 ± 0.052 to 0.315 ± 0.072 mm for uncross linked inserts and 0.329±0.013 to 0.331 ± 0.033 mm for crossed linked inserts respectively (Table 2). Thickness of inserts slightly increased as the concentration of drug increased.

Uniformity of weight:

The average weight of the inserts ranged from 1.09 ±0.073 mg to 1.31±0.003 mg. The maximum weight was observed with 30% drug-loaded insert, where as the average weight of the cross linked films ranged from 1.22 ±0.003 mg to 1.22 ±0.007 mg for two hours and four hours cross-linking, respectively (Table 2). The uniformity of the weights of the inserts indicates good distribution of the drug and polymer.

Folding endurance:

Folding endurance of the inserts were more than 150 times indicate that the inserts have

good film properties. It was found that folding endurance of the inserts was decreased by increase in the drug concentration and cross linking, 30% drug containing cross linking inserts exhibited minimum folding endurance as compared to other films. The folding endurance of all the films was optimum and therefore the films exhibit good physical and mechanical properties.

Tensile strength:

Tensile strength measures the ability of film to withstand rupture. The tensile strength of plain film and drug-loaded films were studied (Table 2). The tensile strength of the inserts ranged from 1.09 ±0.055 to 3.132 ±0.018 kg, for uncross linked films and 3.26±0.108 to 3.80±0.109 kg for cross linked films. The tensile strength was minimum for plain inserts than the drug loaded inserts, thus 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 cross linked films, probably due to the increased toughness and rigidity of the polymeric film following cross linking.

% Moisture loss

The percentage moisture loss values were shown in (Table 2). The uncross linked films showed more moisture loss compared to cross linked films.

Table 2: Physicochemical characteristics of satranidazole loaded chitosan Periodontal inserts

Strip code	Thickness (mm)	Weight uniformity(mg)	Tensile strength (kg)	% moisture loss	Drug content (µg)
CP	0.223±0.0521	1.09±0.0732	1.092±0.055	8.2±1.04	-
STZ-10	0.266±0.0432	1.180±0.0087	1.407±0.051	8.4±0.98	153.48 ± 0.008
STZ-20	0.298±0.0532	1.216±0.0075	2.214±0.025	7.8±1.08	307.27 ± 0.006
STZ-30	0.315±0.0721	1.314±0.0032	3.132±0.018	6.9±1.04	459.36 ± 0.003
STZ-30 2 hrs	0.329±0.0133	1.228±0.0038	3.261±0.108	6.3±1.09	431.61 ± 0.007
STZ-30 4 hrs	0.331±0.0332	1.229±0.0071	3.800±0.109	6.5±1.05	409.79 ± 0.008

Drug content

For the various formulations, drug content was found to vary between 153.48 ± 0.008 to 459.36 ± 0.003 mg (Table 2). The drug content was found to be almost same with their low standard deviation values.

***In vitro* release study**

In the present study, *in vitro* drug release was carried out in triplicate by a static diffusion method. At different time intervals, the sample was withdrawn and cumulative percentage drug release was calculated on the basis of mean amount of drug present in the respective films. *In vitro* drug release study revealed that inserts released the drug in biphasic manner with initial

burst release followed by slow release as shown in figure 8. The release profile showed that there was rapid initial release of the drug on day one, that is, 28.31, 30.00, and 29.61 for 10, 20, and 30% of the drug loaded inserts respectively. An initial burst effect may be due to elution of the drug 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. At the end of nine days the amount of drug release was found to be 91.63, 95.19, and 93.47% for 10, 20, and 30% of the drug loaded inserts, respectively.

