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DEVELOPMENT AND ASSESSMENT OF AN ANTIBIOTIC INTRA-POCKET DEVICE FOR PERIODONTAL DISEASE

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ABSTRACT

Periodontal disease causes destruction of adjuvant structures of the teeth predominate in all groups, ethnicities, races and both genders. It is generally agreed that gram negative anaerobic germs residing in periodontal vacuities are responsible for periodontitis. Systemic antibiotic therapy is employed in treating this diseased condition, but it has limited due to the lack of accessibility to periodontopathic organisms in the periodontal pocket. Different modes of local delivery devices are developed to deliver the drug locally into periodontal pits. These controlled intra-pocket devices also help in the maintenance of therapeutic drug concentration for the desired period of time. The goal of this research was to fabricate controlled release dental films, loaded with satranidazole as an antimicrobial agent which consists of a common biodegradable polymer and a co-polymer in different proportions for targeted delivery of drug. About 6 formulations of satranidazole dental inserts were prepared with gelatin as the principle biodegradable polymer and sodium alginate as a co-polymer in different ratios. Formulated periodontal films were then crosslinked with 2% v/v glutaraldehyde for 2 and 4 hours respectively inorder to upgrade the formulations for extended release. The dental inserts produced in such a manner were evaluated for their thickness uniformity, folding endurance, weight uniformity, % drug content, surface pH, swelling index, *in-vitro* drug release, *in-vitro* permeation, *in-vitro* antibacterial activity and stability studies. Based on these findings best film was selected as the one which is fabricated with gelatin and sodium alginate (1:1 ratio) i.e. F2 (2 hour crosslinked strip), since it can deliver the drug over MIC in each day of therapy and it is having an adequate drug content and other necessitate film characteristics. The kinetic models of the formulation F2 (2 hour crosslinked film) was then found and it denoted that the formulation undergoes zero order release kinetics and model fits to Higuchi which is reflective of the diffusion mechanism of

medication release. The mechanism of drug delivery was found to be Non-Fickian. From the assessments of the results, it is possible to easily predict the fact that gelatin and sodium alginate in a definite concentration can be used to prepare films utilizing the antibacterial activity of satranidazole for healing periodontitis.

KEYWORDS: Periodontitis, Satranidazole, Gelatin, Sodium alginate, Periodontal films, Intra-pocket device.

1. INTRODUCTION

Periodontitis alludes to a provocative malady with essential bacterial etiology in the gingival fissure, which influences the auxiliary organs encompassing the teeth like periodontal tendon, connective tissue and bone. It is one of the world's most prevalent oral chronic disease and approximately 70 million adults suffer from this disease. Periodontitis involves progressive bone loss around the teeth, which may result in loosening and subsequent loss of teeth and is characterized by periodontal cavity formation. Pre-existing gingivitis with the colonization of a wide range of microorganisms often triggers the emergence of periodontal disease. It is a multifactorial infection with great complexity in the mechanisms of pathogenesis. One of the clinical features of the periodontal disease is the formation of a periodontal pocket. Normally the gap between the gingiva and the tooth is 1 to 3 mm deep but it usually exceeds 5mm to 10mm during diseased condition.[1,5] The warm and moist atmosphere around the pocket fosters the growth of gram-negative, anaerobic bacteria that proliferate in the sub gingival space. The most commonly grown anaerobic pathogenic bacteria's that can cause periodontal diseases include Actinobacillus actinomycetencomitans, Bacteroides gingivalis, Bacteroides melaninogenicus sub species intermedius, Porphyromonas gingivalis and Prevotella intermedia.[1] Some of the clinical signs such as gingival bleeding, bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa and localized pain are indicative of the presence of periodontal pockets.

Effective treatment for periodontitis as well as gingivitis has been evolved appreciably in the last decade. The point of dental human services is to control the number of inhabitants in microorganisms either by moderating or capturing of the oro-dental contaminations. Conventional therapy performed for periodontal disease is scaling and root planning followed by systemic antibiotic therapy. The systemic administration of antibiotics leads to therapeutic concentrations at the site of infection, only for a short period of time for controlling sub gingival flora. Therefore long term antimicrobial therapy should be ensured for complete

eradication of microorganisms. Antibiotics administration at higher doses are required to achieve effective concentrations in the pocket, but high doses given for long periods of time eventually causes development of bacterial resistance, super infection, gastrointestinal and central nervous system disturbances. To overcome the disadvantages of systemic chemotherapy with antibiotics, local delivery of antibiotics have been investigated. Drug targeting to a desired site contributes in minimizing the distribution of antimicrobials to other body parts or organs. The central objective of this research was to fabricate and evaluate biodegradable controlled release dental films containing Satranidazole for placement into the periodontal pockets of periodontitis patients for targeted delivery of drug in order to reduce periodontal pathogens. The effects of various formulation variables on the drug release profiles from the films were studied to determine optimum formulation.

Dental films (periodontal films) are far more widely used form of intra-pocket delivery devices which are prepared either by solvent casting or direct milling methods. Films of various polymers have been made for the controlled release of antimicrobials. Depending upon the nature of polymers we can prepare both biodegradable and non-degradable films. Periodontal films that release drugs by diffusion alone are prepared using water insoluble non-degradable polymers, whereas those that release by diffusion and matrix erosion or dissolution use soluble or biodegradable polymers.

In the form of films, we can directly place the drug in to the pocket so as to attain therapeutic drug levels in the gingival crevicular fluid and thus reduce the adverse effects of antibacterial agent on other non oral body parts. Films are matrix delivery systems in which drugs are distributed throughout the polymer and delivery occurs by drug diffusion and/or matrix dissolution or erosion. This dosage form has a few invaluable physical properties for intra-pocket use. The dimensions and shape of the films can be easily controlled according to the dimensions of the pocket to be treated. It can be rapidly inserted into the base of the pocket with minimal discomfort to the patient. The convenient size of the film or sheet may be 0.1-0.5 mm in thickness, 0.5-3 mm in width and 5-50 mm in length and it has sufficient adhesiveness, it will remain submerged without any noticeable interference with the patient's oral hygiene habits.[3] Once a film is inserted in periodontal pocket, the polymer adheres, swells, expands, and reaches narrow crevices and furcations of the cavity, carrying therapeutic agent throughout the cavity.[3] This provides most desirable and advanced treatment for dental diseases. Although this type of drug delivery is still young, it has

attracted much attention and has proved to be a most promising alternative method of treatment. There is great potential in the treatment offered by local drug delivery devices in the field of both pharmacy as well as dentistry.

