Research Article

Design and *In Vitro* Evaluation of Controlled Release Satranidazole Subgingival Films for Periodontitis Therapy

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ABSTRACT

An objective of periodontal treatment is to suppress or eliminate sub gingival periodontal pathogens. Local delivery devices for periodontitis are designed to deliver the drug locally into periodontal pocket. Satranidazole is a 5-nitroimidazole derivative active against gram negative anaerobic periodontal infections. For site specific one time continuous delivery of Satranidazole, an antimicrobial agent in the treatment of periodontal disease was prepared by solvent casting technique using different concentration of chitosan with glycerol as plasticizer. FTIR and DSC studies revealed no interaction between Satranidazole and polymer. The physicochemical parameters along with *in vitro* release characteristics, ex vivo permeation studies and in vitro antibacterial studies were evaluated. All the prepared films have good physicochemical properties and possess consistent sustained release of drug. The in vitro release studies showed an initial burst release of the drug by more than 40% followed by marked fall in release by day two, and progressive moderate sustained release profile to maintain therapeutic level follows zero order. Formulation F5 released 94.104 % of drug in 96 hours and was considered as best controlled release formulation. Ex vivo permeation studies indicated slow and sustained permeation of the drug for 24 hrs. The *in vitro* antibacterial activity studies explained the positive effect on periodontal pathogen. The surface topography of the film was studied by Scanning electron microscopy. The stability studies showed that there were no significant physical changes. The study suggests that chitosan films containing Satranidazole is a potential drug delivery device for the local treatment of periodontitis.

Keywords: Satranidazole, periodontal pocket, chitosan, local delivery, *in vitro* release.

INTRODUCTION

Periodontitis is a disease associated with periodontium in which there is irreversible step of loss of attachment of teeth occurs[1]. The effective use of antimicrobial agent for the treatment of periodontal disease requires an adequate drug concentration at the site of action and a means to maintain that level for a sufficient duration to allow the agent to act. Topical applications like mouthwashes, dentifrices and gels have been successfully tried in controlling the microbial plaque[2]. Topical agents follows an exponential concentration profile while blood and crevicular fluid levels remains at zero, initial salivary concentration reach levels 20 to 50 times bactericidal levels, following expectoration, salivary drug level rapidly fall to approximately one tenth of their initial concentration. Topical agent fail to penetrate deep into periodontal pockets, hence their effectiveness is limited to supragingival areas. So to overcome all these limitations various controlled drug delivery systems, administrating therapeutic levels of antibacterial agents directly into periodontal pocket have been tested as a way to minimize total body dosage and resulting side effects and to maintain therapeutic drug levels in the gingival crevicular fluid [3,4].

One of the clinical features of the periodontal disease is the formation of a periodontal pocket, which is pathologically deepened sulcus. In normal sulcus, the gap between the gingiva and the tooth is normally between1and 3 mm deep. However, during periodontitis, the depth of pocket usually exceeds 5mm[5]. The most commonly grown anaerobic pathogenic bacteria are Actinobacillus actinomycetencomitans, Bacteroides gingivalis, Bacteroides melaninogenicus sub species intermedius, Porphyromonas gingivalis and Prevotella intermedia[6]. Clinical signs such as bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets [7].

Chitosan is a natural, cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin. It is the second most abundant natural polysaccharide and originates from shells of crustaceans. Chitosan is a biodegradable, biocompatible, positively charged nontoxic mucoadhesive biopolymer. Since Chitosan is immobile when left in cavity and degrades as the time advances. It is used as film forming agent, gel forming agent, with its immunostimulatory activities, anticoagulant properties, antibacterial and antifungal action and for its action as a promoter to be used in periodontitis [8].

Satranidazole, a novel nitroimidazole possessing a C-N linkage at C2 of the imidazole ring. The drug produces extensive DNA damage during reduction, characterized by helix destabilization and strand breakage [9]. It exhibits a low bioavailability which is related to its poor aqueous

solubility. Satranidazole falls under class II compounds as per the biopharmaceutical classification system [10, 11]. It is rapidly absorbed and exhibits higher plasma and liver concentration than metronidazole. The MIC90 of Satranidazole against 50 clinical isolates of anaerobes was 0.25 mg/l which was four fold lower than the MIC90 of metronidazole, tinidazole and ornidazole (MIC90 = 1.0 mg/l[12]. Hence an attempt was made to develop polymeric films containing Satranidazole for local drug delivery for the treatment of periodontitis.