In comparison, the cross-linked films showed a decreased burst effect initially followed by sustained release of the drug, but up to 18 days, with more uniformity of drug release per day. The two hour and four hour cross-linked films showed 93.40 and 93.87% drug release, respectively, at the end of 18th days

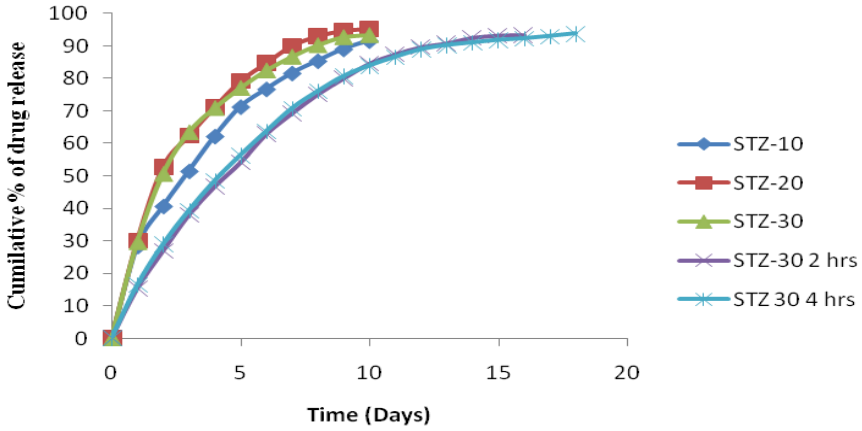

Figure 8: *In-vitro* drug release profile of satranidazole from various periodontal inserts

The *in vitro* release profile was analyzed by various kinetic models. The kinetic models used were zero order, first order, Higuchi and Krosmeyer Peppas equations. The release constants were calculated from the slope of the respective plots (Table 3). Higher correlation was observed with first order plots (r^2 > 0.994 – 0.998) than zero order and Higuchi equation. It was observed from zero order plots that the drug diffused slowly from inserts from the third day. In the present systems, the value for n was found to be in the range of 0.479 to 1.204 indicating that the release mechanisms followed anomalous (non-fickian) transport and zero order release (case II transport).

Stability study:

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 as shown in the (Table 3).

Table: 3. Stability studies of periodontal inserts at various temperatures

Temp.°C	Strip code	Initial drug conc.(µg)	After 30 days
5-8 °C	STZ-10	153.48 ± 0.008	152.55 ± 0.017
	STZ -20	307.27 ± 0.006	304.21 ± 0.060
	STZ -30	459.36 ± 0.003	455.34 ± 0.113
	STZ-30 2hrs	431.61 ± 0.007	427.55 ± 0.088
	STZ-30 4hrs	409.79 ± 0.008	401.63 ± 0.093
27 ± 2 °C	STZ-10	153.48 ± 0.008	152.98 ± 0.013
	STZ -20	307.27 ± 0.006	305.11 ± 0.058
	STZ -30	459.36 ± 0.003	457.21 ± 0.043
	STZ-30 2hrs	431.61 ± 0.007	429.38 ± 0.018
	STZ-30 4hrs	409.79 ± 0.008	405.35 ± 0.063
40 ± 2 °C	STZ-10	153.48 ± 0.008	151.75 ± 0.085
	STZ -20	307.27 ± 0.006	303.28 ± 0.088
	STZ -30	459.36 ± 0.003	454.67 ± 0.120
	STZ-30 2hrs	431.61 ± 0.007	427.88 ± 0.067
	STZ-30 4hrs	409.79 ± 0.008	403.53 ± 0.075

Each value is an average of 3 determinations. STZ – Satranidazole inserts

CONCLUSION:

Periodontal inserts of Satranidazole were prepared by solvent casting method and characterized. The inserts exhibited burst release, which is desirable considering the initial pathogenic load in periodontal pockets, but provided sustained release for more number of days in the local area. The formulation is bio-degradable and would be able to offer benefits such as i.e., increasing residence time, prolonging drug release, reducing frequency of administration, and thereby may help to improve patient compliance. Further work may be carried out to establish the therapeutic utility of this system in patients suffering from periodontitis.

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