Satranidazole (SZ), a novel nitro-imidazole possessing a C-N linkage at C2 of the imidazole ring. It has been found to be more active against aerobic, microaerophilic and anaerobic bacteria than Metronidazole (MZ). The drug produces extensive DNA damage during reduction, characterized by helix destabilization and strand breakage. 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. It is rapidly absorbed and exhibits higher plasma and liver concentration than MZ. 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). Hence an attempt was made to develop an oral-degradable films containing Satranidazole for local drug delivery for the treatment of periodontitis.[1]

2. MATERIALS AND METHODS

Satranidazole was obtained from Alkem Laboratories Ltd, Mumbai, Gelatin was obtained from Sara fine chemicals, Baroda and Sodium Alginate was obtained from Lobachemie pvt. Ltd, Mumbai. Polyethylene glycol (PEG)-400, was purchased from Merck Ltd, Mumbai. Other materials used in the study were of analytical grade and UV spectrometer (Shimadzu 2401/PC Japan) was used for analytical purpose.

2.1. Preformulation studies

Preformulation study of the 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, pH 6.6 solution ranging from 1-3.5µg/ml were scanned from 200-400 nm using UV spectrophotometer.

2.2. Drug –Excipient Interaction Study[2,3,4,7,9]

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 7800 and 350 cm^{-1}. The spectra obtained for Satranidazole and physical mixtures of Satranidazole with polymers were compared to check compatibility of drug with polymers.

2.3. Development of Satranidazole Dental Films[2,3,4,7]

2.3.1. Preparation of Satranidazole Films

Periodontal films were prepared by solvent casting technique. Natural biodegradable polymers were used for the preparation of dental films. Formulations were designed as shown in the table 1, in which weighed quantity of polymer; gelatin was sprinkled on the surface of distilled water and kept aside for 30 minutes to hydrate. After hydration it is subjected to continuous stirring by using a magnetic stirrer and a temperature of 60ºC was maintained until gelatin was dissolved. The above solution was then introduced with co-polymer, sodium alginate and PEG 400 as a plasticizing agent with continuous stirring. Anti-bacterial agent, satranidazole was dissolved separately in small quantity of methanol and added to the polymeric solution for homogenous mixing. After complete mixing for about 30 minutes, the solution was slowly poured into the clean levelled glass moulds of 9 cm diameter. The solution is then allowed to stand for 15 minutes without any further disturbances inorder to remove the entrapped air bubbles. Petri dishes were then placed on ice bath for 30 minutes to become gel and they were dried for 3 days at room temperature. After drying, the cast strips were cut into appropriate dimension of 0.5×0.5 cm and each wrapped in aluminium foils and stored in desiccators at room temperature in dark place for further assessments.

2.3.2. Preparation of satranidazole loaded cross-linked periodontal films[4,7,8]

The films were cross-linked by placing in a chromatographic chamber, which was previously saturated for almost 24 hours with vapours of 2% v/v glutaraldehyde solution for extended release. The films were exposed to vapours for 2 and 4 hours respectively in the chromatographic chamber and then dried. The dried cross-linked strips were wrapped in aluminium foil and placed in desiccators for further evaluation studies.

Table 1: Formulation chart for the development of Satranidazole dental films.

Ingredients	Formulation code					
	F1	F2	F3	F4	F5	F6
Satranidazole(mg)	100	100	100	100	100	100
Gelatin(mg)	300	375	450	525	600	675
Sodium alginate(mg)	450	375	300	225	150	75
PEG 400 (ml)	0.15	0.15	0.15	0.15	0.15	0.15
Distilled water (ml)	20	20	20	20	20	20

Table 2: Crosslinking of Satranidazole loaded dental films using Glutaraldehyde solution.

Formulation code	% of crosslinking agent	Duration of crosslinking (hrs)
F1	2	All films are exposed to glutaraldehyde solution for 2 and 4 hours respectively.
F2	2	
F3	2	
F4	2	
F5	2	
F6	2	

2.4. EVALUATION

Characterization of the films[1,2,3,4]

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. Physicochemical 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.

Thickness uniformity[2,3,4]

The thickness of each periodontal film was measured using screw gauge at different positions of the film and the average was calculated.

Uniformity of weight[2,3,4]

Film pieces of same size (0.5 × 0.5 cm) were taken from different areas of film. The individual weights were determined and the average weight was calculated.

Estimation of percentage moisture loss[3,4]

The percentage moisture loss was carried out to examine the integrity of films at dry conditions. Films of known weight and of predetermined size (0.5 × 0.5 cm) were kept in a desiccator containing anhydrous calcium chloride for 3 days. The films were taken out then and re-weighed to calculate the percentage moisture loss as per the formula.

% Moisture loss was calculated by formula:

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

Folding endurance studies[2,3,4]

The folding endurance of the films was determined by repeatedly folding one film at the same place up to 300 times till it broke or folded, 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.

Surface pH[2,3,4]

The surface pH of the films was determined in order to check out 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 distilled water 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 minute.

Swelling Index[3,4]

The studies for Swelling Index of the films were conducted in simulated salivary fluid of pH 6.6. The film sample (0.5 × 0.5 cm) was weighed and placed in a pre-weighed stainless steel wire sieve. 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[2]

The drug-loaded dental films of known weight (0.5 × 0.5 cm) dimension were dissolved in distilled water and kept aside for overnight. The solution was then filtered with a whatmann filter papper. From the filtrate 5ml was taken and diluted with distilled water in 100ml standard flask. The absorbance was measured at 319nm. The polymer solution without the drug served as a blank.