MATERIALS AND METHODS

SZ was obtained as a kind gift sample from Alkem, Mumbai, India, and Chitosan from Central Institute of Fisheries Technology, Cochin. All other chemicals used in this study are of analytical reagent grade.

Preformulation studies

Preformulation study of drug was carried out to establish its identity and purity which includes

λmax of the drug

An absorption maximum of Satranidazole was determined using phosphate buffer pH6.6. Solution ranging from 1-20 µg/ml were scanned from 200-400 nm using UV spectrophotometer [13].

Analytical Method Development

Standard Calibration curve of Satranidazole was prepared in phosphate buffer pH 6.6. PBS pH 6.6 simulates GCF thus facilitates the extrapolation of results to in vivo conditions. The graded concentrations were chosen within the range in of beer-lambert's law [14].

FTIR Studies

Compatibility of Satranidazole with the excipient and physical mixture of main formulation was established by Infrared Absorption Spectral Analysis (FTIR). Any changes in the chemical composition after combining with the excipient were investigated with IR spectral analysis [13].

Differential scanning calorimetry (DSC)

To study the compatibility between drug and polymer, DSC studied were carried. Calorimetric measurements were made with empty cell (high purity gold discs of Du Pont Company) as the reference. The instrument was calibrated using high purity indium metal as standard. The thermo grams of the samples were obtained at a scanning rate of 10°C/min conducted over a temperature range of 30 – 300°C, respectively [13].

Preparation of films

Chitosan was weighed accurately for the preparation of 1% films of polymer and then was soaked in 100 ml each of aqueous lactic acid solution (2 % v/v) for 24 hour to get a clear solution, which was later filtered through a muslin cloth to remove undissolved polymer (chitin). Satranidazole was incorporated in chitosan solution and vortexed (Standard Vortex Mixer) for 15 min. The viscous dispersion was kept aside for 30 min for complete expulsion of air bubbles. Films were cast by pouring the drug-polymer solution into the centre of glass moulds and allowed to dry at room temperature. The solvent was allowed to evaporate slowly by inverting a glass funnel with a cotton plug in the stem of the funnel was placed on the mould at room temperature for 24 h. After complete evaporation of solvent, cast films were obtained. Inverted funnel was continuously kept on the mould to control drying rate. The dry films were cut into strips of (7 × 2 mm), wrapped in aluminium foil and stored in calcium chloride desiccators at room temperature pending evaluation. Preparation of chitosan films of different concentrations (2%, 3%, 4% and 5%) containing satranidazole follows the same procedure [15]. To accommodate different variables, batches of cast films were prepared. The compositions of films are given in Table 1.

Table 1: Composition of different formulations containing satranidazole

Ingredients	F1	F2	F3	F4	F5
Satranidazole	1%	1%	1%	1%	1%
Chitosan	1%	2%	3%	4%	5%
Glycerol	20%	20%	20%	20%	20%
Lactic acid solution (2%)	10 ml	10 ml	10 ml	10 ml	10 ml

Characterization of the films

Formulated films were subjected to the preliminary evaluation tests. Films with any imperfections, entrapped air, or differing in thickness, weight (or) content uniformity were excluded from further studies. Physico-chemical properties such as thickness, weight uniformity, percentage moisture loss, folding endurance, surface pH, swelling index and drug content uniformity of the prepared films were determined [16 – 19].

Thickness uniformity

The thickness of each film was measured using screw guage (thickness tester) at different positions of the film and the average was calculated.

Uniformity of weight

Film (size of 1 cm^2) was taken from different areas of film. The weight variation of each film was calculated.

Estimation of percentage moisture loss

The 20 films of different concentrations of size (7 × 2 mm) are weighed accurately and then they are kept in desiccators for 3 consecutive days and then reweighed. % moisture loss was calculated by formula:

Moisture loss = (initial wt – final wt / initial wt) ×100.

Folding endurance studies

The folding endurance of the films was determined by repeatedly folding one film at the same place till it broke or folded up to 350 times, which is considered

satisfactory to reveal good film properties. The film was folded number of times at the same place without breaking gave the value of the folding endurance. This test was done on all the films for five times.

Surface pH

The surface pH of the films was determined in order to investigate the possible side effects due to change in pH in vivo, since an acidic or alkaline pH may cause irritation to the periodontal mucosa. The film to be tested was placed in a Petri dish and was moistened with 0.5 ml of Ph 6.6 buffer and kept for 1 h. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibrating for 1 min.

Swelling Index

The studies for Swelling Index of the films were conducted in simulated salivary fluid of pH 6.6. The film sample ($1\times1cm^{2)}$ was weighed and placed in a preweighed stainless steel wire sieve of approximately 800 µm mesh. The mesh containing the film sample was then submerged into 15 ml of the simulated salivary medium contained in a porcelain dish. At definite time intervals, the stainless steel mesh was removed, excess moisture removed by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula:

$$S.I = (w_t - w_0) / w_0$$

Where S.I is the Swelling Index, w_t is the weight of film at time t and w_0 is the weight of the film at time 0.

Drug content uniformity

The drug-loaded films of known weight (7 × 2 mm) were dissolved in 10 ml of 2% aqueous lactic acid, suitably diluted and the amount of drug present was estimated by UV/VIS spectrophotometer (shimadzu) at 319 nm.

***In Vitro* Drug Release Studies**

The pH of gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid. Also, since the film should be immobile in the periodontal pocket, a static dissolution model was adopted for the dissolution studies. Sets of three films of known weight and dimension were placed separately in small sealed test tubes containing 1.0 ml of phosphate buffer (pH 6.6) and 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 was determined by UV/VIS spectrophotometer (shimadzu) at 319 nm (20). The procedure was continued for 4 consecutive days for different polymer concentrations (1%, 2%, 3%, 4% and 5%) respectively.

Mass Balance Study

Following the *in-vitro* release studies, the test films were further analyzed for the drug content left in the film. Each film was dissolved in lactic acid 2% v/v and diluted suitably. The absorbance was measured at 319 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.

***In vitro* Drug Permeation Studies**

For the permeation studies, bovine periodontal mucosa was used as the model membrane. The periodontal mucosa of the freshly sacrificed cattle was procured from the local slaughter house and used in within two hours of slaughter. The mucosa was excised and trimmed evenly from the sides. The epithelium was separated from the underlying connective tissue by surgical method and the delipidized membrane was allowed to equilibrate approximately for one hour in receptor buffer to gain the lost elasticity. The Franz cells that have the surface area of 3.14 cm^2 were used and the receptor compartment had a capacity of approximately 15 ml. The receptor compartment was filled with pH 6.6 phosphate buffer. The epithelial mucosa was exposed to donor compartment, while the opposite side was bathed with receptor solution. A static permeation model was adopted throughout the study. To mimic the body condition during the experiment, the temperature was maintained as 37±0.5°C with an external constant water circulator. Sink conditions were maintained A 1 cm^2 film under study was placed in intimate contact with the excised epithelium, was applied to the donor compartment of the prepared Franz diffusion cell. At a predetermined time, like 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours, 0.5 ml of the sample were withdrawn and the cell was refilled with same amount of the fresh receptor solution. Withdrawn samples were analyzed spectrophotometricaly at 319 nm. Each permeation experiments were replicated three times and from the concentration of the drug the withdrawn solution, the amount of drug permeated to the receptor compartment was calculated [21,22].

***In vitro* Antibacterial Activity**

The procedure follows agar diffusion assay method. *P. gingivalis* should be inoculated directly with clinical material or a broth that has been previously inoculated from clinical material. Inoculated plates should be streaked to obtain isolated colonies, immediately placed in an anaerobic atmosphere and incubated at 35-37°C for 18-48 hours. The samples were tested at different concentration. Sterile PGA plates were prepared and 0.1 ml of the inoculums of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 6 mm and the samples (sample A and B) at different concentration (5µl, 10µl, 15µl and 20µl) were added in each well separately. Sample A is the optimized F5 film and sample B is the blank film. The plates were incubated at 35-37°C for 18-48 hours, a period of time

sufficient for the growth. The zone of inhibition of microbial growth around the well was measured in cm and statistical analysis was performed [23].

Scanning Electron Microscopy (SEM)

The morphology and surface topography of the optimized film F5 were examined by SEM (Joel jsm-6490la analytical SE). Spherical samples (5 mm^2) were mounted on the SEM sample stab using a double sided sticking tape. The samples were coated with gold (200 A°) under reduced pressure (0.001 torr) for 2 min using an ion sputtering device (model JFC-1100 E, Jeol, Japan). The gold coated samples were observed under the SEM at room temperature and photomicrographs of suitable magnifications were obtained [24].