***In-vitro* drug release studies**[2,3,4]

A static dissolution model was adopted for the dissolution studies, since the film should be immobile in the periodontal pocket. As the pH of gingival fluid lies between 6.5 – 6.8, phosphate buffer of pH 6.6 was used as simulated gingival fluid. Sets of six films of known

weight and dimension were placed separately in small test tubes containing 1.0 ml of phosphate buffer (pH 6.6) and kept at 37^0 C for 24 h. The test tubes were gently sealed with small sheets of aluminium foil. The sample solution of 1.0 ml was collected at definite interval of time and replaced with a fresh 1.0 ml of buffer. The procedure was followed until no more drug release take place. The concentration of drug in the buffer was measured at 319nm by using a UV/VIS spectrophotometer (Shimadzu).

Mass balance study[3,4]

Following the in-vitro release studies, the test films were further assayed for the drug content left in the film. Each film was dissolved in distilled water 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* permeation studies of satranidazole loaded films**[4]

The model membrane used for penetration studies was conducted on a bovine periodontal mucosa. It was obtained from a local slaughter house and used for the study within two hours of slaughter. The epithelium was separated from the underlying connective tissue by surgical method and the delipidized membrane was allowed to equilibrate for almost 1 hour in phosphate buffer, pH 6.6 to gain the lost elasticity.

A 1 cm^2 dental film of optimised formulation F2 (crosslinked with 2% v/v glutaraldehyde for 2 hour) and a control (drug solution) was selected for the permeation study. It was placed in intimate contact with the excised epithelium, was applied to the donor compartment of prepared Franz diffusion cell. From the receptor compartment 1ml of the sample were withdrawn at a pre-determined time intervals like, 1, 2, 3, 4, 5, 6, 24 and 48 hours. The receptor cell was refilled with same amount of the fresh phosphate buffer, pH 6.6. Withdrawn samples were analyzed spectrophotometricaly at 319nm. The study was replicated for 3 times for both the optimised formulation (F2) and control (standard drug solution). From the withdrawn samples the concentration of drug permeated to the receptor compartment was calculated.

Scanning Electron Microscopy (SEM)[3,4]

A Scanning electron microscope (model JFC-1100 E, Jeol, Japan) was used to examine the surface characteristics of the optimized film F2 (crosslinked with 2% v/v glutaraldehyde for 2

hour). 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^0) 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.

***In-vitro* Antibacterial studies[3]**

80 ml of nutrient agar media was prepared and sterilized at 15 lb pressure for 20 min in an autoclave. Under aseptic condition 20 ml of nutrient agar media was transferred into 4 sterile petri plates. After solidification 0.1 ml of microbial suspension of both *E.coli* & *S.aureus* of known concentration was spread on the media. Wells were prepared by using a sterile borer of diameter 6 mm and the samples were added in each well separately. The optimized film F2 (crosslinked with 2% v/v glutaraldehyde for 2 hour) and the standard drug solution sample were tested. The plates were then incubated at 37^0C for 48 hrs. Then the zone of inhibition was measured & compared.

Stability studies[2,3]

The stability of the optimised drug loaded polymer films were studied at different temperatures using the reported procedure. The films of size (0.5 × 0.5 cm) were weighed in three sets (9 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 different temperatures like 40±2°C/75±5%RH and 25±2°C/60±5%RH for a period of 30 days. All the polymeric films were observed for any physical changes, such as colour, appearance, flexibility, or texture and the drug content was estimated at an interval of 10 days.

3. RESULTS AND DISCUSSION

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

3.1. Determination of λ_{max}

The λ_{max} of the drug was found to be **319 nm**.

3.2. FTIR Studies

As described in the methodology section the Fourier Transform Infrared spectroscopy studies were carried out for pure drug (Satranidazole) and for the Satranidazole-Polymer physical

mixtures. The results are summarized in the Figures 1 to 4. There were no changes in the major peaks of Satranidazole in the presence of various polymers such as gelatin and sodium alginate. This revealed that the drug and the polymers are compatible with each other.

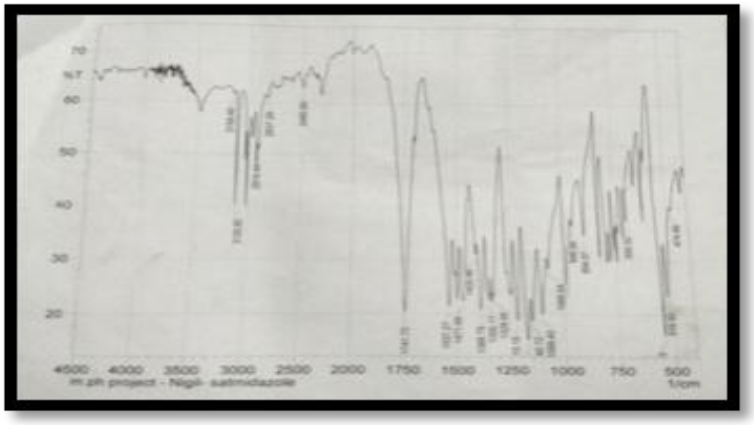


Fig.1. FTIR Spectrum of Satranidazole.

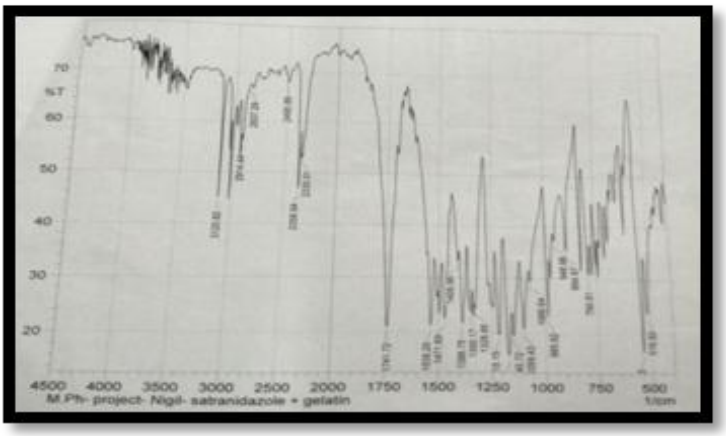


Fig.2. FTIR Spectrum of Satranidazole: Gelatin physical mixture.

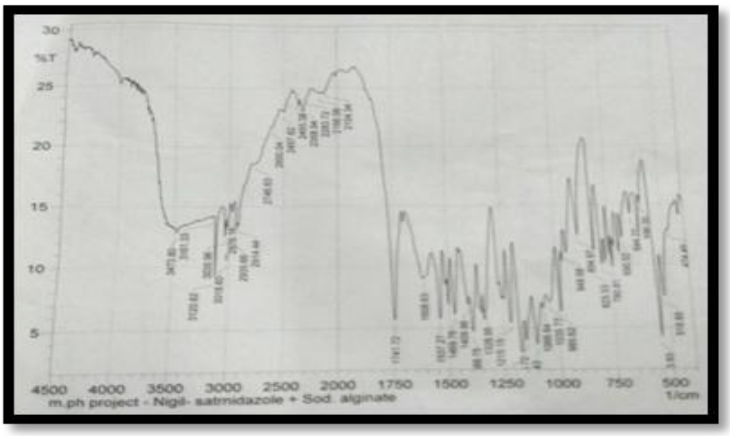


Fig.3. FTIR Spectrum of Satranidazole: Sodium alginate physical mixture.

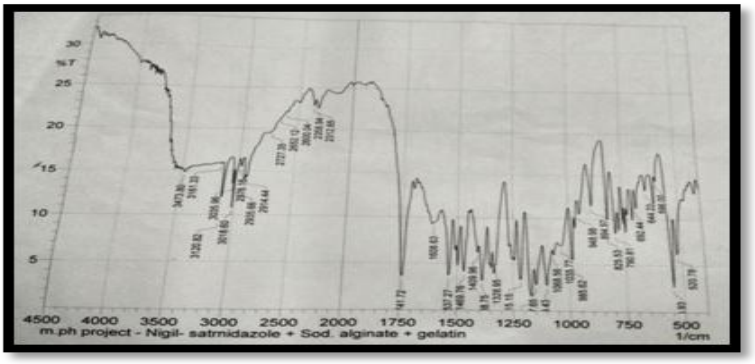


Fig.4. FTIR Spectrum of satranidazole: Sod alginate: gelatin physical mixture.

3.3. Physicochemical evaluation data

Thickness of the Films

The thickness of each film was measured at 6 different points and the average thickness was calculated. The thickness of various films is given in table 3. The data of films thickness indicated that there was no much difference in the thickness within the formulations.

Table 3: Measurement of thickness.

Formulation code	Thickness*(mm)(Mean±SD)
F1	0.15±0.01
F2	0.17±0.02
F3	0.15±0.03
F4	0.17±0.03
F5	0.15±0.03
F6	0.18±0.01

Weight Uniformity test

Drug loaded films (0.5 × 0.5 cm) were tested for uniformity of weight and the results of weight uniformity are given in table 4. The values indicated that the films were uniform in weight.

Table 4: Measurement of Weight Uniformity.

Formulation Code	Weight Uniformity*(mg) (Mean±SD)
F1	6.2±0.2
F2	6.3±0.3
F3	6.1±0.1
F4	6.4±0.2
F5	6.3±0.3
F6	6.6±0.2

Percentage moisture loss

Moisture loss studies were conducted on all the formulations of 2 and 4 hour, 2% v/v glutaraldehyde crosslinked films and reported in table 5. It was observed that the formulation F2 from both 2 and 4 hour crosslinked showed highest moisture loss whereas F 6 formulation has lowest moisture loss. It is because as the concentration of gelatin increases moisture loss decreases, since gelatin has low moisture content. From this study it was observable that all the crosslinked films of 4 hour showed lower moisture loss than 2 hour crosslinked films and it may be due to greater compactness and lower porosity of the 4 hour crosslinked films.

Table 5: Measurement of % Moisture loss.

Formulation code	% Moisture loss(Mean±SD) (2 hour crosslinked films)	% Moisture loss(Mean±SD) (4 hour crosslinked films)
F1	12.69±0.03	7.93±0.04
F2	12.90±0.1	8.06±0.06
F3	11.29±0.2	7.57±0.05
F4	10.93±0.07	7.81±0.03
F5	9.52±0.1	6.55±0.05
F6	9.0±0.1	6.34±0.04

Folding endurance

The folding endurance value for all films was > 250; it indicates that all formulations had ideal film properties.

Table 6: Measurement of folding endurance.

Formulation code	Folding endurance*(Mean±SD)
F1	317±0.57
F2	309±1.0
F3	297±0.57
F4	304±1.0
F5	294±1.0
F6	296±0.57

Surface pH

Surface pH of all the formulations (2 and 4 hour crosslinked films) was determined as described in the methodology chapter. All the formulations were found to have pH between 6 –7. This reveals that the prepared films would not alter the pH of the gingival fluid in the periodontal pocket and therefore may not cause any irritation.

Table 7: Measurement of Surface pH.

Formulation code	Surface pH*(Mean±SD) (2 hour crosslinked films)	Surface pH*(Mean±SD) (4 hour crosslinked films)
F1	6.71±0.07	6.67±0.12
F2	6.74±0.05	6.73±0.07
F3	6.87±0.03	6.78±0.09
F4	6.80±0.08	6.70±0.16
F5	6.74±0.09	6.58±0.05
F6	6.65±0.07	6.72±0.19

Swelling Index

Swelling index of all formulations (2 and 4 hour crosslinked films) were calculated and it was in the range of 32.4%-38.4% and 24.3%-33.1% respectively. Dental Film containing more concentration of sodium alginate showed maximum swelling property than the other films. As the concentration of polymer increased, swelling index also increased. Swelling is very essential before the drug is released from the dosage form.