Stability studies

The stability of the entire drug loaded polymer films were studied at different temperatures using the reported procedure. The films of size (7 × 2 mm) were weighed in three sets (12 films in each set). The films were wrapped individually in aluminium foil and also in butter paper and placed in Petri dishes. These containers were stored at room temperature (27 ± 2°C), and in a refrigerator (5–8 ± 2°C) for a period of 45 days. All the polymeric films were observed for any physical changes, such as colour, appearance, flexibility, or texture, and the % drug release was estimated at an interval of one week.[25, 26]

RESULTS

To confirm the identity, purity and suitability of drug for formulation and to establish a drug profile, preformulation studies were undertaken.

λmax of the drug

The λmax of the drug was found to be 319 nm and it was in accordance with the official standard.

Analytical Method Development

The calibration curve of satranidazole in phosphate buffer pH 6.6, was constructed at a λmax of 319 nm with UV-VIS spectrophotometer. Beer's law obeyed to construct the calibration curve in the concentration range of 1-15 µg/ml. Analyses were done in triplicate.

Drug-Polymer Compatibility

FTIR spectra of Satranidazole, chitosan alone and its combination with polymer are shown in Figure 1. From the spectra confirmed the absence of any chemical incompatibility between the drug and the polymer.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of drug - polymer physical mixture are shown in Figure 2. The DSC thermogram of Drug - Chitosan mixture showed two endothermic peaks, a sharp peak at 186.71°C corresponding to drug and another peak at 60.78°C corresponding to polymer. The sharp endothermic peak between 186 and 187°C (corresponding to satranidazole) in the DSC thermogram of physical mixture indicates absence of drug- carrier interaction, hence compatible.

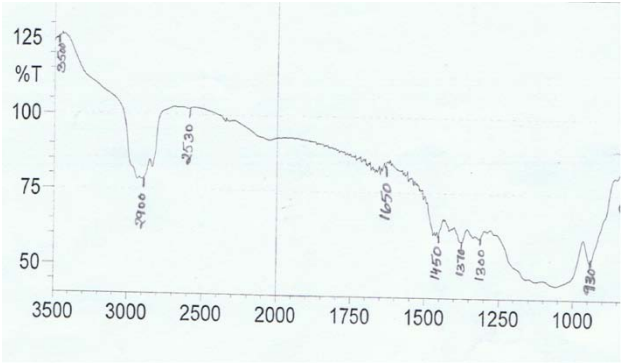

Figure 1: FTIR of Drug - Chitosan physical mixture

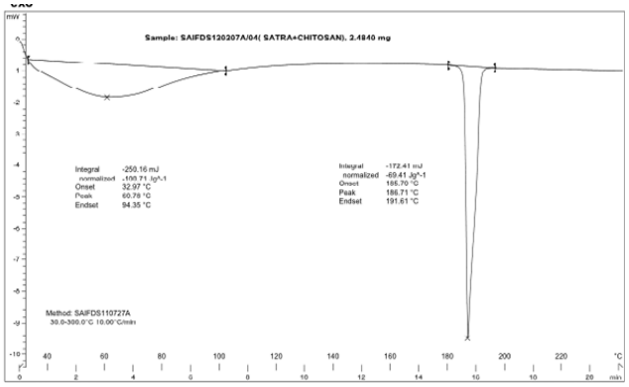

Figure 2: DSC of Drug – Chitosan physical mixture

Preparation of films

As per the basic requirement of easy to insert and flexible films are prepared for periodontitis treatment. The optimum loading for the present investigation, Satranidazole films with chitosan are prepared with five different concentrations, 1 to 5 % were prepared by solvent casting method. Addition of the plasticizer produced a film of good strength. The films were translucent and visually smooth surfaced. The developed procedure to prepare the films was reproducible. The amount of drug added to the polymer solution altered the film characteristics. The optimum drug loading was found to be 1%. At higher drug loadings (i.e., > 1%), the films were stiff and brittle. The prepared films were characterized for physicochemical properties, including in vitro release.