Table 8: Measurement of swelling index.

Formulation code	Swelling index*(%)(Mean±SD) (2 hour crosslinked films)	Swelling index*(%)(Mean±SD) (4 hour crosslinked films)
F1	38.4±0.6	33.1±0.5
F2	35.6±0.4	31.2±0.7
F3	34.6±0.7	29.5±0.9
F4	35.2±0.3	26.8±0.2
F5	33.9±0.8	25.7±0.4
F6	32.4±0.7	24.3±0.4

Drug content uniformity

The percentage drug content in various formulations (2 and 4 hour crosslinked films) ranged from 80.30% – 93.68% and 78.81% - 90.7% respectively given in table 9. It was observed from the drug content data that there was no significant difference in the uniformity of the drug content.

However, when compared with the theoretical drug content the estimated drug content was slightly less; it may be due to the drug loss during fabrication of the films. The percentage of drug content in the 2 hour crosslinked formulations ranked in the order F2>F3>F4>F1>F5>F6, whereas for 4 hour crosslinked formulations it is in the order of F2>F1>F3>F5>F4>F6.

Table 9: Measurement of percentage drug content.

Formulation code	% Drug content*(Mean±SD) (2 hour crosslinked films)	% Drug content*(Mean±SD) (4 hour crosslinked films)
F1	87.73±0.29	89.96±0.47
F2	93.68±0.52	90.7±0.46
F3	91.45±0.11	88.48±0.08
F4	89.22±0.99	81.78±0.92
F5	84.0±0.96	82.53±0.35
F6	80.30±0.16	78.81±0.19

3.3.1. *In-vitro* drug release

The releases of Satranidazole from the dental films were varied according to the concentration and time taken for crosslinking of polymer. The release of the drugs from both the 2 and 4 hour crosslinked film formulations ranked respectively in the order F2 > F1 >F3>F4>F5>F6 and F2>F1>F3>F4>F5>F6. The amounts of the drug released after 4days for both the time were 79.12%, 77.12%, 74.77%, 72.12%, 65.55%, 57.36% and 65.29%, 62.62%, 57.87%, 57.38%, 54.30%, 52.48% respectively. The cumulative % drug release profile of all the formulation batches has been shown in table 10 and 11. Graphs are plotted between cumulative % drug releases versus time as shown in Figures 5-8.

Table 10: Percentage cumulative drug release of formulations crosslinked with 2% v/v glutaraldehyde for 2 hour.

Time (Days)	% Cumulative drug release*(Mean±SD) (2 hour crosslinked films)					
	F1	F2	F3	F4	F5	F6
1	24.09±0.79	24.0±0.30	23.77±0.12	26.85±0.15	23.71±0.29	22.34±0.40
2	56.14±0.86	58.76±0.12	56.55±0.33	58.99±0.12	53.41±0.46	44.04±0.16
3	69.37±0.51	70.96±0.04	70.98±0.02	70.25±0.29	62.93±0.17	52.82±0.18
4	77.12±0.24	79.12±0.12	74.77±0.23	72.12±0.43	65.55±0.42	57.36±0.64

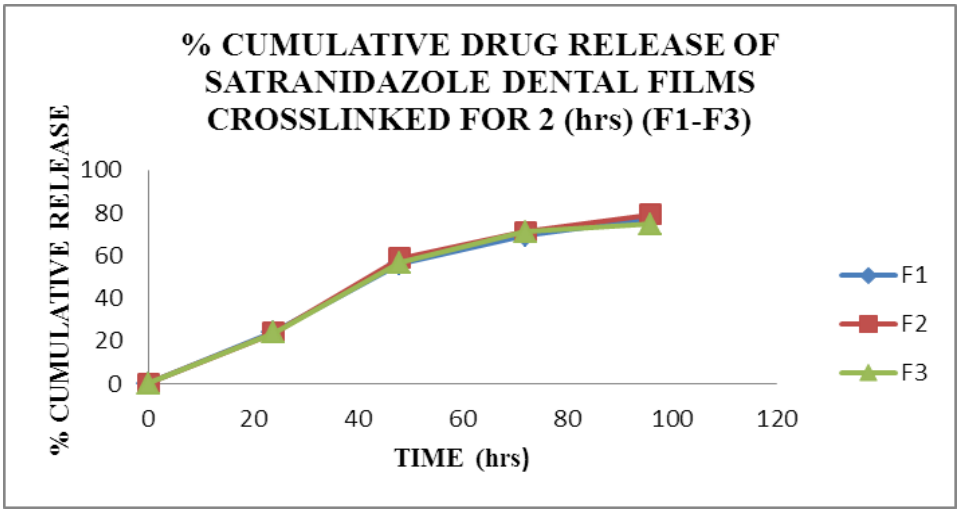


Fig.5. Comparison of *in-vitro* % cumulative release profile of Dental films F1-F3.

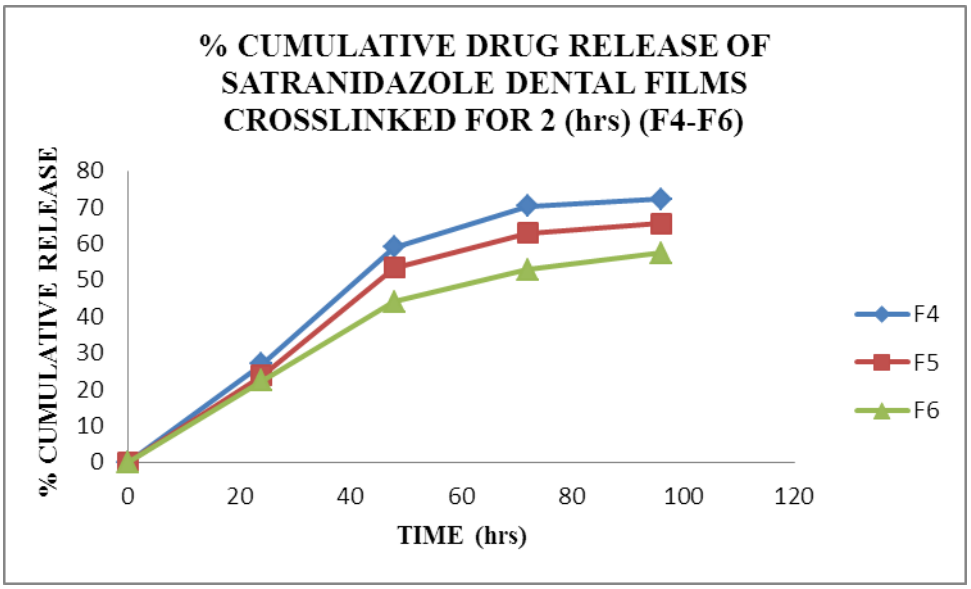


Fig.6. Comparison of *in-vitro* % cumulative release profile of Dental films F4-F6.

Table 11: Percentage cumulative drug release of formulations crosslinked with 2% v/v glutaraldehyde for 4 hour.

Time (Days)	% Cumulative drug release*(Mean±SD) (4 hour crosslinked films)					
	F1	F2	F3	F4	F5	F6
1	22.30±0.30	21.57±0.46	14.08±0.19	15.54±0.46	23.14±0.38	16.60±0.40
2	46.77±0.23	49.75±0.25	35.90±0.22	37.40±0.20	37.75±0.37	36.18±0.31
3	53.18±0.38	59.33±0.61	51.02±0.15	52.23±0.46	47.92±0.21	46.04±0.25
4	62.62±0.32	65.29±0.33	57.87±0.15	57.38±0.50	54.30±0.34	52.48±0.52

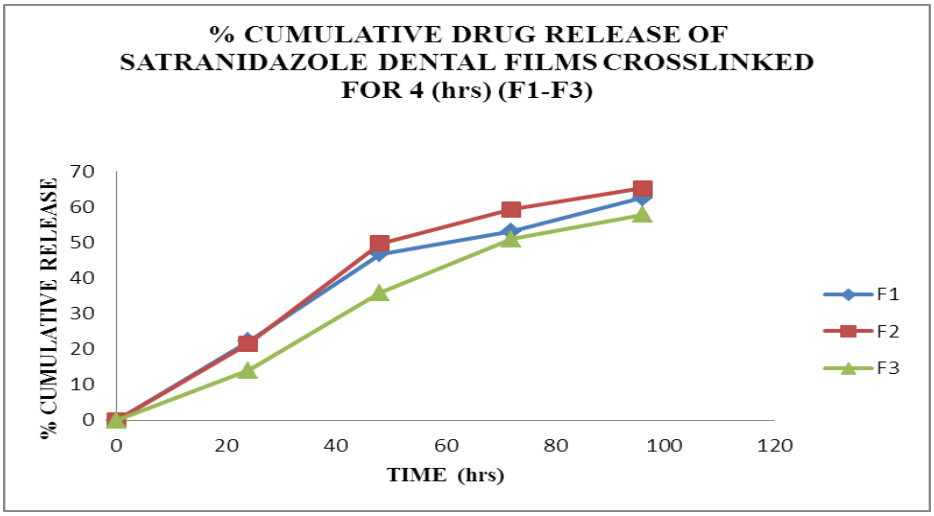


Fig.7. Comparison of in-vitro % cumulative release profile of Dental films F1-F3.

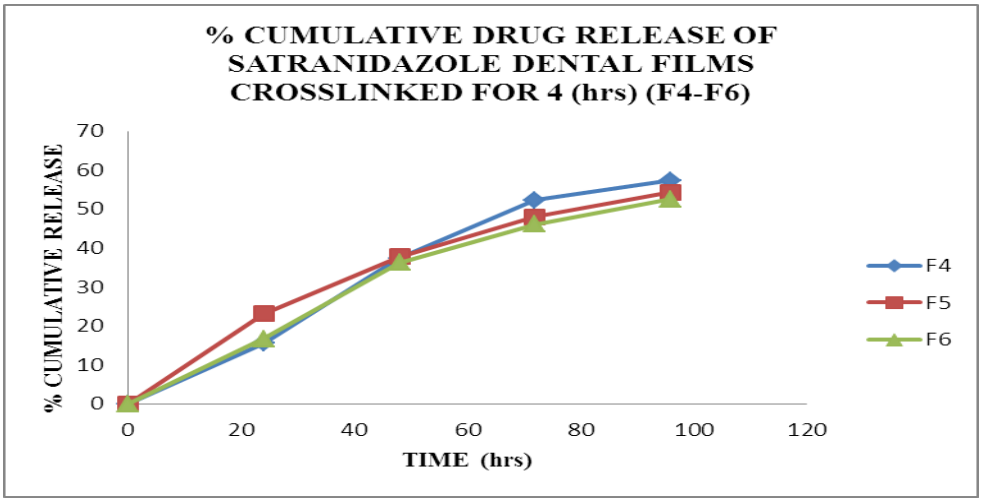


Fig.8. Comparison of in-vitro % cumulative release profile of Dental films F4-F6.

3.3.2. Mass balance study

This shows how much amount of drug remains in the film after 4 days of release. The values obtained from the in-vitro release studies and mass balance studies indicated that the drug content did not that much differs from the experimental drug content.

Table 12: Determinations of Mass balance study.

Formulation code	% Massbalance*(Mean±SD) (2 hour crosslinked films)	% Massbalance*(Mean±SD) (4 hour crosslinked films)
F1	14.71±0.18	22.14±0.21
F2	16.74±0.17	23.76±0.18
F3	16.30±0.07	25.92±0.19
F4	19.0±0.16	23.59±0.20
F5	20.12±0.07	23.39±0.15
F6	20.15±0.07	23.59±0.05

3.3.3. *In-vitro* permeation studies of satranidazole loaded films

In-vitro permeation study is carried out on the best formulation F2 (crosslinked with 2% v/v glutaraldehyde solution for 2 hour). This study shows how much amount of drug permeated through the mucosa after 48 hours of release. The values obtained from the study depicited that the % cumulative drug release did not that much differs from the *in-vitro* dissolution study of optimised formulation F2 (2 hour crosslinked). At the same time the optimised film showed a well extended release, when compared to that of the control (standard drug solution).