Evaluation of films

The Table 2 shows the physicochemical properties of various formulation of Satranidazole films, reveals that the thickness of dental films increases as the concentration of the polymer increases from 0.05 ±0.01 to 0.1 ±0.02 mm for F1 to F5.The average weight of films varies from1.3 ±0.03 to1.7 ±0.03 mg for F1 to F5. Films did not show any cracks even after folding for more than 350 times. Hence it was taken as the end point. Surface pH for all formulations of chitosan (F1 to F5) ranges from 5.6 ±0.02 to 6.1 ±0.01. Swelling index value increases as the concentration of polymer increases from 24.7 ±0.01

to 32.8 ±0.01 % for F1 to F5.The percentage moisture loss decreased from 12 ±0.02 to 8.2 ±0.01 for F1 to F5. All the formulations exhibited good drug content. The residual drug content in the film after 4 days was determined and it did not differ from the experimental drug content by more than 3 %. The in vitro drug release studies showed maximum % drug release of 49.5067 % to 94.10413% from day 1 to day 4 for F5. The release time profile of Satranidazole from different concentration of Chitosan films are shown in Figure 3. After four days, the film had lost their integrity and hence was not fit for further release study. The release kinetics of drug was found to be zero order. Highuchi's diffusion model gave a better and best fit of release data indicating diffusion dominating as the plots showed maximum linearity with R^2 values of 0.998 for F5. The Korsmeyer Peppas model was found to be linear with the correlation coefficient values of 0.998 for F5 and "n" value for F5 was 1.050 suggests zero order release profile. The permeation data proved that the increase in the polymer concentration caused increased in the permeation rate. Ex vivo permeation studies indicated slow and sustained permeation of the drug for 1 to 24 hrs. The rank order of drug permeation from various formulations was found to be F5 > F4 > F3> F2 > F1, are represented graphically in Figure 4.The in vitro antibacterial activity demonstrated a significant antibacterial profile of the optimized film F5 against Porphyromonas gingivalis, shown in Figure 5. Scanning electron microscopy (SEM) showed the upper surface of film containing Satranidazole (F5) was rough, as shown in Figure 6. From the stability studies it was confirmed that the optimized film F5 of Satranidazole remained stable at room temperature (30 ± 2°C) and at refrigerator temperature (4 ± 2°C).

Table 2: The physicochemical properties of Satranidazole sub gingival films

Film code	TN (mm)	W.U (mg)	FE	Surface PH	S.I (%)	Most.lss (%)	Drg.Cnt U(%)	Mass B.S
F1	0.05±0.01	1.3±0.03	> 350	5.6±0.02	24.7±0.01	12±0.02	91.5±0.34	38.2±0.24
F2	0.06±0.02	1.55±0.01	> 350	5.7±0.01	23±0.01	11.2±0.01	92.8±0.42	34.4±0.21
F3	0.08±0.02	1.63 ±0.02	> 350	5.6±0.05	24.5±0.04	10.2±0.32	93.6±0.56	21.8±0.54
F4	0.09 ±0.02	1.7±0.01	> 350	6 ±0.01	28.8±0.03	8±0.04	94.5±0.55	13.5±0.32
F5	0.1±0.02	1.7±0.03	> 350	6.1±0.01	32.8 ±0.01	8.2±0.01	98±0.15	5.73±0.23

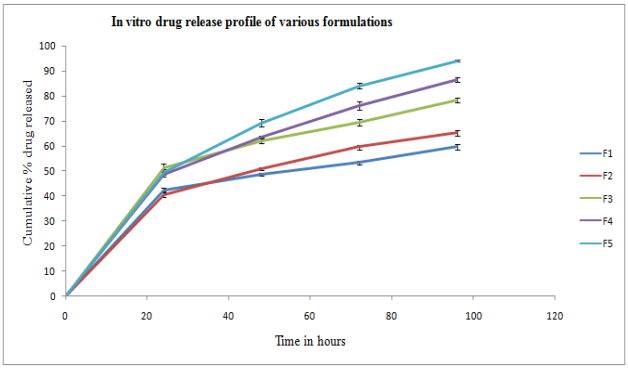

Figure 3: *In vitro* release studies of various formulations. Error bars represent ± SD, n = 3.

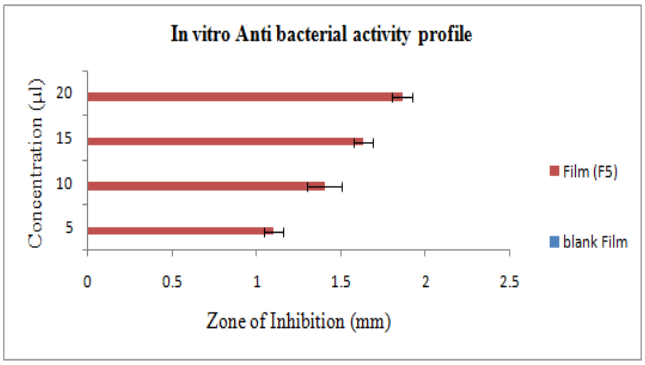

Figure 5: *In vitro* Antibacterial Activity of optimized F5 film and blank film. Error bars represent ± SD, n = 3.