Table 13: *In-vitro* comparison of percentage cumulative drug release of selected formulation F2 (2 hour crosslinked) with control.

Time (hours)	% Cumulative drug release*(Mean±SD)	
	F2 (2 hour crosslinked films)	Control (standard drug solution)
1	3.19±0.19	41.64±0.36
2	5.24±0.32	65.97±0.21
3	7.11±0.35	76.46±0.54
4	9.33±0.20	81.43±0.53
5	10.06±0.21	82.50±0.43
6	11.17±0.17	83.02±0.13
24	23.89±0.19	83.63±0.16
48	55.12±0.74	83.77±0.12

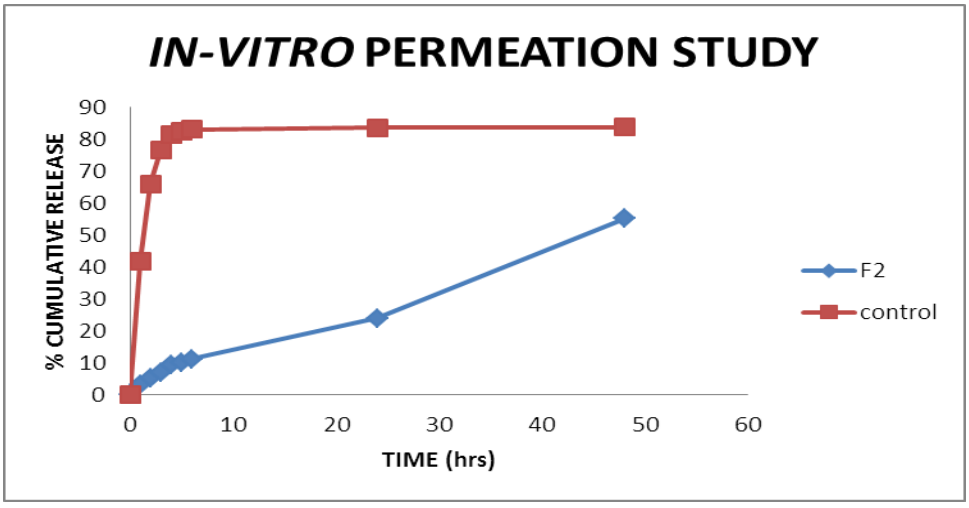


Fig.9. Comparison of % cumulative drug release between F2 (2hour crosslinked film) and control by *in-vitro* permeation study.

3.3.4. Scanning Electron Microscopy (SEM)

Scanning electron micrographs indicate that prior to drug release, the top surface of the films is smooth which exhibits pores as can be seen in Figure 10. These pores indicated that the

film F2 (2 hour crosslinked) had sufficient drug loading capacity. After the release of the drug, the surface became more rough and irregular as can be seen in Figure 11. The reason for this is the coagulation of polymers after the release of the drug from the pores.

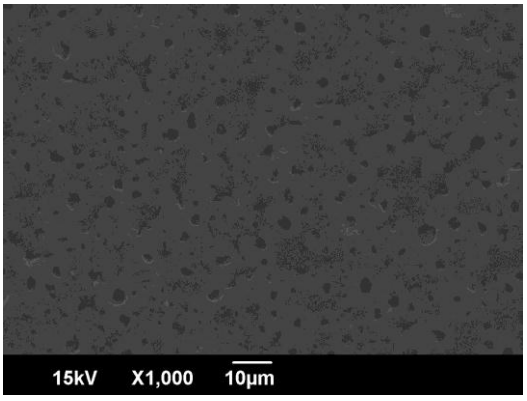


Fig.10. SEM images of dental film F2 (2 hour crosslinked) before drug release.

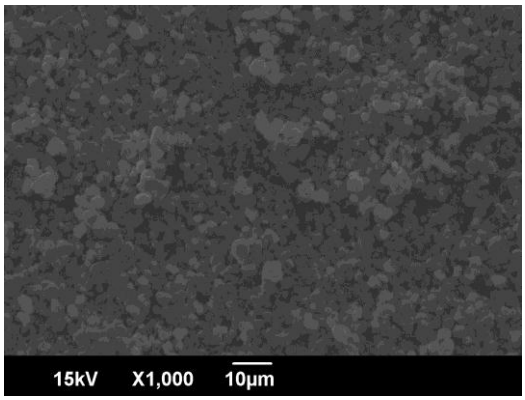


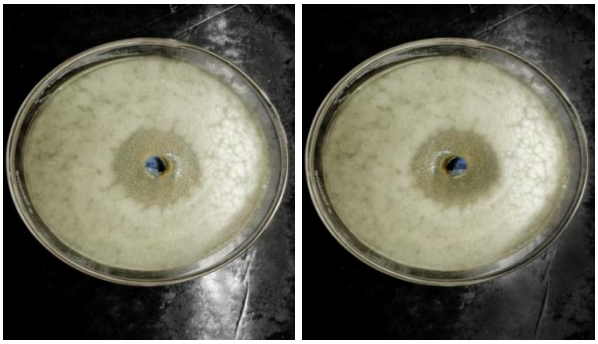
Fig.11. SEM images of dental film F2 (2 hour crosslinked) after drug release.

3.3.5. *In-vitro* antibacterial study

In-vitro antibacterial activity was performed as mentioned in methodology on *S.aureus* and *E.coli* organisms. The results of antibacterial activity were shown in table 14. The study indicates that the formulated polymeric film F2 (2 hour crosslinked) containing Satranidazole retained their antibacterial activity and also it shows the same effect as the standard Satranidazole drug.