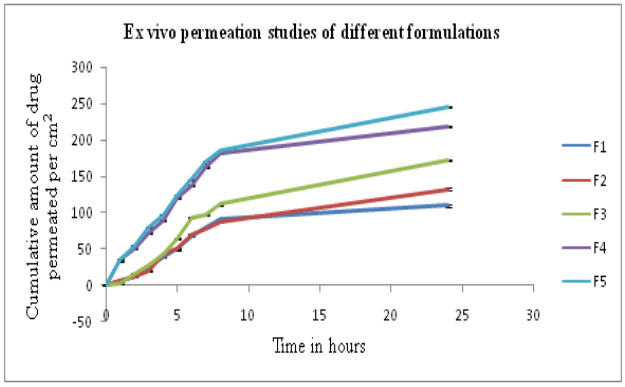

Figure 4: *Ex vivo* permeation studies. Error bars represent ± SD, n = 3.

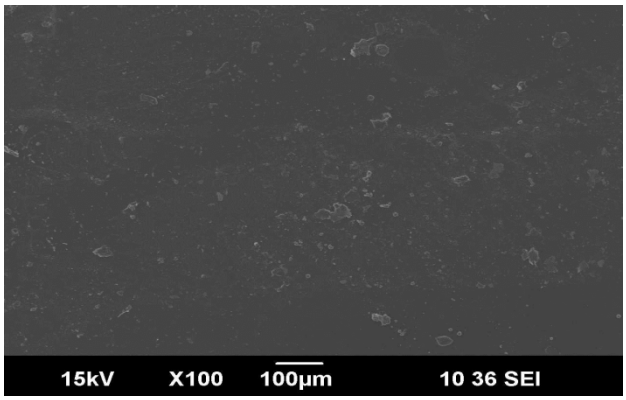

Figure 6: SEM of optimized F5 film

DISCUSSION

Satranidazole subgingival films, were prepared by solvent casting technique using lipophilic polymer with the incorporation of glycerol as a plasticizer. The FTIR studies and the DSC studies confirmed the absence of chemical interaction between the drug and the polymer. The physicochemical evaluation data indicates that thickness and average weight of strips increases as the concentration of the polymer increases, surface pH of the all the formulations was very close to the neutral pH, indicated negligible irritation to the mucosal membrane as evident from the pH of formulations. The percentage moisture loss decreases as the concentration of chitosan increases in the formulation due to hydrophobic characteristics. The folding endurance was found to be greater than 350 folds due to hydrophobic characteristics of chitosan. The drug content studies showed uniform and homogeneous distribution of drug into the films. Mass balance studies indicated that, the drug content did not differ from the experimental drug content by more than 3%. The in vitro drug release studies showed slow and controlled drug release for 4 days. Since the chip remains immobile in the periodontal pocket, a static dissolution model was adopted in this work. 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 four days. The percentage cumulative drug release was greater in F5 formulations. The release profile also shows that there is statistically significant difference ($p<0.05$) in the release profile from the formulation when the polymer concentration was changed. Drug permeation studies through bovine periodontal mucosa conducted on the PBS 6.6 solution of the pure drug showed slow and sustained permeation in less than 24 h indicating the suitability of formulating Satranidazole in controlled release local delivery for longer periods of time. Throughout the permeation study, the average permeation rate was found to be above the minimum inhibitory concentration of Satranidazole. Satranidazole film F5 confirmed good anti bacterial activity against Porphyromonas gingivalis. The SEM study indicates that the upper surface of the films containing Satranidazole (F5) was rough with pores inside and was loosely arranged. The drug remained intact and stable in the periodontal films during storage, with no significant chemical interaction between the drug and the excipients.

CONCLUSION

The greatest advantages associated with the use of intra pocket delivery systems over systemic delivery are that the administration is less time consuming than mechanical debridement and a lesser amount of drug is sufficient to achieve effective concentration at the site. The periodontal mucosa offers several advantages for controlled drug delivery for extended periods of time. The drug loaded chitosan films were flexible and demonstrated satisfactory physicochemical characteristics. On the basis of in vitro characterization it was concluded that Satranidazole could be incorporated in a slow release device for the local treatment of periodontitis. Further, detailed investigation is required to establish in-vivo efficiency of these films.

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