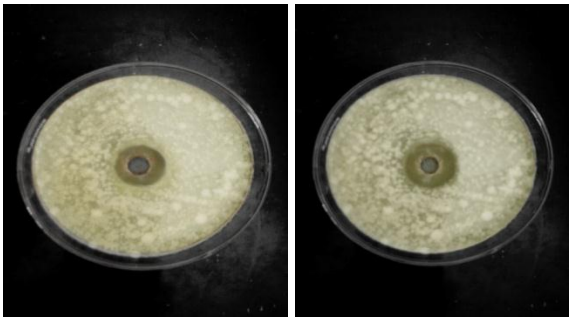
Table 14. Measurement of Zone of Inhibition using *E.coli* and *S.aureus.*

ORGANISM	SAMPLE	ZONE OF INHIBITION(mm)
E.coli	Pure drug	25
	Dental film F2	25
S.aureus	Pure drug	38
	Dental film F2	40



Control **F2 (2 hour crosslinked film)**

Fig.12. Antibacterial zone with *S.aureus* (with control and F2).



Control **F2 (2 hour crosslinked film)**

Fig. 13. Antibacterial zone with *E.coli* (With control and F2).

3.3.6. Stability studies

The selected formulation F2 (2 hour crosslinked film) was subjected to stability testing. Changes in the appearance, surface pH, folding endurance and drug content of the stored films were investigated at a period of 10 days and after 1 month. The folding endurance and drug content of the formulation were found to be decreasing but there was no much deviation from the original value. The results at different temperatures are shown in tables 15 & 16.

Table 15: Stability study data at 40±2°C/75±5%RH.

Time(days)	Physical appearance	Folding endurance	Surface pH	% Drug content
0	No changes	309±1.0	No change	91.20±0.78
10	Slight changes	264±1.0	No change	87.18±0.29
20	Slight changes	257±0.57	No change	84.96±0.14
30	Slight changes	253±1.0	No change	83.76±0.32

Table 16: Stability study data at 25±2°C/60±5%RH.

Time(days)	Physical appearance	Folding endurance	Surface pH	% Drug content
0	No changes	303±0.57	No change	88.56±0.57
10	No changes	299±1.0	No change	87.72±0.42
20	No changes	288±1.0	No change	85.22±0.35
30	No changes	282±0.57	No change	84.45±0.62

4. CONCLUSION

The present examination manages the improvement of Satranidazole stacked crosslinked dental films that can be utilized for local supported medication conveyance framework into the contaminated periodontal area, utilizing polymers. The biodegradable polymer gelatin is most appropriate alongside a co-polymer sodium alginate in various proportions for the treatment of periodontitis. Various evaluation studies were conducted for selecting the best formulation. Materials were at first subjected to different preformulation contemplates. All characters of the immaculate medication conformed to the norms. FTIR studies demonstrated that there was no interaction between drug and polymers. Satranidazole loaded crosslinked dental films were formulated with appropriate biodegradable polymers in different ratios and were subjected to various physicochemical studies.

- Measurement of thickness and consistency of weight ponders showed that there was no much contrast in thickness and weight within formulations.
- Percentage moisture loss studies showed that F2 (2 hour crosslinked film) has highest moisture loss whereas F6 (4 hour crosslinked film) has minimum % moisture loss. This may be due to the concentration increase of gelatine, since gelatine has low moisture content. From this examination it was also discernible that all the crosslinked films of 4 hour indicated lower moisture loss than 2 hour crosslinked films and it might be because of more prominent minimization and lower porosity of the 4 hour crosslinked films.
- Folding endurance studies guarantee that all the formulations had good film properties. Films exhibiting folding endurance above 300 indicate that they are tough and flexible.
- The pH of all the formulations was in the scope of 6.58 to 6.87, which lies in the typical pH range of the periodontal mucosa and would not create any mucosal bothering.
- Swelling index showed extreme swelling for the films made with more concentration of sodium alginate. As the convergence of polymer expanded, the swelling index additionally expanded.

- The formulation F2 (2 hour crosslinked film) indicated similarly high Percentage drug content than the others. This demonstrates the uniform and homogenous dissemination of medication all through the film which might be because of high ensnarement of drug in the polymer extent of 1:1.
- *Invitro* release studies indicated a cumulative release of 79.12% for 2 hour crosslinked film F2, after 4 days of medicament release. Optimum proportion of polymer and copolymer containing formulation ensures the best drug release.
- *Invitro* permeation studies showed a cumulative release of 75.74% for optimised film F2 (2 hour crosslinked film) revealed that the permeation of drug through the bovine mucosa exhibited slow and sustained permeation of the drug for 1 to 48 hours.
- The periodontal film F2 (2 hour crosslinked film) was chosen as the optimised formulation based on *invitro* release studies, *invitro* permeation studies, drug content and other physicochemical evaluation data since, it can integrate more quantity of drug and better drug release characters for 4 days.
- SEM analysis before drug release studies of this optimized film showed that the film have sufficient drug loading and release capability.
- In vitro antimicrobial studies carried out on F2 (2 hour crosslinked film) formulation signified that the drug can hold its antibacterial property in film form also. The activity of the satranidazole loaded film and pure drug were identical.
- Stability studies denoted that the formulation F2 (2 hour crosslinked film) is stable in different temperatures.

The above results validate the advantage of using satranidazole for the therapy of periodontitis in the form of films made up of gelatin as the main biodegradable polymer and sodium alginate as the copolymer in an optimized concentration for sustained release of drug. The major advantages of this dental film results in aggregation of drug at its site of action, which lowers possible side effects and enhance the pharmacological benefits for the extended period of time. It increases patient compliance and reduce the systemic side effects of conventional therapy. Crosslinking with 2% v/v glutaraldehyde had a defined credit on the release rate of the medication. At long last, bioerodable type of crosslinked dental films, loaded with satranidazole was effectively created and that could be easily placed in the unhealthy region and be capable of delivering therapeutic concentration of drug for extended period at a controlled rate with lower dose.

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